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Long-term salinity tolerance is accompanied by major restructuring of the coral bacterial microbiome

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Abstract

Scleractinian corals are assumed to be stenohaline osmoconformers, although they are frequently subjected to variations in seawater salinity due to precipitation, freshwater runoff, and other processes. Observed responses to altered salinity levels include differences in photosynthetic performance, respiration, and increased bleaching and mortality of the coral host and its algal symbiont, but a study looking at bacterial community changes is lacking. Here we exposed the coral *Fungia granulosa* to strongly increased salinity levels in short- and long-term experiments to disentangle temporal and compartment effects of the coral holobiont (i.e. coral host, symbiotic algae, and associated bacteria). Our results show a significant reduction in calcification and photosynthesis, but a stable microbiome after short-term exposure to high salinity levels. By comparison, long-term exposure yielded unchanged photosynthesis levels and visually healthy coral colonies indicating long-term acclimation to high salinity levels that were accompanied by a major coral microbiome restructuring. Importantly, a bacterium in the family *Rhodobacteraceae* was succeeded by *Pseudomonas veronii* as the numerically most abundant taxon. Further, taxonomy-based functional profiling indicates a shift in the bacterial community towards increased osmolyte production, sulfur oxidation, and nitrogen fixation. Our study highlights that bacterial community composition in corals can change within days to weeks under altered environmental conditions, where shifts in the microbiome may enable adjustment of the coral to a more advantageous holobiont composition.

Introduction

Coral reefs are among the most diverse and productive ecosystems on the planet (Reaka-Kudla *et al.* 1996) and provide a wide range of goods and services to approximately 500 million people in more than 100 countries (Wilkinson 2008). Coral reef ecosystems rely on the three-dimensional carbon skeleton framework built by scleractinian corals. Scleractinian

corals are metaorganisms - so-called coral holobionts - consisting of the coral host, its dinoflagellate endosymbionts (genus *Symbiodinium*), and a diverse microbial assemblage consisting of fungi, bacteria, archaea, and viruses (microbiome) (Rohwer *et al.* 2002). The bacterial microbiome has been shown to play important roles in coral health (Rosenberg *et al.* 2007), immunity (Ritchie 2011), and carbon, sulfur, and nitrogen cycling (Lema *et al.* 2012; Raina *et al.* 2009; Rohwer *et al.* 2002). Moreover, it has been suggested that microbial assemblages facilitate the acclimation of coral holobionts to new environmental conditions (the probiotic hypothesis, Reshef *et al.* 2006). However, knowledge about the specific role of the vast majority of microbes, and in particular bacteria, to holobiont function is still limited (Barott *et al.* 2011; Bourne & Webster 2013; Lesser *et al.* 2004; Morrow *et al.* 2012).

Although frequently exposed to changes in seawater salinity, e.g. due to precipitation, freshwater runoff, periods of prolonged drought, or desalination processes (Chartrand *et al.* 2009; Edge *et al.* 2013; Hédouin *et al.* 2015; Lirman & Manzello 2009; Roberts *et al.* 2010), scleractinian corals are assumed to be stenohaline osmoconformers with a limited ability to adjust to salinity fluctuations (Ferrier-Pages *et al.* 1999; Hédouin *et al.* 2015; Hoegh-Guldberg & Smith 1989; Kerswell & Jones 2003; True 2012). Nevertheless, studies show that some coral species are able to tolerate greater salinity fluctuations than others. For instance, *Stylophora pistillata* showed decreased respiration and photosynthetic rates at minor salinity decreases; whereas *Siderastrea radians* displayed a high resilience towards salinity changes (Ferrier-Pages *et al.* 1999; Lirman & Manzello 2009). Further, Coles (2003) reported high salinity tolerance of corals in the Arabian Gulf and Red Sea, with some species surviving salinities of 48-50 PSU. While higher animals possess excretory systems to adjust for changes in salinity, most marine invertebrates, similar to scleractinian corals, are considered osmoconformers (Evans 2008; Yancey *et al.* 2002). Yet, in sea anemones changes in free amino acid pools are involved in osmoregulation (Shick 1991). Similarly, marine

microorganisms (i.e. algae and bacteria) accumulate diverse molecules that serve as osmolytes upon increased salinity exposure (Csonka & Hanson 1991; Mayfield & Gates 2007). However, osmoregulation in the coral-algal endosymbiosis represents a challenging scenario. The coral animal host has to equilibrate the external osmotic pressure with its intracellular environment, which is determined by its own metabolism and that of its algal symbionts (Mayfield & Gates 2007). In this context the role of the coral-associated bacteria is virtually unknown.

To elucidate the role of coral-associated bacteria to salinity changes, we exposed the coral *Fungia granulosa* to strongly increased salinities resulting from seawater reverse osmosis (SWRO) desalination concentrate. By collecting data from all holobiont compartments (i.e. coral host, symbiont algae, bacterial microbiome) using a combination of short- (4 h) and long-term (29 d) experimental treatments, we aimed to disentangle compartment-specific responses and to assess potential adaptation/acclimation processes by characterizing the initial response and long-term effects on the coral holobiont.

Material and Methods

Study site

We used concentrated salt brine from a seawater reverse osmosis (SWRO) desalination plant located at King Abdullah University of Science and Technology (KAUST, Saudi Arabia) to test the effects of increased salinity to *Fungia granulosa*. The SWRO currently discharges an average concentrated salt brine of 41 358 $m^3 day^{-1}$. The submerged discharge structure is located at 22°17.780N, 39°04.444E at 18 m depth with four discharge screens (1,800 × 1,000 mm) about 6 m above the seafloor (Figure S1, Supporting Information) (van der Merwe *et al.* 2014b).

Short-term hypersalinity treatment

Hypersaline water of 55 Practical Salinity Unit (PSU) was collected directly from the desalination plant discharge (see above). A hose was used to collect the concentrate via SCUBA and pumped into a container on an accompanying boat. The collected water was then transferred into 50 L opaque plastic bins and salinity was determined (WTW Cond 3310, WTW, Weilheim, GER). Ambient water (39 PSU) was collected from the top of the desalination discharge structure. We collected 14 specimens of *Fungia granulosa* (6-8 cm) on the same day (March 2014) in about 8 km distance at Fsar reef (22°13.945N, 39°01.783E; 14-18 m). The corals were sampled separately in zip-lock bags and transferred to 50 L opaque plastic containers filled with ambient reef water (39 PSU) upon return to the boat. Four specimens were immediately rinsed with filtered seawater (FSW; 0.22 µm), wrapped in aluminum foil, and flash frozen in liquid nitrogen for further analyses (freshly collected corals). The remaining ten coral specimens were photo-acclimated to constant experimental light conditions of 120 µmol m^{-2} s^{-1} (measured with DIVING-PAM, Walz, Effeltrich, GER) for 30 min at 27°C. Each coral specimen was measured thrice via pulse-amplitude modulated fluorometry (PAM; measured with DIVING-PAM) for photochemical efficiency. Subsequently, ten specimens were transferred into one 1 L glass beaker each: five were placed in a 39 PSU 'control' bin and five in a 55 PSU 'high salinity' bin, each holding 50 L of water. Four 50 mL water samples were taken from the control and treatment bin each over a 0.45 µm filter attached to a syringe for total alkalinity (TA) measurements. Oxygen concentration (WTW Multi 3500i, WTW), salinity, and temperature (both WTW Cond 3310) were measured within each of the coral beakers prior to incubation start. Incubations were stopped after 4 h beaker by beaker. Again, oxygen and photochemical efficiency were measured and 50 mL water samples from each beaker for TA measurements were taken in

duplicate. Corals were rinsed with FSW, wrapped in aluminum foil, and frozen in liquid nitrogen until further analysis.

Long-term hypersalinity treatment

We selected the KAUST SWRO discharge site (see above) for an *in situ* transplantation experiment, further described in van der Merwe *et al.* (2014b). Previous measurements suggested increased salinities also at a greater distance (>15 m) from the discharge structure (van der Merwe *et al.* 2014a). In order to cover a range of salinities resulting from the brine discharge, to assess the discharge dilution pattern, and the salinity impact on the coral *F. granulosa*, six stations were chosen along a transect: station 1 in the discharge screen, all other stations on the seafloor at 0, 2.5, 5, 15, and 25 m distance northwards from the discharge structure (Figure S1, Supporting Information). Environmental parameters (i.e. salinity, temperature, dissolved oxygen, photosynthetically active radiation (PAR)) along the transect were assessed at each station (van der Merwe *et al.* 2014b). For the long-term experiment, 18 specimens of *F. granulosa* were collected from 14-18 m at Fsar reef (January 2014). Collected corals were acclimated at ambient salinity on the desalination discharge structure roof for 20 h and tagged with nylon fishing line and labels. Three corals were randomly distributed to each of the six stations. The corals were fixed onto bricks and to the screen grid (station 1), respectively (van der Merwe *et al.* 2014b). All corals were measured thrice via PAM 1 h after attachment and visually assessed for any signs of bleaching; additionally water samples (1 L cubitainer) and PAR measurements were taken at each station. To test for the degree of correlation between changes in ambient light levels and effective quantum yield, we used SigmaPlot 11 (SYSTAT Software, Point Richmond, CA, USA) to conduct a linear regression analysis. All water samples were measured for dissolved oxygen and salinity immediately upon return to the boat. The sampling procedure was repeated after 1, 4, 6, 8, 15, and 29 days and routinely took place between 11:00h and 12:00h.

After 29 days water samples from each station were taken for assessment of reef water bacterial community. All coral specimens were collected into separate zip-lock bags, rinsed with FSW on board, wrapped in aluminum foil, and flash frozen in liquid nitrogen until further analysis.

Coral physiology

We determined coral calcification rate (G) according to the alkalinity anomaly method (Schneider & Erez 2006). Each 50 mL water sample was analyzed for total alkalinity (TA) using an automated titrator (Titrando 888, Metrohm AG, Herisau, CH) with 0.01 M HCl. Following the Gran approximation, we used the second endpoint of the titration curve to estimate TA (Grasshoff *et al.* 2009). We used the difference in TA between initial and final sample, normalized over incubation time (T) and coral tissue surface area (SA) to calculate G as follows:

$$G\ [\mu mol\ CaCO_3 cm^{-2} h^{-1}] = \frac{\frac{\Delta TA}{2} * (V_{jar} - V_{coral})\ [L] * \text{water density}\ [kg\ L^{-1}]}{T\ [h] * SA\ [cm^2]}$$ (Schneider & Erez 2006).

We used the difference of dissolved oxygen concentration at incubation start and end, and normalized over T and SA to calculated net photosynthesis (P_n):

$$P_n\ [\mu g\ O_2 cm^{-2} h^{-1}] = \frac{\Delta O_2\ [\mu g L^{-1}] * (V_{beaker} - V_{coral}\ [L])}{T\ [h] * SA\ [cm^2]}$$ (Schneider & Erez 2006).

Corals were modeled as cylinders as this approximates their shape well; diameter and height were measured using a caliper. Coral volume (V) and surface area (A) were calculated following: Surface area $A=\pi r^2$, Volume $V=\pi hr^2$.

Additionally, we assessed the photochemical efficiency of *F. granulosa* via PAM fluorometry (DIVING-PAM). The effective quantum yield,

$$\Phi PSII = \frac{Fm' - F}{Fm'} = \frac{\Delta F}{Fm'}$$ (Genty *et al.* 1989).

reflects the efficiency of photosystem II (PSII) under ambient light adapted conditions (Ralph & Gademann 2005). Ambient light conditions were logged with the PAM's fiber quantum sensor. The light intensity for the incubation experiment (about 120 μmol $m^{-2}s^{-1}$) represents roughly the measured light intensity at the collection site (15 m; 100-150 μmol $m^{-2}s^{-1}$).

16S rRNA gene sequencing

Flash frozen corals were stored at -80°C until DNA extraction. Each specimen was carefully unwrapped on ice, transferred into a sterile zip-lock bag and doused with 5 mL Qiagen RLT buffer (Qiagen AllPrep kit, Hilden, GER). While thawing, buffer and coral tissue were carefully blasted off using tap air pressure and pipette barrier tips. The buffer-tissue mixture was transferred into 15 mL Falcon tubes and vortexed. A 500 µL aliquot was used for DNA extraction following the manufacturer's protocol (Qiagen AllPrep kit). DNA concentrations were quantified on a NanoDrop 2000C spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Water samples were transported to the lab on ice in the dark. We filtered 1 L sample over 0.22 µm Durapore PVDF filters (Millipore, Billerica, MA, USA). Filters were frozen at -80°C. Half of each filter was cut into small stripes with sterile razorblades and transferred into 2 mL Eppendorf vials. After adding 400 µl Qiagen RLT buffer, the samples were incubated on a rotating wheel for 20 min. Subsequent extraction steps were conducted following the manufacturer's protocol (Qiagen AllPrep kit).

For PCR amplification we used about 60 ng DNA for coral samples and about 5 ng DNA for water samples. We used the primers 784F [5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGGATTAGATACCCTGGTA-3'] and 1061R [5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCRRCACGAGCTGACGAC-3'] that target variable regions 5 and 6 of the 16S rRNA gene (Andersson *et al.* 2008) and have been shown to amplify well with coral DNA (Bayer *et al.* 2013). The primers contain

Illumina adapter overhangs (underlined above; Illumina, San Diego, CA, USA). All PCRs were performed in triplicates using Qiagen Multiplex PCR kit with 0.2 μM of each primer adjusted to a total volume of 20 μl with RNase free water. The amplification cycling temperatures were set to one cycle at 95°C for 15 min, 25 cycles each at 95°C for 30 s, 55°C for 90 s, and 72°C for 30 s; a final extension step at 72°C for 10 min. 10 μL of each sample were used for visual quality check via 1% agarose gel electrophoreses. The triplicate PCRs of each sample were then pooled and cleaned with Agencourt AMPure XP magnetic bead system (Beckman Coulter, Brea, CA, USA). The clean PCR product then underwent an indexing PCR to add Nextera XT indexing and sequencing adapters (Illumina) according to the manufacturer's protocol. Indexed PCR products were cleaned up using the Agencourt AMPure XP protocol, quantified on the BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA) and QuBit (Quant-IT dsDNA Broad Range Assay Kit; Invitrogen, Carlsbad, CA, USA), and pooled in equimolar ratios. The final pooled library was purified on a 2% agarose gel to remove any excess primer dimers. The library was sequenced at 8 pM with 10% phiX on the Illumina MiSeq, 2 x 300 bp paired end version 3 chemistry according to the manufacturer's specifications. Sequences determined in this study have been deposited in the NCBI Sequence Read Archive under accession number PRJNA282461 (http://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA282461).

Bacterial community analysis

For amplicon analysis, we used mothur (http://www.mothur.org/ version 1.16.1, Schloss *et al.* 2009). Sequence reads were split according to barcodes, assembled to contigs, and quality trimmed. Duplicates, i.e. identical sequences, were merged using the 'unique.seqs' command in order to save computation time, and the command 'count.seqs' were used to keep a count of the number of sequences over samples represented by the remaining representative sequence. Rare sequence reads were removed (n<10 over all samples), remaining sequences

were aligned against SILVA (release 119, Pruesse *et al.* 2007), and a pre-clustering step (2 bp difference) was performed (Huse *et al.* 2010). Chimeric sequences were removed using UCHIME as implemented in mothur (Edgar *et al.* 2011). Additionally, chloroplasts, mitochondria, archaea, eukaryotes, and unknown reads were removed. Sequences were classified against Greengenes (McDonald *et al.* 2012) using a bootstrap of 60 and the sample compositions were compared on a family level. For further analyses, sequences were subsampled to 10 000 sequence reads and a 97% similarity cutoff level was chosen to obtain OTUs. Chao1 (Chao 1984), Simpson Evenness, Inverse Simpson Index, Principal Coordinate Analysis (PCoA), and Analysis of Molecular Variance (AMOVA; Excoffier *et al.* 1992) were performed as implemented in mothur. PCoA data were plotted in SigmaPlot 11. To identify the main contributing OTU for similarity within each of the groups, (i.e. freshly collected coral, short-term ambient salinity, short-term high salinity, long-term ambient salinity, and long-term high salinity), a Similarity Percentages (SIMPER) analysis was performed using PRIMER v6 software (Clarke & Gorley 2006). To identify OTUs that were significantly different between long-term hypersaline conditions and all other treatments we used the statistical package indicspecies (Cáceres & Legendre 2009) in R (Team 2014). We employed the BEST (BIO-ENV) routine in PRIMER in order to identify the 'best' match between OTU distributions and associated environmental variables (i.e., salinity, temperature, dissolved oxygen, light levels, effective quantum yield, and depth). OTU abundances and environmental data were square root transformed and environmental data were normalized. Bray-Curtis similarity was used to calculate an OTU resemblance matrix, Euclidean distance was used for the environmental parameter resemblance matrix. The Weighted-Spearman rank correlation method was used to identify the parameter (or combination of parameters) providing the highest ρ.

In order to assess putative functional profiles based on the 16S community composition, we used METAGENassist for automated taxonomic-to-phenotypic mapping (Arndt *et al.* 2012). Input files were created in mothur using the 'make.shared' and 'classify.otu' commands based on all coral samples. During data processing in METAGENassist, all 2152 distinct OTUs were assigned, mapped, and condensed into 400 functional taxa and filtered based on interquantile range (Hackstadt & Hess 2009). After filtering, 360 functional taxa remained and were normalized over samples by sum and over taxa by range scaling. We then analyzed the data for 'metabolism-by-phenotype', and used Euclidean distance measure and average clustering algorithm to visualize the results in a heatmap. To confirm patterns obtained by METAGENassist, we investigated specific genes associated with identified processes via PICRUSt (Langille *et al.* 2013). Input files for PICRUSt were created in mothur using the 'make.biom' command including all coral samples. Clusters of orthologous groups were created by predicting the metagenomes in PICRUSt.

Results

Coral and *Symbiodinium* physiology after short-term hypersalinity treatment

To assess short-term effects of strongly increased salinity on coral holobiont function we determined physiological parameters from the coral host and *Symbiodinium* at the end of 4 h incubations in ambient (39 PSU) and hypersaline treatments (55 PSU) (Table 1). We could not visually detect any signs of bleaching (Table 1), but we observed increased mucus production including small bubbles in the high salinity treatments (Figure S2, Supporting Information). Corals displayed an about 8-fold decreased calcification rate (G) under hypersaline compared to ambient conditions (0.031±0.073 compared to 0.243±0103 $CaCO_3$ µmol cm^{-2} h^{-1}, $P_{t\text{-}test}<0.05$). Oxygen net production (i.e. oxygen-producing photosynthesis mainly by *Symbiodinium*, respiration mainly by coral host and *Symbiodinium*, bacterial

contribution to both) was higher under ambient (start: 8.03±0.05 mgL^{-1}; stop: 10.47±1.15 mgL^{-1}) than under hypersaline (start: 7.14±0.11 mgL^{-1}; stop: 8.44±0.45 mgL^{-1}) conditions. Net photosynthesis-based oxygen production (P_n) was significantly higher in ambient condition (13.49±4.87 compared to 8.28±3.98 $\mu gO_2cm^{-2}h^{-1}$, $P_{t\text{-}test}<0.05$). As expected, the effective quantum yield (ϕPII) of PSII in *Symbiodinium* showed no distinct differences between corals from ambient and high salinity conditions at experiment start (0.709±0.019 and 0.710±0.019). In contrast, ϕPII was significantly decreased for the hypersalinity treatment after the 4 h incubation period (0.681±0.018 compared to 0.718±0.018 under ambient conditions, $P_{t\text{-}test}<0.05$).

Coral and *Symbiodinium* physiology after long-term hypersalinity treatment

F. granulosa specimens showed no signs of impaired photosynthetic performance during and after a 29 day *in situ* experiment (van der Merwe *et al.* 2014b). We found a rapid dilution of the discharged brine: Only salinity levels at station 1 (i.e. at the SWRO discharge screen) were increased (average 49.4±2.0 PSU) and significantly ($P_{ANOVA}<0.05$) different from all other stations (2-6), where salinity was on average 41.3±0.7 PSU and not significantly different between stations ($P_{ANOVA}>0.05$). Therefore, corals from station 1 were assigned to the hypersaline long-term treatment (n=3) and corals from all other stations were pooled into the long-term ambient group (n=15). Water temperature decreased slightly with increasing distance from the discharge, but differences did not exceed 0.5°C across average temperatures (stations 1-6, AVG: 26.3°C, 26.1°C, 26.0°C, 26.0°C, 25.8°C, 25.8°C). Dissolved oxygen varied between 5.75 and 6.37 mgL^{-1} and was not significantly different ($P_{ANOVA}>0.05$) between stations. *Symbiodinium* effective quantum yields (ϕPII) were stable for all corals regardless of high or ambient salinity conditions. Average ϕPIIs were within the natural range measured at the collection site (0.682-0.709) with 0.705±0.009 (station 1), 0.694±0.008 (station 2), 0.687±0.007 (station 3), 0.687±0.019 (station 4), 0.693±0.017

(station 5), and 0.690±0.02 (station 6). Differences across the stations - and between daily measurements - coincided with light levels as compared by PAR values (van der Merwe *et al.* 2014b), as shown by a linear regression analysis (R^2=0.80, P<0.001). ϕPII measurements from station 1, reflecting ambient levels from the collection site, indicate that high salinity did not impair the photosynthetical efficiency of *Symbiodinium in hospite*.

Seawater and coral bacterial communities

We produced 41 16S rRNA gene libraries with a total of 4 784 475 reads distributed over 9 water samples, 4 freshly collected *F. granulosa* colonies, 10 short-term incubation corals (5 high and 5 ambient salinity), and 18 long-term incubation corals (3 high salinity at station 1 and 15 ambient, i.e. 3 from each of stations 2-6). After quality trimming, chimera detection, and removal of undesired (e.g. chloroplasts) and rare sequences (n<10 over all samples), 2 612 132 reads with an average length of 292 bp were retained for further analyses. In order to assess sample and condition specific differences in bacterial composition we classified all sequences to the family level (Figure 1). Water samples were dominated by *Pelagibacteriaceae* (~60-70%) and markedly different from all coral samples, but similar to each other. Coral samples were dominated by bacteria from the family *Rhodobacteraceae* (~20-80%), except for long-term hypersaline coral samples, which appeared more even and were not dominated by a distinct bacterial family (Figure 1).

Coral microbiome restructuring after long-term salinity exposure

In order to reveal differences in bacterial community composition between treatments and over time, we subsampled to 10 000 reads and clustered sequence data to operational taxonomic units (OTUs, similarity cutoff≤0.03). Good's estimator (Good 1953) ranged between 0.98 and 1.0 coverage for all samples indicating that the majority of bacterial diversity was represented. Chao1 estimates of species richness, Simpson evenness, and Inverse Simpson Index (diversity measure) were all highest for long-term hypersaline

samples compared to all other samples - with an approximate average 10-fold increase for diversity (Inverse Simpson Index) and a 3-fold increase for Chao1 and Simpson Evenness (Table 2). We compared microbial community abundance profiles over coral colonies, treatments, and time points in a Principal Coordinate Analysis (PCoA) based on Bray-Curtis dissimilarity (Figure 2). Water samples were clustering closely together and differed significantly from all coral samples ($P_{AMOVA} \leq 0.005$), but not between each other ($P_{AMOVA}=1$) confirming that bacterial communities from hypersaline water samples did not exhibit distinct bacterial communities in comparison to ambient water samples (Figure S3, Supporting Information). Importantly, we observed a distinct grouping among coral samples. Long-term hypersaline coral samples clustered significantly away from all other coral samples (i.e. freshly collected, short-term incubations, and long-term ambient) ($P_{AMOVA} \leq 0.001$), and all other coral samples were not significantly different from each other ($P_{AMOVA} \geq 0.05$). In order to uncover the main environmental driver underlying the coral microbiome changes, we employed a biological-environmental matching routine that compares OTU distribution with environmental parameters. This analysis revealed a significant correlation between environmental data (i.e., salinity, temperature, dissolved oxygen, light levels, effective quantum yield, and depth) and the coral microbiomes. From all considered environmental parameters and combinations thereof, salinity as a single factor best explained variations in OTU abundance and distribution ($\rho=0.639$, $P \leq 0.01$) (Table S1, Supporting Information).

To assess overall similarity and to identify the main contributing OTU within each of the groups (i.e. freshly collected coral, short-term ambient salinity, short-term high salinity, long-term ambient salinity, and long-term high salinity), we looked for the presence of 'core' microbiome OTUs with SIMPER (Similarity Percentages) analysis. All groups showed a consistent 'within-group' similarity of about 50% (Bray-Curtis) (Table 3). Looking at the main contributing OTU within long-term hypersaline samples, we identified OTU0010

(*Pseudomonas veronii*) (Table 3). The average abundance of this OTU was increased by about 3-fold in corals from the long-term hypersaline treatment compared to all other coral samples (836.7 vs. 270.7 average read counts) (Table S2, Supporting Information). Strikingly, corals from all other groups revealed the same single, unclassified OTU in the family *Rhodobacteraceae* (OTU0001) to be the numerically abundant bacterial taxon (Table 3). The average abundance of OTU0001 for these groups was 5105.6 reads (relative abundance 51.1%) compared to an average abundance of only 133 reads (relative abundance 1.33%) of this OTU in long-term hypersaline coral samples (Table 3; Table S2, Supporting Information).

Based on the similarity of diversity estimates, proximity in PCoA clustering, and an identical main contributing OTU for the microbial communities of freshly collected coral, short-term ambient salinity, short-term high salinity, and long-term ambient salinity treatments, we jointly compared corals from these treatments to corals from the long-term high salinity treatment. Differentially abundant OTUs were determined with indicspecies and revealed that 523 OTUs were significantly ($P \leq 0.01$) different between corals from the hypersaline long-term treatment in comparison to all other coral samples. Interestingly, only 3 OTUs (of a total of 4 OTUs) were highly significantly ($P \leq 0.001$) enriched in all coral groups, but absent in corals from the hypersaline long-term treatment. These were OTU0005, OTU0003 (both unclassified spp., order *Cytophagales*), and OTU0009 (unclassified sp., family *Rhodobacteraceae*) (Table S3, Supporting Information). In contrast, indicspecies identified 519 significantly enriched OTUs in corals from the hypersaline long-term treatment, which together made up a relative abundance of 55.6%. Of those, 5 OTUs had an average abundance of at least 100 reads and 104 OTUs had an average abundance of at least 10 reads and were highly significantly enriched in corals from the hypersaline long-term treatment ($P \leq 0.001$; Table S3, Supporting Information). The highly significant enrichment of hundreds

of OTUs under hypersaline conditions over a course of 29 days in connection with the parallel decrease of the otherwise numerically abundant OTU (i.e. OTU0001) demonstrates a major restructuring of the coral microbiome. In contrast to previous findings (Bouvier & del Giorgio 2002), bacterial communities in the seawater did not change upon high salinity exposure, presumably because of a rapid brine dilution (van der Merwe *et al.* 2014b) and thus short residence times of associated bacteria therein.

Taxonomy-based functional profiling of bacterial communities

We used METAGENassist to predict putative changes in bacterial community function based on differences in bacterial community composition. In line with our taxonomy-based analysis, the long-term hypersaline samples formed a distinct group from all other coral samples (Figure 3). In these three samples we found a pronounced upregulation of polyhydroxybutyrate storage, sulfur oxidizer, syntrophic bacteria, and xylan, alkane, and biomass degraders (Figure 3). At the same time dehalogenation, ammonium oxidizer, and nitrite and sulfate reducer were down-regulated. Next, we used PICRUSt to analyze specific genes underlying identified processes (i.e., PHB metabolism, sulfur cycling, and nitrogen cycling) affected in the long-term hypersaline samples. Further supporting the results from METAGENassist, we found decreased abundance of the enzyme polyhydroxybutyrate depolymerase associated with PHB metabolism, decreased anaerobic DMSO reductase and sulfite reductase associated with sulfur cycling, and decreased nitrite reductase associated with the process of nitrite reduction (Figure S4, Supporting Information). Besides these processes, some samples were enriched for other functions, e.g. ‘iron oxidizer’, ‘sugars fermentor’, ‘chitin degradation’, ‘selenate reducer’ (among others) in METAGENassist. However, samples enriched for these functions did not reveal any apparent patterns in regard to treatment (i.e. control, ambient, or high salinity) or exposure time (i.e., 4 h or 29 days).

Discussion

In this study we assessed the effect of short- and long-term hypersalinity exposure on the Red Sea coral *Fungia granulosa*. Our results indicate distinct short- and long-term reactions of the coral holobiont. The short-term experiment, aimed to measure the initial response, was characterized by an absence of changes in the bacterial community structure, but a significant reduced calcification and photosynthesis under strongly increased salinity levels. In contrast, the long-term transect experiment indicated a putative acclimation response, since corals exposed to high salinity for 29 days did not exhibit measureable (photo) physiological effects or signs of bleaching (van der Merwe *et al.* 2014b), but rather, displayed a significant shift in the associated bacterial community.

Short-term and long-term coral physiology

Fungia granulosa is a single-polyp scleractinian coral that has been demonstrated to possess a slow growth rate (Chadwick-Furman *et al.* 2000). Accordingly, our values for the calcification rate (G) were lower than those reported for other corals, e.g. for *Stylophora subseriata* (1.05-1.73 µmol $CaCO_3$ $cm^{-2}h^{-1}$) (Sawall *et al.* 2011). It is of note that calcification effectively stopped at high salinities in the short-term treatment. Unfortunately, calcification rates are not available for the long-term treatment. However, it would be interesting to see whether calcification rates are not influenced in long-term hypersaline exposure – as observed for photosynthetic efficiency.

In line with previous studies, our short-term incubation showed an overall oxygen increase, which was significantly lower for high salinity conditions (Gattuso *et al.* 1999; Lirman & Manzello 2009; Manzello & Lirman 2003). Further, a reduction of photosynthetic rates has been documented for hyper- and hyposaline scenarios before (Alutoin *et al.* 2001; Chartrand *et al.* 2009; Ferrier-Pages *et al.* 1999; Moberg *et al.* 1997; Muthiga & Szmant 1987). However, long-term ϕPII values from our coral samples were in the same range as

photosynthetic yields originating from corals in their natural environment. We could not observe any differences between hypersaline and ambient conditions in regard to the coral's photophysiology or bleaching status (van der Merwe *et al.* 2014b).

In accordance with data collected from our short- and long-term experiments, an initial sharp decline in photosynthetic performance with subsequent recovery has been suggested as an acclimation pattern (Lirman & Manzello 2009; Manzello & Lirman 2003). Mayfield and Gates (2007) interpreted these patterns as an indication for osmoregulatory processes, also considering corals to generally tolerate slow salinity changes better than more rapid ones (Muthiga & Szmant 1987). Our physiological data are supporting corals as being able to adjust to salinity. We report distinct physiological effects on coral host (calcification) and *Symbiodinium* (decreased photosynthesis) in our 4 h incubation. In the long-term *in situ* experiment we could not find any influence on the (photo) physiology of *F. granulosa* indicating acclimation of the coral holobiont to prevailing salinity levels.

Coral microbiome restructuring

To our knowledge, this is the first study that assesses coral bacterial microbiome structure under short- and long-term exposure of corals to salinity changes. Healthy corals maintain mostly specific, stable, and uneven microbial assemblages indicating selected microbiomes (Bayer *et al.* 2013; Bourne *et al.* 2008; Bourne & Webster 2013; Jessen *et al.* 2013; Kelly *et al.* 2014; Meron *et al.* 2011a; Meron *et al.* 2012). The microbial communities are diverse and contribute to pathogen inhibition due to production of antimicrobial substances as well as competition for space and nutrients (Klaus *et al.* 2007; Rosenberg *et al.* 2007; Thurber *et al.* 2009). In our experiments we found no distinct bacterial community changes after 4 h salinity exposure, which contrasts the measured physiological reactions of coral host and algal symbiont. At the same time, Apprill *et al.* (2009) measured doubling times of 10+ hours for coral associated bacteria, which may have affected our ability to determine a microbiome

response in the short-term experiment. Conversely, we found no physiological reaction, but pronounced microbial community changes after a 29 d hypersaline treatment. All corals, except those from the long-term hypersaline treatment, revealed highly uneven bacterial microbiomes that were numerically dominated by a single, distinct OTU (i.e. OTU0001) that could only be identified to the level family, namely *Rhodobacteraceae*. Bacteria from this family were repeatedly observed in healthy corals (Bayer *et al.* 2013; Ceh *et al.* 2012; Kellogg *et al.* 2014; Li *et al.* 2014; Morrow *et al.* 2012; Sunagawa *et al.* 2009), even though they have also been found to be associated with stressed corals and stressed sea urchins (Buchan *et al.* 2005; Godwin *et al.* 2012; Meron *et al.* 2011a; Meron *et al.* 2011b; Meron *et al.* 2012; Pantos *et al.* 2015; Sunagawa *et al.* 2009). Additionally, bacteria in the family *Rhodobacteraceae* have been found to be enriched in corals isolated from deeper habitats (27 m) compared to their shallow counterparts (6 m) (Pantos *et al.* 2015), which may explain their dominance in healthy *F. granulosa* collected in this study from a depth of 15-18 m. Taken together, the presence of *Rhodobacteraceae* in a range of hosts denotes environmental flexibility. For this reason, it is challenging to assign a specific role. However, the high abundance of a distinct OTU of this bacterial family in *F. granulosa* specimens from all treatments but the high salinity long-term treatment indicates that this taxon likely provides an important function to the coral holobiont.

In contrast, *Pseudomonas veronii*, the 'core' microbiome member and the most abundant taxon in corals from the hypersaline long-term treatment, was present at a much lower abundance in corals from all other treatments (i.e. freshly collected coral, short-term ambient salinity, short-term high salinity, and long-term ambient salinity). Since *P. veronii* was present in all corals, albeit at lower abundance, we argue that its increase under high salinity might signify a change of selection regime for this taxon under the altered environmental conditions, and not an opportunistic association. The uniformity of all water samples, i.e. no

significant differences in water samples over different treatments or time points, further supports a selective process for the changes in the coral microbiomes. The specific function of *P. veronii* remains to be determined. However, it seems to be a versatile taxon that has been isolated from distinct environments, e.g. natural freshwater springs, soil samples, and wastewater filters where it has been shown to degrade a variety of simple aromatic organic compounds making it a beneficial bacterium for bioremediation of contaminated environments (Elomari *et al.* 1996; Nam *et al.* 2003; Onaca *et al.* 2007). More generally, bacteria in the genus *Pseudomonas* have repeatedly been shown to be abundant in hypersaline environments and display broad metabolic capacity (Brusa *et al.* 2001; Fendrich 1988; Isnansetyo & Kamei 2009; Sass *et al.* 2001).

Among other bacterial taxa that increased in abundance in the long-term hypersalinity treatment, we identified the coral pathogen *Vibrio shilonii* (OTU0264) and also some unclassified *Alteromonadaceae* taxa (Table S3, Supporting Information). These taxa are presumably associated with coral stress and disease, but are known to reside in healthy corals as well (Rosenberg & Falkovitz 2004; Sunagawa *et al.* 2009). Taken together, bacterial microbiome restructuring under high salinity levels as signified by loss of the numerically dominant bacterial taxon (i.e. OTU0001), the increase in *P. veronii* (i.e. OTU0010), as well as an overall increase in richness, evenness, and diversity possibly indicates stress (Bourne *et al.* 2008; Garren *et al.* 2009; Meron *et al.* 2011a; Meron *et al.* 2011b; Sunagawa *et al.* 2009; Zhang *et al.* 2015). At the same time, major microbiome restructuring induced by environmental stress (i.e. high salinity) in the absence of a measurable physiological reaction of the coral holobiont may give support to the probiotic hypothesis (Reshef *et al.* 2006), i.e. a change of the microbiome to facilitate coral holobiont acclimation.

Functional changes of bacterial communities indicate metabolic adjustment

Mapping of differences in bacterial community composition to putative functional differences revealed a prominent increase in polyhydroxybutyrate (PHB) storage as well as changes in nitrogen and sulfur cycling in long-term hypersaline samples in comparison to all other coral samples. PHB can be synthesized by microorganisms as a carbon reservoir in cells (Roberts 2005) and may be produced in response to variable stressors, such as nutrient limitation, e.g. under nitrogen limiting conditions (Ayub *et al.* 2004; Soto *et al.* 2012). Interestingly, PHB has also been identified as an osmolyte in microorganisms (Arora *et al.* 2006; Doronina *et al.* 2000; Martin *et al.* 2002; Soto *et al.* 2012). Additionally, PHB production in rhizobia bacteria with a potential benefit for plant cultivation in saline soil has been suggested (Ali *et al.* 2014; Arora *et al.* 2006). It is striking that *Pseudomonas* strains closely related to *P. veronii* are shown to produce PHB (Ayub *et al.* 2004; Soto *et al.* 2012; Yan *et al.* 2008), but even more so, the genome of *P. veronii* harbors the enzyme 3-hydroxyisobutyryl dehydrogenase (Ramírez-Bahena *et al.* 2015), which is part of the PHB metabolism (Hügler & Sievert 2011). This provides a putative functional link to the numerical dominance of *P. veronii* in the long-term hypersaline samples and potentially indicates functional adaptation of the coral holobiont by alteration of its microbiome. Such functional changes were shown in the aphid *Acyrthosiphon pisum* where replacing the native gut bacteria *Buchnera* line LSR1 with line 5AY from a more thermotolerant aphid matriline conferred a dramatic increase in thermal tolerance (Moran & Yun 2015).

Changes in sulfur cycling as suggested by an up-regulation of ‘Sulfur oxidizer’ and a down-regulation of ‘Sulfate reducer’ presumably indicate the enrichment of oxidized products in the sulfur metabolism. The coral holobiont is a major contributor to the production of dimethylsulphide (DMS), a central compound of the global sulphur cycle (Raina *et al.* 2013), which can become oxidized to dimethylsulphoxide (DMSO) (Sunda *et al.* 2002). DMSO has

a stronger reactivity towards reactive oxygen species (ROS) than DMS, is more hydrophilic (allowing higher cellular concentrations), and can be further oxidized to the water-soluble antioxidant methane sulphinic acid (Sunda *et al.* 2002). Hence, an increased production (accompanied by a decreased reduction) of DMSO acting as an ROS scavenger may enable the coral to cope with increased oxidative stress in *Symbiodinium*. In agreement with these patterns, increased oxidative stress accompanied by antioxidant production as a response to high salinity has been shown for algae and other plants (Fadzilla *et al.* 1997; Gossett *et al.* 1996; Hernández *et al.* 2000; Jahnke & White 2003).

Another distinct pattern emerged from the metabolic profile of nitrogen-related functions. We found processes that increase nitrogen availability for the holobiont to be enhanced (i.e., 'dinitrogen-fixing' and 'nitrogen fixation'), whereas processes that require the availability of fixed nitrogen are reduced (i.e., 'ammonia oxidizer' and 'nitrite reducer'). This suggests an enhanced nutrient limitation of the coral holobiont (Rädecker *et al.* 2015). Nutrient limitation may be a consequence of an increased metabolism with enhanced nutrient requirements.

Long-term coral holobiont response may indicate acclimation

Changes of the coral microbiome under changed environmental conditions were previously described (e.g. Jessen *et al.* 2013; Kelly *et al.* 2014; Klaus *et al.* 2007; Meron *et al.* 2012; Pantos *et al.* 2015), and that these changes are relevant to holobiont function was demonstrated by Moran & Yun (Moran & Yun 2015). In line with these studies, we interpret the here-observed prevalent change of the coral microbiome in combination with a lack of an apparent stress response by the coral or symbiont in the long-term high salinity treatment as indication for an acclimation response. This is supported by the putative functional changes we detected in the microbial community, i.e. upregulation of PHB as an osmolyte, alterations to the nitrogen cycle to compensate for nutrient deficiency, and synthesis of DMSO as a ROS scavenger. It is important to consider that the adjustments of the *Fungia granulosa* coral

holobiont to a high salinity environment presumably require considerable energy and these energy requirements need to be taken into account when assessing the response of corals to changes in salinity. Taking the large biomass of the solitary coral *F. granulosa* into account, energy reserves may be sufficient for supposedly initial stress periods (as a response to the changing environmental conditions) and simultaneous acclimation. By comparison, commonly employed setups using small coral fragments in short-term experiments may considerably underestimate coral resilience towards (salt) stress and might miss acclimation due to insufficient energy reserves of the coral fragment to sustain and acclimate to the stressor (Chartrand *et al.* 2009; Ferrier-Pages *et al.* 1999; Kerswell & Jones 2003; Lirman & Manzello 2009; Manzello & Lirman 2003; Seveso *et al.* 2013).

Taken together, we argue that changes in salinities lead to (small) changes in the holobionts internal environment, which in turn affect microbiome structure by selecting for a more advantageous bacterial community composition as posited by the coral probiotic hypothesis (Reshef *et al.* 2006). Future studies should target the temporal stability of restructured coral microbiomes accompanied by physiological measures under enduring 'stress' conditions to unequivocally confirm the importance of the microbiome to coral holobiont function.

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Conflict of Interest

The authors declare no conflict of interest.

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Data Accessibility

Sequences determined in this study have been deposited in the NCBI Sequence Read Archive under accession number PRJNA282461 (http://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA282461).

Author contributions

TR, CRV, RvdM designed and conceived the experiments. TR and MAO generated data. TR, CRV, MAO, and AR analyzed and interpreted data. CRV and RvdM contributed reagents/materials/analysis tools. TR and CRV wrote the manuscript.

Tables

Table 1. Physiological response of coral host and algal symbiont to short- and long-term hypersalinity exposure (*=$P_{t\text{-}test}$<0.05; NA=not available, values are shown as means±SD).

	short-term (4 h)		long-term (29 d)	
	39 PSU	55 PSU	~41 PSU	~49 PSU
Calcification rate [$CaCO_3$ µmol $cm^{-2}h^{-1}$]	0.243±0.103	0.031±0.073*	NA	NA
Photosynthetic efficiency (ϕPII)	0.718±0.018	0.681±0.018*	0.690±0.015	0.705±0.009
Coral tissue discoloration (bleaching)	Not visually apparent	Not visually apparent	Not visually apparent	Not visually apparent

Table 2. Summary statistics of 16S rRNA gene amplicon sequencing detailing reef water and microbial communities associated with *Fungia granulosa* under ambient and hyper-saline conditions (*=subsampled to n=10000; ST=short-term incubation; LT=long-term incubation; A=ambient; H=hypersaline; Numbers in the LT sample names denote transect station; WS=water sample.). Total number of OTUs=2,235.

Group	Sample	Treatment	No. of reads	Coverage*	No. of OTUs*	Chao1*	Simpson Evenness*	Inverse Simpson Index*
reef water	reef_WS	-	41,689	1.00	136	195	0.026	3.540
	ST_A_WS	39 PSU	47,773	1.00	132	159	0.033	4.364
	ST_H_WS	55 PSU	37,985	0.99	181	280	0.016	2.920
	LT_1_WS	~49 PSU	42,098	0.99	185	266	0.018	3.373
	LT_2_WS	~41 PSU	68,342	1.00	158	217	0.019	2.968
	LT_3_WS	~41 PSU	49,940	0.99	158	236	0.016	2.546
	LT_4_WS	~41 PSU	41,004	1.00	171	213	0.021	3.524
	LT_5_WS	~41 PSU	44,270	0.99	177	234	0.026	4.560
	LT_6_WS	~41 PSU	37,508	1.00	158	186	0.023	3.622
freshly collected corals	reef coral_R1	-	105,511	1.00	129	161	0.021	2.646
	reef coral_R2	-	29,560	1.00	98	119	0.025	2.408
	reef coral_R3	-	20,358	1.00	61	80	0.027	1.661
	reef coral_R4	-	158,984	0.99	168	260	0.014	2.343
short-term ambient salinity	ST_A_R1	4 h at 39 PSU	92,228	0.99	206	237	0.019	3.984
	ST_A_R2		132,436	1.00	98	129	0.040	3.939
	ST_A_R3		65,695	1.00	64	106	0.021	1.370
	ST_A_R4		99,762	1.00	78	112	0.023	1.808
	ST_A_R5		51,040	1.00	84	111	0.033	2.814
short-term hypersaline	ST_H_R1	4 h at 55 PSU	51,437	1.00	113	118	0.041	4.685
	ST_H_R2		69,779	0.99	174	223	0.022	3.809
	ST_H_R3		76,435	1.00	69	96	0.022	1.534
	ST_H_R4		10,393	1.00	139	149	0.077	10.670
	ST_H_R5		55,964	1.00	110	125	0.037	4.050

long-term ambient salinity	LT_A_2_R1	29 d at ~41 PSU	37,060	0.99	348	525	0.009	3.291
	LT_A_2_R2		77,465	0.99	140	227	0.019	2.688
	LT_A_2_R3		75,949	0.99	239	300	0.009	2.068
	LT_A_3_R1		39,101	0.99	295	376	0.035	10.420
	LT_A_3_R2		15,655	0.99	282	334	0.015	4.188
	LT_A_3_R3		59,872	1.00	141	184	0.017	2.391
	LT_A_4_R1		63,348	0.99	272	314	0.033	9.006
	LT_A_4_R2		123,875	0.99	310	495	0.011	3.317
	LT_A_4_R3		72,038	0.99	276	399	0.017	4.752
	LT_A_5_R1		68,602	0.99	264	371	0.011	2.823
	LT_A_5_R2		106,800	0.98	446	631	0.009	4.147
	LT_A_5_R3		55,622	0.99	228	279	0.008	1.903
	LT_A_6_R1		121,617	0.99	228	377	0.019	4.338
	LT_A_6_R2		47,763	0.99	265	327	0.013	3.565
	LT_A_6_R3		64,844	0.99	328	424	0.042	13.754
long-term hypersaline	LT_H_1_R1	29 d at ~49 PSU	32,455	0.99	608	685	0.091	55.242
	LT_H_1_R2		63,823	0.98	736	835	0.076	56.261
	LT_H_1_R3		56,052	0.98	864	968	0.042	36.288

Table 3. Summary of SIMPER analyses showing the main contributing OTU in each treatment group. Displayed are Bray-Curtis similarity measures between group members based on OTU abundances within a sampling group, the main contributing OTU, the average abundance of this OTU, and the average contribution of this OTU to the overall group similarity.

Treatment group (Bray-Curtis similarity)	main contributing OTU (bootstrap value)	AVG read abundance	AVG contribution [%]
freshly collected coral (51.19%)	OTU0001 unclassified sp.(100), family Rhodobacteraceae(100)	6530	36.53
short-term 39 PSU (49.98%)	OTU0001 unclassified sp.(100), family Rhodobacteraceae(100)	5627.2	30.65
short-term 55 PSU (47.95%)	OTU0001 unclassified sp.(100), family Rhodobacteraceae(100)	4594.4	23.42
long-term ~41 PSU (44.03%)	OTU0001 unclassified sp.(100), family Rhodobacteraceae(100)	4722.3	19.29
long-term ~49 PSU (56.20%)	OTU0010 *Pseudomonas veronii*(93), family Pseudomonadaceae(100)	836.7	2.22

Figure legend

Figure 1. Bacterial taxonomy stack plot on the phylogenetic level of family (Greengenes database, bootstrap≥60). Each color represents one of the 16 most abundant families in all samples. All other taxa are grouped under category ‘others’. ST=short-term incubation; LT=long-term treatment; A=ambient; H=hypersaline; numbers in the LT sample names denote transect station; WS=water sample.

Figure 2. Clustering of coral samples based on Bray-Curtis dissimilarity of microbial

community abundances in a Principal Coordinate Analysis (PCoA) (R^2=0.91). Water samples=reef water, reef=freshly collected *F. granulosa*, short-term ambient=4 h at 39 PSU, short-term hyper=4 h at 55 PSU, long-term ambient=29 d at 41 PSU, long-term hyper=29 d at 49 PSU, percentages on axes indicate variations explained by the two coordinates.

Figure 3. Taxonomy-based functional profiling of bacterial communities. Heatmap created in METAGENassist displaying changes in putative functional profiles based on the 16S community composition. Changes are displayed on a relative scale with enrichment in red and depletion in blue. Data were analyzed for metabolism by phenotype with an Euclidean distance measure and average clustering algorithm. ST=short-term incubation; LT=long-term treatment; A=ambient; H=hypersaline; Numbers in the LT sample names denote transect station.

Supporting Information

Figure S1. Seawater Reverse Osmosis (SWRO) desalination plant discharge structure and 25 m transect. Stations are marked with X (Station 1: discharge screen, Station 2: 0 m, Station 3: 2.5 m, Station 4: 5, Station 5: 5 m, Station 6: 25 m).

Figure S2. Increased mucus production and bubble formation of the coral *Fungia granulosa* after 4 hours incubation at a) 39 PSU b) 55 PSU.

Figure S3. Clustering of water samples based on Bray-Curtis dissimilarity of microbial community abundances in a Principal Coordinate Analysis (PCoA) (R^2=0.89). ST=short-term incubation; LT=long-term treatment; A=ambient; H=hypersaline, numbers depict station number in long-term experiment, WS=water sample, control_Fsar=reef water sample, asterisks denote hypersaline samples, percentages indicate variations explained by the two coordinates.

Figure S4. Relative absence/presence of microbial genes associated with (A, B) PHB metabolism and (C, D) sulfur cycling via PICRUSt. ST=short-term incubation; LT=long-term treatment; A=ambient; H=hypersaline, numbers depict station number in long-term experiment, reef_coral=controls, asterisks denotes hypersaline long-term samples.

Table S1. Correlation between coral microbiomes and environmental parameters. The BEST routine in PRIMER was used to identify the 10 'best' matches between among-sample patterns of the OTU distribution and associated environmental variables (i.e., salinity, temperature, dissolved oxygen, light levels, effective quantum yield, and depth). No. of permutations=999, $P \leq 0.001$).

Table S2. OTU abundance over samples with annotation and reference OTU sequence. WS=water sample, FC=freshly collected corals, ST=short-term incubation; LT=long-term treatment; A=ambient; H=hypersaline; Numbers in the LT sample names denote transect station.

Table S3. OTUs enriched in fresh, short-term ambient & hypersaline, and long-term ambient corals ($P \leq 0.001$, average abundance$\geq$10); OTUs enriched in long-term hypersaline corals ($P \leq 0.001$, average abundance$\geq$10; abundance count in each of the 3 replicates). FC=freshly collected corals, ST=short-term incubation; LT=long-term treatment; A=ambient; H=hypersaline; Numbers in the LT sample names denote transect station.