***Supplementary methods***

**Materials and methods**

*Species studied*

We used the Red Sea jellyfish *C. andromeda* as a model cnidarian holobiont for climate change studies because (a) this species hosts a dense population of Symbiodiniaceae cells (Hofmann et al., 1996; Verde and McCloskey, 1998; Lampert, 2016), (b) it is a benthic species, usually populating shallow coastal waters in the tropics (*e.g.,* coral reefs, mangroves and seagrass meadows; Lampert, 2016; Klein et al., 2017; Ohdera et al., 2018), (c) it inhabits environments characterized by extreme O2 fluctuations (external and internal; Gray et al., 2012 and Arossa et al., 2021, respectively), and (d) it is remarkably resistant to extreme temperature levels (Gray et al., 2012, Klein et al., 2017). Recent work has also highlighted similarities between the microbiomes of *Cassiopea* and scleractinian corals, further emphasizing the species’ potential as an ideal model to investigate the role of the microbiome in cnidarian holobiont functioning (Carabantes et al., 2022).

*Seawater chemistry manipulation*

To achieve the nominal O2 treatments, four sets of three mass flow controllers (MFCs; Omega®) delivered mixtures of pure oxygen (O2), nitrogen (N2), and CO2 gas to the seawater. Two sets of MFCs and were allocated to each O2 treatment and controlled via LabVIEW Software (*sensu* Klein et al., 2017). For each treatment, the gases were mixed in stainless-steel manifolds and delivered in equal quantities before entering secondary stainless-steel manifolds that were used to adjust the flow rates manually. Air stones were used to gently diffuse the gas mixtures in the seawater of each replicate aquaria. Automatic transitioning between the two O2 levels of the second treatment was accomplished over 2 hours via the LabVIEW Software.

*Biological responses*

Symbiodiniaceae density analyses were performed following a protocol adapted from Klein et al. (2019). Briefly, we processed 18 oral arms (three from each medusa at each timepoint) as follows: once defrosted, the oral arms were homogenized using a pestle and a glass tissue grinder (Wheaton Science Products, Millville, NJ) until no tissue chunks were visible. Homogenized tissues were diluted to meet working range requirements of the analyses and dilutions were taken into account during subsequent calculations. Symbionts were counted using a hemocytometer in a 10 μL volume (n = 3) under a fluorescent microscope (Leica DM6000 B).

Chlorophyll-*a* content was measured via 100% ethanol overnight extraction. Birefly, these samples were centrifuged at 3000 × *g* at 4 ºC for 10 min, and the supernatant was discarded. The pelleted symbionts were resuspended in 1 mL of 100% ethanol and chlorophyll-*a* was extracted overnight in darkness at 4 ºC. Samples were then centrifuged at 13000 × *g* for 5 min. The supernatant was transferred to a 96-wells plate and the absorption of the supernatant was determined at 629 and 665 nm (Ritchie, 2006) using a microplate reader spectrophotometer (SpectraMax® Paradigm®, Molecular Devices, CA). Blanks (100% ethanol) were used as controls. Total chlorophyll concentrations were determined using coefficients from spectrophotometric equation for chlorophyll-*a* (μg mL-1) (-2.6094 × A629 + 12.4380 × A665) for dinoflagellates in ethanol (Ritchie, 2006). The total protein content of the samples (expressed in μg mL-1) was measured using the PierceTM BCA Protein Assay Kit (Thermo Fisher Scientific). Chlorophyll-*a* content was standardized to the total protein content of the oral arm and reported as μg mg-1 protein-1.

The bell diameter of each holobiont was measured through the transparent bottom of the glass aquaria and during water change (at ~6.00 AM) to minimize disturbance. Percent change in bell size was calculated as follows (Equation 1) on the holobionts that lasted until the end of the measurements:

(1)

Where Sizef is the end size in cm and Sizei is the initial size in cm.

*DNA extraction, ITS2 sequencing and Symbiodiniaceae identification*

One mL of the homogenate used for measuring the main biological responses was processed for Illumina sequencing of the Internal Transcriber Space 2 (ITS2) region for the characterization of the symbionts community inside *C. andromeda* oral arms (modified from Herrera et al., 2020). Briefly, oral arm samples were cut into small pieces and stored at -20 ºC until further processing. Samples were homogenized on ice using a pestle and a glass tissue grinder (Wheaton Science Products, Millville, NJ) until no tissue chunks were visible. DNA was extracted using DNeasy® Plant Mini Kit (Qiagen) following manufacturer instructions. A Nanodrop (Thermo Fisher Scientific) was used to validate DNA extractions for quality and quantity. PCR amplification was performed in triplicates on the supernatant using primers (Illumina adapters) SYM\_VAR\_5.82S2 (5′TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG‐GAATTGCAGAACTCCGTGAACC3′) and SYM\_VAR\_REV (5′GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG‐CGGGTTCWCTTGTYTGACTTCATGC3′; Hume et al., [2018](https://onlinelibrary.wiley.com/doi/full/10.1111/gcb.15263#gcb15263-bib-0047)).

Each PCR reaction was run with 10 μL of Master Mix (Qiagen Multiplex PCR Kit, Qiagen), 1 μM of each primer, and 8 µL of sample for a final reaction volume of 20 μL. The thermocycler was set as follows: 15 min at 95 ºC, followed by 45 cycles of 30 s at 95 ºC, 90 s at 56 ºC, and 30 s at 72 ºC, with a final extension step of 10 min at 72 ºC. For each sample, PCR products were run on a 1% agarose electrophoresis gel using 20 μL of sample. The PCR product was purified by cutting the gel bands and using the QIAquick® gel extraction kit (Qiagen) following manufacturer instructions and DNA quantified using Nanodrop.

Indexing was performed using the Nextera XT Index Kit v2 set A (Illumina), according to the manufacturer's instructions followed by sample normalization and final library pooling. A SequalPrep Normalization Plate Kit (Invitrogen, Thermo Fisher Scientific) was used for normalization, avoiding the more labor‐intensive process of quantifying and aliquoting each individual sample. The final pooled library was quantified on a BioAnalyzer (Agilent Technologies) and sequenced at 7 pM with 20% phiX on the Illumina MiSeq, 2 × 300 bp end version 3 chemistry according to the manufacturer's specifications at the Bioscience Core Lab at KAUST, Saudi Arabia. The online database SymPortal (SymPortal; Hume et al., 2018) was utilized to identify the Symbiodiniaceae communities using these sequencing results. In brief, the Symbiodiniaceae communities within the C. andromeda used in this study were identified using ITS2 sequencing data. The SymPortal analytical framework (Hume et al., 2018), which employs ITS2 profiles to resolve and categorize Symbiodiniaceae diversity, was used to analyze MiSeq ITS2 sequencing reads. Symbiodiniaceae communities within the C. andromeda were resolved using the SymPortal analytical framework pipeline (Klein et al., 2019). Raw sequence of ITS2 sequencing data have been deposited in the Sequence Read Archives (NCBI BioProject accession no. PRJNA966908).

***Total DNA extraction for microbiome analyses***

The oral arms that had not been utilized for previous analyses were immediately snap-frozen and kept at -80 ºC until processing. They were then thoroughly homogenized in ceramic mortars under sterile conditions after being completely thawed on ice (Shuett and Doepke, 2010). Homogenates were transferred in 1.5 mL sterile tubes, snap-frozen, and stored at –80 ºC. Samples were thawed on ice the day of the extraction, and a modified version of the technique outlined by Robbins and colleagues (Robbins et al., 2019) was used to extract total DNA from the samples. Up to 1.5 mL of thawed sample was added with 2 mL of lysis buffer (0.75 M sucrose, 50 mM EDTA, 50 mM Tris pH 8.3) and incubated at room temperature for 5 min. Three hundred µL of lysozyme solution (100 mg mL-1 in sterile water) were added to each sample, and tubes were incubated for 1 h at 37 ºC with shaking at 100 rpm. Samples were liquid nitrogen snap-frozen and thawed at 70 ºC three times, then added with 600 µL of SDS 25% solution and incubated for 10 min at 70 ºC. 100 µL of PK-SDS solution (20 mg/mL proteinase K in SDS 10%) were added to each tube and incubated for 1 h at 55 ºC with shaking at 100 rpm. Samples then underwent two other cycles of liquid nitrogen snap-freezing and thawing at 70 ºC. The digested mixtures were added with 1 volume of Phenol:Chloroform:Isoamyl alcohol (25:24:1), incubated for 5 min at 37 ºC, then centrifuged at 16.000 × g for 5 min. Supernatants were transferred in new tubes and added with 1 volume of Chloroform:Isoamyl alcohol (24:1) and centrifuged at 16,000 g for 5 min. The supernatant was transferred into a new tube and added with one volume of ice-cold isopropanol. One-tenth of the final volume of 3 M sodium acetate solution was added, and samples were incubated at –20 ºC overnight. Tubes were centrifuged for 30 min at 14.000 × g at 4 ºC, and the supernatant was discarded. The pellet was gently washed twice with 80% ethanol without resuspending and centrifuged again for 10 min at 14.000 × g at 4 ºC. Ethanol was discarded, and the pellet was left to dry in sterile conditions for 1 h at 21 ºC. DNA was resuspended in 150 µL of ultrapure H2O.

***Amplification of the bacterial 16S rRNA gene,*** *metabarcoding library preparation and sequencing*

The nested PCR for the amplification of the hypervariable V3–V4 regions of the bacterial 16S rRNA were run with the following settings: in the first PCR the 27f (3′-AGAGTTTGATCMTGGCTCAG-5′) – 907r (3′-CCGTCAATTCCTTTGAGTTT-5′) primers and in the second PCR the I341f (5’-CCTACGGGNGGCWGCAG-3’) and I785r (5’- GACTACHVGGGTATCTAATCC-3’) primers synthesised with the appropriate overhang adapter sequences for the Illumina MiSeq system. Both PCR reactions were performed in a final volume of 25 μL containing: 2.5 μL of 1 × High Fidelity Buffer, 1.5 mM of MgSO4, 0.75 μL of 0.3 mM dNTPs, 0.75 μL of each primer (0.3 mM), 0.2 μL (1 U) of Platinum® High Fidelity DNA Polymerase (Invitrogen, Waltham, USA), and 2 μL of DNA template (ca. 10 ng) along with the addition of 0.5 μM each of universal pPNA and mPNA clamps to reduce the amplification of host and dietary chloroplasts and mitochondria (Fitzpatrick et al., 2018; Reigel et al., 2020). The used thermal protocol was designed as follows: 95 ºC for 3 min, followed by respectively 20 and 24 cycles for the first and the nested PCR at 94 ºC for 30 s, 72 ºC for 20 s, 55 ºC for 55 s, and 68 ºC for 45 s and a final extension at 68 ºC for 4 min.

*Seawater chemistry characterization*

TA, pHNBS, temperature and salinity values were used to calculate *p*CO2, using CO2SYS (Pierrot et al., 2006), with K1 and K2 constants from Mehrbach et al. (1973), as revised by Dickson and Millero (1987), and the KHSO4 constant from Dickson (1990). Dissolved oxygen (*d*O2) and pH were measured according to Table S1 using a Mettler Toledo portable meter (Mettler Toledo, SevenGO Duo). The pH probe was calibrated using commercial pH standard solutions (pH 4.0, 7.0, and 10.0), whereas the *d*O2 sensor was calibrated following manufacturer’s instructions using a one-point calibration (100% air saturation) obtained using a Mettler Toledo calibration tube. The schedule in Table S1 was designed to ensure consistent conditions as expected throughout the entire experiment. Salinity was measured at the beginning, mid, and end of the experiment.

*Data analysis*

*Environmental and biological data*

Data were tested for normality performing a Shapiro-Wilk’s test (*p*>0.05) (Shapiro and Wilk, 1965; Razali and Wah, 2011), then by analysing Skewness and Kurtosis, and finally by visually inspecting histograms, normal Q-Q plots and box plots. If normality assumptions were not met, a transformation was applied. All applied transformations are listed in the tables summarizing the statistical analyses performed.

Linear Mixed Models (LMMs) used to analyse the dependent variables ofsize percent change, bell pulsation rate, photochemical efficiency, chlorophyll, symbiont density, glutathione-S-transferase, superoxide dismutase, and microbiome diversity were run as follows. For size percent change, bell pulsation rate, and photochemical efficiency, O2 treatment (with two levels: (“Nighttime supersaturation” and “100% air saturation”) was the fixed factor and temperature (with 9 levels: 30 ºC to 37 ºC) was the repeated measure. For chlorophyll*-a*, symbiont density, glutathione-S-transferase, and superoxide dismutase, O2 treatment (with two levels: (“Nighttime supersaturation” and “100% air saturation”) was the fixed factor and temperature (with 3 levels: 30 ºC, 34 ºC, and 36 ºC) was the repeated measure. In the microbiome analyses, an extra level for temperature was included (38 ºC) because some extra samples were collected for this purpose after the holobionts died but were not used for the other biological variables. Water bath number was used as random term to test for potential biases associated with individual water baths. Potential redundant terms were tested using the Wald Z test of simultaneous coefficients and estimated covariance parameters. If a significant effect on the fit of the model was observed, the tested terms were retained to account for associated variance. If a term was found to be redundant, it was removed, and the analysis was rerun. For all the analyses with LMMs the following covariance structures were tested: AR[1], AR[1]: heterogeneous, and Compound Symmetry [CS]. The best model was chosen using the smaller-is-the-better approach by comparing the goodness-of-fit statistics terms (*i.e.,* –2 log likelihood, Akaike information criterion [AIC], and Bayesian information criterion [BIC]). *Post hoc* analyses with multiple pairwise comparisons were performed using IBM® SPSS® Statistics software (Version 27.0.1.0) as necessary to test differences among the means of our data.

Results were interpreted and presented following the results of the LMM analyses and based on the highest-order, significant terms. If no significant term was observed, response variables were reported as average ± 1SE across all replicates (see Supplementary materials for figures representing analyses with non-significant results).

*Microbiome amplicon sequence data*

Raw sequences were trimmed and primers were removed using cutadapt with default parameters and by removing untrimmed reads (Martin, 2011). The plugin demux was further used to visualise interactive quality plots and assess read quality, and based on this, the forward and reverse reads were truncated at 260/280 and 180/200 bp, respectively. Using DADA2 with default parameters (Callahan et al., 2016), the reads were denoised and joined to produce amplicon sequence variants (ASVs, average length 416 bp). ASVs were clustered at 97% sequence similarity to obtain operational taxonomic units (OTUs). OTUs’ taxonomy was assigned against the SILVA 138 database using the plugin classify-sklearn (Quast et al., 2012). OTUs not identified as bacteria (*i.e.,* chloroplast, mitochondria, archaea and unclassified), singletons and OTUs detected in PCR and DNA blank controls were removed from the dataset (number of reads and OTUs removed per compartment are reported in Supplementary Table S11). Diversity accumulation curves are shown in Supplementary Fig. S13.

After taxonomic assignment and removal of non-bacterial reads, a total of 2,119,602 reads were classified in 1,426 bacterial OTUs (97% of sequence identity). The sequencing effort was sufficient to capture also the less abundant taxa (1,361 OTUs [95.4%] with relative abundance < 0.1%; Fig. S13). The compositional similarity matrix (Bray-Curtis, BC) of the log-transformed OTU table was calculated in PRIMER v.6.1, and the homogeneity of multivariate dispersions (PERMDISP) was tested to evaluate the dispersion of samples. Principal coordinates analysis (PCoA) and multivariate generalised linear models (many GLM, main and multiple comparison tests, using negative binomial family errors) were first performed to evaluate differences between seawater and holobionts oral arms by using PRIMER v.6.1 (Anderson et al., 2008) and the R package *mvabund* (Wang et al., 2012), respectively. The same approach was used to test the effect of ‘Treatment’ (“100% air saturation” *vs* “Nighttime supersaturation”), ‘Temperature’ (30 ºC, 34 ºC, 36 ºC and 38 ºC) and their interaction on bacterial compositional variability in holobionts samples. The factor aquarium was used as our random factor to account for spatial pseudo-replication. Pairwise comparisons were performed for the statistically significant term. The occurrence of temperature decay patterns in the “100% air saturation” and “nighttime supersaturation” was tested using the linear regression (GraphPad Prism 7 software, La Jolla, California, USA) between the distance from the centroid obtained from PERMDISP of each oral arm group and the temperature and between the similarity of bacterial communities (BC) and the temperature.

1. Anderson MMJJ, Gorley RNRN, Clarke KRR. 2008 PERMANOVA + for PRIMER: Guide to Software and Statistical Methods. Plymouth, UK: PRIMER-E.
2. Arossa S, Barozzi A, Callegari M, Klein SG, Parry AJ, Hung SH, Steckbauer A, Aranda M, Daffonchio D, Duarte CM. 2021 The Internal Microenvironment of the Symbiotic Jellyfish Cassiopea sp. From the Red Sea. Frontiers in Marine Science, 8, p.705915. https://doi.org/10.3389/fmars.2021.705915
3. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016 DADA2: High-resolution sample inference from Illumina amplicon data. Nature methods, *13*(7), 581-583. https://doi.org/10.1038/nmeth.3869
4. Carabantes N, Cerqueda-García D, García-Maldonado JQ, Thomé PE. 2022 Changes in the bacterial community associated with experimental symbiont loss in the mucus layer of *Cassiopea xamachana* jellyfish. Frontiers in Marine Science, p.762. https://doi.org/10.3389/fmars.2022.879184
5. Dickson AG, Millero FJ. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep Sea Research Part A. Oceanographic Research Papers. 1987 Oct 1;34(10):1733-43 https://doi.org/10.1016/0198-0149(87)90021-5
6. Fitzpatrick CR, Lu-Irving P, Copeland J, Guttman DS, Wang PW, Baltrus DA, et al. 2018 Chloroplast sequence variation and the efficacy of peptide nucleic acids for blocking host amplification in plant microbiome studies. Microbiome 6: 144. https://doi.org/10.1186/s40168-018-0534-0
7. Gray SE, Degrandpre MD, Langdon C, Corredor JE. 2012 Short‐term, & seasonal pH, pCO2, & saturation state variability in a coral‐reef ecosystem. Global Biogeochemistry Cycles. 26, GB3012. https://doi.org/10.1029/2011GB004114
8. Herrera M, Klein SG, Schmidt‐Roach S, Campana S, Cziesielski MJ, Chen JE, Duarte CM, Aranda M. Unfamiliar partnerships limit cnidarian holobiont acclimation to warming. Global Change Biology. 2020 Oct;26(10):5539-53. https://doi.org/10.1111/gcb.15263
9. Hofmann DK, Fitt WK, Fleck J. 1996 Checkpoints in the life-cycle of *Cassiopea* spp.: control of metagenesis and metamorphosis in a tropical jellyfish. Int. J. Dev. Biol. 40, 331–338. https://doi.org/10.1387/ijdb.8735945
10. Hume BC, Smith EG, Ziegler M, Warrington HJ, Burt JA, LaJeunesse TC, Wiedenmann J, Voolstra CR. 2019 SymPortal: A novel analytical framework and platform for coral algal symbiont next‐generation sequencing ITS2 profiling. Molecular ecology resources. 2019 Jul;19(4):1063-80. https://doi.org/10.1111/1755-0998.13004
11. Klein SG, Pitt KA, Nitschke MR, Goyen S, Welsh DT, Suggett DJ, Carroll AR. 2017 *Symbiodinium* mitigate the combined effects of hypoxia and acidification on a noncalcifying cnidarian. Global Change Biology 23(9), 3690-3703. *Symbiodinium* mitigate the combined effects of hypoxia and acidification on a noncalcifying cnidarian. https://doi.org/10.1111/gcb.13718
12. Klein SG, Pitt KA, Lucas CH, Hung SH, Schmidt-Roach S, Aranda M, Duarte CM. 2019 Night-time temperature reprieves enhance the thermal tolerance of a symbiotic cnidarian. Frontiers in Marine Science, p.453. https://doi.org/10.3389/fmars.2019.00453
13. Lampert KP. 2016 *Cassiopea* and its zooxanthellae. In The Cnidaria, Past, Present and Future. 415-423. https://doi.org/10.1007/978-3-319-31305-4\_26
14. Martin M. 2011 Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J.; 17:10. https://doi.org/10.14806/ej.17.1.200
15. Mehrbach C, Culberson CH, Hawley JE, Pytkowicx RM. 1973 Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure 1. Limnology and oceanography. Nov;18(6):897-907.
16. Ohdera AH, Abrams MJ, Ames CL, Baker DM, Suescún-Bolívar LP, Collins AG, Freeman CJ, Gamero-Mora E, Goulet TL, Hofmann DK, Jaimes-Becerra A. 2018 Upside-down but headed in the right direction: review of the highly versatile *Cassiopea xamachana* system. Frontiers in Ecology and Evolution 6, 35. https://doi.org/10.3389/fevo.2018.00035
17. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, ... Glöckner FO. 2012 The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic acids research, 41(D1), D590-D596. https://doi.org/10.1093/nar/gks1219
18. Razali NM, Wah YB. Power comparisons of shapiro-wilk, kolmogorov-smirnov, lilliefors and anderson-darling tests. Journal of statistical modeling and analytics. 2011 Jan 1;2(1):21-33.
19. Reigel AM, Owens SM, Hellberg ME. 2020 Reducing host DNA contamination in 16S rRNA gene surveys of anthozoan microbiomes using PNA clamps. Coral Reefs, 39(6), 1817-1827. https://doi.org/10.1007/s00338-020-02006-5
20. Ritchie RJ. 2006 Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. Photosynthesis research. Jul;89:27-41. https://doi.org/10.1007/s11120-006-9065-9
21. Robbins SJ, Song W, Engelberts JP, Glasl B, Slaby BM, Boyd J, Marangon E, Botté ES, Laffy P, Thomas T, Webster NS. 2021 A genomic view of the microbiome of coral reef demosponges. The ISME journal. Jun;15(6):1641-54. https://doi.org/10.1038/s41396-020-00876-9
22. Schuett C, Doepke H. 2010 Endobiotic bacteria and their pathogenic potential in cnidarian tentacles. Helgoland marine research. Sep;64(3):205-12. https://doi.org/10.1007/s10152-009-0179-2
23. Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). Biometrika. 1965 Dec 1;52(3/4):591-611. https://doi.org/10.2307/2333709
24. Verde EA, McCloskey LR. 1998 Production, respiration, and photophysiology of the mangrove jellyfish *Cassiopea xamachana* symbiotic with zooxanthellae: effect of jellyfish size and season. Marine Ecology Progress Series, 168, pp.147-162. https://doi.org/10.3354/meps168147
25. Wang Y, Naumann U, Wright ST, Warton DI. 2012 mvabund – an R package for model-based analy- sis of multivariate abundance data. Methods in Ecology and Evolution 3: 471–474. https://doi.org/10.1111/j.2041-210X.2012.00190.x