

Safe applications of ozone in tropical marine RAS: Determining impacts of elevated total residual oxidants (TRO) on *Stylophora pistillata* and *Xenia* sp. holobionts

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ABSTRACT

Ozone-aided treatment of seawater creates secondary oxidants, which can be toxic for aquaculture animals. We aimed to define safe limits of ozonation-derived total residual oxidants (TRO) for *Stylophora pistillata* and *Xenia* sp. by conducting a 96 h acute toxicity experiment. In a chronic exposure experiment we then determined the impacts of three sub-lethal levels of TRO on animal health over four weeks by monitoring respiration, net photosynthesis, photosynthetic efficiency, and three oxidative stress biomarkers (CAT, SOD, LPO). We further documented the changes in coral surface microbiomes exposed to elevated TRO with 16S rRNA amplicon gene sequence analysis. We showed that *Xenia* sp. is more susceptible to elevated TRO, with first mortalities occurring within 24 h at 0.188 mg L⁻¹ and higher. In *Stylophora*, mortalities were observable within 24 h at 1.272 mg L⁻¹ and after 72 h at 0.353 mg L⁻¹ TRO. Sub-lethal TRO levels caused higher respiration and lower photosynthetic efficiency in *Xenia*, and increased catalase activity by 29–42 % in *Stylophora* and by 38–45 % in *Xenia*, while not impacting lipid peroxidation or superoxide dismutase activity. Microbiomes displayed TRO-induced changes in both species, with a decline in Saprospiraceae as a potential indicator for ozone-inflicted mucus layer degradation. Vibrionaceae fully disappeared from *Stylophora pistillata* microbiomes after 26 days of exposure to 0.131 mg L⁻¹ TRO, suggesting ozonation as a potential tool to treat pathogens in coral aquaculture. Our study represents the first analysis of ozonation-induced impacts on coral holobionts, thereby providing a guideline for the safe application of ozone in coral cultivation.

1. Introduction

The growing demand for corals in ornamental aquaculture, reef restoration, and natural product development has fuelled a thriving coral husbandry industry (Leal et al., 2016). Tropical soft and hard corals are cultivated either in ocean-based nurseries or land-based systems such as flow-through systems (FTS) and recirculating aquaculture systems (RAS). In these controlled environments, water quality management is critical to ensure optimal coral growth and prevent disease outbreaks. Seawater—whether natural or artificial—must be disinfected to eliminate pathogens, which may enter via newly introduced coral specimens, live rock, or vector species (Sheridan et al., 2013).

Nevertheless, a wide range of coral diseases have been reported in cultivation settings (Mohamed and Sweet, 2019), highlighting the

urgent need for effective water treatment strategies. While coral diseases in natural reef environments remain poorly understood (Sheridan et al., 2013; Sweet et al., 2012), even less is known about their etiology and treatment in land-based aquaculture. Coral-associated microbes are highly responsive to environmental conditions, and many members of the microbiome may turn pathogenic under stress (Thurber et al., 2009). Moreover, emerging evidence suggests that several coral diseases are likely polymicrobial in origin rather than caused by a single pathogen (Meyer et al., 2019; Sweet et al., 2012, 2019).

Common ailments of cultivated corals include white syndrome (WS), characterised by progressive tissue loss and often linked to *Vibrio* spp., and brown jelly disease, which is exclusive to RAS and thought to be caused by the ciliate *Helicostoma nonatum* (Mohamed and Sweet, 2019). Although coral disease research is still in its infancy, promising

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approaches such as probiotic administration (Delgadillo-Ordóñez et al., 2024), phage therapy (Cohen et al., 2012; Efrony et al., 2007), and other microbiome-based strategies are emerging. In practice, however, the most common method of disease management in aquaculture is the fragmentation and removal of affected colonies. Anecdotal reports from hobbyist communities also describe the use of freshwater dips, iodine, or antibiotics, although peer-reviewed validation of these treatments is still largely lacking (Mohamed and Sweet, 2019).

Disease prevention in RAS depends not only on direct antimicrobial interventions but also on maintaining stable environmental conditions to avoid stress-induced dysbiosis in coral microbiomes. As coral aquaculture is still an emerging field, it presents a valuable opportunity for the adoption of novel, evidence-based water treatment practices. Among these, ozone-aided disinfection has gained considerable attention. Land-based RAS are increasingly appreciated in modern aquaculture for their high controllability, low water usage, and reduced environmental footprint (Badiola et al., 2012; Martins et al., 2010). However, the continual reuse of water calls for the implementation of advanced mechanical and biological filtration systems, coupled with efficient disinfection protocols (Timmons and Ebeling, 2013). Ozone is particularly attractive in this context, as it improves water quality by increasing oxygen levels and eliminating suspended solids, microbes, and dissolved organic compounds, thereby enhancing the health and survival of aquatic organisms (Grotmol and Totland, 2000; Liltved et al., 2006; Tango and Gagnon, 2003).

Despite its benefits, seawater ozonation needs to be practiced with caution. Unlike freshwater systems, the ozonation of seawater leads to the formation of ozone-produced oxidants (OPO), which result from the reaction between ozone and naturally occurring bromide ions (Br^-). These reactive by-products are transient and eventually degrade, but excessive ozone dosing or prolonged water recirculation can lead to the accumulation of toxic concentrations, which can be measured in seawater as total residual oxidants (TRO) with the DPD method (Buchan et al., 2005). TRO tolerance is highly species-specific, with existing research largely focused on commercially valuable organisms such as whiteleg shrimp (*Litopenaeus vannamei*) (Schroeder et al., 2010), tilapia (*Oreochromis niloticus*) (Wuertz et al., 2023), sole (*Solea senegalensis*) (Guilherme et al., 2019), and turbot (*Psetta maxima*) (Reiser et al., 2010). In contrast, there is limited knowledge regarding safe TRO thresholds for anthozoans, despite the frequent use of ozone in tropical marine aquaria.

The status of ozone-produced oxidants as secondary oxidants effectively extends the disinfecting properties of ozone beyond the point of application, thereby providing an advantage over disinfection methods like ultraviolet (UV) treatment, which are often limited to localised effects (Summerfelt, 2003). These oxidants persist in the water column and continue to exert antimicrobial activity as water circulates through the system. Notably, they have been shown to influence biofilm formation and alter microbial community composition in aquaculture systems (Aalto et al., 2022; Wietz et al., 2009). This is particularly advantageous, as biofilms are widely recognised as reservoirs for pathogens, offering protection from environmental stressors and antimicrobial agents (Cai and Arias, 2017). However, the germicidal action of ozonation-derived oxidants is not limited to system infrastructure; it likely also affects the biofilms associated with cultivated organisms themselves and thereby the host-associated microbiomes of marine macrophytes and animals, although direct evidence remains scarce. This raises the possibility of using ozone to deliberately modify dysbiotic or disease-associated microbiomes through the action of ozone-produced oxidants. At the same time, oxidant formation calls for caution in ozone water treatment, as unintended disruptions to beneficial microbial communities could compromise coral health and ecosystem stability.

1.1. Objectives

With the following experiments we aimed to define safe TRO concentrations for corals by exposing two model species to an increasing series of ozonation levels (400–800 mV) for 96 h, with TRO (measured by the DPD method) serving as a proxy for ozone-produced oxidants (OPO). We furthermore tested their long-term physiological reactions by exposing them to sublethal ozone-elevated TRO levels (C-350mV, C-480mV, C-600mV) over the course of four weeks by monitoring respiration, net photosynthesis, and photosynthetic efficiency. Oxidative stress management was analysed by measuring lipid peroxidation (LPO), catalase (CAT) activity, and superoxide dismutase (SOD) activity, assessing both cellular defense mechanisms and the extent of damage induced by TRO exposure. To investigate species-specific responses to oxidative water treatment, we focused on two commonly cultured but physiologically distinct coral species: the hard coral *Stylophora pistillata*, a widely used model in coral research known for its experimental tractability (Shefy and Rinkevich, 2021), and the soft coral *Xenia* sp., a fast-growing octocoral representative often found in aquaculture settings (Ellis, 1999). Considering the potential of ozone-produced oxidants to modify microbial communities of water and biofilms, we hypothesised that they would also alter coral surface-associated microbiomes. To test this hypothesis, we sampled the corals' surface mucus layer (SML) microbiomes and filtered tank seawater and applied 16S rRNA gene amplicon sequencing to monitor changes in the microbial communities.

2. Materials and methods

2.1. Coral husbandry and maintenance

Stylophora pistillata colonies used in the following experiments originate from Hagenbeck's Tierpark (Germany). *Xenia* sp. colonies were supplied by DeJong Marinelife (Netherlands). Both species had been in cultivation since before 2014 and were kept in the aquaria facilities at the Centre for Tropical Marine Research (ZMT) for at least a year prior to this study. Mother colonies were cut into fragments of 2–5 cm two weeks before the first experiment and attached to coral plugs with coral glue (Fauna Marin, Germany). They were housed in a 600 L aquarium filled with artificial seawater (Tropic Marin Pro Reef, Germany), with an Absolute Salinity (S_A) of 35 g kg⁻¹. During this phase, they were fed with live copepods ad libitum every 3–4 days.

2.2. Experimental setup

We conducted two experiments using the same experimental setup. Experiments took place in indoor recirculating systems consisting of a main 200 L tank holding the experimental organisms, and a water treatment unit of 100 L located underneath. The latter contained a protein skimmer (Deltec i450, Deltec, Germany) and a return pump (UP 2000, 2000 L h⁻¹, aquabee Aquarientechnik, Germany) that transferred water from the treatment unit to the experimental tank. An overflow valve in the experimental tank led the water back into the treatment unit. The experimental tank was equipped with an additional submersible pump (Turbelle® nanostream 6025, 2800 L h⁻¹, TUNZE Aquarientechnik GmbH, Germany) to create water movement. Temperature was set to 25 °C and regulated automatically through an aquarium heater controlled by an UW thermometer (TSic™-506F, IST, Switzerland). An ozone generator (Ozone 90, AquaMedic, Germany) was connected to the protein skimmer in all but the control tanks, and ozone was sucked into the skimmer's air inlet of the skimmer pump. Ozonation levels were defined by the redox values in the experimental tanks. The ozone generator was connected to a redox controller (Redox Potential Mess- und Regelgeraet, Sander, Germany), which switched the generator on when the redox value measured by a connected sensor placed in the experimental tank fell beneath a predefined value. Artificial seawater (S_A 35 g kg⁻¹), prepared using reverse osmosis water and

commercial sea salt (Tropic Marin, Germany), was used in all experiments. Light was supplied in a 12:12 h light:dark cycle by two Hydra 52 HD LED lamps per tank (Aquaillumination, USA) at an intensity of $629.8 \pm 102.6 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ measured 20 cm above the water surface with a RAMSES spectral radiometer (TriOS, Germany).

2.3. Acute toxicity experiment

The experimental design comprised seven ozone-controlled redox levels (control, 400, 500, 600, 700, 750, and 800 mV), with two biological replicates per level ($n = 2$). 10 colonies of *Stylophora pistillata* and *Xenia* were placed in each experimental tank 7 days prior to the beginning of the experiment. Sangokai (SANGOKAI BASIS-System, SANGOKAI, Germany) was administered according to the producer's recommendations to facilitate acclimation by providing core nutrients, trace elements and amino acids. Corals were fed with live copepods ad libitum every 3–4 days during the acclimation phase. At the start of the experiment, redox levels were gradually ramped up over an 8-h period to reach their target values, after which they were maintained at those final setpoints for 96 h of exposure. Mortalities were determined daily according to visual appearance and by applying tactile stimuli to polyps and documenting reactions. Photos of colonies were taken at the end of each day using an underwater camera (9Black, GoPro, US). TRO concentrations were measured twice per day in triplicates, using the DPD method following the producer's instruction (Lovibond, Germany); one finely ground tablet was dissolved in 10 mL of water taken from the experimental tank and absorbance was measured at 510 nm after an incubation time of 3 min (Buchan et al., 2005). TRO concentrations were expressed as Cl_2 equivalents. Oxygen and pH were measured daily with a handheld multisensor (WTW Multiprobe, Xylem, US).

2.4. Chronic toxicity experiment

An experiment testing the impacts of sublethal ozonation levels was conducted over the course of 30 days, with four sub-lethal redox levels and corresponding mean TRO concentrations chosen based on the mortalities recorded in the previous experiment: 'control' (~167 mV), 'C-350mV' (low) – corresponding to routinely applied levels in tropical marine aquaria -, 'C-480mV' (medium), and 'C-600mV' (high). Although two *Xenia* mortalities occurred in the acute trial at a redox setpoint of 600 mV, this concentration was retained as the highest treatment level, as it could still be regarded as sub-lethal for *Stylophora* and reflected a realistic scenario of accidental ozone overdosing in a tropical marine RAS setting. Five replicates were installed for each treatment ($n = 5$). Samples were taken before the beginning of ozonation (Day 0), after 1–2 days for short-term effects, after 13–14 days for intermediate effects, and after 26–27 days for long-term effects. Sampling was conducted over two consecutive days due to time constraints, with one coral species sampled per day, starting with *Stylophora*. One individual was sampled per tank and sampling point. The parameters we recorded were respiration, net photosynthesis, and photosynthetic efficiency (Fv/fm). Additionally, visual appearances of colonies were documented every 3–4 days with a waterproof camera (9Black, GoPro, US). TRO and other water parameters were measured every 3–4 days, as in the previous experiment (see Supplemental Material, Table S1 for pH and O_2 saturation on selected dates). For respiration and photosynthesis rate measurements, coral fragments were placed in 200 mL plastic screw top containers filled with seawater from their respective tanks after carefully cleaning the coral stubs with a toothbrush to remove microalgae and bacterial biofilms. Containers were equipped with a magnetic stir bar and closed underwater in the experimental tanks to exclude air bubbles. Containers were placed in a water bath (25 °C) on top of a magnetic stirrer and connected to an optode (Fiber-Optic Oxygen Meter, Pyroscience, Germany) via a stick-on oxygen-sensor spot (OXSP5, Pyroscience, Germany), measuring O_2 levels in the container. For respiration measurements, the containers were incubated in the dark for

20 min, and O_2 concentrations were logged in intervals of 2 s. Subsequently, they were exposed to light conditions matching those of the experimental tanks for 20 min, recording O_2 -production resulting from photosynthesis in 2-s intervals. Corals were then transferred to 1000 mL PVC containers filled with seawater (25 °C), and photosynthetic efficiency (Fv/fm) was measured following 15 min of dark incubation using a PAM-2500 chlorophyll fluorometer (Waltz, Germany). The fluorescence probe was placed 1 cm above the base of the coral colony. *Stylophora* colonies were then weighed using an analytical balance (Sartorius, Germany) with a custom suspension setup to record buoyant weight. *Xenia* colonies were placed on a scale, and their biomass was recorded as milligrams of wet weight. Following these measurements, corals were sacrificed by shock-freezing in liquid nitrogen for later assessment of oxidative stress biomarkers (LPO, CAT, SOD) in coral tissues. They were stored at -80°C until further processing.

One colony of each *Stylophora* and *Xenia* in every experimental tank was designated for monitoring the surface-associated microbiome throughout the experiment. To this end, samples were taken by removing the colony briefly from the tank and swabbing the same area every time from base to tip with a sterile cotton swab. *Stylophora* swab samples were taken from the treatments 'control', 'C-350mV' and 'C-600mV' at Day 0 and Day 26 of the experiment for assessing the impacts of long-term ozonation, and the 'C-600mV' treatment was additionally sampled on Day 1 to assess short-term impacts. *Xenia* swab samples were taken at Day 0 and Day 26 of the treatments 'control', 'C-350mV', and 'C-480mV'. For the sampling of tank water microbiomes, 1.8 L of water from all 'control' and 'C-350mV' treatments were filtered through 0.22 μm Whatman filters (Sigma-Aldrich, Germany) on Day 0 and Day 27. The swabs and filters were placed in microtubes and shock-frozen in liquid nitrogen, then stored at -80°C until DNA extraction.

2.5. Biomarker analyses: oxidative stress damage & response

2.5.1. Homogenisation of coral tissues

Xenia sp. Whole colonies were thawed and homogenised in 0.1 M K-phosphate buffer (dilution 1:5) for 3×10 s using an ultra turrax (Sigma-Aldrich, US), with samples put on ice between rounds of homogenisation. Aliquots were taken for LPO (lipid peroxidation) quantification assays. Samples were then centrifuged for another 20 min at 10,000g, and aliquots were taken for CAT (catalase) and SOD (superoxide dismutase) activity assays, as well as for protein quantification. All aliquots were stored at -80°C until processing.

Stylophora pistillata. Pieces of colonies between 1.8 and 3.5 g were homogenised to a fine powder with a pre-chilled mortar and pestle and mixed with 1500 μL of 0.1 M K-phosphate buffer. Samples were centrifuged for 3 min at 3000g to remove coral skeletons. 1 mL of supernatant was transferred to a 2 mL Eppendorf tube. Homogenisation and aliquoting were then performed as described for *Xenia*.

2.5.2. Biomarker measurements

All biomarkers were measured using a spectrophotometer (Tecan, Switzerland) and transparent F-bottom 96-microwell plates (Greiner, Austria). Readings were performed in triplicates or quadruplicates. Blank readings were performed using homogenisation buffer (K-phosphate buffer, 0.1 M, pH 7.4) and subtracted from the sample readings before analysis. Protein content of both crude homogenate and post-mitochondrial supernatant (supernatant obtained after centrifugation at 10,000g for 20 min) was measured following Bradford (1976). LPO was assessed following the method by Ohkawa et al. (1979) and modified by Bird and Draper (1984), adapted for microwell plate readers. Malondialdehyde (MDA)-thiobarbituric acid (TBA) complexes were measured at 535 nm, and results standardised to protein content of the crude homogenate. Values were expressed as nmol TBARS mg^{-1} protein. SOD activity was measured following the method after McCord and Fridovich (1969), adapted for microwell plate readers. A xanthine-xanthine oxidase system was used to generate superoxide radicals,

and SOD activity in the sample inhibited their reduction of cytochrome c, which was measured at 550 nm over 5 min. A standard curve was prepared using SOD with a known concentration of 1.5 U mL⁻¹. Catalase (CAT) was assessed following the method of Claiborne (1985), adapted for microwell plates. CAT activity was measured at 240 nm for 3 min by tracking the decrease in absorbance as hydrogen peroxide (H₂O₂) was converted into water (H₂O) and oxygen (O₂). Results of both SOD and CAT were standardised to protein content of the post-mitochondrial supernatant, yielding values in SOD U mg⁻¹ protein and $\mu\text{mol min}^{-1}$ mg⁻¹ protein.

2.6. DNA extraction and 16S rRNA gene amplicon sequencing

DNA from swab samples and water filters was extracted using the Qiagen Blood & Tissue kit (Qiagen, Germany) following the producer's instructions. For sequencing, variable regions V3 and V4 of the 16S rRNA gene were amplified using the universal primer pair 341F (5'-CCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWCTAAT-3') (Caporaso et al., 2012; Muyzer et al., 1993). PCR products were normalised using the SeqPrep Normalisation Plate Kit (Thermo Fischer Scientific, Waltham, MA, USA), pooled equimolarly, and sequenced on the Illumina MiSeq v3 2x300bp (Illumina Inc., San Diego, CA, USA). Demultiplexing after sequencing was based on 0 mismatches in the barcode sequences. Amplification and sequencing work were carried out in the microbiome lab of the IKMB at Kiel University, Germany. Sequences were processed using Qiime2 (Bolyen et al., 2019). After primer removal with cutadapt (Martin, 2011), dada2-plugin was used with default settings to filter and merge demultiplexed raw reads (Callahan et al., 2016). Taxonomical assignments were computed using the feature-classifier plugin (classify sklearn) with default settings and SILVA classifiers (release 138) pre-trained with the respective primers for prokaryotes (Bokulich et al., 2018; Pedregosa et al., 2012; Quast et al., 2013). All raw sequence data have been deposited under NCBI BioProject PRJNA1301290.

2.7. Statistical analyses

2.7.1. Respiration, net photosynthesis, photosynthetic efficiency, molecular biomarkers for oxidative stress (CAT, SOD, LPO)

O₂ production (light measurement) and consumption rates (dark measurements) were calculated from the O₂ data using linear regression over at least 400 s where rates were stable. Net photosynthesis was calculated by subtracting respiration from gross O₂ production. All data were standardised by dividing by buoyant weight (*Stylophora*) and wet weight (*Xenia*) of the colonies. All statistical analyses were done in RStudio (version 2023.6.0.421), engaging the packages 'emmeans' (Lenth et al., 2025), 'dplyr' (Wickham et al., 2023), 'car' (Fox and Weisberg, 2019), 'rstatix' (Kassambara, 2023), and 'MASS' (Venables and Ripley, 2002). Data were visualised using the 'ggplot2' (Wickham, 2016) and 'patchwork' (Pedersen, 2022) packages. Data were checked for normality by visual inspection of histograms and with Shapiro Wilk tests, and homogeneity of variance was tested with Levene's test. In the photosynthetic efficiency dataset for *Xenia*, outliers were removed using the interquartile range (IQR) method, excluding values lying more than 1.5 times the IQR below the first quartile or above the third quartile. This step was necessary to meet the assumptions of normality and homogeneity of variance required for parametric tests. Data transformations were applied as follows to fulfill the assumptions of two-way ANOVA: log transformation was used for net photosynthesis (*Stylophora*), respiration (*Xenia*), SOD (*Stylophora*), and CAT (*Xenia*); square root transformation for net photosynthesis (*Xenia*) and SOD (*Stylophora*); Box-Cox transformation for CAT (*Stylophora*); and inverse transformation for LPO (*Xenia*). If the design was balanced, data were analysed using two-way ANOVA (Type 1), with sampling day and treatment as fixed factors, and their interaction included in the model. Type 2 ANOVA with only main effects included in the model was used if the

design was unbalanced. This applied to all physiological measurements in *Xenia*, where high mortalities occurred in some replicates, and to all biomarker measurements in both *Stylophora* and *Xenia*, where mortalities and occasional sample mishandling led to unbalanced designs. Post-hoc pairwise *t*-tests with Tukey's adjustment of *p*-values were performed to show differences between treatments at specific sampling days. *P*-values below 0.05 were considered statistically significant.

2.7.2. Multivariate statistics: 16S rRNA gene amplicon sequence data

All multivariate analyses of data deriving from 16S rRNA gene amplicon sequencing were performed in RStudio, using the 'phyloseq' (McMurdie and Holmes, 2013), 'emmeans' (Lenth, 2024), 'rstatix' (Kassambara, 2023), 'vegan' (Oksanen et al., 2024), 'pairwiseAdonis' (Martinez Arbizu, 2020), 'FSA' (Ogle et al., 2025), and 'DESeq2' (Love et al., 2014) packages. Plots were created using the 'ggplot2' (Wickham, 2016), 'ggVennDiagram' (Gao et al., 2021), and 'patchwork' (Pedersen, 2022) packages. A feature table, a metadata table and a taxonomy table containing all samples (*Stylophora*, *Xenia* and cultivation water) were imported into RStudio and combined to a phyloseq object using the 'phyloseq' package. 3467 mitochondrial and 105,512 cyanobacterial sequences were filtered from the set. A Bray-Curtis distance matrix was calculated, and a PCoA analysis performed. A PCoA plot was created by plotting PC1 and PC2. The tankwater samples were thereafter removed from the set, and the phyloseq object was split up into two separate phyloseq objects '*Stylophora*' and '*Xenia*'. Low abundance reads were removed from both objects by applying a threshold of 10 minimum reads and 0.01 % relative abundance. Good's coverage index was calculated to evaluate sequencing depth. Data sets were rarefied to the respective minimum sequencing depth (5920 reads in *Stylophora*, 7054 in *Xenia*) before determining alpha diversity through the calculation of Observed richness, Shannon index and Pielou's evenness. Differences between treatments were tested for with Kruskal-Wallis tests, and Dunn tests with Benjamini-Hochberg correction were applied to identify differences between treatments at single timepoints. Bray-Curtis distance matrices were created for *Stylophora* and *Xenia* data sets, and we performed PERMANOVA to test for the impacts of treatment and sampling day on differences in community composition. Pairwise post-hoc comparisons were performed using the pairwise.adonis() function of the 'pairwiseAdonis' package, with Benjamini-Hochberg correction for multiple comparisons. A differential abundance analysis was carried out on ASV level for C-600mV-treatment *Stylophora* samples, contrasting Day 0 and Day 1, to identify the short-term impacts of high TRO-exposure.

Log-scaled mean raw abundance counts of families indicative of coral health (Flavobacteriaceae (McDevitt-Irwin et al., 2017), Rhodobacteraceae (McDevitt-Irwin et al., 2019; Rosales et al., 2020), Alteromonadaceae, Rhizobiaceae (Becker et al., 2022; McDevitt-Irwin et al., 2019; Rosales et al., 2020)) and mucus layer integrity (Saprospiraceae (Weidlich et al., manuscript under review)), as well as potential symbionts (Rhizobiaceae and Alteromonadaceae), and pathogens (Vibrionaceae (Glasl et al., 2016; Thurber et al., 2009), Sphingomonadaceae (Rosales et al., 2019), Pseudoalteromonadaceae (MacKnight et al., 2021)), were plotted as barplots displaying the experimental start and end. Kruskal-Wallis tests were used to identify the impacts of ozone treatment at the experimental end. Dunn's pairwise post-hoc tests with Holm correction were used to assess treatment differences. The 15 most abundant ASVs in both species across all treatments were plotted as horizontal bar charts showing experimental start and end. A Venn diagram was created to illustrate the overlap among the 25 most abundant ASVs identified in each treatment group at the experimental end. Shared and unique ASVs between treatments were depicted to illustrate the core and treatment-specific microbial signatures.

3. Results

3.1. Acute toxicity experiment

3.1.1. TRO measurements

Mean TRO concentrations ranged from 0.057 (C1, 400 mV) to 1.272 mg L⁻¹ (C6, 800 mV). Notably, there was an exponential increase in TRO concentration with rising redox values, resulting in large differences among the higher ozonation levels. In addition, variability increased with redox potential, as higher treatments were associated with greater variance in TRO concentrations across sampling points.

Table 1. Acute toxicity experiment – TRO measurements in experimental tanks. The redox setpoint (mV) and the mean concentrations of total residual oxidants (TRO) measured using the DPD method, reported as mean ± standard error (SE) for every treatment level. TRO values are expressed as equivalent chlorine concentrations (mg Cl₂ L⁻¹).

3.1.2. Mortalities

Within the first 24 h of the experiment, all colonies of both *Stylophora pistillata* and *Xenia* sp. perished in both replicates of the highest treatment level (800 mV), as well as *Xenia* sp. in the 750 and 700 mV treatments. Two additional *Xenia* sp. mortalities were recorded in one replicate of the 600 mV treatment within the subsequent 24 h. In *Stylophora*, another 5 and 7 mortalities occurred in the 750 mV treatment replicates within the last 24 h of the experiment. No mortalities occurred in controls, 400 mV and 500 mV treatments. Total mortality counts after 96 h of exposure are summarised in **Table 2**.

Table 2. Mortalities of *Stylophora pistillata* and *Xenia* sp. after 96 h exposure to seven ozone-controlled redox levels ($n = 2$ replicate tanks per level, 10 colonies per species per tank).

3.1.3. Visual appearances

Stylophora pistillata responded to the ramping up of ozone levels by temporarily retracting tentacles across all ozone treatments. However, tentacle extension resumed in all but the highest two treatments within a few hours, indicating a degree of acclimatisation to moderate TRO exposure. Within 48 h, *Stylophora* displayed fully extended tentacles in the 400 mV treatment, while tentacle extension remained partial in the 500 mV and 600 mV treatments for the rest of the experiment. In the 750 and 800 mV treatments, colonies showed visible tissue necrosis and lesions after 24 h and 96 h, respectively. Notably, no bleaching occurred prior to the mortalities in these treatments, suggesting that tissue damage progressed rapidly, bypassing the typical bleaching phase often associated with sublethal stress.

In *Xenia* sp., colonies exposed to TRO at redox levels of 500 mV and higher developed a darker, greyish coloration, differing distinctly from the light pink appearance of control individuals. The intensity of the colour change correlated with increasing ozone levels. Colonies in the ≥600 mV treatments exhibited clear signs of physiological distress, including contraction of the body and loss of tentacles. *Xenia* also temporarily retracted its tentacles in response to ozone exposure, with re-extension occurring slower compared to *Stylophora*. While mortality

Table 1

Acute toxicity experiment – TRO measurements in experimental tanks. The redox setpoint (mV) and the mean concentrations of total residual oxidants (TRO) measured using the DPD method, reported as mean ± standard error (SE) for every treatment level. TRO values are expressed as equivalent chlorine concentrations (mg Cl₂ L⁻¹).

Treatment	Control	C1	C2	C3	C4	C5	C6
Redox Setpoint (mV)	NA	400	500	600	700	750	800
mean TRO (mg Cl ₂ L ⁻¹) ± SE	NA	0.057 ± 0.010	0.092 ± 0.013	0.099 ± 0.009	0.188 ± 0.014	0.353 ± 0.040	1.272 ± 0.193

Table 2

Mortalities of *Stylophora pistillata* and *Xenia* sp. after 96 h exposure to seven ozone-controlled redox levels ($n = 2$ replicate tanks per level, 10 colonies per species per tank).

	Control	C1 (400 mV)	C2 (500 mV)	C3 (600 mV)	C4 (700 mV)	C5 (750 mV)	C6 (800 mV)
<i>Stylophora</i>							
Replicate 1	0	0	0	0	0	5	10
Replicate 2	0	0	0	0	0	7	10
<i>Xenia</i>							
Replicate 1	0	0	0	2	10	10	10
Replicate 2	0	0	0	0	10	10	10

was limited in the 600 mV group, colonies appeared in poor condition by the end of the experiment, showing multiple missing polyp heads per colony and a dark coloration. In contrast, *Xenia* colonies in the 400 mV treatment appeared healthy overall after 96 h, with morphology and coloration similar to controls and tentacles fully extended.

3.2. Chronic toxicity experiment

3.2.1. TRO measurements

Ranges of redox values in the experimental tanks and mean TRO values (mg Cl₂ L⁻¹) calculated from all 5 replicates of each treatment are shown in **Table 3**. The high range of measured redox values resulted from the spatial distance between the redox probe and the treatment tank, where ozone was injected. A boxplot showing TRO-concentrations for each individual experimental tank can be found in the supplemental material (Supplemental material, Fig. S1).

Table 3. Chronic toxicity experiment – TRO measurements in experimental tanks. The table presents the redox setpoint (mV), the observed range of redox values recorded every 3–4 days, and the mean concentrations of total residual oxidants (TRO) measured using the DPD method, reported as mean ± standard error (SE) for every treatment level. TRO values are expressed as equivalent chlorine concentrations (mg Cl₂ L⁻¹).

3.2.2. Mortalities

Only for *Xenia* sp. mortality was observed. Mortalities occurred in 6 of the 20 experimental units: one in a control treatment, two cases of two mortalities each in C-350mV treatments, and three cases in C-600mV treatments, where we recorded 6, 5, and 3 mortalities—making C-600mV the group with the highest mortality rate. There were no incidences of mortality in any C-480mV treatment (Table S2, Supplemental material).

3.2.3. Physiological responses

As in the previous experiment, both species initially retracted their

Table 3

Chronic toxicity experiment – TRO measurements in experimental tanks. The table presents the redox setpoint (mV), the observed range of redox values recorded every 3–4 days, and the mean concentrations of total residual oxidants (TRO) measured using the DPD method, reported as mean ± standard error (SE) for every treatment level. TRO values are expressed as equivalent chlorine concentrations (mg Cl₂ L⁻¹).

Treatment	Control	C-350mV	C-480mV	C-600mV
Redox setpoint (mV)	NA	350	480	600
Redox range (mV)	167–190	326–404	413–554	542–671
Mean TRO (mg Cl ₂ L ⁻¹) ± SE	0.03 ± 0.008	0.057 ± 0.019	0.078 ± 0.014	0.131 ± 0.027

tentacles following the activation of the ozone generators but extended them again after a few hours. In *Stylophora pistillata*, tentacles remained only partially extended in C-480mV and C-600mV treatments throughout the experiment, whereas in controls and C-350mV, full extension was observed after several days of acclimatisation. *Xenia* sp. colonies showed visible signs of stress in the higher treatments (C-480mV and C-600mV), characterised by body contraction, retracted or partially retracted tentacles, and a shift to a greyish coloration. These symptoms were less pronounced in C-350mV. By the end of the experiment, most C-600mV *Xenia* colonies displayed a distinct greyish colouration and several missing tentacle heads. Colonies in the C-480mV treatments showed only little colour change compared with light pink individuals in the control tanks and were not missing tentacle heads. Notably, C-350mV *Xenia* seemed to adapt to oxidant concentrations after about 2 weeks, and most colonies looked as healthy as those in control tanks by the end of the experiment. In *Stylophora pistillata*, there was no significant effect of ozone treatment on net photosynthesis ($F(3, 64) = 2.4, p = 0.08$) and respiration ($F(3, 64) = 2.35, p = 0.08$) (Fig. 1). There was no effect of sampling day, nor factor interactions. *Stylophora* photosynthetic efficiency was not affected by TRO ($F(3, 64) = 0.41, p =$

0.75), however slightly, but significantly ($F(3,64) = 5.19, p < 0.01$) increased from Day 0 (0.574–0.595) to Day 26 (0.593–0.625). In *Xenia*, exposure to TRO significantly elevated respiration ($F(3,61) = 4.07, p = 0.01$) and lowered photosynthetic efficiency ($F(3, 56) = 3.75, p = 0.02$). The impact on net photosynthesis was not significant ($F(3, 61) = 2.64, p = 0.057$). There was also a significant effect of sampling day on respiration ($F(3, 61) = 3.59, p = 0.01$), with respiration rates increasing over time — from 0.086 to 0.138 $\mu\text{mol O}_2 \text{ min}^{-1} \text{ g}^{-1}$ on Day 0 to 0.096–0.220 $\mu\text{mol O}_2 \text{ min}^{-1} \text{ g}^{-1}$ by Day 26. Pairwise post hoc testing showed elevated respiration by 63.5 % in C-480mV ($p = 0.07$) and 23.6 % in C-600mV ($p = 0.07$) on Day 27. Photosynthetic efficiency was reduced by 12 % in C-350mV ($p = 0.03$) and by 13 % in C-600mV ($p = 0.03$) on Day 14 (Fig. 1).

There was a significant impact of ozone treatment on CAT activity in both *Stylophora pistillata* ($F(3, 62) = 4.22, p = 0.011$) and *Xenia* sp. ($F(3, 60) = 6.42, p < 0.001$) (Fig. 2). In *Stylophora*, pairwise comparisons showed a 21 % increase in C-480mV ($p = 0.003$) and a 26 % increase in C-600mV ($p = 0.015$) of CAT activity on Day 13 compared with controls, and there was a strong impact of sampling day ($F(3, 62) = 10.27, p = 0.001$). In *Xenia*, there was an additional impact of sampling day ($F(3,$

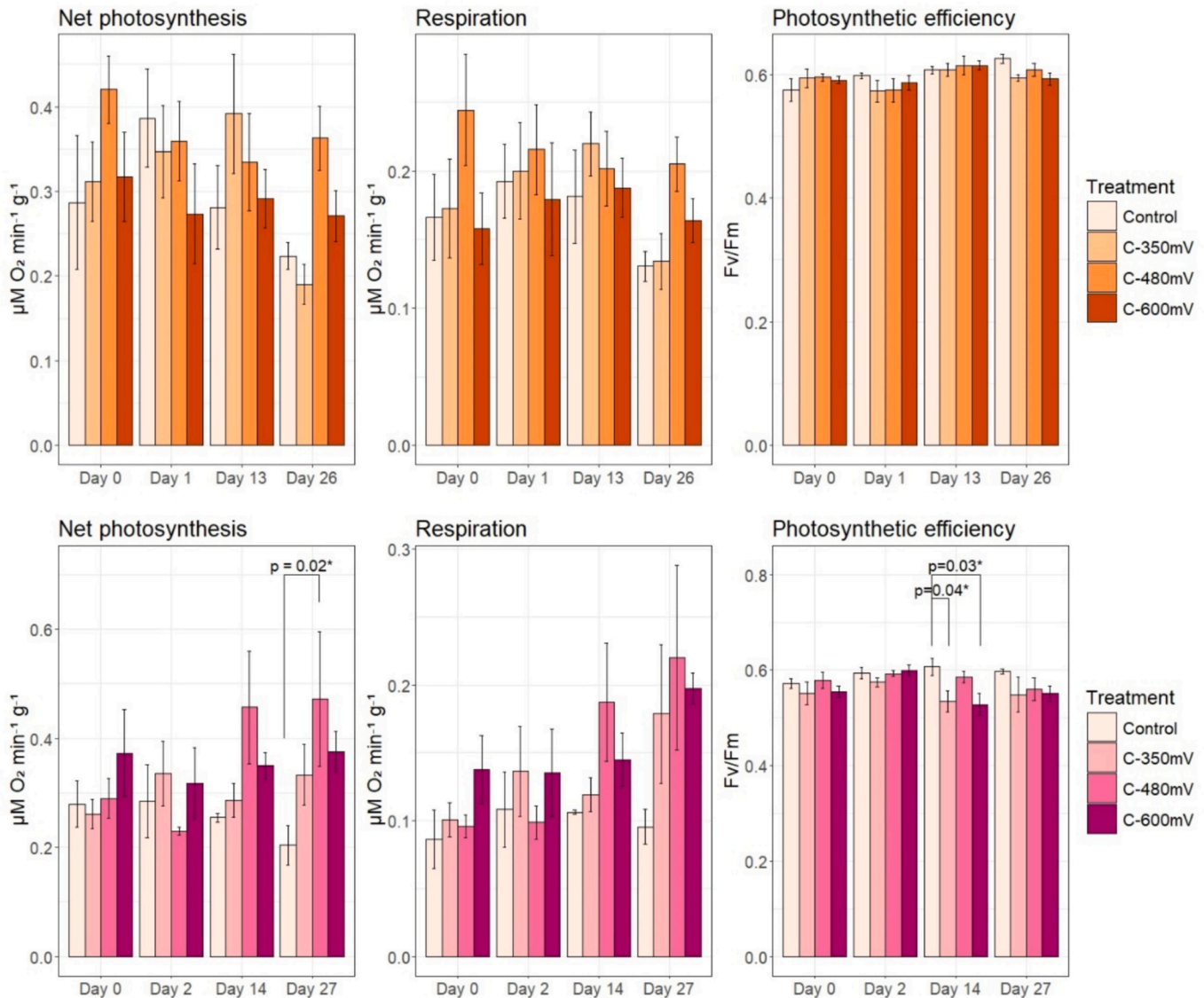


Fig. 1. Net photosynthesis, respiration, and photosynthetic efficiency in *Stylophora* sp. (upper row) and *Xenia* sp. (lower row). Displayed are mean values (bars) and standard errors (error bars). Ozone treatment increased net photosynthesis ($p = 0.02$) and lowered photosynthetic efficiency in *Xenia* ($p = 0.02$). Significant ($p < 0.05$) p -values of pairwise post-hoc comparisons are displayed in the graph. ($n = 5$ for all treatments).

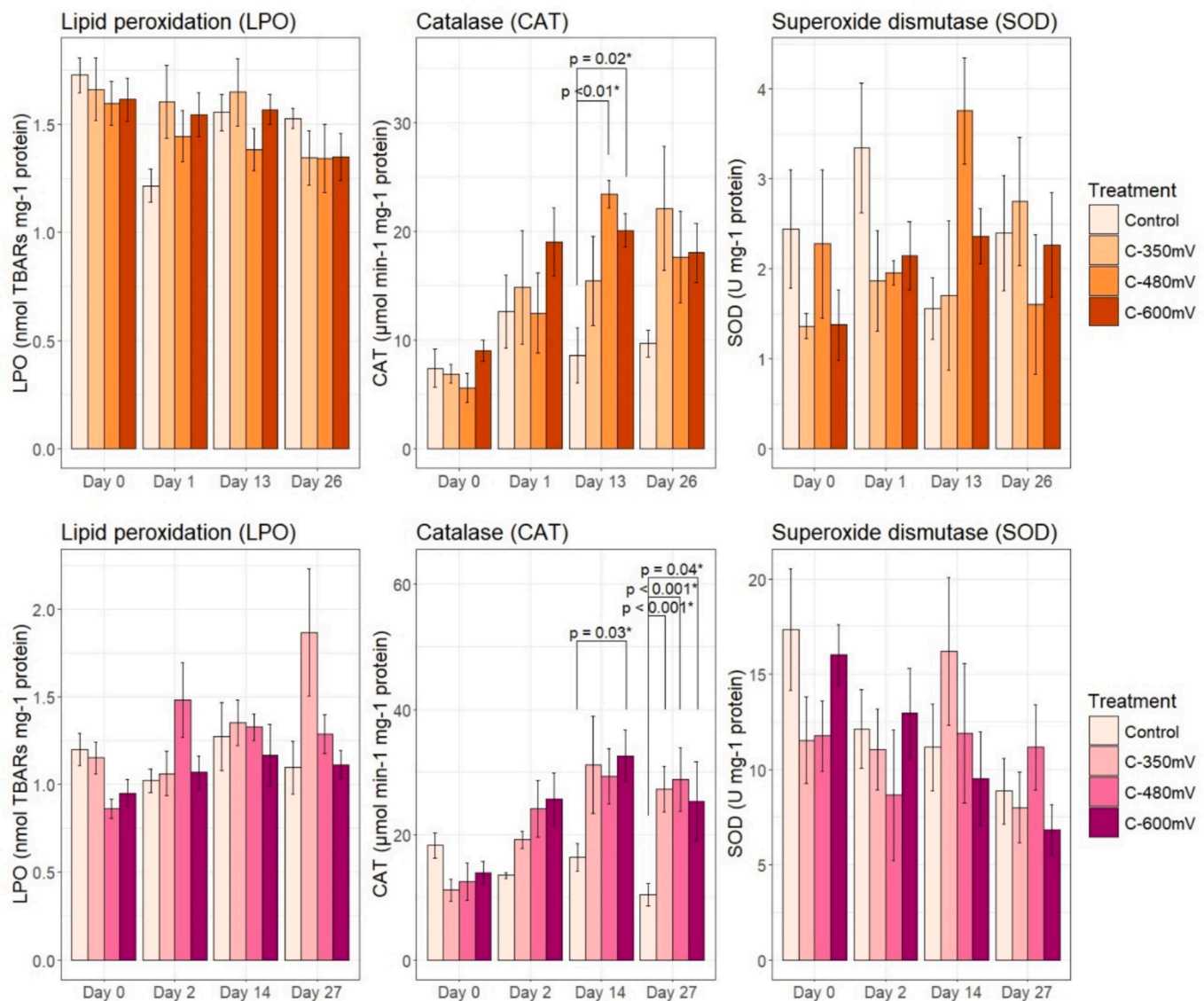


Fig. 2. Lipid peroxidation (LPO) levels, Catalase (CAT), and Superoxide dismutase (SOD) activity in *Stylophora pistillata* (upper row) and *Xenia sp.* (lower row). Displayed are mean values (bars) and standard errors (error bars). Significant ($p < 0.05$) p -values of pairwise post-hoc comparisons are displayed. There was a significant impact of ozone treatment on catalase activity in both *Stylophora pistillata* ($p < 0.01$) and *Xenia sp.* ($p < 0.001$), but no impact on LPO or SOD in either species. ($n = 5$ for all treatments).

60) = 7.96, $p < 0.001$). Pairwise comparisons showed a 26 % increase of CAT activity in C-600mV compared with controls already after two days of TRO exposure ($p = 0.07$). CAT activity was between 38 and 77 % and 29–42 % higher than controls on Day 14 ($p = 0.028$ – 0.09) and Day 27 ($p = 0.004$ – < 0.001), respectively. No impact of ozone treatment on LPO levels or SOD activity was detected in either species. There was a general downward trend in LPO levels in *Stylophora*, with a significant effect of sampling day ($F(3, 59) = 3.60$, $p = 0.018$). SOD activity in *Xenia* was strongly impacted by sampling day ($F(3, 61) = 3.13$, $p = 0.03$), with a general downward trend over time in all treatments and the control (Fig. 2).

3.3. Surface mucus layer (SML) microbiomes: *Stylophora pistillata* And *Xenia sp.*

3.3.1. Sample statistics

After filtering, a total of 681,012 reads of 521 ASVs were retained in the *Stylophora pistillata* data set. The number of reads per sample ranged from 5920 to 46,775, with a mean of 20,030 reads per sample. 514,735

reads of 503 ASVs were retained in the *Xenia sp.* microbiome data set. Reads per sample ranged from 7054 to 33,104, with a mean of 17,158 reads per sample. Good's coverage index exceeded 0.99 in all samples post-filtering, indicating sufficient sequencing depth across datasets.

3.3.2. Alpha diversity

In *Stylophora pistillata*, observed richness differed significantly on Day 0 ($p = 0.035$), with the highest mean ASV count in C-600mV and the lowest in controls (Fig. 3a). However, by Day 26, the trend reversed ($p = 0.017$), and richness in the C-600mV treatment fell below those of the controls (Dunn test, $Z = -2.82$, $p = 0.015$). We further observed a significant difference in the Shannon index at Day 0 ($p = 0.038$) and on Day 26 ($p = 0.05$), following the same general pattern: on Day 0, C-600mV treatment samples exhibited higher diversity, while on Day 26, the trend reversed, with nearly all C-600mV samples showing a Shannon index value below 3. Evenness differed only on Day 0 ($p = 0.027$), with a significantly higher value in C-600mV than the control (Dunn test, $Z = 2.40$, $p = 0.049$; Fig. 3a).

In contrast, *Xenia* surface-associated microbiomes showed no

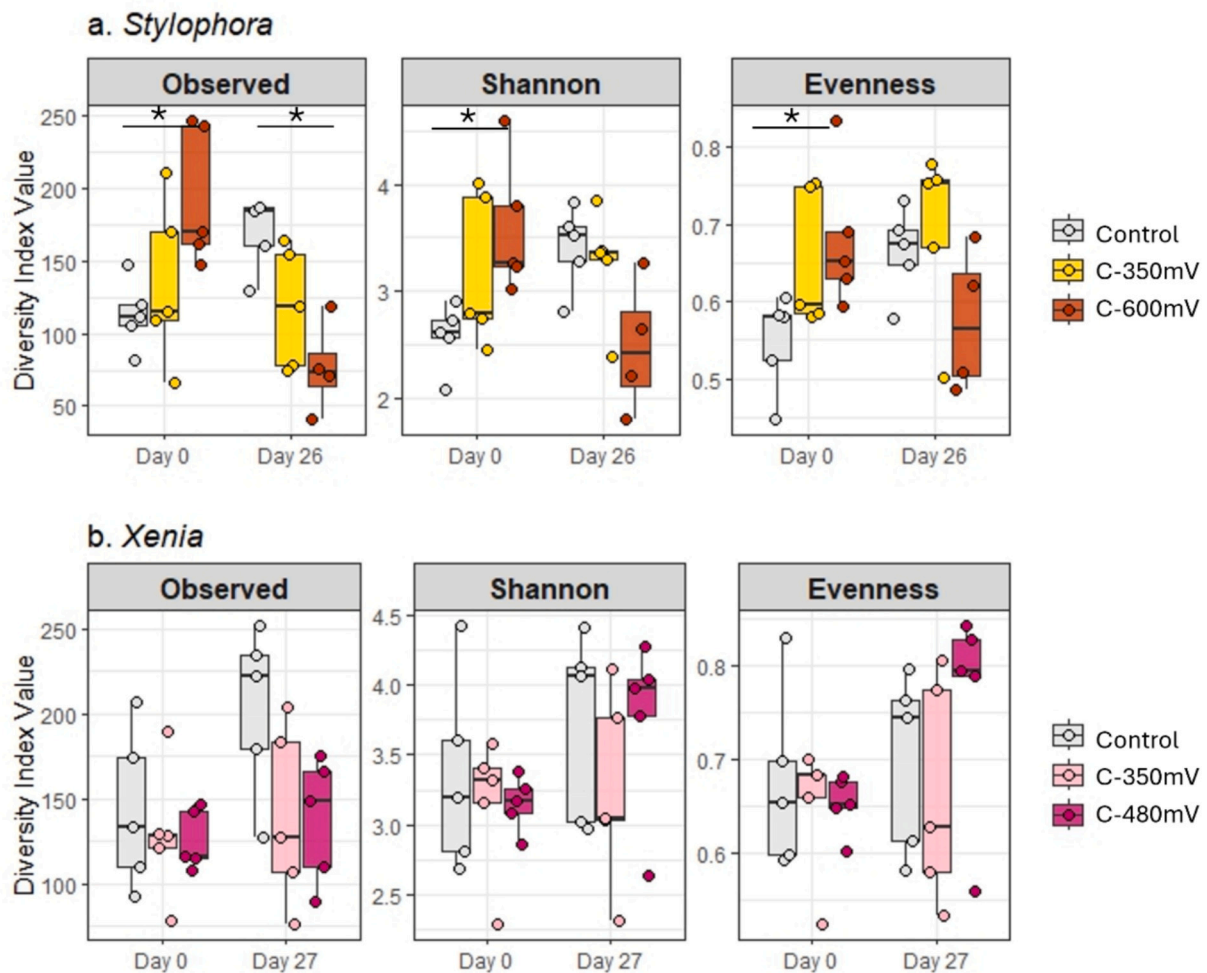


Fig. 3. Alpha diversity of a. *Stylophora pistillata* and b. *Xenia* sp. surface mucus layer (SML) microbiomes under ozone treatments and control conditions. Metrics shown include Observed richness, Shannon index, and Pielou's evenness calculated following rarefaction across samples. Horizontal bars indicate treatment medians; boxes represent interquartile ranges (IQR), and whiskers extend to 1.5 times the IQR. Shannon index and Pielou's evenness are shown as diversity measures, while Observed richness reflects the number of ASVs. Bars and asterisks denote statistically significant ($p < 0.05$) differences. ($n = 5$ for all treatments).

significant differences in any diversity measure (p -values 0.099–0.887; Fig. 3b).

3.3.3. Beta-diversity

Principal Coordinate Analysis (PCoA), based on the Bray-Curtis distance matrix of community data from all samples (*Stylophora* control, C-350mV, and C-600mV; *Xenia* control, C-350mV, and C-480mV; tank water control, C-350mV), revealed distinct patterns in community succession throughout the experiment (Fig. 4). On Day 0, *Stylophora* samples clustered separately from *Xenia* and tank water samples, indicating initial differences in community composition. Effects of ozone treatments became visible as early as Day 1 and got more pronounced by Day 26, as the *Stylophora* samples moved along PC1. By Day 26, *Stylophora* samples from C-350mV and C-600mV treatments formed separate sub-clusters, highlighting the differential impacts of treatment levels. *Xenia* and tank water samples initially overlapped on Day 0, but by Day 27, TRO (C-350mV and C-480mV treatments) caused them to cluster together further along PC1, suggesting that ozonation homogenised their microbial communities. Control samples of *Stylophora*, *Xenia*, and tank water moved along PC2, forming a distinct cluster of the respective Day 26/Day 27 samples, indicating that their microbiomes became more similar to each other over time, but diverged from those of the ozone treatments.

PERMANOVA analysis based on Bray-Curtis distance revealed a

significant interactive impact of timepoint and treatment on the microbial community composition in both corals (Tables 4, 5). Pairwise post hoc testing showed that in *Stylophora*, C-600mV communities on Day 0 and Day 1 differed significantly ($F = 2.45$, $R^2 = 0.23$, $p = 0.038$), as did Day 1 and Day 26 communities ($F = 2.79$, $R^2 = 0.28$, $p = 0.036$). After 26 days, control ($F = 5.99$, $R^2 = 0.43$, $p = 0.025$), C-350mV ($F = 2.87$, $R^2 = 0.26$, $p = 0.021$), and C-600mV ($F = 4.94$, $R^2 = 0.41$, $p = 0.021$) had formed distinctly different communities from their respective Day 0 states. Furthermore, on Day 26, both C-350mV and C-600mV differed significantly from the control (C-350mV vs control: $F = 4.10$, $R^2 = 0.34$, $p = 0.025$; C-600mV vs control: $F = 7.47$, $R^2 = 0.52$, $p = 0.021$), but not from each other ($F = 1.21$, $R^2 = 0.15$, $p = 0.291$). In *Xenia*, all treatments differed on Day 27 from their respective Day 0 communities (control: $F = 3.14$, $R^2 = 0.28$, $p = 0.018$; C-350mV: $F = 2.74$, $R^2 = 0.26$, $p = 0.018$; C-480mV: $F = 3.83$, $R^2 = 0.32$, $p = 0.018$). Day 27 communities of C-350mV and C-480mV also differed significantly from the control (C-350mV vs control: $F = 3.13$, $R^2 = 0.28$, $p = 0.018$; C-480mV vs control: $F = 3.86$, $R^2 = 0.33$, $p = 0.020$), but did not differ from each other ($F = 0.77$, $R^2 = 0.088$, $p = 0.869$).

3.3.4. Bacterial families linked to health, symbiosis and coral mucus integrity in *Stylophora pistillata* SML

Of the 8 bacterial families assessed, only Flavobacteriaceae (Kruskal-Wallis $\chi^2(2) = 8.54$, $p = 0.014$), Saprospiraceae (Kruskal-Wallis

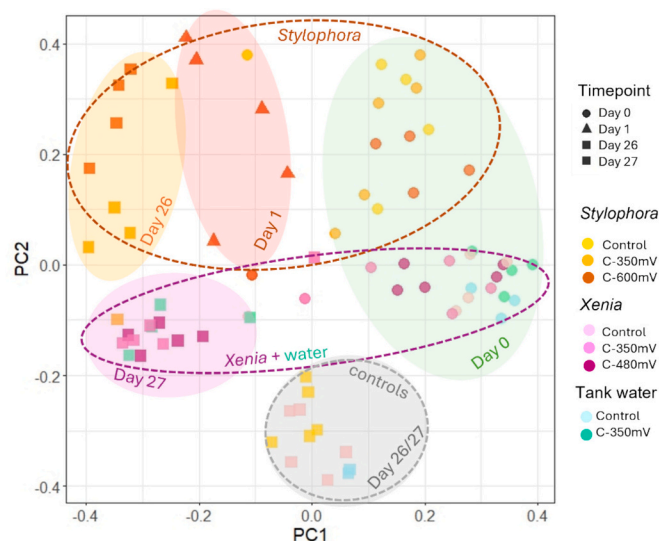


Fig. 4. PCoA plot (PC1 vs PC2) of all samples (*Stylophora*, *Xenia*, tank water) based on Bray-Curtis distance matrix. *Stylophora* samples (control = ●, C-350mV = ●, C-600mV = ●), *Xenia* samples (control = ●, C-350mV = ●, C-480mV = ●), and filtered tank water samples (control = ●, C-350mV = ●) are shown throughout the experiment (start and end). Day 0 samples are shown as circles, Day 1 samples as triangles, and Day 26/Day 27 samples as squares. TRO had a clear community-shaping effect, with *Stylophora* samples separating distinctly by Day 1 and diverging further by Day 26. *Xenia* and water samples formed overlapping clusters on Day 0 and a combined cluster on Day 27, indicating a similar response to TRO. All control samples (*Stylophora*, *Xenia*, water) clustered together after 26/27 days.

Table 4

PERMANOVA results of 16S rRNA gene amplicon sequencing data from the surface mucus layer (SML) of *Stylophora pistillata*. The table shows the degrees of freedom (Df), sum of squares (SUMOFSQS), R-squared (R^2), F-statistic (F), and p-value (PR(>F)) for the effects of Timepoint, Treatment, and the interaction between Timepoint and Treatment.

	Df	SumOfsqs	R^2	F	pr(>F)
Timepoint	2	1.995	0.205	4.762	0.001 ***
Treatment	2	0.910	0.093	2.171	0.003 **
Timepoint*	2	1.187	0.122	2.832	0.001 ***
Treatment					
Residual	27	5.657	0.580		
Total	33	9.750	1.000		

Table 5

PERMANOVA results of 16S rRNA gene amplicon sequencing data from the surface mucus layer (SML) of *Xenia* sp. The table shows the degrees of freedom (Df), sum of squares (SUMOFSQS), R-squared (R^2), F-statistic (F), and p-value (PR(>F)) for the effects of Timepoint, Treatment, and the interaction between Timepoint and Treatment.

	Df	SumOfsqs	R^2	F	pr(>F)
Timepoint	1	1.581	0.175	6.526	0.001 ***
Treatment	2	0.874	0.097	1.803	0.012 *
Timepoint*	2	0.748	0.083	1.543	0.027 *
Treatment					
Residual	24	5.815	0.645		
Total	29	9.019	1.000		

$\chi^2(2) = 39.2$, $p < 0.001$), and Vibrionaceae (Kruskal–Wallis $\chi^2(2) = 7.44$, $p = 0.024$) showed significant changes in abundance in response to ozone treatment ($p < 0.05$; Fig. 5). On Day 26, Flavobacteriaceae exhibited a mean abundance of 75.4 ± 31.8 counts in controls, while 22.3 ± 9.6 in C-350mV and 14.7 ± 7.33 in C-600mV (Control vs. C-

600mV: $Z = -2.91$, $p = 0.011$, Dunn test). Saprospiraceae were highly abundant in controls (mean 136 ± 43), and significantly lower in both C-350mV (mean 16.8 ± 9.04 ; $Z = -4.83$, $p < 0.001$, Dunn test) and C-600mV (mean 1.15 ± 0.76 ; $Z = -5.84$, $p < 0.001$, Dunn test). Vibrionaceae were not present in C-600mV, thereby differing significantly from C-350mV ($Z = 2.29$, $p = 0.044$, Dunn test) and the control ($Z = -2.51$, $p = 0.037$, Dunn test).

3.3.5. 15 top abundant ASVs across treatments and sampling days:

Stylophora pistillata

On Day 0, the most abundant ASVs were SAR324 (Marine Group B), *Altererythrobacter* sp., and *Maritimimonas* sp. By Day 26, these ASVs declined markedly in controls, while *Marivita* sp., NRL2, and *Candidatus* *Kaiserbacteria* increased. In C-350mV, *Maritimimonas* sp. fell below 1 %, *Altererythrobacter* sp. disappeared, and SAR324 remained stable (16.5 %), alongside increases in *Maribacter* sp. and *JGI_0000069* P22 (class Gracilibacteria). In C-600mV, declines resembled C-350mV, but *Vibrio* sp. was also lost. Notably, SAR324 expanded drastically to 46.9 %, with *JGI_0000069* P22 (class Gracilibacteria) as the second most abundant group (Fig. 7a). High relative sequence abundances in *Maritimimonas* sp. (20.3 %), *Ruegeria* sp. (5.6 %), and a not closer identified Francisellaceae (ASV582) (3.7 %) were observed in Day 1 samples of the C-600mV treatments (Fig. 7a). The SAR324 (Marine Group B) exhibited a relative abundance of 17.9 %, nearly double of what was detected on Day 0. Differential abundance analysis identified the short-term effects of high TRO levels (Day 1) in the C-600mV treatment to be a complete disappearance of *Altererythrobacter* sp., *Porticoccus litoralis*, *Maribacter* sp. and NRL2 sequences from *Stylophora pistillata* SML microbiomes. One highly abundant uncultured *Lewinella* sp. (baseMean = 154) displayed a $\log_2$ fold change of -34 . Similarly, a *Coxiella* sp. (baseMean = 77) exhibited a $\log_2$ fold change of -23 , and an uncultured *Phaeodactylibacter* (baseMean = 307) showed a decline of $-5 \log_2$ fold change. Only two ASVs were found to increase in relative abundance on Day 1 compared to Day 0: an uncultured *Candidatus* *Cryptoproductis* (Rickettsiaceae) and an uncultured *Lawsonella* (Corynebacteriaceae). However, both ASVs remained at low abundances.

3.3.6. 25 top abundant ASVs across treatments on Day 26: similarities and differences in *Stylophora pistillata* SML microbiomes

On the final sampling day, the 25 most abundant ASVs accounted for 73.95 %, 66.73 %, and 82.18 % of the total relative abundance in control, C-350mV, and C-600mV treatments, respectively. Fig. 8a presents a Venn diagram based on these taxa, illustrating overlaps and unique ASVs across the three treatments. A total of 19 ASVs were unique to the control group, including three Saprospiraceae (*Phaeodactylibacter* sp. and two uncultured taxa), three Flavobacteriaceae (*Tenacibaculum* sp., an NS3a group bacterium, and *Maritimimonas* sp.), two Magnetospiraceae, as well as *Legionella* sp., *Marivita* sp., and *Pseudohongikella* sp.

Ten ASVs were exclusive to the C-350mV treatment, including two Rhodobacteraceae (*Limibaculum* sp. and one uncultured), as well as *Lewinella* sp., *Vibrio* sp., *Margulisbacteria* sp., and *Aestuariuspira* sp. In the C-600mV treatment, 11 ASVs among the top 25 were uniquely represented, such as *Algisphaera agarilytica*, *Pseudodidymarina sediminum*, *Ruegeria* sp., *Oceanicaulis* sp., and one representative each of the K189A clade and Pla4 lineage. Two ASVs—*Rubritalea* sp. and one uncultured Micavibrionaceae—were shared between the control and C-350mV treatments, while one unidentified bacterial taxon was found in both the control and C-600mV. An overlap of 10 ASVs was observed between C-350mV and C-600mV, including two Hyphomonadaceae (*Filomicrobium* sp. and one uncultured), *Maribacter* sp., *Fulvivirga* sp., *Cutibacterium* sp., *Streptococcus* sp., and *Staphylococcus* sp. Three ASVs were shared across all treatments: one unidentified Rhodobacteraceae, a SAR324 clade (Marine group B) bacterium, and an unknown Gammaproteobacterium.

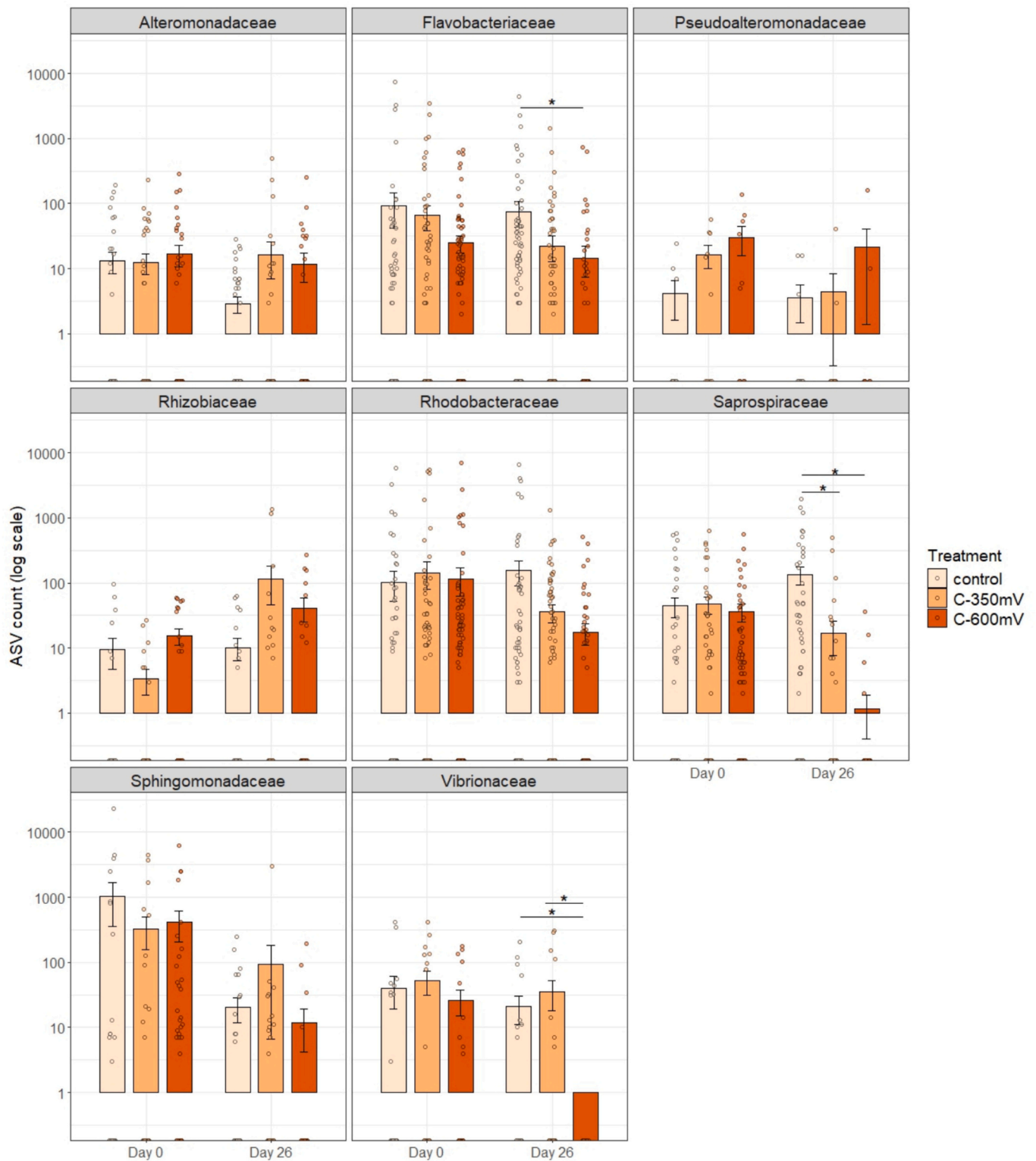


Fig. 5. Log-transformed raw abundance of bacterial families linked to health, symbiosis, and coral mucus integrity in *Stylophora pistillata*. Data are shown for the three treatments: control, C-350mV, and C-600mV on experimental start (Day 0) and end (Day 26). Bars represent mean ASV counts (log scale); whiskers indicate $1.5 \times$ the interquartile range (IQR). Dots represent individual samples. Horizontal lines and asterisks denote significant pairwise differences ($p < 0.05$) from post-hoc tests. Ozone treatment significantly affected the abundance of Flavobacteriaceae ($p = 0.014$), Saprospiraceae ($p < 0.001$), and Vibrionaceae ($p = 0.025$). ($n = 5$ for all treatments).

3.3.7. Bacterial families linked to health, symbiosis and coral mucus integrity in *Xenia* sp. SML

The only bacterial family that exhibited a significant change in

sequence abundance in response to ozone treatments was Saprospiraceae (Kruskal–Wallis $\chi^2(2) = 33.5$, $p < 0.001$). On Day 27, Saprospiraceae showed a marked decline in mean abundance from 117.0 ± 50.3

in controls to 6.35 ± 2.32 in C-350mV and 9.08 ± 3.78 in C-480mV. Post-hoc testing revealed significantly lower abundances in both C-350mV ($Z = -5.63$, $p < 0.001$, Dunn test) and C-480mV ($Z = -5.25$, $p < 0.001$, Dunn test) compared to the control (Fig. 6).

3.3.8. 15 top abundant ASVs across treatments and sampling days: *Xenia* sp

In *Xenia*, Day 0 samples across all treatments were dominated by high relative abundances of Rhodobacteraceae ASV24, *Altererythrobacter* sp., and a Gammaproteobacterium ASV31. Other ASVs of notable abundance included NRL2 and *Vibrio* sp..

On Day 27, control samples showed relative abundance declines in

Altererythrobacter sp., Rhodobacteraceae ASV24, Gammaproteobacterium ASV31, *Phaeodactylibacter* sp., and *Vibrio* sp., while *Marivita* sp., NRL2, and Saprospiraceae ASV75 increased. In C-350mV and C-480mV treatments, Gammaproteobacterium ASV31 declined, several taxa (e.g., *Altererythrobacter* sp., *Phaeodactylibacter* sp., NRL2) disappeared, and *Vibrio* sp. increased, most strongly in C-480mV (Fig. 7b).

3.3.9. *Xenia* SML 25 top abundant ASVs across treatments on Day 27: similarities and differences

The 25 most abundant ASVs accounted for 67.51 %, 68.92 %, and 59 % of total abundances in control, C-350mV, and C-480mV treatments, respectively. Fig. 8b displays a Venn diagram depicting overlaps and

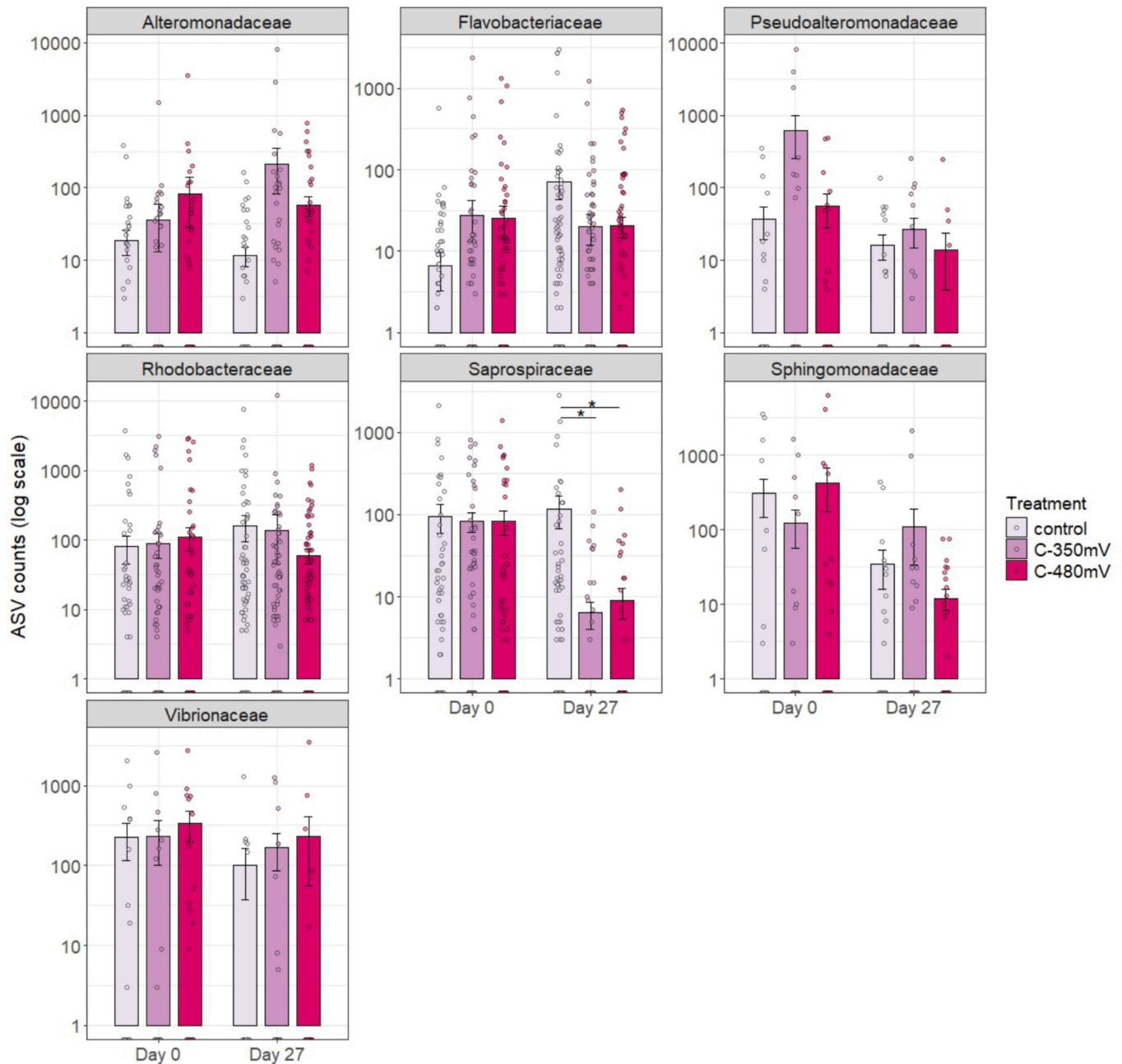


Fig. 6. Log-transformed raw abundance of bacterial families linked to health, symbiosis, and coral mucus integrity in *Xenia* sp. Data are shown for the three treatments control, C-350mV, and C-480mV on Day 0 and Day 27. Bars represent mean ASV counts (log scale); whiskers indicate 1.5× the interquartile range (IQR). Dots represent individual samples. Horizontal bars and asterisks indicate significant ($p < 0.05$) differences from pairwise post-hoc testing. Ozone treatment had a significant impact only on Saprospiraceae ($p < 0.001$). Note that y-axis scales vary between panels.

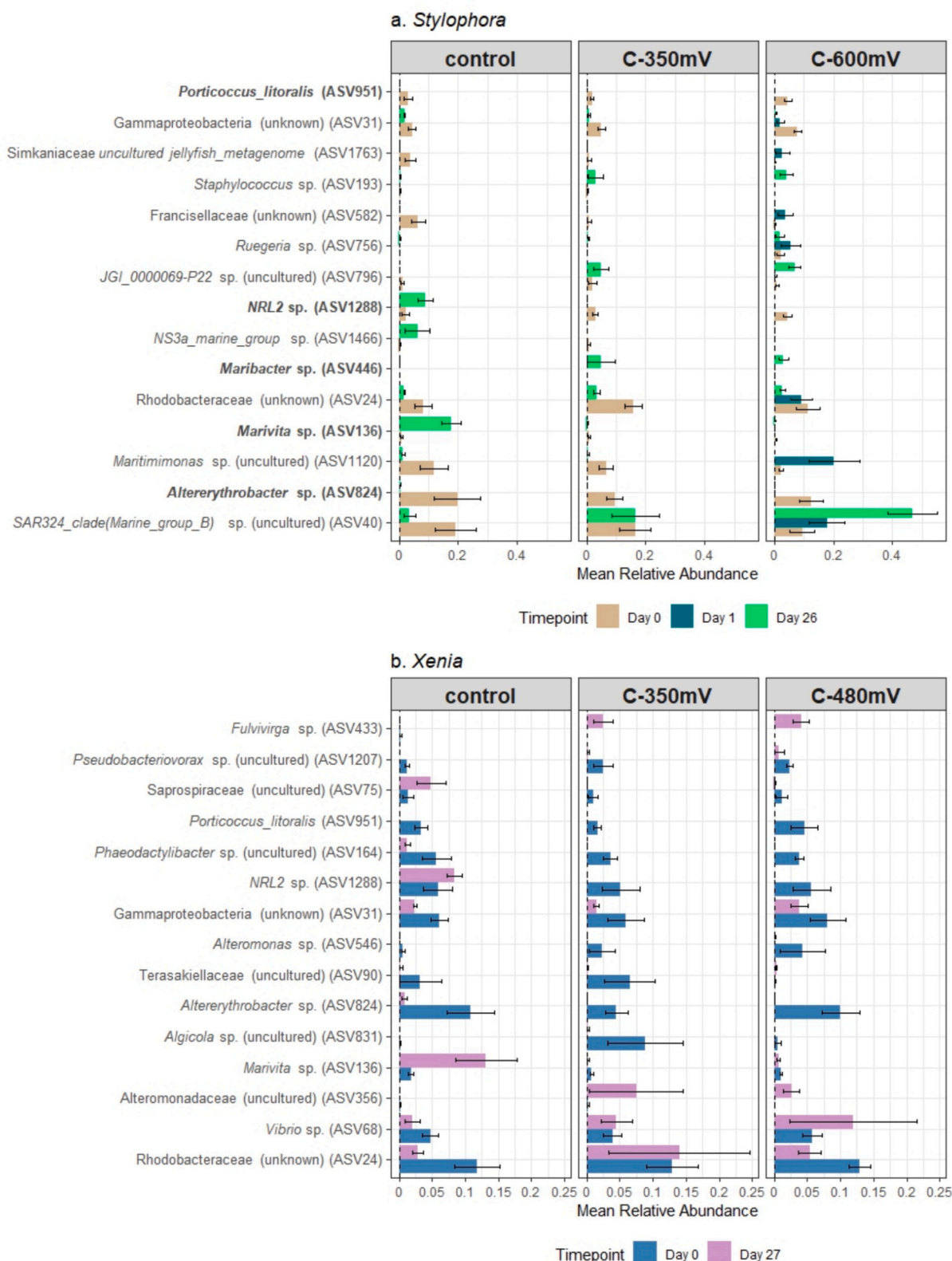


Fig. 7. Mean relative abundances of the top 15 most abundant ASVs across all treatments and sampling days in a. *Stylophora pistillata* and b. *Xenia* sp. SML microbiomes. a. *Stylophora* samples include control, C-350mV, and C-600mV treatments on Day 0 and Day 26 with additional data for Day 1 in C-600mV. Significant ASVs identified by DESeq2 analysis (contrasting Day 0 and Day 1) are indicated in bold. b. *Xenia* samples include control, C-350mV, and C-480mV treatments, with data presented for Day 0 and Day 27. Bars represent mean relative abundances; error bars indicate standard error (SE).

unique ASVs among treatments. In control samples, 19 of the 25 ASVs were unique, including three Saprospiraceae (two uncultured and one *Phaeodactylbacter* sp.), three Flavobacteriaceae (*Maritimimonas* sp., *Tenacibaculum* sp., and an NS3a group bacterium), two Magnetospiraceae, *Legionella* sp., *Marivita* sp., and *Pseudohongiella* sp..

Nine ASVs were exclusive to the C-350mV treatment, seven of which belonged to Proteobacteria, including *Pseudoalteromonas* sp., *Haliangium* sp., and *Peredibacter* sp.. In the C-480mV treatment, eight ASVs were unique, such as *Oleispira* sp., *Tropicibacter* sp., *Roseivivax* sp., and *Flexithrix* sp. C-350mV and C-480mV shared 11 ASVs, including three Alteromonadaceae (*Aestuuriibacter* sp., one uncultured, and one unclassified), *Thalassospira* sp., *Fulvivirga* sp., and *Limibaculum* sp. One uncultured Micavibrionaceae was shared between the control and C-480mV treatments. Five ASVs were common to all treatments—each a member of the Proteobacteria—including *Vibrio* sp., *Ruegeria* sp., one Rhodobacteraceae, one Hyphomonadaceae, and one unclassified Gammaproteobacterium (Fig. 8b).

4. Discussion

4.1. Oxidants induce oxidative stress and modify SML microbiomes in both *Stylophora* and *Xenia*

Our findings provide first insights into the tolerance of two tropical coral species to ozone-elevated TRO, revealing a generally higher resilience in the hard coral *Stylophora pistillata* compared to the soft coral *Xenia* sp.. Long-term exposure to 500mV and above induced visible stress responses and discoloration in *Xenia*. Both species responded to TRO exposure by increasing catalase activity, indicating activation of antioxidant defense mechanisms. While low-level TRO concentrations of 0.057 mg Cl₂ L⁻¹ (C-350mV) did not affect metabolic rates in *Stylophora*, it significantly altered the microbial composition of the surface mucus layer (SML) in both corals. Signs of SML degradation were observed in both species even under mild ozonation. We showed that mean TRO levels of 0.131 mg Cl₂ L⁻¹ (C-600mV) can rapidly and profoundly restructure *Stylophora* SML microbiomes, with pronounced changes

detectable after just one day of exposure. These changes included a complete reduction of Vibrionaceae abundance in *Stylophora* SML microbiomes after 26 days, demonstrating the potential of TRO as a tool for pathogen control in tropical coral aquaculture systems. However, lower ozone levels did not suppress Vibrionaceae in either species.

4.2. Oxidant exposure and coral health: Increased metabolic demand and compensatory photosynthesis in *Stylophora pistillata* and *Xenia* sp

Photosynthesis and respiration can serve as proxies for health and metabolic status of anthozoans (Ferrier-Pagès et al., 2010; Paradis et al., 2019; Simancas-Giraldo et al., 2021), while photosynthetic efficiency is more indicative of symbiont health and performance (Bhagooli and Hidaka, 2003; Ferrier-Pagès et al., 2007). Different environmental stressors can vary in their impact on these parameters (Brown, 1997; Ferrier-Pagès et al., 2007; Meziere et al., 2021). Our results showed a similar pattern in both *Stylophora pistillata* and *Xenia* sp., where increases in respiration and net photosynthesis were observed following TRO exposure at various levels, accompanied by a loss of photosynthetic efficiency in *Xenia*. Since no bleaching was observed at any of the treatment levels tested in our experiments, and the photosynthetic efficiencies in the ozone treatments remained well within what is considered normal for hard –and soft corals (Cohen and Dubinsky, 2015; Winters et al., 2003), we conclude that the stress exerted by TRO was probably more severe for the coral hosts than for their symbionts. Although oxidative stress can impair both hosts and symbionts, symbiotic dinoflagellates (Symbiodiniaceae) may exhibit greater tolerance to oxidative agents such as hydrogen peroxide (H₂O₂) and other reactive oxygen species (ROS), likely due to their robust antioxidant systems and cellular compartmentalisation (Gardner et al., 2017). Future studies using isolated *Symbiodinium* cultures exposed to comparable TRO concentrations could provide more detailed insights into their direct sensitivity to ozonation by-products.

Species-specific patterns in physiological parameters were evident: In *S. pistillata*, low-level TRO concentrations (C-350mV, 0.057 mg Cl₂ L⁻¹) did not significantly affect metabolic performance relative to

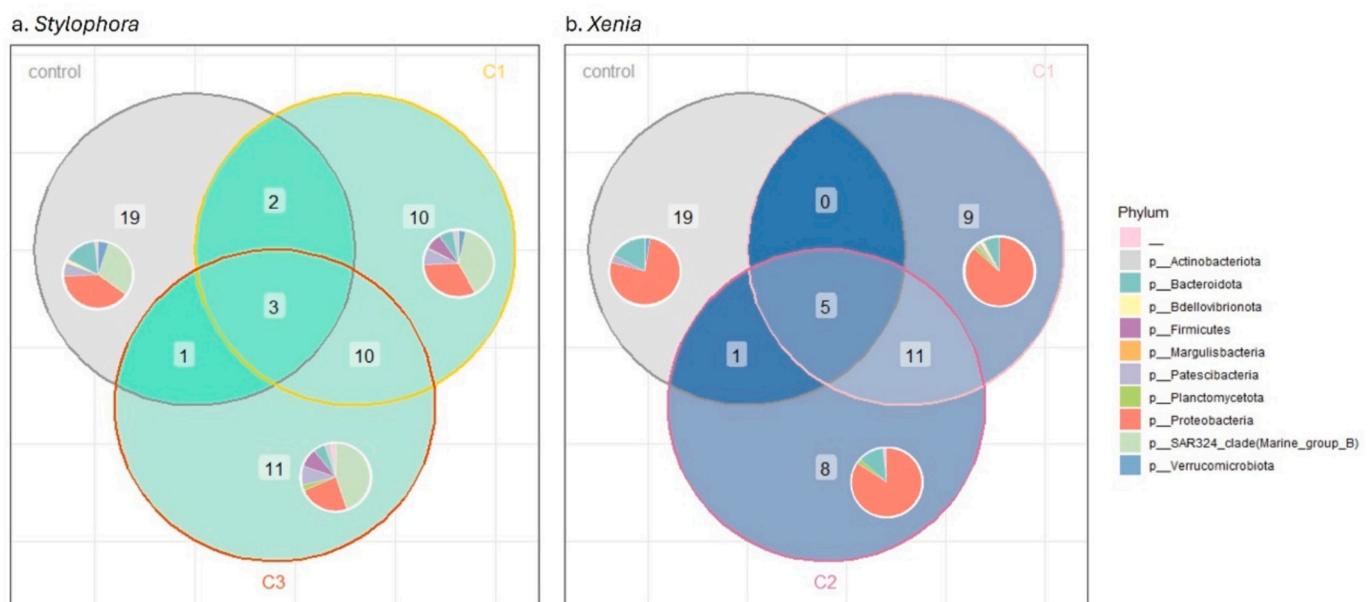


Fig. 8. Venn diagrams depicting the overlap of the 25 most abundant ASVs among treatments on the final sampling day. Pie charts show the relative abundance of bacterial phyla represented by ASVs in the respective treatment group. a. *Stylophora pistillata* SML microbiomes on Day 26 across three treatments: Control, C-350mV, and C-600mV. A high number of unique ASVs could be found in controls, while C-350mV and C-600mV shared more ASVs than were unique to each treatment. b. *Xenia* sp. SML microbiomes on Day 27 across three treatments: Control, C-350mV, and C-480mV. Similar to *Stylophora pistillata* SML microbiomes, a large share of the 25 top ASVs were unique to controls, while C-350mV and C-480mV had more common than unique taxa. In both species, some ASVs were highly abundant irrespective of treatment (3 in *Stylophora pistillata*, 5 in *Xenia* sp.).

controls, while medium and high levels induced marked responses. In contrast, *Xenia* sp. showed no significant physiological differences among ozone treatments (C-350mV, C-480mV, C-600mV), despite visible signs of stress at higher levels such as loss of tentacles and colour changes. This suggests that *Xenia*'s stress response mechanisms may have been fully activated at 350 mV, with no further physiological change detectable at higher concentrations—possibly indicating a threshold response or early saturation of its oxidative stress pathways. Alternatively, the tested redox range may not have been broad enough to induce clearly distinguishable metabolic effects. Fluctuations in redox levels and corresponding TRO concentrations may have introduced overlap between treatments (see Fig. S1 in the Supplemental material), thus obscuring more subtle treatment-specific effects.

Our results point toward a generalised stress response of the coral holobionts to oxidants, with increased respiration rates likely reflecting heightened metabolic demand for stress mitigation processes such as enzymatic activity (e.g., CAT), antioxidant production, or cellular repair mechanisms. The coral symbionts likely compensated for energy losses by increasing their photosynthetic rate, albeit operating with impaired efficiency, indicating a shift from optimal function to compensatory metabolism under TRO-induced stress.

4.3. Elevated TRO levels stimulated catalase activity in corals, but had no effect on superoxide dismutase activity and did not induce lipid peroxidation

Tropical corals activate enzymatic defences such as catalase (CAT) and superoxide dismutase (SOD) in response to various environmental stressors (Chen et al., 2022; Costa et al., 2021; Dias et al., 2019a; Dias et al., 2019b; Downs et al., 2000; Levy et al., 2006; Ramos and García, 2007; Tang et al., 2018). Additionally, a range of peroxidases, glutathione transferases, heat shock proteins, as well as non-enzymatic antioxidants can be activated or upregulated to deal with high ROS (reactive oxygen species) levels. ROS formation beyond the cell's enzymatic ability to degrade them causes oxidative stress, as ROS damage cell components such as lipids, proteins and DNA (Lesser, 2006). The interaction of ROS with lipids is a major pathway of cellular damage, and lipid degradation products such as malondialdehyde can be measured as a proxy of oxidative stress damage (Halliwell and Gutteridge, 1984). The combination of SOD, CAT and LPO level measurements is therefore considered adequate to assess a coral's primary response to oxidative stress, and furthermore its effectiveness by estimating damages inflicted by ROS (Costa et al., 2021; Dias et al., 2019a; Teixeira et al., 2013). There have been relatively few studies examining oxidative stress biomarkers in response to TRO formation in ozonated seawater. Guilherme et al. (2019) reported DNA damage in *Solea senegalensis* exposed to ozonation by-products, and two earlier studies investigated oxidative stress responses in finfish: Ritola et al. (2000) assessed glutathione levels in ozone-exposed Arctic charr, while Ritola et al. (2002) measured SOD, CAT, and lipid peroxidation (LPO) in ozonated blood of *Oncorhynchus mykiss*. Beyond these, most research on the effects of ozonation by-products in aquaculture has focused primarily on endpoints such as mortality, behaviour, histological changes, physiological parameters, and disease resistance (X. Li et al., 2014; Reiser et al., 2010; Schroeder et al., 2010). In our study, elevated TRO levels had a more pronounced impact on the soft coral *Xenia* sp. compared to the hard coral *Stylophora pistillata*, yet both species exhibited a similar oxidative stress response: increased catalase (CAT) activity without corresponding changes in superoxide dismutase (SOD), and no detectable lipid peroxidation (LPO), suggesting that antioxidant defences were effectively mitigating ROS-related damage. However, CAT activity was much more pronounced in *Xenia* sp. than in *Stylophora pistillata*, suggesting that the soft coral mounted a more sensitive oxidative stress response. It reacted more intensely to oxidant exposure, yet not necessarily with greater resilience. Additionally, there were no significant differences in CAT activity between ozone treatments, which may imply that the

antioxidant response was not sufficiently scalable to match the elevated stress levels at higher TRO concentrations. This could potentially explain the observed mortalities and overall less favourable visual condition of *Xenia* sp. in that treatment—effects that, despite being physiologically relevant, were not reflected in LPO levels. This discrepancy could suggest that CAT activity alone may not fully capture the biological costs of exposure to ozone-produced oxidants, particularly in sensitive soft coral species.

Our results contrast with the response observed in *O. mykiss*, where elevated blood and plasma LPO levels were recorded following in vitro exposure of blood samples to ozone, despite simultaneous upregulation of CAT and SOD activity (Ritola et al., 2002). In an experiment with juvenile turbot (*Psetta maxima*), a rise in blood cortisol levels was observed after only one day of exposure to TRO levels of 0.1 and 0.15 mg L⁻¹ (Reiser et al., 2010). Although comparisons between whole-organism exposure in marine invertebrates and in vitro exposure of fish blood to gaseous ozone, as well as between molecular oxidative stress biomarkers such as CAT and SOD, and blood cortisol levels must be made with caution, our results suggest a higher tolerance to ozone-induced oxidative stress in hard corals like *Stylophora* than in finfish. This may help explain their resilience to ozonation-caused TRO concentrations that are known to be lethal to many fish species.

SOD and CAT activity often go hand-in-hand, with one enzyme converting the products of the other, yet the sole upregulation of CAT activity is not unprecedented. Air exposed octocoral *Veretillum cymorium* increased CAT but not SOD activity in a study by Teixeira et al. (2013). In *Galaxea fascicularis*, the exposure to H₂O₂ increased CAT, but not SOD activity, which was interpreted by the authors of the study as an effect of increased levels of H₂O₂ in the cytosol, but no actual formation of superoxide ions (Higuchi, 2008). Since ozonation of seawater also produces considerable amounts of hydrogen peroxide (H₂O₂)—which, due to its uncharged state, is membrane-permeable—it is possible that the observed increases in catalase (CAT) activity in our study reflect a corresponding rise in cytosolic H₂O₂ concentrations.

Taken together, our findings show species-specific differences in coral responses to oxidative stress inflicted by elevated TRO. Both *Stylophora pistillata* and *Xenia* sp. demonstrated the ability to activate antioxidant defences through an increase in CAT activity, and the absence of lipid peroxidation suggests that these mechanisms were largely effective in preventing oxidative damage. This points toward a certain degree of resilience of tropical coral species to purposefully or accidentally inflicted sublethal TRO concentrations and, importantly, allows for the possibility of using ozone as a tool for microbiome manipulation without necessarily compromising coral health. Future research should aim to further elucidate the thresholds of oxidative stress tolerance across a wider range of coral species and explore the long-term implications of repeated or chronic ozone exposure on coral health and physiology.

4.4. Ozone as a tool for microbiome modulation in coral aquaculture: impacts on *Stylophora pistillata* and *Xenia* sp. SML microbial communities

Closed aquaculture systems are not influenced by natural fluctuations or pollution events; however, environmental parameters shaped by water treatment methods, the presence of macrophytes and animals, and the feeding regime can significantly influence the microbiomes of cultivated organisms (Lorgen-Ritchie et al., 2023; Moschos et al., 2022; Steiner et al., 2021). As a rule, host-associated microbial communities tend to be less diverse than those of their wild counterparts (Kooperman et al., 2007; Schöttner et al., 2009). In our study, the microbiomes of both coral species, as well as the tank water samples, became increasingly similar over the course of the experiment in the controls but not in the ozone treatment groups. The substantial and immediate impact of just a single day of high TRO concentrations (0.131 mg L⁻¹, C-600mV) on the *Stylophora* SML furthermore suggests that ozone-produced oxidants directly affected coral-associated microbial communities. This

short-term effect raises concerns about the potential consequences of ozone system malfunctions, which could lead to similar spikes in TRO concentration and, in turn, severe alterations to coral-associated microbiomes. On the other hand, it highlights the potential of ozone to selectively and effectively modulate SML microbiomes without requiring prolonged exposure.

Notably, even low-level TRO in the C-350mV treatment (0.057 mg Cl₂ L⁻¹) induced changes in microbial composition like those seen under medium ozonation (C-480mV) in *Xenia* sp., demonstrating that routinely applied ozone levels in tropical aquaria can distinctly shape coral-associated microbiomes. High TRO concentrations corresponding to a redox potential of 600mV moderately reduced both Shannon index and Observed richness in *Stylophora* SML microbiomes. While these effects were not severe, they do suggest caution in applying high TRO concentrations for microbiome modulation, as prolonged exposure could amplify community-level impacts. In contrast, ozonation did not significantly affect Shannon diversity index in *Xenia* sp., although a slight reduction in observed richness reflects a selective loss of rare taxa. Despite this, microbial communities retained a substantial degree of diversity even after 26/27 days of ozone exposure. This indicates that while ozone may selectively remove low-abundance or sensitive taxa, the core structure and functional potential of the microbial community likely remain intact—albeit with a shifted taxonomic profile. Such an altered community may also exhibit increased susceptibility to infection by pathogens. This could be addressed in future studies, for example by monitoring SML microbial communities over a prolonged period following ozone-produced oxidant exposure, with or without targeted pathogen challenges.

Given that dissolved ozone has previously been shown to alter the composition of bacterial biofilms (Marino et al., 2018; Shichiri-Negoro et al., 2021; Tachikawa et al., 2009), its impact on animal-associated microbiomes is not unexpected. Nevertheless, most prior research has focused almost exclusively on the physiological impacts of ozone-produced oxidants on aquaculture animals or on waterborne microbial communities. Our study demonstrates that all tested sublethal levels of ozonation significantly altered the microbial composition of coral SML microbiomes, while only inducing low stress in *Stylophora pistillata*. Meanwhile, *Xenia* sp. was shown to be more sensitive, with several mortalities in the highest (C-600mV) treatment, but moderate to good performances in intermediate (C-480mV) and low (C-350mV) ozone treatments. This opens up possibilities for the targeted use of ozone as a tool to manage coral-associated microbiomes in aquaculture, offering a non-antibiotic strategy to shape microbial communities. However, further research is necessary to determine long-term effects, optimise dosage, and assess species-specific responses to ensure the safety and efficacy of ozone application in coral husbandry. Similarly, changes in the microbiomes of other coral tissues were not assessed here and need to be addressed in future studies.

4.5. Impact on indicator families: degradation of mucus layer even at low TRO concentrations and disappearance of Vibrionaceae from *Stylophora pistillata* SML microbiomes only in the highest ozone treatment

Coral microbiomes are typically dominated by Alpha- and Gammaproteobacteria, Bacteroidota, Bacillota, and Cyanobacteria (Hernandez-Agreda et al., 2017; Voolstra et al., 2024), yet environmental changes can alter their relative proportions, and environmental stress can shift SML microbiomes from mutualistic toward potentially opportunistic or pathogenic communities (Thurber et al., 2009). Conversely, the coral-associated microbiomes can play a pivotal role in modulating host responses to environmental change (Avila-Magaña et al., 2021; Marangon et al., 2025), underscoring the importance of investigating environmental stress impacts at the holobiont level. Despite extensive research, knowledge about the specific functional roles of microbial taxa within the coral holobiont remains limited (Ainsworth et al., 2017). This is especially true for octocorals (Cnidaria:

Octocorallia), which are less frequently studied than reef-building scleractinian corals. Consequently, most insights into coral-associated microbiomes are derived from compositional data, with functional roles often inferred rather than directly observed. Here, we focused on a subset of bacterial families previously reported to shift in relative abundance under environmental stress across various tropical hard- and soft coral species. By doing so, we aimed to draw parallels to earlier findings about the effects of environmental stressors on coral holobionts and evaluate the effects of ozone-induced stress on the *Stylophora pistillata* and *Xenia* sp. holobionts under closed-system cultivation conditions.

Flavobacteriaceae have been associated with coral stress responses and are often considered opportunistic. They have been reported in higher relative abundances in diseased (Certner and Vollmer, 2018) and temperature-stressed (Li et al., 2021) scleractinian corals, and in necrotic tissue of the soft coral *Eunicella gazella* (Keller-Costa et al., 2021). Flavobacteriaceae abundance has further been linked to oxygen gradients, being higher in oxygen-rich environments (*Acropora cervicornis*; Swaminathan et al., 2023). In our study, Flavobacteriaceae significantly declined under high ozonation (C-600mV) in *Stylophora pistillata* SML microbiomes, with a similar, though non-significant, trend in *Xenia* sp., where relative abundances in both C-350mV and C-480mV were roughly 3.5 times lower than in controls. This pattern suggests a general susceptibility of this family to TRO, becoming more pronounced with increasing concentrations. Notably, there was already a downward trend (Control - > C-350mV - > C-600mV) in Day 0 samples, and relative abundances did not change considerably over time within treatments. In the surface mucus layer (SML) of *Stylophora pistillata*, we identified five ASVs belonging to the Flavobacteriaceae family, including two classified as *Tenacibaculum* spp.—a genus harbouring known pathogens of Atlantic salmon and other finfish, which may cause tenacibaculosis (Avendaño-Herrera et al., 2006; Frisch et al., 2018). *Tenacibaculum* spp. showed high susceptibility to both C-350mV and C-600mV ozone treatments in our study (Supplemental material, Fig. S6) while being among the top 25 ASVs of both *Stylophora pistillata* and *Xenia* sp. control SML microbiomes, demonstrating ozone's potential to combat this and possibly other Flavobacteriaceae pathogens. However, since fish are generally more sensitive to TRO, prolonged exposure like in our setup is not feasible. As an alternative, ozone nanobubble dip-baths—with shorter exposure times—could be a more practical solution (Nghia et al., 2022).

Members of Alteromonadaceae, Pseudoalteromonadaceae, and Vibrionaceae have been associated with diseased coral microbiomes (Glasl et al., 2016; MacKnight et al., 2021; Sekar et al., 2006). Some Vibrionaceae species have been directly implicated as disease agents (Gignoux-Wolfsohn and Vollmer, 2015; Luna et al., 2010; Ushijima et al., 2023). In our dataset, Vibrionaceae contained 5 ASVs, with one *Vibrio* sp. comprising 97.15 % of total Vibrionaceae abundance in Day 26 *Stylophora* samples. Vibrionaceae relative abundances showed no significant differences but tended to increase under low TRO concentrations (C-350mV, 0.057 mg L⁻¹) in both *Stylophora pistillata* and *Xenia*, and under moderate (C-480mV, 0.078 mg L⁻¹) ozonation in *Xenia*. However, under high ozonation (C-600mV, 0.131 mg L⁻¹), Vibrionaceae were completely undetectable in *Stylophora* SML microbiomes after 26 days. These findings may indicate a state of microbial dysbiosis under low and medium ozone exposure, possibly favouring opportunistic *Vibrio* proliferation. However, when TRO concentrations are sufficiently high, *Vibrio*'s antioxidant defences are overwhelmed (Feng et al., 2018; Pumkaew et al., 2021). While this finding highlights the need for caution when applying ozone in tropical marine aquaria it also suggests that high-level ozonation could be a tool for pathogen control in aquaria and land-based coral culture systems, given the infected host is sufficiently resilient toward elevated levels of TRO.

In a recent study, we found evidence that exposure to high TRO concentrations resulting from ozonation degraded the microbial biofilm on *Ulva* sp. in integrated multitrophic aquaculture (IMTA), leading to a

reduction in ASVs associated with polysaccharide degradation (Weidlich et al., under review). Among these, members of the Saprospiraceae family stood out due to their decline, likely because they inhabit the outer biofilm layer where they metabolise complex carbohydrates. In our present study, Saprospiraceae were the only bacterial family that declined significantly in both *Stylophora pistillata* and *Xenia* sp. SML microbiomes. This supports a similar role of Saprospiraceae in coral mucus layers, which are likely susceptible to oxidant-inflicted degradation of their food source in a similar way as in *Ulva* biofilms. Coral mucus has various important functions, among others acting as a defense line against environmental stressors, regulating immune response, and harbouring symbiont microbes (Brown and Bythell, 2005; Wild et al., 2004). Its reduction even under the lowest ozone treatment, which produced mean TRO concentrations below those typically considered safe for many aquaculture organisms (Guilherme et al., 2019; Reiser et al., 2010; Schroeder et al., 2010), reveals a critical knowledge gap regarding TRO impacts on host-associated microbiomes in aquaculture. Regarding corals in particular, mucus secretion—both in consistency and quantity—varies greatly between species; consequently, oxidant-derived degradation of mucus likely affects different species in distinct ways. Further research is needed to clarify both the hazards and potential applications of ozone-aided seawater treatment in coral aquaculture systems.

5. Conclusions

With this study we provided first insights into the tolerance of two coral species common to tropical aquaria for by-products of ozone-aided seawater treatment, thereby providing guidelines for the safe limits of ozone use in tropical coral aquaculture. On short-term (96 h), *Stylophora pistillata* could persist in ozonation levels of up to 700 mV (0.188 mg L⁻¹ TRO), while *Xenia* sp. displayed first mortalities already at 600 mV (0.099 mg L⁻¹ TRO). Long-term exposure to 350 mV (0.057 mg L⁻¹ TRO) was shown to impact physiological parameters in *Xenia*, but not *Stylophora pistillata*, while triggering catalase activity in both species, asking for caution in routinely applied ozonation levels in tropical coral aquaculture and aquaria. Moreover, the impacts of ozone-derived elevated TRO levels on the composition of microbial communities in the SML show the potential of ozone as a tool to modify SML microbiomes in coral aquaculture. The disappearance of potential pathogens like *Vibrio* sp. and *Tenacibaculum* spp. from *Stylophora* SML in the highest ozone treatment were an especially interesting finding in that context. Our findings, however, also call for caution in the use of ozone in coral aquaculture, as even low levels of ozone-elevated TRO were found to impact community composition, potentially causing microbial dysbiosis.

CRedit authorship contribution statement

Sabine Weidlich: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sabine Keuter:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Tania Aires:** Writing – review & editing. **Aschwin Engelen:** Writing – review & editing, Supervision, Resources, Methodology. **Andreas Kunzmann:** Writing – review & editing, Supervision, Resources, Funding acquisition.

Author statement

Sabine Weidlich: Conceptualisation (lead); methodology (lead); investigation (lead); data curation (lead); formal analysis (lead); writing – original draft (lead). Sabine Keuter: methodology (equal); investigation (equal); formal analysis (equal); writing – review and editing (equal). Tania Aires: review and editing (equal). Aschwin Engelen: supervision (equal); resources (equal); writing – review and editing (lead). Andreas Kunzmann: supervision (lead); resources (equal); writing –

review and editing (equal); funding acquisition (lead).

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT in order to improve flow and clarity of certain text parts. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aquaculture.2025.743369>.

Data availability

The sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA123456.

References

- Aalto, S.L., Syropoulou, E., de Jesus Gregersen, K.J., Tirola, M., Pedersen, P.B., Pedersen, L.F., 2022. Microbiome response to foam fractionation and ozonation in RAS. *Aquaculture* 550. <https://doi.org/10.1016/j.aquaculture.2021.737846>.
- Ainsworth, T.D., Fordyce, A.J., Camp, E.F., 2017. The other microeukaryotes of the coral reef microbiome. *Trends Microbiol.* 25 (12), 980–991. <https://doi.org/10.1016/j.TIM.2017.06.007>.
- Avendaño-Herrera, R., Toranzo, A.E., Magariños, B., 2006. Tenacibaculosis infection in marine fish caused by *Tenacibaculum maritimum*: a review. *Dis. Aquat. Org.* 71 (3), 255–266. <https://doi.org/10.3354/DAO071255>.
- Avila-Magaña, V., Kamel, B., DeSalvo, M., Gómez-Campo, K., Enríquez, S., Kitano, H., Rohlf, R.V., Iglesias-Prieto, R., Medina, M., 2021. Elucidating gene expression adaptation of phylogenetically divergent coral holobionts under heat stress. *Nat. Commun.* 12 (1), 1–16. <https://doi.org/10.1038/s41467-021-25950-4>.
- Badiola, M., Mendiola, D., Bostock, J., 2012. Recirculating aquaculture systems (RAS) analysis: Main issues on management and future challenges. *Aquac. Eng.* 51, 26–35. <https://doi.org/10.1016/j.AQUAENG.2012.07.004>.
- Becker, C.C., Brandt, M., Miller, C.A., Apprill, A., 2022. Microbial bioindicators of stony coral tissue loss disease identified in corals and overlying waters using a rapid field-based sequencing approach. *Environ. Microbiol.* 24 (3), 1166–1182. <https://doi.org/10.1111/1462-2920.15718>.
- Bhagooli, R., Hidaka, M., 2003. Comparison of stress susceptibility of in hospite and isolated zooxanthellae among five coral species. *J. Exp. Mar. Biol. Ecol.* 291 (2), 181–197. [https://doi.org/10.1016/S0022-0981\(03\)00121-7](https://doi.org/10.1016/S0022-0981(03)00121-7).
- Bird, R.P., Draper, H.H., 1984. Comparative studies on different methods of malonaldehyde determination. *Methods Enzymol.* 105 (C), 299–305. [https://doi.org/10.1016/S0076-6879\(84\)05038-2](https://doi.org/10.1016/S0076-6879(84)05038-2).
- Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., Huttley, G.A., Gregory Caporaso, J., 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* 6 (1). <https://doi.org/10.1186/s40168-018-0470-z>.

- Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnord, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Caporaso, J.G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* 37 (8), 852–857. <https://doi.org/10.1038/s41587-019-0209-9>.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72 (1–2), 248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3).
- Brown, B.E., 1997. Adaptations of reef corals to physical environmental stress. *Adv. Mar. Biol.* 31 (31), 221–299. [https://doi.org/10.1016/S0065-2881\(08\)60224-2](https://doi.org/10.1016/S0065-2881(08)60224-2).
- Brown, B.E., Bythell, J.C., 2005. Perspectives on mucus secretion in reef corals. *Mar. Ecol. Prog. Ser.* 296, 291–309. <https://doi.org/10.3354/MEPS296291>.
- Buchan, K.A.H., Martin-Robichaud, D.J., Benfey, T.J., 2005. Measurement of dissolved ozone in sea water: a comparison of methods. *Aquac. Eng.* 33 (3), 225–231. <https://doi.org/10.1016/j.aquaeng.2005.02.002>.
- Cai, W., Arias, C.R., 2017. Biofilm formation on aquaculture substrates by selected bacterial fish pathogens. *J. Aquat. Anim. Health* 29 (2), 95–104. <https://doi.org/10.1080/08997659.2017.1290711>.
- Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat. Methods* 13 (7), 581–583. <https://doi.org/10.1038/nmeth.3869>.
- Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* 6 (8), 1621–1624. <https://doi.org/10.1038/ismej.2012.8>.
- Certner, R.H., Vollmer, S.V., 2018. Inhibiting bacterial quorum sensing arrests coral disease development and disease-associated microbes. *Environ. Microbiol.* 20 (2), 645–657. <https://doi.org/10.1111/1462-2920.13991>.
- Chen, Y.T., Ding, D.S., Lim, Y.C., Singhanian, R.R., Hsieh, S., Chen, C.W., Hsieh, S.L., Dong, C.D., 2022. Impact of polyethylene microplastics on coral *Goniopora columna* causing oxidative stress and histopathology damages. *Sci. Total Environ.* 828, 154234. <https://doi.org/10.1016/j.scitotenv.2022.154234>.
- Claiborne, A., 1985. Catalase activity. In: Greenwald, R.A. (Ed.), *CRC Handbook of Methods for Oxygen Radical Research*. CRC Press, Boca Raton, pp. 283–284.
- Cohen, I., Dubinsky, Z., 2015. Long term photoacclimation responses of the coral *Stylophora pistillata* to reciprocal deep to shallow transplantation: photosynthesis and calcification. *Front. Mar. Sci.* 2 (JUN), 140583. <https://doi.org/10.3389/fmars.2015.00045/BIBTEX>.
- Cohen, Y., Joseph Pollock, F., Rosenberg, E., Bourne, D.G., 2012. Phage therapy treatment of the coral pathogen *Vibrio coralliilyticus*. *MicrobiologyOpen* 2 (1), 64. <https://doi.org/10.1002/MB03.52>.
- Costa, A.P.L., Silva, D.A.M., Rodrigues, A.C.M., Marques, C.R., Soares, A.M.V.M., Rocha, R.J.M., 2021. Species-specific oxidative stress responses and cellular energy allocation after coral shipping. *Aquac. Rep.* 19, 100623. <https://doi.org/10.1016/j.aqrep.2021.100623>.
- Delgadillo-Ordoñez, N., Garcías-Bonet, N., Raimundo, I., García, F.C., Villela, H., Osman, E.O., Santoro, E.P., Curdia, J., Rosado, J.G.D., Cardoso, P., Alsagga, A., Barno, A., Antony, C.P., Bocanegra, C., Berumen, M.L., Voolstra, C.R., Benzoni, F., Carvalho, S., Peixoto, R.S., 2024. Probiotics reshape the coral microbiome in situ without detectable off-target effects in the surrounding environment. *Commun. Biol.* 7 (1), 1–16. <https://doi.org/10.1038/s42003-024-06135-3>.
- Dias, M., Ferreira, A., Gouveia, R., Madeira, C., Jøge, N., Cabral, H., Diniz, M., Vinagre, C., 2019a. Long-term exposure to increasing temperatures on scleractinian coral fragments reveals oxidative stress. *Mar. Environ. Res.* 150, 104758. <https://doi.org/10.1016/j.marenvres.2019.104758>.
- Dias, M., Madeira, C., Jøge, N., Ferreira, A., Gouveia, R., Cabral, H., Diniz, M., Vinagre, C., 2019b. Oxidative stress on scleractinian coral fragments following exposure to high temperature and low salinity. *Ecol. Indic.* 107, 105586. <https://doi.org/10.1016/j.ecolind.2019.105586>.
- Downs, C.A., Mueller, E., Phillips, S., Fauth, J.E., Woodley, C.M., 2000. A molecular biomarker system for assessing the health of coral (*Montastraea faveolata*) during heat stress. *Mar. Biotechnol.* 2 (6), 533–544. <https://doi.org/10.1007/S10126000038/METRICS>.
- Efrony, R., Loya, Y., Bacharach, E., Rosenberg, E., 2007. Phage therapy of coral disease. *Coral Reefs* 26 (1), 7–13. <https://doi.org/10.1007/S00338-006-0170-1/FIGURES/9>.
- Ellis, S., 1999. Farming soft corals for the marine aquarium trade. In: *Center for Tropical and Subtropical Aquaculture Publication Number*, 140.
- Feng, L., Zhang, K., Gao, M., Shi, C., Ge, C., Qu, D., Zhu, J., Shi, Y., Han, J., 2018. Inactivation of *Vibrio parahaemolyticus* by aqueous ozone. *J. Microbiol. Biotechnol.* 28 (8), 1233–1246. <https://doi.org/10.4014/JMB.1801.01056>.
- Ferrier-Pagès, C., Richard, C., Forcioli, D., Allemand, D., Pichon, M., Shick, J.M., 2007. Effects of temperature and UV irradiation increases on the photosynthetic efficiency in four scleractinian coral species, 213 (1), 76–87. <https://doi.org/10.2307/25066620>.
- Ferrier-Pagès, C., Rottier, C., Beraud, E., Levy, O., 2010. Experimental assessment of the feeding effort of three scleractinian coral species during a thermal stress: effect on the rates of photosynthesis. *J. Exp. Mar. Biol. Ecol.* 390 (2), 118–124. <https://doi.org/10.1016/J.JEMBE.2010.05.007>.
- Fox, J., Weisberg, S., 2019. *An R Companion to Applied Regression*, Third edition. Sage, Thousand Oaks CA. <https://www.john-fox.ca/Companion/>.
- Frisch, K., Småge, S.B., Vallestad, C., Duesund, H., Brevik, J., Klevan, A., Olsen, R.H., Sjaatil, S.T., Gauthier, D., Brudeseth, B., Nylund, A., 2018. Experimental induction of mouthrot in Atlantic salmon smolts using *Tenacibaculum maritimum* from Western Canada. *J. Fish Dis.* 41 (8), 1247–1258. <https://doi.org/10.1111/JFD.12818>.
- Gao, C.H., Yu, G., Cai, P., 2021. ggVennDiagram: an intuitive, easy-to-use, and highly customizable R package to generate Venn diagram. *Front. Genet.* 12. <https://doi.org/10.3389/FGENE.2021.706907>.
- Gardner, S.G., Raina, J.B., Nitschke, M.R., Nielsen, D.A., Stat, M., Motti, C.A., Ralph, P.J., Petrou, K., 2017. A multi-trait systems approach reveals a response cascade to bleaching in corals. *BMC Biol.* 15 (1), 117. <https://doi.org/10.1186/S12915-017-0459-2>.
- Gignoux-Wolfsohn, S.A., Vollmer, S.V., 2015. Identification of candidate coral pathogens on white band disease-infected staghorn coral. *PLoS One* 10 (8), e0134416. <https://doi.org/10.1371/JOURNAL.PONE.0134416>.
- Glasl, B., Herndl, G.J., Frade, P.R., 2016. The microbiome of coral surface mucus has a key role in mediating holobiont health and survival upon disturbance. *ISME J.* 10 (9), 2280–2292. <https://doi.org/10.1038/ismej.2016.9>.
- Grotmol, S., Totland, G.K., 2000. Surface disinfection of Atlantic halibut *Hippoglossus hippoglossus* eggs with ozonated sea-water inactivates nodavirus and increases survival of the larvae. *Dis. Aquat. Org.* 39 (2), 89–96. <https://doi.org/10.3354/DAO039089>.
- Guilherme, S., Crespo, R., Azevedo, D., Marques, A., Santos, M.A., Serradeiro, R., Pacheco, M., 2019. DNA and chromosomal damage in Senegalese sole (*Solea senegalensis*) as side effects of ozone-based water treatment - contribution to optimization of fish-farming practices. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* 219, 68–76. <https://doi.org/10.1016/J.CBPC.2019.02.001>.
- Halliwell, B., Gutteridge, J.M.C., 1984. Lipid peroxidation, oxygen radicals, cell damage, and antioxidant therapy. *Lancet* 323 (8391), 1396–1397. [https://doi.org/10.1016/S0140-6736\(84\)91886-5](https://doi.org/10.1016/S0140-6736(84)91886-5).
- Hernandez-Agreda, A., Gates, R.D., Ainsworth, T.D., 2017. Defining the Core microbiome in corals' microbial soup. *Trends Microbiol.* 25 (2), 125–140. <https://doi.org/10.1016/J.TIM.2016.11.003>.
- Higuchi, T., Activities of antioxidant enzymes (SOD and CAT) in the coral *Galaxea fascicularis* against increased hydrogen peroxide concentrations in seawater. <https://www.researchgate.net/publication/273635123>.
- Kassambara, A., 2023. Rstatix: pipe-friendly framework for basic statistical tests. In: R Package Version 0.7.2. <https://cran.r-project.org/package=rstatix>.
- Keller-Costa, T., Lago-Lestón, A., Saraiva, J.P., Toscan, R., Silva, S.G., Gonçalves, J., Cox, C.J., Kypides, N., Nunes da Rocha, U., Costa, R., 2021. Metagenomic insights into the taxonomy, function, and dysbiosis of prokaryotic communities in octocorals. *Microbiome* 9 (1), 1–21. <https://doi.org/10.1186/S40168-021-01031-Y/FIGURES/5>.
- 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 (1), 106–113. <https://doi.org/10.1111/J.1574-6968.2007.00921.X>.
- Leal, M.C., Ferrier-Pagès, C., Petersen, D., Osinga, R., 2016. Coral aquaculture: applying scientific knowledge to ex situ production. *Rev. Aquac.* 8 (2), 136–153. <https://doi.org/10.1111/raq.12087>.
- Lesser, M.P., 2006. Oxidative stress in marine environments: biochemistry and physiological ecology. *Annual Rev. Physiol.* 68, 253–278. <https://doi.org/10.1146/ANNUREV.PHYSIOL.68.040104.110001>.
- Levy, O., Achituv, Y., Yacobi, Y.Z., Stambler, N., Dubinsky, Z., 2006. The Impact of Spectral Composition and Light Periodicity on the Activity of Two Antioxidant Enzymes (SOD and CAT) in the coral *Favia fava*. <https://doi.org/10.1016/j.jembe.2005.06.018>.
- Li, X., Blancheton, J.P., Liu, Y., Triplett, S., Michaud, L., 2014. Effect of oxidation-reduction potential on performance of European sea bass (*Dicentrarchus labrax*) in recirculating aquaculture systems. *Aquac. Int.* 22 (4), 1263–1282. <https://doi.org/10.1007/s10499-013-9745-3>.
- Li, J., Long, L., Zou, Y., Zhang, S., 2021. Microbial community and transcriptional responses to increased temperatures in coral *Pocillopora damicornis* holobiont. *Environ. Microbiol.* 23 (2), 826–843. <https://doi.org/10.1111/1462-2920.15168>.
- Lilved, H., Vogelsang, C., Modahl, J., Dannevig, B.H., 2006. High resistance of fish pathogenic viruses to UV irradiation and ozonated seawater. *Aquac. Eng.* 34 (2), 72–82. <https://doi.org/10.1016/J.AQUAENG.2005.05.002>.
- Lorgen-Ritchie, M., Uren Webster, T., McMurtrie, J., Bass, D., Tyler, C.R., Rowley, A., Martin, S.A.M., 2023. Microbiomes in the context of developing sustainable intensified aquaculture. *Front. Microbiol.* 14, 1200997. <https://doi.org/10.3389/fmicb.2023.1200997/XML/NLM>.
- Lenth, R.V., Banfai, B., Bolker, B., Buerkner, P., Giné-Vázquez, I., Herve, M., Jung, M., Love, J., Miguez, F., Piskowski, J., Riebl, H., Singmann, H., 2025. *emmeans: Estimated Marginal Means, aka Least-Squares Means* (Version 1.11.1) [Computer software]. <https://cran.r-project.org/web/packages/emmeans/index.html>.
- Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15 (12), 550. <https://doi.org/10.1186/s13059-014-0550-8>.
- Luna, G.M., Bongiorno, L., Gili, C., Biavasco, F., Danovaro, R., 2010. *Vibrio harveyi* as a causative agent of the white syndrome in tropical stony corals. *Environ. Microbiol. Rep.* 2 (1), 120–127. <https://doi.org/10.1111/J.1758-2229.2009.00114.X>.
- MacKnight, N.J., Cobleigh, K., Lasseigne, D., Chaves-Fonnegra, A., Gutting, A., Dimos, B., Antoine, J., Fuess, L., Ricci, C., Butler, C., Muller, E.M., Mydlarz, L.D., Brandt, M., 2021. Microbial dysbiosis reflects disease resistance in diverse coral species. *Commun. Biol.* 4 (1), 1–11. <https://doi.org/10.1038/s42003-021-02163-5>.
- Marangon, E., Rådecker, N., Li, J.Y.Q., Terzin, M., Buerger, P., Webster, N.S., Bourne, D. G., Laffy, P.W., 2025. Destabilization of mutualistic interactions shapes the early heat stress response of the coral holobiont. *Microbiome* 13 (1), 1–18. <https://doi.org/10.1186/S40168-024-02006-5/FIGURES/7>.

- Marino, M., Maifreni, M., Baggio, A., Innocente, N., 2018. Inactivation of foodborne Bacteria biofilms by aqueous and gaseous ozone. *Front. Microbiol.* 9 (AUG). <https://doi.org/10.3389/FMICB.2018.02024>.
- Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J.* 17 (1), 10–12. <https://doi.org/10.14806/EJ.17.1.200>.
- Martinez Arbizu, P., 2020. pairwiseAdonis: Pairwise Multilevel Comparison using Adonis (R Package Version 0.4).
- Martins, C.I.M., Eding, E.H., Verdegem, M.C.J., Heinsbroek, L.T.N., Schneider, O., Blancheton, J.P., d'Orbcastel, E.R., Verreth, J.A.J., 2010. New developments in recirculating aquaculture systems in Europe: a perspective on environmental sustainability. *Aquac. Eng.* 43 (3), 83–93. <https://doi.org/10.1016/J.AQUAENG.2010.09.002>.
- McCord, J.M., Fridovich, I., 1969. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J. Biol. Chem.* 244 (22), 6049–6055.
- McDevitt-Irwin, J.M., Baum, J.K., Garren, M., Vega Thurber, R.L., 2017. Responses of coral-associated bacterial communities to local and global stressors. *Front. Mar. Sci.* 4 (AUG), 286253. <https://doi.org/10.3389/FMARS.2017.00262/PDF>.
- McDevitt-Irwin, J.M., Garren, M., McMinds, R., Vega Thurber, R., Baum, J.K., 2019. Variable interaction outcomes of local disturbance and El Niño-induced heat stress on coral microbiome alpha and beta diversity. *Coral Reefs* 38 (2), 331–345. <https://doi.org/10.1007/S00338-019-01779-8/FIGURES/7>.
- McMurdie, P.J., Holmes, S., 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 8 (4). <https://doi.org/10.1371/JOURNAL.PONE.0061217>.
- Meyer, J.L., Castellanos-Gell, J., Aebly, G.S., Häse, C.C., Ushijima, B., Paul, V.J., 2019. Microbial community shifts associated with the ongoing stony coral tissue loss disease outbreak on the Florida reef tract. *Front. Microbiol.* 10, 2244. <https://doi.org/10.3389/FMICB.2019.02244/FULL>.
- Meziere, Z., Rich, W.A., Carvalho, S., Benzoni, F., Morán, A.G., Berumen, M.L., 2021. *Stylophora* Under Stress: A Review of Research Trends and Impacts of Stressors on a Model Coral Species. <https://doi.org/10.1016/j.scitotenv.2021.151639>.
- Mohamed, A.R., Sweet, M., 2019. Current Knowledge of Coral Diseases Present within the Red Sea, pp. 387–400. https://doi.org/10.1007/978-3-319-99417-8_21.
- Moschos, S., Kormas, K.A., Karayanni, H., 2022. Prokaryotic diversity in marine and freshwater recirculating aquaculture systems. *Rev. Aquac.* 14 (4), 1861–1886. <https://doi.org/10.1111/RAQ.12677>.
- Muyzer, G., De Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* 59 (3), 695–700. <https://doi.org/10.1128/aem.59.3.695-700.1993>.
- Nghia, N.H., Nguyen, N.T., Binh, P.T., May, L.T., Huy, T.T., Giang, P.T., St-Hilaire, S., Van, P.T., 2022. Effect of nanobubbles (oxygen, ozone) on the Pacific white shrimp (*Penaeus vannamei*), *Vibrio parahaemolyticus* and water quality under lab conditions. *Fish. Aquat. Sci.* 25 (8), 429–440. <https://doi.org/10.47853/FAS.2022.E39>.
- Ogle, D.H., Doll, J.C., Wheeler, A.P., Dinno, A., 2025. Simple Fisheries Stock Assessment Methods [R package FSA version 0.10.0]. CRAN: Contributed Packages. <https://doi.org/10.32614/CRAN.PACKAGE.FSA>.
- Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 95 (2), 351–358. [https://doi.org/10.1016/0003-2697\(79\)90738-3](https://doi.org/10.1016/0003-2697(79)90738-3).
- Oksanen, J., Blanchet, F.G., Minchin, P.R., 2024. (954). *Vegan: Community Ecology Package*. R Package Version 2.0–2. <https://github.com/vegandevs/vegan>.
- Paradis, B.T., Henry, R.P., Chadwick, N.E., 2019. Compound effects of thermal stress and tissue abrasion on photosynthesis and respiration in the reef-building coral *Acropora cervicornis* (Lamarck, 1816). *J. Exp. Mar. Biol. Ecol.* 521, 151222. <https://doi.org/10.1016/J.JEMBE.2019.151222>.
- Pedersen, T.L., 2022. Patchwork: The Composer of Plots. R Package Version 1.1.3. <https://CRAN.R-project.org/package=patchwork>.
- Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., Blondel, M., Müller, A., Nothman, J., Louppe, G., Prettenhofer, P., Weiss, R., Dubourg, V., Vanderplas, J., Passos, A., Cournapeau, D., Brucher, M., Perrot, M., Duchesnay, É., 2012. Scikit-learn: Machine Learning in Python. <http://arxiv.org/abs/1201.0490>.
- Pumkaew, M., Taweephitakthai, T., Satanwat, P., Yocawibun, P., Chumtong, P., Pungrasmi, W., Powtongsook, S., 2021. Use of ozone for *Vibrio parahaemolyticus* inactivation alongside nitrification biofilter treatment in shrimp-rearing recirculating aquaculture system. *J. Water Process Eng.* 44, 102396. <https://doi.org/10.1016/J.JWPE.2021.102396>.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41 (D1). <https://doi.org/10.1093/nar/gks1219>.
- Ramos, R., García, E., 2007. Induction of mixed-function oxygenase system and antioxidant enzymes in the coral *Montastraea faveolata* on acute exposure to benzo(a) pyrene. *Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol.* 144 (4), 348–355. <https://doi.org/10.1016/J.CBPC.2006.11.006>.
- Reiser, S., Schroeder, J.P., Wuertz, S., Kloas, W., Hanel, R., 2010. Histological and Physiological Alterations in Juvenile Turbot (*Psetta maxima*, L.) Exposed to Sublethal Concentrations of Ozone-Produced Oxidants in Ozonated Seawater. <https://doi.org/10.1016/j.aquaculture.2010.07.007>.
- Ritola, O., Lyytikäinen, T., Pylkkö, P., Mölsä, H., Lindström-Seppä, P., 2000. Glutathione-dependent defence system and monooxygenase enzyme activities in Arctic charr *Salvelinus alpinus* (L.) exposed to ozone. *Aquaculture* 185 (3–4), 219–233. [https://doi.org/10.1016/S0044-8486\(99\)00355-5](https://doi.org/10.1016/S0044-8486(99)00355-5).
- Ritola, O., Peters, L.D., Livingstone, D.R., Lindström-Seppä, P., 2002. Effects of in vitro exposure to ozone and/or hyperoxia on superoxide dismutase, catalase, glutathione and lipid peroxidation in red blood cells and plasma of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquac. Res.* 33 (3), 165–175. <https://doi.org/10.1046/J.1365-2109.2002.00649.X>.
- Rosales, S.M., Miller, M.W., Williams, D.E., Traylor-Knowles, N., Young, B., Serrano, X. M., 2019. Microbiome differences in disease-resistant vs. susceptible *Acropora* corals subjected to disease challenge assays. *Sci. Rep.* 9 (1), 1–11. <https://doi.org/10.1038/s41598-019-54855-y>.
- Rosales, S.M., Clark, A.S., Huebner, L.K., Ruzicka, R.R., Muller, E.M., 2020. Rhodobacterales and Rhizobiales are associated with stony coral tissue loss disease and its suspected sources of transmission. *Front. Microbiol.* 11, 517258. <https://doi.org/10.3389/FMICB.2020.00681/BIBTEX>.
- Schöttner, S., Hoffmann, F., Wild, C., Rapp, H.T., Boetius, A., Ramette, A., 2009. Inter- and intra-habitat bacterial diversity associated with cold-water corals. *ISME J.* 3 (6), 756–759. <https://doi.org/10.1038/ISMEJ.2009.15>.
- Schroeder, J.P., Gärtner, A., Waller, U., Hanel, R., 2010. The toxicity of ozone-produced oxidants to the Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture* 305 (1–4), 6–11. <https://doi.org/10.1016/J.AQUACULTURE.2010.03.030>.
- Sekar, R., Mills, D.E.K., Remily, E.R., Voss, J.D., Richardson, L.L., 2006. Microbial communities in the surface mucopolysaccharide layer and the black band microbial mat of black band-diseased *Siderastrea siderea*. *Appl. Environ. Microbiol.* 72 (9), 5963–5973. <https://doi.org/10.1128/AEM.00843-06/ASSET/69955362-2AB9-4F83-919D-EE0ECA842985/ASSETS/GRAPHIC/ZAM0090671140003.JPEG>.
- Shely, D., Rinkevich, B., 2021. *Stylophora pistillata* - a model colonial species in basic and applied studies. In: *Handbook of Marine Model Organisms in Experimental Biology: Established and Emerging*, pp. 195–216. <https://doi.org/10.1201/9781003217503-11/STYLOPHORA-PISTILLATA>.
- Sheridan, C., Kramarsky-Winter, E., Sweet, M., Kushmaro, A., Leal, M.C., 2013. Diseases in coral aquaculture: causes, implications and preventions. In: *Aquaculture*, Vols. 396–399, pp. 124–135. <https://doi.org/10.1016/j.aquaculture.2013.02.037>.
- Shichiri-Negoro, Y., Tsutsumi-Arai, C., Arai, Y., Satomura, K., Arakawa, S., Wakabayashi, N., 2021. Ozone ultrafine bubble water inhibits the early formation of *Candida albicans* biofilms. *PLoS One* 16 (12 December 2021). <https://doi.org/10.1371/JOURNAL.PONE.0261180>.
- Simancas-Giraldo, S.M., Xiang, N., Kennedy, M.M., Nafeh, R., Zelli, E., Wild, C., 2021. Photosynthesis and respiration of the soft coral *Xenia umbellata* respond to warming but not to organic carbon eutrophication. *PeerJ* 9. <https://doi.org/10.7717/peerj.11663>.
- Steiner, K., Heasman, K., Laroche, O., Pochon, X., Preece, M., Bowman, J.P., Walker, S.P., Symonds, J.E., 2021. The microbiome of Chinook salmon (*Oncorhynchus tshawytscha*) in a recirculation aquaculture system. *Aquaculture* 534, 736227. <https://doi.org/10.1016/J.AQUACULTURE.2020.736227>.
- Summerfelt, S.T., 2003. Ozonation and UV irradiation—an introduction and examples of current applications. *Aquac. Eng.* 28 (1–2), 21–36. [https://doi.org/10.1016/S0144-8609\(02\)00069-9](https://doi.org/10.1016/S0144-8609(02)00069-9).
- Swaminathan, S.D., Meyer, J.L., Johnson, M.D., Paul, V.J., Bartels, E., Altieri, A.H., 2023. Divergent responses of the coral holobiont to deoxygenation and prior environmental stress. *Front. Mar. Sci.* 10, 1301474. <https://doi.org/10.3389/FMARS.2023.1301474/BIBTEX>.
- Sweet, M., Jones, R., Bythell, J., 2012. Coral diseases in aquaria and in nature. *J. Mar. Biol. Assoc. U. K.* 92 (4), 791–801. <https://doi.org/10.1017/S0025315411001688>.
- Sweet, M., Burian, A., Fifer, J., Bulling, M., Elliott, D., Raymundo, B., 2019. Compositional homogeneity in the pathobiome of a new, slow-spreading coral disease. *Microbiome* 7 (1), 1–14. <https://doi.org/10.1186/S40168-019-0759-6/TABLES/1>.
- Tachikawa, M., Yamanaka, K., Nakamuro, K., 2009. Studies on the disinfection and removal of biofilms by ozone water using an artificial microbial biofilm system. *Ozone Sci. Eng.* 31 (1), 3–9. https://doi.org/10.1080/01919510802586566/ASSET/F6CAA912-3F41-4380-BFDE-1D7C0FC4C952/ASSETS/IMAGES/BOSE_A_358824_O_F00066.JPG.
- Tang, J., Ni, X., Zhou, Z., Wang, L., Lin, S., 2018. Acute microplastic exposure raises stress response and suppresses detoxification and immune capacities in the scleractinian coral *Pocillopora damicornis*. *Environ. Pollut.* 243, 66–74. <https://doi.org/10.1016/J.ENVPOL.2018.08.045>.
- Tango, M.S., Gagnon, G.A., 2003. Impact of ozonation on water quality in marine recirculation systems. *Aquac. Eng.* 29 (3–4), 125–137. [https://doi.org/10.1016/S0144-8609\(03\)00061-X](https://doi.org/10.1016/S0144-8609(03)00061-X).
- Teixeira, T., Diniz, M., Calado, R., Rosa, R., 2013. Coral physiological adaptations to air exposure: heat shock and oxidative stress responses in *Veretillum cymonarium*. *J. Exp. Mar. Biol. Ecol.* 439, 35–41. <https://doi.org/10.1016/j.jembe.2012.10.010>.
- Thurber, R.V., Willner-Hall, D., Rodriguez-Mueller, B., Desnues, C., Edwards, R.A., Angly, F., Dinsdale, E., Kelly, L., Rohwer, F., 2009. Metagenomic analysis of stressed coral holobionts. *Environ. Microbiol.* 11 (8), 2148–2163. <https://doi.org/10.1111/j.1462-2920.2009.01935.x>.
- Timmons, M.B., Ebeling, J.E., 2013. *Recirculating Aquaculture System*, 3rd edition. Ithaca Publishing Company.
- Ushijima, B., Gunasekera, S.P., Meyer, J.L., Tittl, J., Pitts, K.A., Thompson, S., Sneed, J. M., Ding, Y., Chen, M., Jay Houk, L., Aebly, G.S., Häse, C.C., Paul, V.J., 2023. Chemical and genomic characterization of a potential probiotic treatment for stony coral tissue loss disease. *Commun. Biol.* 6 (1), 1–13. <https://doi.org/10.1038/s42003-023-04590-y>.
- Venables, W.N., Springer, B.D.R., 2025. *Modern Applied Statistics with S*, Fourth edition. Retrieved June 4, 2025, from <http://www.insightful.com>.
- Voolstra, C.R., Raina, J.B., Dörr, M., Cárdenas, A., Pogoreutz, C., Silveira, C.B., Mohamed, A.R., Bourne, D.G., Luo, H., Amin, S.A., Peixoto, R.S., 2024. The coral microbiome in sickness, in health and in a changing world. *Nat. Rev. Microbiol.* 22 (8), 460–475. <https://doi.org/10.1038/s41579-024-01015-3>.

- Wickham, H., 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag, New York. ISBN 978-3-319-24277-4. <https://ggplot2.tidyverse.org>.
- Wickham, H., François, R., Henry, L., Müller, K., 2023. Dplyr: a grammar of data manipulation. In: R Package Version 1.1.4. <https://CRAN.R-project.org/package=dplyr>.
- Wietz, M., Hall, M.R., Høj, L., 2009. Effects of seawater ozonation on biofilm development in aquaculture tanks. *Syst. Appl. Microbiol.* 32, 266–277. <https://doi.org/10.1016/j.syapm.2009.04.001>.
- Wild, C., Huettel, M., Klueter, A., Kremb, S.G., Rasheed, M.Y.M., Jørgensen, B.B., 2004. Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. *Nature* 428 (6978), 66–70. <https://doi.org/10.1038/nature02344>.
- Winters, G., Loya, Y., Röttgers, R., Beer, S., 2003. Photoinhibition in shallow-water colonies of the coral *Stylophora pistillata* as measured in situ. *Limnol. Oceanogr.* 48 (4), 1388–1393. <https://doi.org/10.4319/LO.2003.48.4.1388>.
- Wuertz, S., Schulz, C., Klatt, S., Kleiner, W., Schroeder, J.P., 2023. Adverse effects of ozonation on the animal welfare of tilapia *Oreochromis niloticus* in recirculation aquaculture. *Aquac. Rep.* 32, 101737. <https://doi.org/10.1016/J.AQREP.2023.101737>.