The ISME Journal (2012) 6, 1775–1785

www.nature.com/ismej

ORIGINAL ARTICLE

Changes in coral microbial communities in response to a natural pH gradient

Dalit Meron[1,2], Riccardo Rodolfo-Metalpa[3], Ross Cunning[4], Andrew C Baker[4], Maoz Fine[1,5,6] and Ehud Banin[1,2,6]

[1]*The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel;* [2]*The Institute for Nanotechnology and Advanced Materials, Bar-Ilan University, Ramat Gan, Israel;* [3]*Marine Institute, Marine Biology and Ecology Research Centre, University of Plymouth, Plymouth, UK;* [4]*Division of Marine Biology and Fisheries, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL, USA and* [5]*The Interuniversity Institute for Marine Science in Eilat, Eilat, Israel*

Surface seawater pH is currently 0.1 units lower than pre-industrial values and is projected to decrease by up to 0.4 units by the end of the century. This acidification has the potential to cause significant perturbations to the physiology of ocean organisms, particularly those such as corals that build their skeletons/shells from calcium carbonate. Reduced ocean pH could also have an impact on the coral microbial community, and thus may affect coral physiology and health. Most of the studies to date have examined the impact of ocean acidification on corals and/or associated microbiota under controlled laboratory conditions. Here we report the first study that examines the changes in coral microbial communities in response to a natural pH gradient (mean pH_T 7.3–8.1) caused by volcanic CO_2 vents off Ischia, Gulf of Naples, Italy. Two Mediterranean coral species, *Balanophyllia europaea* and *Cladocora caespitosa*, were examined. The microbial community diversity and the physiological parameters of the endosymbiotic dinoflagellates (*Symbiodinium* spp.) were monitored. We found that pH did not have a significant impact on the composition of associated microbial communities in both coral species. In contrast to some earlier studies, we found that corals present at the lower pH sites exhibited only minor physiological changes and no microbial pathogens were detected. Together, these results provide new insights into the impact of ocean acidification on the coral holobiont.
The ISME Journal (2012) **6,** 1775–1785; doi:10.1038/ismej.2012.19; published online 22 March 2012
Subject Category: microbial ecology and functional diversity of natural habitats
Keywords: holobiont; pH; ocean acidification; bacteria; coral; microbial community

Introduction

Corals comprise a dynamic and highly diverse consortium of microorganisms, including bacteria, archaea, fungi, viruses, endolithic autotrophs and symbiotic dinoflagellates, and as such represent the paradigm of a holobiont (Rohwer *et al.*, 2002; Bourne and Munn, 2005; Rosenberg *et al.*, 2007; Wegley *et al.*, 2007). Interactions among coral associates influence holobiont physiology and health. The diversity and distribution of host–symbiont relationships are altered by environmental changes such as increased temperature (Banin *et al.*, 2000; Ben-Haim *et al.*, 2003; Bourne *et al.*, 2007), nutrient enrichment (Szmant, 2002; Garren *et al.*, 2009) and diseases (Sunagawa *et al.*, 2009). Such alterations in the coral resident microbial community structure may either present as a stress response, or facilitate more rapid and versatile adaptation of the holobiont to changes in environmental conditions. The latter supposition is termed the 'probiotic hypothesis' (Reshef *et al.*, 2006) and should be considered when examining the influence that environmental changes have on corals.

One environmental change extensively researched in recent years is the projected increase in atmospheric CO_2. Atmospheric CO_2 partial pressure (pCO_2) has been increasing since the pre-industrial period, presently exceeds 380 p.p.m., and is expected to increase to 700 p.p.m. or more by the end of this century (IPCC, 2007). One of the direct outcomes of rising atmospheric pCO_2 is ocean acidification. In light of these atmospheric CO_2 projections, global ocean models predict surface pH reductions of 0.2–0.4 pH units by 2100 (Caldeira and Wickett, 2005).

Changes in ocean pH are expected to impact marine ecosystems and cause shifts in community structure (Ishimatsu *et al.*, 2005; Bibby *et al.*, 2007; Hoegh-Guldberg *et al.*, 2007; Kuffner *et al.*, 2007; Martin *et al.*, 2008), especially among calcifying

Correspondence: E Banin, Bar Ilan University, The Mina and Everard Goodman Faculty of Life Sciences, Institute for Advanced Materials and Nanotechnology, Ramat Gan, Israel 52900.
E-mail: banine@mail.biu.ac.il
[6]These authors contributed equally to this work.
Received 18 August 2011; revised 21 December 2011; accepted 10 February 2012; published online 22 March 2012

organisms (Gattuso *et al.*, 1998; Orr *et al.*, 2005). Several studies have shown that reduced pH produces a variety of physiological effects in the holobiont, such as altered dinoflagellate productivity (Crawley *et al.*, 2010), increased susceptibility to bleaching (Anthony *et al.*, 2008) and decreased coral calcification (Fine and Tchernov, 2007; Anthony *et al.*, 2008). However, other studies have reported no change, or even an increase, in coral calcification in response to lower pH (Ries *et al.*, 2009; Jury *et al.* 2010; Rodolfo-Metalpa *et al.*, 2010).

Bacterial associates are also expected to respond to changes in pH. Indeed, studies examining the response of coral-associated microbial communities to decreased pH conditions (Vega-Thurber *et al.*, 2009; Meron *et al.*, 2011) have reported changes in bacterial species composition. Specifically, these studies have found an increase in bacteria belonging to families that include known coral pathogens, such as *Vibrionaceae* and *Alteromonadaceae* (Bourne and Munn, 2005; Ritchie, 2006; Arboleda and Reichardt, 2009; Sunagawa *et al.*, 2009; Meron *et al.*, 2011), as well as families previously isolated from diseased, injured or stressed marine invertebrates, such as *Rhodobacteraceae* (Meron *et al.*, 2011). In addition, increased levels of secondary metabolites and stress resistance factors (for example, antibiotics and toxins) were observed at low pH (Vega-Thurber *et al.*, 2009; Meron *et al.*, 2011), suggesting that the coral holobiont exhibits a stress response to reduced pH.

These previous studies were all done under controlled laboratory conditions. Here, we used a natural pH gradient off the coast of Ischia (Gulf of Naples, Italy) to examine the impact of pH on the coral microbial community. This gradient results from a natural marine CO_2 flux from volcanic vents (Hall-Spencer *et al.*, 2008). We monitored the response of the microbial communities of two Mediterranean coral species, *Balanophyllia europaea* and *Cladocora caespitosa*, to this pH gradient. Our results provide insight into the potential effects of predicted ocean acidification on corals, at both the holobiont and community level.

Materials and methods

Experimental design

The experiment was carried out in Castellod' Aragonese off Ischia Island, Italy (40°043.84′N; 013°57.08′E) (Supplementary Figure S1), where volcanic CO_2 emissions from the seabed acidify a large shallow-water area. The vent gas comprises 90–95% CO_2, 3–6% N_2, 0.6–0.8% O_2, 0.2–0.8% CH_4 and 0.08–0.1% Ar (no sulfur) (Hall-Spencer *et al.*, 2008). The experimental setup was designed to compare ambient pH (site C, pH 8.1) with three reduced pH sites: site B1 (mean pH_T 7.38, min 6.83), site B2 (mean pH_T 7.53, min 7.10) and site B3 (mean pH_T 7.77, min 7.22). See Supplementary Table S1 for additional data and carbonate chemistry.

In March 2008, 28 polyps of *B. europaea* and 12 colonies of *C. caespitosa* at 3–7 m depth were collected from around Castello Aragonese. These corals do not occur normally in the area of the vent plume. The corals were transported to a nearby laboratory (Stazione Zoologica A. Dohrn), where they were maintained in flow-through aquaria continuously supplied with seawater pumped from 3 m depth at ambient temperatures. The turnover rate of seawater in the 150-l aquaria was 30% h^{-1}. Light irradiance of $\sim$100 μmol photons m^{-2} s^{-1} was provided by True-Lite (NRG Saver Supply, Henderson, TX, USA) and Sunlux (Sunlux Technologies, Bangalore, India) fluorescent tubes, and measured using an Li-Cor 4π spherical underwater quantum sensor (LI-193SA; Lambda Instruments, Lincoln, NE, USA). The photoperiod was 12:12 h light/dark. After 4 days recovery, samples were secured using epoxy glue (HoldFast, Cleveland, OH, USA) onto individual tagged plastic plates (4–6 × 4–6 cm^2).

Seven *B. europaea* polyps and three colonies of *C. caespitosa* were transplanted to 3–4 m depth at each test site (sites B1-B3) on the south side of the Castello near the CO_2 vents, where pH_T were reported to vary from 7.2–7.9 units (Hall-Spencer *et al.*, 2008). In addition, seven *B. europaea* polyps and three colonies of *C. caespitosa* were transplanted to a reference site 100–150 m away from the vents where the pH was normal (Site C, mean pH_T 8.0–8.2). In summary, two PVC plates (30 × 60 cm) were positioned at each of the four sites, and each plate was considered an experimental block (block A and B). Samples were collected after 7 months and frozen immediately in liquid nitrogen for subsequent molecular work, or brought back to the laboratory as live animals for further analysis.

Measurements of seawater carbonate chemistry

Seawater temperature was measured at 15 min intervals in 2008 and 2009 using Hobo Onset loggers positioned near the coral sites. Total alkalinity (A_T) and pH in total scale (pH_T) were measured frequently *in situ* (Supplementary Table S1). Water samples were collected in glass bottles next to the transplants or in aquaria, and the pH_T was immediately measured using a meter accurate to 0.01 pH units (Methrom pH mobile) calibrated using TRIS/HCl and 2-aminopyridine/HCl buffer solutions (Dickson *et al.*, 2007). Seawater samples were then passed through 0.7 μm pore size filters (GF/F Whatman) and treated with 0.05 ml of 50% $HgCl_2$ (Merck, Analar, Whitehouse, NJ, USA), and stored in the dark at 4 °C pending analysis. Three replicate 20 ml sub-samples were analyzed at 25 °C using a titration system composed of a pH meter with a Methrom pH electrode and a 1-ml automatic burette (METHROM, Herisau, Switzerland). pH was measured at 0.02 ml increments of 0.1 N HCl. A_T was calculated from the

Gran function applied to pH variations from 4.2 to 3.0, as mEq l^{-1} from the slope of the curve HCl volume versus pH. Titrations of total alkalinity standards provided by A.G. Dickson (batch 99 and 102) were within 0.7 μmol kg^{-1} of the nominal value. Parameters of the carbonate system (pCO_2, CO_3^{2-}, HCO_3^-, C_T and saturation state of calcite (Ω_c) and aragonite (Ω_a)) were calculated from pH_T, mean A_T, temperature and mean salinity (38) using the free-access CO_2sys package. A_T and salinity were virtually constant throughout.

Chlorophyll fluorometry, protein and chlorophyll concentration assays

To study the photosynthetic capacity of symbiotic dinoflagellates we used an imaging pulse amplitude modulated fluorometer (Walz GmbH, Effeltrich, Germany). After collection, each sample was dark-adapted for 20 min before various parameters of individual polyps were assayed: the maximum quantum yield of photosystem II (F_v/F_m), electron transport rate (ETR) and non-photochemical quenching (NPQ). Maximum quantum yield of photosystem II was calculated using the Genty equation (Genty *et al.*, 1989): $F_v/F_m = (F_m - F_0)/F_m$.

After taking these measurements, all fragments were collected, frozen and kept in the dark. Subsequently, the coral tissue was removed using an air brush with filtered seawater. The slurry was homogenized using a DIAX 100 homogenizer (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) for 10 s before centrifugation at 4000 g for 5 min. The dinoflagellate pellet was re-suspended, and aliquots removed for cell counts and chlorophyll measurements. Chlorophyll was extracted in 100% acetone at 4 °C for 5 h, and chlorophyll *a* measured using a spectrophotometer (Ultrospec 2100 pro, GE Bioscience, Maryland Heights, MO, USA) and appropriate equations (Jeffrey and Humphrey, 1975). Cell counts were obtained from images obtained from a digital camera (Digital Sight, Nikon, Tokyo, Japan) attached to a microscope (D-Eclipse, Nikon), and image analysis software (MATROX Inspector 2.1, Matrox Imaging, Dorval, QC, Canada) was used to count cells in each of four fields per sample. Chlorophyll concentration per cell was calculated by dividing the total chlorophyll by the counted *Symbiodinium* cells. Total protein concentration and host protein concentrations were analyzed using the Quick Start Bradford Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA). Optical density at 595 nm was read using a spectrophotometer (Ultrospec 2100 pro, GE Bioscience). Owing to the morphology of the studied species (a single polyp in *B. europaea* and phaceloid in the case of *C. caespitose*) the number of symbiotic dinoflagellates and protein concentration were normalized per polyp. The differences in chlorophyll fluorometry, protein and chlorophyll concentration between pH treatments were tested by one-way analysis of variance (ANOVA) and *post-hoc* Tukey's HSD test.

Coral fractionation and DNA extraction

Corals were separated into tissue and skeleton fractions; the tissue was extracted from the coral using an air brush and centrifuged at 9300 *g* for 15 min. To minimize any cross contamination of tissue in the skeleton fraction, the skeletons were repeatedly cleaned and washed with sterile seawater, before being crushed with a mortar and pestle. For each pH treatment, the replicates of the separated fraction (that is, tissue or skeleton) were pooled together, homogenized and then DNA was extracted using the UltraClean Soil DNA kit according to the manufacturer's guidelines (MoBio, Carlsbad, CA, USA).

PCR amplification of 16S rRNA genes, clone libraries and sequencing

Bacterial primers 8F and 1492R (Lane, 1991) were used to amplify the 16S rRNA gene from the various fractions. 16S rRNA genes were amplified in a 25 μl reaction mixture comprising 2.5 μl of 10 × buffer, 2.5 μl of dNTP mixture (2.5 mM), each primer at 5 μM, 1 ng of template DNA, and 2.5 U of Dream Taq DNA polymerase (Fermentas, International Inc., Burlington, ON, Canada). Amplification conditions for the PCR included an initial denaturation step of 94 °C for 3 min, followed by 29 cycles of 94 °C for 35 s, 56 °C for 35 s, and 72 °C for 1.5 min and a final extension step of 72 °C for 7 min. Reaction products were checked for size and purity on a 1% agarose gel. PCR products from tissue and skeleton samples were ligated into the pCRII-TOPO-TA cloning vector (Invitrogen, Madison, WI, USA). Cloning of the 16S rRNA libraries and sequencing was carried out at The Genome Center (Washington University, St Louis, MO, USA) using the reverse primer 907R (5′-GTCAATTCMTTTGA GTTT-3′), as described by Lane *et al.* (1985). The nucleotide sequence data for clones reported in this paper were deposited in the GenBank nucleotide sequence database under accession numbers JQ235845–JQ236575.

Phylogenetic and statistical analysis

Operational taxonomic unit (OTU) analysis of the sequences was done using MOTHUR software (http://www.mothur.org) (Schloss *et al.*, 2009), rarefaction and Shannon–Wiener index of diversity were calculated. In addition, representative sequences of the OTUs classified by MOTHUR were compared with the NCBI database (National Center for Biotechnology Information; www.ncbi.nlm.nih.gov) and RDP Classifier (Ribosomal database project; http://rdp.cme.msu.edu) using the BLASTn algorithm (Wang *et al.*, 2007). Phylogenetic trees were calculated using the neighbor-joining method and constructed using the ARB phylogenetic program software (Ludwig *et al.*, 2004). The extent of similarity between the clone libraries obtained from

each fraction was assessed by Unifrac analysis using *Unifrac P-value* (Lozupone *et al.*, 2006). The Correlation and regression analyses were formed using STATISTICA (version 7.1) software (Stat Soft Inc., Tulsa, OK, USA).

Symbiodinium *identification*

Dinoflagellate *Symbiodinium* were identified using denaturing gradient gel electrophoresis (DGGE) of the internal transcribed spacer-2 region of ribosomal DNA (rDNA). Briefly, the ITS-2 region was amplified using the primers 'ITSintfor2' and 'ITS2clamp' (LaJeunesse and Trench, 2000) with the following thermal cycling conditions: an initial denaturation step of 94 °C for 3 min followed by 35 cycles of 1 min at 94 °C, 1 min at 55 °C and 1 min at 74 °C. Amplification success was confirmed by gel electrophoresis on a 1.5% agarose gel. Subsequently, products were separated by DGGE (35–75% gradient) using a CBS scientific system and visualized, after SYBR gold staining, using a UV transilluminator. Dominant bands were excised from the gel and reamplified using the same primer set without the GC clamp. The resulting amplicons were purified and sequenced using BigDye Terminator v3.1 cycle sequencing kit and an Applied Biosystems 3130xl Genetic Analyzer (Foster City, CA, USA). Sequences were identified using the NCBI database and the BLASTn algorithm.

Results

Photophysiology of B. europaea *and* C. caespitosa *along the pH gradient*

All coral fragments from both species survived collection, recovery, transplantation and experimentation without exhibiting any visual signs of disease. At the end of the experiment, both species at the reference site exhibited similar F_v/F_m values. The only variation observed was that of *C. caespitosa* in the low pH site (pH_T 7.3) that displayed lower F_v/F_m values (one-way ANOVA with *post-hoc* Tukey's HSD test $P<0.001$, Figure 1), no significant difference was observed for *B. eruopeae* (one-way ANOVA $P>0.05$). Similarly, no significant differences in ETR and NPQ were detected in either specie between the different pH sites (one-way ANOVA $P>0.05$, data not shown).

In *C. caespitosa*, chlorophyll concentration per *symbiodinium* cell was higher in the lower pH sites (pH_T 7.3 and 7.5) as compared with the higher pH site (pH_T 7.8) and the control site (pH_T 8.1). Statistically significant differences were detected between lower pH (pH_T 7.3) and pH_T 7.8 (one-way ANOVA, with *post-hoc* Tukey's HSD test ($P<0.05$), Figure 2a). *B. europeae* did not show such a change (one-way ANOVA, $P>0.05$), although the overall levels (mean 5.212E-06 ± 1.087E-06) were higher than those obtained for *C. caespitosa* (1.577E-06 ± 2.153E-07).

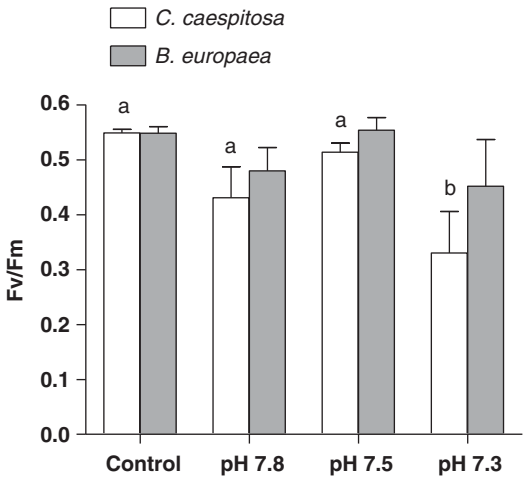

Figure 1 Maximum dark-adapted F_v/F_m values for *C. caespitosa* (gray) and *B. europaea* (white) exposed to a natural pH gradient. The reference site (C) is ~300 m from B1. Means were compared by one-way ANOVA, with *post-hoc* Tukey's HSD test. Different letters represent significant differences ($P<0.05$). Note that only in *C. caespitosa* a significant difference was observed.

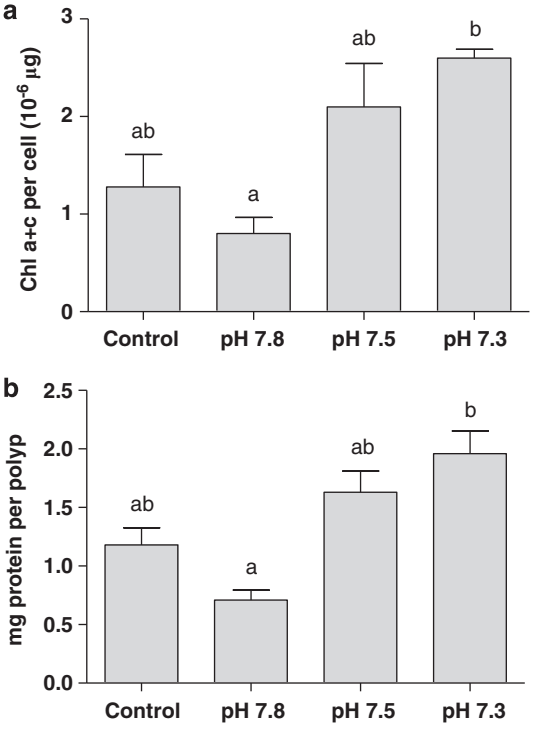

Figure 2 Chlorophyll levels (**a**) and protein concentrations (**b**) in *C. caespitosa* exposed to a natural pH gradient. Protein concentration was calculated per polyp, whereas the chlorophyll concentration was calculated per *symbiodinium* cell as described in Materials and methods. Data are expressed as means ± s.e.m. Means were compared by one-way ANOVA, with *post-hoc* Tukey's HSD test. Different letters represent significant differences ($P<0.05$).

Symbiodinium *identity and density in* B. europaea *and* C. caespitosa *across the* CO_2 *vent*

Symbiodiunium analysis revealed that *C. caespitosa* was found to be dominated by *Symbiodinium* B2 (that is, there were no other *Symbiodinium* taxa

detectable at the resolution of DGGE, which typically detects symbionts present at 5–10% of the total symbiont community (Thornhill *et al.*, 2006)). In contrast, *B. europaea* contained *Symbiodinium* B2 mixed with a novel *Symbiodinium* that was 95% similar to 'Mediterranean A' (Visram *et al.*, 2006; Hunter *et al.*, 2007).

Symbiodinium densities (cells per polyp) did not vary between the sites in both corals (one-way ANOVA, $P>0.05$). For *C. caespitosa* the average density was 1.81E+06 ± 2.6E+05 and for *B. europaea* average was 1.23E+07 ± 6.33E+05. It should be noted that a significant increase in protein concentration (mg per polyp) was observed for *C. caespitosa* taken from the site at the lowest pH (pH_T 7.3) compared with pH_T 7.8 (one-way ANOVA, with *post-hoc* Tukey's HSD test ($P<0.05$); Figure 2b).

Composition and diversity of bacterial communities associated with B. europaea *and* C. caespitosa *along the pH gradient*

To test whether the bacterial community associated with each coral species was influenced by pH, microbial DNA was isolated from the tissues and skeletons of *B. europaea* and *C. caespitosa* from each site, and 16S rRNA clone libraries were constructed and sequenced ($n=2652$). Rarefaction analyses were performed on the clone libraries to determine the number of unique bacterial clones as a proportion of the estimated total diversity within each library. The rarefaction curves (cutoff 97%) did not reach an asymptote (Supplementary Figure S2a, b), indicating the actual total diversity could be much higher. Nevertheless, pattern comparisons revealed significant differences in bacterial community composition between the two corals species (*Unifrac P-value,* $P<0.01$ (Figure 3). In general, there was a subdivision according to fraction, tissue versus skeleton, for each species, rather than pH, although these distributions were not found to be statistically significant (*UniFrac P-value,* $P>0.05$).

The composition and the diversity of the bacterial communities associated with the tissues and skeletons of the two corals at each site were examined in more detail by inspecting the 16S rRNA sequences in the clone libraries (Figures 4 and 5 and Supplementary Table S2–4). The first parameter examined was the Shannon–Wiener index, which measures species diversity (richness and evenness). The correlation between the pH sites and the Shannon–Wiener index values of each fraction was significant only in *C. caespitosa* skeleton ($r^2=0.968$, $P<0.05$) for which the Shannon–Wiener values decreased from 4.8 to 3.8 (ambient and lowest pH, respectively) (Supplementary Table S4).

When examining the composition of the bacterial community, 26 class groups were found associated with *C. caespitosa*. In all pH conditions, the three dominant groups were: *Alphaproteobacteria, Bacteroidetes* and *Gammaproteobacteria* (Figure 4 and

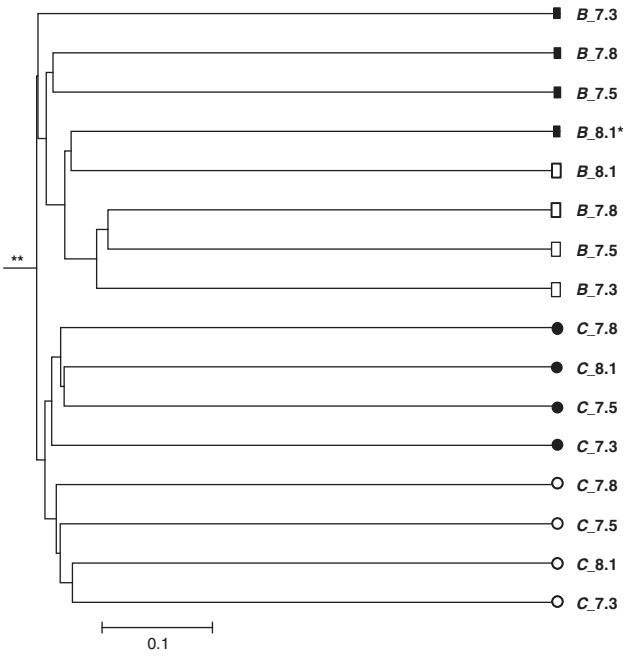

Figure 3 Cluster analysis of microbial community 16S rRNA clone libraries generated from the tissues and skeletons of *B. europaea* and *C. caespitosa* exposed to four pH conditions. Cluster analysis was performed by cluster environment using unifrac *P*-value (Unifrac Metric analysis) (Lozupone and Knight, 2005) $n=2652$. Squares represent the coral *B. europaea* (*B*), circles represent *C. caespitosa* (*C*), and the fractions are divided into tissue (black) and skeleton (white). *Note that 8.1 (microbes generated from *B. europaea* tissue exposed to ambient pH) clusters with *B. europaea* skeleton fraction communities. **Significant differences between the tree clusters. The bar represents a weighted UniFrac distance of 0.1.

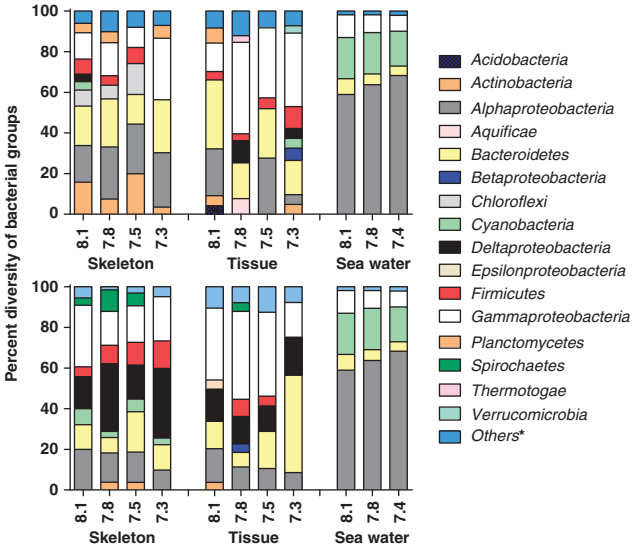

Figure 4 The impact of pH on the bacterial groups associated with tissue and skeleton composition and relative abundance of *C. aespitosa* and *B. europaea*, as well as the surrounding water. The chart was constructed based on the 16S rRNA gene sequences in clone libraries generated from the corals (top) *C. caespitosa* ($n=1354$), (bottom) *B. europaea* ($n=1298$) and sea water ($n=2029$). *Classes under 3%.

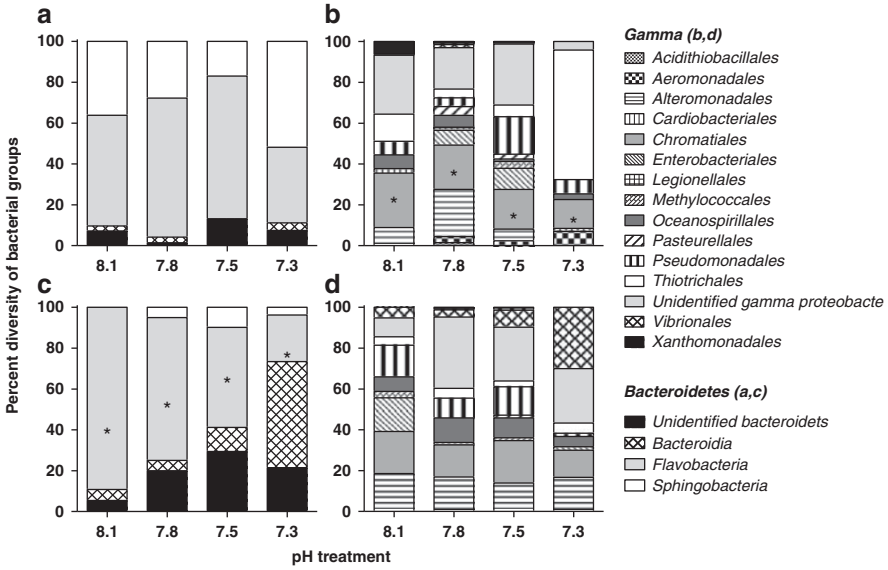

Figure 5 Changes in bacterial composition and relative abundance of the *Bacteroidetes* (left) and *Gammaproteobacteria* (right) groups associated with *C. caespitosa* (**a**, **b**) and *B. europaea* (**c**, **d**) exposed to four pH conditions. The chart was constructed using BLASTn algorithm and RDP Classifier. *Marks significant correlation between the group and pH.

Supplementary Table S2). Interestingly, a negative correlation was observed between the *Alphaproteobacteria* and *Deltaproteobacteria* ($r^2 = 0.917$, $P < 0.05$) and between *Gammaproteobacteria* and *Actinobacteria* ($r^2 = 0.924$, $P < 0.05$). In *Alphaproteobacteria,* (most of which belong to *Rhodobacterales*), the most abundant clones were most similar to coral-associated bacteria FJ952800 and sponge-associated bacteria AM259856 (Supplementary Table S3 and Supplementary Figure S3). *Flavobacteriales* and *Sphingobacteriales* were the main groups in the *Bacteroidetes*, and the abundant clones most closely match marine bacterium GU061250, sponge symbionts EF159908 and plankton bacterium (Supplementary Table S3 and Supplementary Figure S4). Within the *Bacteroidetes* classes, a negative correlation was observed between *Flavobacteria* and *Sphingobacteria* ($r^2 = 0.935$, $P < 0.05$). The abundance of *Flavobacteria* decreased (from 54.0% to 37.0%), whereas that of *Sphingobacteria* increased (from 36% to 52%) (Figure 5a).

Finally in the *Gammaproteobacteria*, the abundant clone was most similar to Mediterranean sponge bacterium AJ581351 (Supplementary Table S3 and Supplementary Figure S6). Although *Gammaproteobacteria* was one of the three dominant groups associated with *C. caespitosa* at each site, the abundance of this group was greater at the sites exposed to the CO_2 vent (13.4% of sequences at ambient pH versus 27.2%, 24% and 32.5% at pH_T 7.8, 7.5 and 7.3, respectively). The increased numbers of *Gammaproteobacteria* were mainly in the tissue fraction, although there was also a greater abundance in the skeleton fraction at pH_T 7.3 (Supplementary Table S2). Notably, there were changes in composition within the dominant groups. For example, within the *Gammaproteobacteria* group, the abundance of *Thiotrichales* was greater at the lowest pH (63.4%), such that it became the major group constituent, whereas *Alteromonadales* disappeared at the lowest pH. It is important to note that a significant correlation was observed between pH and *Chromatiales* whose abundance was reduced with decreasing pH ($r^2 = 0.954$, $P < 0.05$) (Figure 5b).

B. europaea was found to maintain 21 associated bacterial class groups. The four dominant groups were: *Alphaproteobacteria*, *Bacteroidetes, Deltaproteobacteria* and *Gammaproteobacteria* (Figure 4 and Supplementary Table S2). In *Alphaproteobacteria*, many clones belonged to the *Rhizobiales* subgroup, and the most abundant clones were most similar to the *Oculina patagonica* mucus bacterium AY654755 (Supplementary Table S3 and Supplementary Figure S3). A significant correlation between the *Alphaproteobacteria* group and decreasing pH was observed within the tissue fraction in which the abundance of the *Alphaproteobacteria* reduced with decreasing pH ($r^2 = 0.907$, $P < 0.05$) (Supplementary Table S2 and Figure 4). In the *Bacteroidetes* subgroup, the abundant clones displayed the most similarity to the *Paralvinella palmiformis* mucus bacterium AJ441239 and planktonbacterium AY167327 (Supplementary Table S3 and Supplementary Figure S4). The *Desulfovibrionales* and *Desulfobacterales* were the main groups in the *Deltaproteobacteria*, and the abundant clones most closely match *Desulfovibrio sp.* sediment bacteria DQ522111, GQ850564 and *Montipora sp.* bacterium AB470955 (Supplementary Table S3 and Supplementary Figure S5). The abundance of the *Gammaproteobacteria* group was lower at the sites exposed

to the CO_2 vent; at ambient pH it was 32.6%, whereas at pH_T 7.8, 7.5 and 7.3 it was 30.4%, 27.2% and 19.9%, respectively (Figure 4 and Supplementary Table S2). At lower pH the abundance of *Pseudomonadales* and *Enterobacteriales* subgroups was lower or undetectable, whereas the numbers of *Vibrionales* increased from 5% to 30%, at pH_T 8.1 versus 7.3, respectively (Figure 5d). In the *Gammaproteobacteria* group, the *Alteromonadales* and *Chromatiales* were the most dominant groups (Figure 5d). The abundant clones are most similar to marine bacteria GU061987 and GU061172 and uncultured *vibrio sp.* AY785255 (Supplementary Table S3 and Supplementary Figure S6). Unlike what was observed for *C. caespitosa,* only select groups increased in abundance at the sites exposed to the CO_2 vent. The best example of this was the *Bacteroidetes* group, the abundance of which increased from 12.8% (at ambient pH) to 19.2% and 26.3% (at pH_T 7.5 and 7.3, respectively). This increased abundance was most evident in the tissue fraction, increasing from 13.5% to 18.2% and 47.8%, at pH_T 8.1, 7.5 and 7.3, respectively (Table S2). Similar to what was seen for the *Bacteroidetes* group associated with *C. caespitosa*, there were changes in the composition of the *Bacteroidetes* group associated with *B. europaea*, specifically, in *Flavobacteriales* where a pH dependent correlation was found ($r^2 = 0.9737$, $P < 0.05$). The *Flavobacteriales* decreased in abundance from 89.2% at pH_T 8.1 to 70%, 49% and 22.8% at pH_T, 7.8, 7.5 and 7.3, respectively, whereas the *Bacteroidia* subgroup increased in abundance from 5% at ambient pH to 51.9% at pH_T 7.3 (Figure 5c).

Discussion

A predicted global change during the twenty-first century is an increase in CO_2 concentration in the atmosphere and oceans, leading to ocean acidification. The consequences of ocean acidification remain debated and more research is needed to better understand the impact of reduced pH on the ocean environment. A volcanic vent near Ischia Island (Italy) provides a unique natural environment with a pH gradient, enabling research into the influence of ocean acidification on marine organisms, including corals. In an earlier study, it was found that the abundance of organisms that deposit calcium carbonate skeletons declined significantly within vent areas (Hall-Spencer *et al.*, 2008). Corals did not occur naturally at the vent site. However, other organisms thrived, such as seagrasses and other macroalgae, which appear to have outcompeted corals in this environment. On the basis of these observations, it has been proposed that ocean acidification can reduce biodiversity and radically alter marine ecosystems (Hall-Spencer *et al.*, 2008).

The present study takes advantage of the same volcanic vents near Ischia Island to further understand the impact of changes in oceanic pH on various aspects of coral biology, including physiology, the type and density of symbiotic dinoflagellates, and the composition of associated bacterial communities. Notably, we observed that reef coral symbioses from the lower pH sites exhibited only minor changes in photophysiological parameters. Although F_v/F_m was consistently lower for *C. caespitosa* at lower pH compared with control sites (in agreement with the data of Hall-Spencer *et al.* 2008), this reduction was only significant at the lowest pH site (pH 7.3) (Figure 1). pH had even less impact on the F_v/F_m values for *B. europaea*, where none of the sites were significantly different from the controls. In addition, there was no detectable impact of pH on the ETR in either species, indicating that the thylakoid membranes were energetically coupled irrespective of pH. Similarly, NPQ was comparable at the four sites. It is possible that as the corals were collected in a low-light-intensity environment (turbulent and turbid waters) photosystem II was not stressed by high light intensities, and therefore no chronic photo-damage can be detected in any of the pH environments. Therefore, this study found that overall changes in ocean pH did not interfere greatly with the photosynthetic activity of these reef corals. These findings corroborate other studies reporting no changes in photosynthesis under ocean acidification conditions (Reynaued *et al.*, 2003; Schneider and Erez, 2006) or only slightly altered cytoplasmic pH values in response to external pH shifts of similar magnitude (Raven and Smith, 1980; Gimmler, 2000).

A higher protein concentration per polyp was noted for *C. caespitosa* at the lowest pH site (Figure 2b). Previous studies have shown an increase in host protein at reduced pH (Fine and Tchernov, 2007; Anthony *et al.*, 2008; Krief *et al.*, 2010). This may indicate a thicker tissue, which can serve as a barrier between external and internal environments, enabling calcification despite more acidic water conditions and protecting the symbiotic dinoflagellates in the coral gastrodermis. Several recent studies have also reported thicker tissue under lower pH conditions (Anthony *et al.*, 2008; Krief *et al.*, 2010; Fabricius *et al.*, 2011). Furthermore, recent boron measurements on the coral *C. caespitosa* held in aquaria at pH_T 7.8 and from CO_2 vents (Trotter *et al.*, 2011) showed that this species can adjust its internal pH allowing calcification in undersaturated seawater.

Examining the zooxanthellae revealed that both coral species contained *Symbiodinium* B2, but only *B. europaea* contained *Symbiodinium* clade A. No change in *Symbiodinium* diversity was observed following the 7-month exposure to reduced pH. It has been proposed that tolerance to various environmental stresses, such as light or temperature, is influenced by *Symbiodinium* type (Baker, 2003, 2004; Knowlton and Rohwer, 2003; Rodolfo-Metalpa *et al.*, 2006; Jones *et al.*, 2008). Indeed, clade A has

been shown to impart resistance to short-term increases in temperature under experimental conditions to its host (Rodolfo-Metalpa *et al*., 2006).

Although the two coral species were exposed to similar conditions, the bacterial communities associated with each species were significantly different (Figure 3), demonstrating specific coral-bacterial associations, as reported previously (Frias-Lopez *et al*., 2002; Rohwer *et al*., 2002; Ritchie and Smith, 2004; Rohwer and Kelly, 2004; Bourne and Munn, 2005). When examining the microbial community within each coral species some changes in bacterial group composition were observed at the various pH treatments. For example, a subdivision of bacterial groups for each coral can be seen according to fraction, tissue versus skeleton (Figure 3). However, we did not find significant difference between the bacterial cluster and the pH treatments. In addition, in most cases, no correlation was observed between the specific bacterial classes and the pH change. This may indicate that under these tested conditions pH is not a major driving force in determining the bacterial composition and the diversity. Our data, generated from observing corals in a natural pH gradient, contrasts with results from previous studies performed in the laboratory, which indicated a dominant pH-dependent change in the bacterial community that clustered according to pH treatment and not according to coral fraction (Meron *et al*., 2011). It should be emphasized that the corals examined were different in these studies and this may also determine the difference in response.

In our prior laboratory experiments (carried out with a Red Sea coral species) we found that decreased pH resulted in an increase in bacteria linked to coral disease and stress, but not in any external signs of coral disease (Meron *et al*., 2011). Similarly, in the present field study, the corals did not show any signs of disease, but in contrast to the laboratory study, there was also no significant increase in specific pathogens or bacterial communities associated with diseased or stressed corals. For example, *Rhodobacteraceae* (*Alphaproteobacteria)*, a bacterial family isolated from diseased, injured or stressed marine invertebrates (Sekar *et al*., 2006; Sunagawa *et al*., 2009) that increased in abundance in the laboratory experiments (Meron *et al*., 2011), did not increase in abundance at lower pH in the present study, and in fact decreased in *B. europaea* (Figure 4 and Supplementary Table S2). Similarly, *Alteromonadaceae and Vibrionaceae* (of *Gammaproteobacteria*)*,* which are often associated with diseased and stressed corals, increased in abundance at lower pH in the laboratory experiments (Meron *et al*., 2011), but were absent or did not change in abundance in the present study (Figure 5b and d). In the case of *Vibrionaceae* associated with *B. europaea*, an increase in abundance was observed at lower pH, but these bacteria represented only 6% of the total microbial community at pH_T 7.3 and no homologs of known pathogens were detected (Figure 5d). Another bacterial group detected in healthy corals (Sekar *et al*., 2008) but also linked with stress conditions, including pH (Vega-Thurber *et al*., 2009) and with black band disease are the *Bacteroidetes* (Cooney *et al*., 2002; Frias-Lopez *et al*., 2002, 2004; Barneah *et al*., 2007). As with the *Vibrionaceae*, the present study found no significant correlation in the abundance of *Bacteroidetes* associated with *C. caespitosa* with the pH values, although the proportion of *Bacteroidetes* in the microbial population associated with *B. europaea* increased from 12.8% at pH_T 8.1% to 26.3% at pH_T 7.3; this increase again was mainly evident in the tissue fraction (Figure 4 and Supplementary Table S2). It is interesting to note that a significant correlation was observed in *B. europaea* between *Flavobacteriales* group (belonging to *Bacteroidetes*) and reduction in pH (Figure 5c).

The diverse microbial communities associated with the corals may have an important role in the minor pH impact we observed. Kitano and Oda (2006) described a model showing how a host capable of accommodating highly diverse bacterial flora is more likely to survive fluctuating environmental conditions, such as changes in foods and pathogens. A corollary of this model is that the observed dynamic changes in the bacterial communities of the coral holobiont do not necessarily reflect stress or sensitivity but rather enable tolerance, acclimatization and survival, despite variable biotic and abiotic parameters. Further research is required to corroborate and better understand how, and under which circumstances, different coral symbionts can increase tolerance to specific disturbances.

Although in the present study the corals were exposed to a wide range of pH values some of which are much lower than what is expected in the next century, our findings suggests that pH most likely has relatively little impact on coral microbial ecology and coral photophysiology, compared with other environmental stresses, such as increased temperature (Kuhl *et al*., 1995; Furla *et al*., 1998, 2000; Hoegh-Guldberg, 1999; Al-Horani *et al*., 2003). This finding could be explained by the fact that corals are naturally exposed to variable pH conditions (Kuhl *et al*., 1995; Furla *et al*., 1998, 2000; Al-Horani *et al*., 2003). Monitoring of reef waters over time shows that several parameters, including pCO_2, pH, total alkalinity, total inorganic carbon and dissolved oxygen, change diurnally due to photosynthesis and calcification during the day and respiration at night (Kayanne *et al*., 2000). In fact, the differences in $\Omega_{aragonite}$ and pH values that occur within the coelenteron in light versus dark conditions are much greater than those predicted to occur due to ocean acidification within this century (Furla *et al*., 1998, 2000; Al-Horani *et al*., 2003; Venn *et al*., 2011). Indeed Furla *et al*. (1998) and Kuhl *et al*. (1995) showed that the coelenteric pH fluctuates from 7.4 at night to 8.6–8.9 during the day.

A key advantage of studying the impact of pH on corals in natural habitats is the large pool of microbial species in the ocean. Laboratory environments cannot mimic the dynamism and microbial diversity present in nature. Moreover, it is possible that aquarium conditions themselves contribute to stress or disturbance in the microbial community. Kooperman *et al*. (2007) demonstrated that the same coral species has different associated microbial communities in the laboratory compared with field conditions . Despite this, laboratory experiments are critical tools in providing a basic understanding of how pH impacts marine organisms. Naturally occurring CO_2 vent sites provide important complementary systems that can serve as good models for examining the impact of changes in ocean pH in the natural environment. The current study, which examined corals maintained in a natural pH gradient, suggests that, at least for these two coral species, reduced pH does not seem to significantly reduce coral health. Fabricius *et al*. (2011) recently examined coral diversity in a natural pH gradient off the coast of Papua New Guinea. The authors report a decrease in coral diversity, although the existing coral species were found in good health suggesting acclimation to the pH environment. No doubt future studies will continue to take advantage of these unique natural environments to explore further the influence of reduced pH on marine ecosystems.

Acknowledgements

The work was partially supported by the US-Israel Binational Science Foundation grant no. 2006318 to EB and ACB and by the Israel Science Foundation 09/328 to MF. This is a contribution of the European Project 'Mediterranean Sea Acidification under a changing climate' (MedSeA; grant agreement 265103). Thanks are due to all collaborators from StazioneZoologica 'A. Dohrn' for their help during the fieldwork. RR-M was granted by the Journal Experimental Biology travel fellowship. The work contributes to the EU 'Mediterranean Sea Acidification under a changing climate' project (MedSeA; grant agreement 265103). We thank Ayalana Reiss for her critical review of the manuscript and Dr Maya Offek for her help with statistical analyses. This research is part of the requirements for a Ph.D. thesis for Dalit Meron at Bar Ilan University.

References

Al-Horani FA, Al-Moghrabi SM, de Beer D. (2003). The mechanism of calcification and its relation to photosynthesis and respiration in the scleractinian coral *Galaxea fascicularis*. *Mar Biol* **142**: 419–426.

Anthony KR, Kline DI, Diaz-Pulido G, Dove S, Hoegh-Guldberg O. (2008). Ocean acidification causes bleaching and productivity loss in coral reef builders. *Proc Natl Acad Sci USA* **105**: 17442–17446.

Arboleda M, Reichardt W. (2009). Epizoic communities of prokaryotes on healthy and diseased scleractinian corals in Lingayen Gulf, Philippines. *Microb Ecol* **57**: 117–128.

Baker AC. (2003). Flexibility and specificity in coral-algal symbiosis: diversity, ecology and biogeography of *Symbiodinium*. *Ann Rev Ecol Syst* **34**: 661–689.

Baker AC. (2004). *Diversity and Ecology of Symbiodinium on Coral Reefs and its Relationship to Bleaching Resistance and Resilience*. Springer-Verlag: New York/Berlin.

Banin E, Ben-Haim Y, Israely T, Loya Y, Rosenberg E. (2000). Effect of the environment on the bacterial bleaching of corals. *Water Air Soil Pollut* **123**: 337–352.

Barneah O, Ben-Dov E, Kramarsky-Winter E, Kushmaro A. (2007). Characterization of black band disease in Red Sea stony corals. *Environ Microbiol* **9**: 1995–2006.

Ben-Haim Y, Zicherman-Keren M, Rosenberg E. (2003). Temperature-regulated bleaching and lysis of the coral *Pocillopora damicornis* by the novel pathogen *Vibrio coralliilyticus*. *Appl Environ Microbiol* **69**: 4236–4242.

Bibby R, Cleall-Harding P, Rundle S, Widdicombe S, Spicer J. (2007). Ocean acidification disrupts induced defences in the intertidal gastropod *Littorina littorea*. *Biol Lett* **3**: 699–701.

Bourne D, Iida Y, Uthicke S, Smith-Keune C. (2007). Changes in coral-associated microbial communities during a bleaching event. *ISME J* **2**: 350–363.

Bourne D, Munn C. (2005). Diversity of bacteria associated with the coral *Pocillopora damicornis* from the Great Barrier Reef. *Appl Environ Microbiol* **7**: 1162–1174.

Caldeira K, Wickett ME. (2005). Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *Geophys Res* **110**: C09S04.

Crawley A, Kline DI, Dunn S, Anthony K. (2010). The effect of ocean acidification on symbiont photorespiration and productivity in *Acropora formosa*. *Glob Change Biol* **16**: 851–863.

Cooney RP, Pantos O, Le Tissier MD, Barer MR, O'Donnell AG, Bythell JC. (2002). Characterization of the bacterial consortium associated with black band disease in coral using molecular microbiological techniques. *Environ Microbiol* **4**: 401–413.

Dickson AG, Sabine CL, Christian JR. (2007). *Guide to Best Practices for Ocean CO2 Measurements*. North Pacific Marine Science Organization: Sidney, BC, pp 191.

Fabricius KE, Langdon C, Uthicke S, Humphrey C, Noonan S, De'ath G *et al*. (2011). Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. *Nature Clim Change* **1**: 165–169.

Fine M, Tchernov D. (2007). Scleractinian coral species survive and recover from decalcification. *Science* **315**: 1811.

Frias-Lopez J, Klaus JS, Bonheyo GT, Fouke BW. (2004). Bacterial community associated with black band disease in corals. *Appl Environ Microbiol* **70**: 5955–5962.

Frias-Lopez J, Zerkle AL, Bonheyo GT, Fouke BW. (2002). Partitioning of bacterial communities between seawater and healthy, black band diseased, and dead coral surfaces. *Appl Environ Microbiol* **68**: 2214–2228.

Furla P, Bénazet-Tambutté S, Jaubert J, Allemand D. (1998). Functional polarity of the tentacle of the sea anemone *Anemonia viridis*: role in inorganic carbon acquisition. *Am J Physiol Regul Integr Comp Physiol* **274**: R303–R310.

Furla P, Galgani I, Durand I, Allemand D. (2000). Sources and mechanisms of inorganic carbon transport for coral calcification and photosynthesis. *J Exp Biol* **203**: 3445–3457.

Garren M, Raymundo L, Guest J, Harvell CD, Azam F. (2009). Resilience of coral-associated bacterial communities exposed to fish farm effluent. *PLoS One* **4**: e7319.

Gattuso JP, Frankignoulle M, Bourge I, Romaine S, Buddemeier RW. (1998). Effect of calcium carbonate saturation of seawater on coral calcification. *Global and Planetary Change* **18**: 37–46.

Genty B, Briantais JM, Baker NR. (1989). The relationship between the quantum yield of photosynthetic electron-transport and quenching of chlorophyll fluorescence. *Biochimica Et Biophysica Acta* **990**: 87–92.

Gimmler H. (2000). Primary sodium plasma membrane ATPases in salt-tolerant algae: facts and fictions. *J Exp Bot* **51**: 1171–1178.

Hall-Spencer JM, Rodolfo-Metalpa R, Martin S, Ransome E, Fine M, Turner SM *et al.* (2008). Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature* **454**: 96–99.

Hoegh-Guldberg O. (1999). Climate change, coral bleaching and the future of the world's coral reefs. *Mar and Fresh Res* **50**: 839–866.

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Green P, Gomez E *et al.* (2007). Coral reefs under rapid climate change and ocean acidification. *Science* **318**: 1737–1742.

Hunter RL, LaJeunesse TC, Santos SR. (2007). Structure and evolution of the rDNA internal transcribed spacer (ITS) region 2 in the symbiotic dinoflagellates (*Symbiodinium, Dinophyta*). *J Phycol* **43**: 120–128.

Ishimatsu A, Hayashi M, Lee K-S, Kikkawa T, Kita J. (2005). Physiological effects on fishes in a high-CO_2 world. *J Geophys Res* **110**: C09S09.

Jeffrey SW, Humphrey GF. (1975). New spectrophotometry equations for determining Chl *a, b, c1, c2* in higher plants, algae and natural phytoplankton. *Biochem Physiol Pflanzen* **167**: 191–194.

Jones AM, Berkelmans R, van Oppen MJH, Mieog JC, Sinclair W. (2008). A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. *Proc Biol Sci* **275**: 1359–1365.

Jury CP, Whitehead RF, Szmant AM. (2010). Effects of variations in carbonate chemistry on the calcification rates of *Madracis mirabilis* (= *Madracis mirabilis sensu* Wells, 1973): bicarbonate concentrations best predict calcification rates. *Glob Change Biol* **16**: 1632–1644.

Kayanne H, Kudo S, Hata H, Yamano H, Nozaki K, Kato K *et al.* (2000). Integrated monitoring system for coral reef water pCO_2, carbonate system and physical parameters. *Proc 9th Int Coral Reef Symp (Bali, Indonesia)* **2**: 23–27.

Kitano H, Oda K. (2006). Robustness trade-offs and host-microbial symbiosis in the immune system. *Mol Syst Biol* **2**: 2006.0022.

Knowlton N, Rohwer F. (2003). Multispecies microbial mutualisms on coral reefs: the host as a habitat. *Am Nat* **162**: s51–s62.

Kooperman N, Ben-Dov E, Kramarsky-Winter E, Barak Z, Kushmaro A. (2007). Coral mucus-associated bacterial communities from natural and aquarium environments. *FEMS Microbiol Lett* **276**: 106–113.

Krief S, Hendy EJ, Fine M, Yam R, Meibom A, Foster GL *et al.* (2010). Physiological and isotopic responses of scleractinian corals to ocean acidification. *Geochimica et Cosmochimica Acta* **74**: 4988–5001.

Kuffner BI, Andersson JA, Jokiel LP, Rodgers SK, Mackenize TF. (2007). Decreases abundance of crustose coralline algae due to ocean acidification. *Nature* **1**: 114–117.

Kuhl M, Cohen Y, Dalsgaard T, Jorgensen BB, Revsbech NP. (1995). Microenvironment and photosynthesis of zooxanthellae in scleractinian corals studied with microsensors for O_2, pH and light. *Mar Ecol Prog Ser* **117**: 159–172.

LaJeunesse TC, Trench RK. (2000). Biogeography of two species of *Symbiodinium* (Freudenthal) inhabiting the intertidal sea anemone *Anthopleura elegantissima* (Brandt). *Biol Bull* **199**: 126–134.

Lane DJ. (1991). *16S/23S rRNA Sequencing*. John Wiley & Sons: Chichester, UK.

Lane DJ, Pace B, Olsen GJ, Stahlt DA, Sogint ML, Pace NR. (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proc Natl Acad Sci USA* **82**: 6955–6959.

Lozupone C, Hamady M, Knight R. (2006). UniFrac – An online tool for comparing microbial community diversity in a phylogenetic context. *BMC Bioinformatics* **7**: 371.

Lozupone C, Knight R. (2005). UniFrac: a new phylogenetic method for comparing microbial communities. *Appl Environ Microbiol* **71**: 8228–8235.

Ludwig W, Strunk O, Westram R, Richter L, Meier H, Yadhukumar *et al.* (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**: 1363–1371.

Martin S, Rodolfo-Metalpa R, Ransome E, Rowley S, Buia MC, Gattuso JP *et al.* (2008). Effects of naturally acidified seawater on seagrass calcareous epibionts. *Biol Lett* **4**: 689–692.

Meron D, Atias E, Iasur Kruh L, Elifantz H, Minz D, Fine M *et al.* (2011). The impact of reduced pH on the microbial community of the coral *Acropora eurystoma*. *ISME J* **5**: 51–60.

Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC, Feely RA *et al.* (2005). Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* **437**: 681–686.

Raven JA, Smith FA. (1980). Intracellular pH regulation in the giant-celled marine alga *Chaetomorpha darwinii*. *J Exp Bot* **31**: 1357–1369.

Reshef L, Koren O, Loya Y, Zilber-Rosenberg I, Rosenberg E. (2006). The coral probiotic hypothesis. *Appl Environ Microbiol* **8**: 2067–2073.

Reynaued S, Leclercq N, Romaine-Lioud S, Ferrier-Pages C, Jaubert J, Gattuso JP. (2003). Interacting effects of CO_2 partial pressure and temperature on photosynthesis and calcification in a scleractinian coral. *Glob Chang Biol* **9**: 1660–1668.

Ries J, Cohen A, McCorkle D. (2009). Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. *Geology* **37**: 1131–1134.

Ritchie KB. (2006). Regulation of microbial population by coral surface mucus and mucus-associated bacteria. *Mar Ecol Prog Ser* **322**: 1–14.

Ritchie KB, Smith GW. (2004). *Coral Health and Disease*. Springer: New York/Berlin.

Rodolfo-Metalpa R, Martin S, Ferrier-Pagès C, Gattuso J-P. (2010). Response of the temperate coral *Cladocora caespitosa* to mid- and long-term exposure to pCO_2 and temperature levels projected for the year 2100AD. *Biogeosciences* **7**: 289–300.

Rodolfo-Metalpa R, Richard C, Allemand D, Bianchi CN, Morri C. (2006). Response of zooxanthellae in symbiosis

with the Mediterranean corals *Cladocora caespitosa* and *Oculina patagonica* to elevated temperatures. *Mar Biol* **150**: 45–55.

Rohwer F, Kelly S. (2004). *Coral Health and Disease*. Springer: New York/Berlin.

Rohwer F, Seguritan V, Azam F, Knowlton N. (2002). Diversity and distribution of coral-associated bacteria. *Mar Ecol Prog Ser* **243**: 1–10.

Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I. (2007). The role of microorganisms in coral health, disease and evolution. *Nat Rev Microbiol* **5**: 355–362.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB *et al.* (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* **75**: 7537–7541.

Schneider K, Erez J. (2006). The effect of carbonate chemistry on calcification and photosynthesis in the hermatypic coral *Acropora eurystoma*. *Limnol Oceanogr* **51**: 1284–1293.

Sekar R, Kaczmarsky L, Richardson LL. (2008). Microbial community composition of black band disease on the coral host *Siderastrea siderea* from three regions of the wider Caribbean. *Mar Ecol Prog Ser* **362**: 38–98.

Sekar R, Mills DK, Remily ER, Voss JD, Richardson LL. (2006). Microbial communities in the surface mucopolysaccharide layer and the black band microbial mat of black band-diseased *Siderastrea siderea*. *Appl Environ Microbiol* **72**: 5963–5973.

Sunagawa S, DeSantis TZ, Piceno YM, Brodie EL, DeSalvo MK, Voolstra CR *et al.* (2009). Bacterial diversity and White Plague Disease-associated community changes in the Caribbean coral *Montastraea faveolata*. *ISME J* **3**: 512–521.

Szmant AM. (2002). Nutrient enrichment on coral reefs: is it a major cause of coral decline? *Estuaries* **25**: 743–766.

The International Panel on Climate Change (2007) Reporthttp://www.ipcc.ch.

Thornhill DJ, LaJeunesse TC, Kemp DW, Fitt WK, Schmidt GW. (2006). Multi-year, seasonal genotypic surveys of coral-algal symbioses reveal prevalent stability or post-bleaching reversion. *Mar Biol* **148**: 711–722.

Trotter J, Montagna P, McCulloch P, Silenzi S, Reynaud S, Mortimer G *et al.* (2011). Quantifying the pH 'vital effect' in the temperate zooxanthellate coral *Cladocora caespitosa*: Validation of the boron seawater pH proxy. *Earth Planet Sci Lett* **303**: 163–173.

Vega-Thurber R, Willner-Hall D, Rodriguez-Mueller B, Desnues C, Edwards RA, Angly F *et al.* (2009). Metagenomic analysis of stressed coral holobionts. *Environ Microbiol* **11**: 2148–2163.

Venn A, Tambutte E, Holcomb M, Allemand D, Tambutt S. (2011). Live tissue imaging shows reef corals elevate pH under their calcifying tissue relative to seawater. *PLoS ONE* **6**: e20013.

Visram S, Wiedenmann J, Douglas AE. (2006). Molecular diversity of symbiotic algae of the genus *Symbiodinium* (Zooxanthellae) in cnidarians of the Mediterranean Sea. *J Mar Biol Assoc UK* **86**: 1281–1283.

Wang Q, Garrity GM, Tiedje JM, Cole JR. (2007). Naïve bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* **73**: 5261–5267.

Wegley L, Edwards R, Rodriguez-Brito B, Liu H, Rohwer F. (2007). Metagenomic analysis of the microbial community associated with the coral *Porites astreoides*. *Environ Microbiol* **9**: 2707–2719.

Supplementary Information accompanies the paper on The ISME Journal website (http://www.nature.com/ismej)