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Abstract

Reef ecosystems are under increasing pressure from global and local stressors. Rising seawater temperature and high ultraviolet radiation (UVR) levels are the main drivers of the disruption of the coral-dinoflagellate symbiosis (bleaching). Bleaching can also be exacerbated by nitrate contamination in coastal reefs. However, the underlying physiological mechanisms are still poorly understood. Here, we assessed the physiological and oxidative state of the scleractinian coral *Pocillopora damicornis*, maintained eight weeks in a crossed-factorial design including two temperatures (26 °C or 30 °C), and two nitrate (0.5 and 3 µM-enriched), and UVR (no UVR and 25/1.5 Wm^{-2} UVA/B) levels. Nitrate enrichment, and high temperature, significantly impaired coral photosynthesis. However, UVR alleviated the nitrate and temperature-induced decrease in photosynthesis, by increasing the coral's antioxidant capacity. The present study contributes to our understanding of the combined effects of abiotic stressors on coral bleaching susceptibility. Such information is urgently needed to refine reef management strategies.

Keywords

Eutrophication, nitrate, coral, photosynthesis, symbiosis, ultra-violet

Introduction

Coral reefs are among the most diverse and productive marine ecosystems on the planet despite thriving in oligotrophic waters (i.e. low levels of nitrogen and phosphorus; Crossland et al., 1991). They provide a range of important economic services to local population, such as tourism and recreation, fisheries and coastal protection (Moberg and Folke, 1999). Reefs rely on the nutritional and optimized symbiosis between corals and autotrophic dinoflagellates of the Symbiodiniaceae family (LaJeunesse et al., 2018). Symbionts efficiently take up and assimilate dissolved inorganic nutrients from the oligotrophic seawater (Godinot et al., 2009; Muscatine et al., 1984; Rädecker et al., 2015) and are able to recycle the host's metabolic wastes (Rahav et al., 1989). They also transfer most of their photosynthates to the coral host, which relies on this major source of nutrients to sustain its respiratory demand, growth and reproduction (Muscatine et al., 1984; Tremblay et al., 2014).

Despite their ecological and economical value, reef ecosystems are under increasing pressure from multiple global stressors, which disrupt the stability of the coral-dinoflagellate symbiosis (Ellis et al., 2019). The loss of symbionts (i.e. bleaching; Weis 2008) significantly impairs the coral nutritional state and may ultimately lead to significant mortality rates. Coral bleaching is thus one of the greatest threats to reef ecosystems nowadays (Eakin et al., 2019; Suggett and Smith, 2020). Large-scale bleaching events have increased in frequency over the last decades mainly due to heatwaves, and elevated sea surface temperatures (SST) maintained over several weeks (Couch et al., 2017; Hughes et al., 2017). Coral bleaching susceptibility and severity, however, depends on other environmental parameters such as the irradiance levels, both photosynthetically active radiation (PAR) and ultra-violet radiation (UVR), either UVA (400-320 nm) or UVB (320-280 nm). UVR can penetrate the water column down to 150 m depth in very oligotrophic waters (Barron et al., 2009; Kahng et al., 2019; Tedetti and Sempéré, 2006). While the thermotolerance threshold of corals is generally decreased under exposure to high PAR or UVR levels (Downs et al., 2013; Lesser and Farrell, 2004; Torregiani and Lesser, 2007), the contrary has also been observed (Halac et al., 2010; McCauley et al., 2018; Rosic et al., 2020). For example, Rosic et al. (2020) showed a synergetic effect of low PAR (no UVR exposure) and thermal stress that caused higher bleaching levels of the scleractinian coral *Acropora millepora* compared to thermal stress and high PAR. Antagonistic interactions of temperature and UVR were also observed on Caribbean octocorals (McCauley et al., 2018). Finally, two experiments showed an

enhancement of the photosynthetic performances of coral larvae under UVR exposure (Zhou et al., 2017, 2016). This discrepancy in the effects of UVR on coral physiology is most likely due to the different doses of UVR to which organisms are exposed (Barron et al., 2009; Overmans and Agustí, 2020). Therefore, UVR levels may be a tipping point towards postponing or speeding up DNA damage and coral bleaching (Ben-Zvi et al., 2019).

In addition to global change stressors, coral reefs also face local anthropogenic impacts. These include upwelling events (Richardson et al., 2020), nutrient enrichment of coastal seawater through land run off, agriculture and urban wastes (Fabricius, 2011, 2005). Under these conditions, inorganic nitrogen (N) and phosphorus (P) concentrations can be much higher (up to 4 µM N and 0.5 µM P, (Brodie et al., 2011; Govers et al., 2014; Naumann et al., 2015; Rouzé et al., 2015)) than the usual levels measured on reefs (ca. 0.5 µM N and 0.1 µM P (Charpy, 2001; Kinsey and Davies, 1979; Lomas and Lipschultz, 2006; Meeder et al., 2012)). Recent findings have shown that concentrations of 2 to 5 µM of nitrate, which is the main nitrogen compound resulting from human activities, can enhance coral bleaching during thermal stress, (Burkepile et al., 2019; Ezzat et al., 2016; Marangoni et al., 2020; Rosset et al., 2017; Wiedenmann et al., 2013). However, as for UVR exposure, the effect of nitrate on thermal stress-induced bleaching largely varies, depending for example on coral species, symbiont densities, nitrate and other nutrient concentrations (Fabricius et al., 2013; Schlöder and D'Croz, 2004; Serrano et al., 2018).

Variations in coral bleaching susceptibility under either UVR exposure or nitrate enrichment, may depend on changes in UVR or nitrate levels, and more specifically on corals' antioxidant capacity (Ezzat et al., 2015; Krueger et al., 2015; Marangoni et al., 2020; Muller-Parker et al., 2015). Under thermal stress, the concentrations of reactive oxygen species (ROS) and nitrogen species (RNS) within the host and symbiont cells drastically increase (Weis, 2008). These compounds cause cellular damage through DNA degeneration, protein oxidation and lipid peroxidation (Freeman and Crapo, 1982; Suggett and Smith, 2020). In order to cope with high levels of ROS and RNS, corals produce various protecting molecules, such as ascorbate, catalase (CAT), glutathione peroxidases and super-oxide dismutases (Krueger et al., 2014; Liñán- Cabello et al., 2010). Damages to coral tissue and bleaching occur when ROS and RNS production exceed the antioxidant capacity of the coral holobiont. While UVR and nitrate can respectively be sources of ROS and RNS (Lesser, 2006; Moniczewski et al., 2015), the oxidative status of corals under the combination of the two stressors, has never been investigated and thus needs further attention.

The present study aims to investigate the effects of nitrate enrichment on coral bleaching susceptibility, when combined with different temperatures and/or UVR conditions. For this purpose, we used the widespread scleractinian coral species *Pocillopora damicornis*. We measured the changes in the physiological and oxidative status of the corals following exposure to the individual and combined stressors. We used a UVR level that can be received by tropical corals living at 10-15 m depth (daily dose of 125 Wd^{-1} UVA and 7.5 Wd^{-1} UVB), instead of the three times higher doses measured in shallow reefs. We hypothesized that thermally-stressed or UVR-stressed corals exposed to nitrate enrichment would experience increased levels of oxidative stress compared to corals maintained with no nitrate enrichment. Given the increasing exposure of reefs to human pollution and recurrent heat waves events, this study aims to identify the worse combination of factors for the survival of reef building corals such as *Pocillopora damicornis*. A deeper understanding of the impacts of multiple stressors on reef building corals will help identify adaptive strategies to better protect corals and associated coral reefs.

Material and Methods

1. Experimental design

Ten colonies of the scleractinian coral *Pocillopora damicornis* were used to generate 160 nubbins (16 nubbins from each colony), which were then equally divided in 16 aquaria of 25 L (one nubbin per colony, 10 per aquaria). Nubbins were left for three weeks in similar conditions (see below) for healing and were fed once a week with *Artemia salina* nauplii. During this period, aquaria were continuously supplied (at a rate of 8 $L.h^{-1}$) with oligotrophic seawater (0.5 µM nitrate, 0.2 µM phosphorus) and corals were maintained at 25 °C under a photosynthetically active radiation (PAR) of 200 ± 10 µmol $photons.m^{-2}.s^{-1}$ (12:12h photoperiod). PAR was delivered by 400 W metal halide lamps (HPITS, Philips) above the aquaria and PAR intensity was measured using a LI-COR data logger (LI-100) connected to a quantum sensor (LI-I93). Temperature was controlled by heaters connected to an Elliwell PC 902/T controller. Nutrient levels were measured once a week using an AA3 Seal autoanalyzer according to Aminot et al. (2009).

The 16 aquaria were then divided into 4 sets of 4 aquaria, each set being maintained under a different nitrate and UVR level (Fig. 1). The first set was a control condition (C26), which was not enriched with nitrate (0.5 µM nitrate) and not exposed to UVR. A second set of 4 aquaria (UV26) was not enriched with nitrate, but received 25 Wm^{-2} of UVA and 1.5 Wm^{-2} of UVB during 5 hours per day (between 10:00 and 15:00) provided by two Q-panel UVA 340 lamps. The spectral distribution of these lamps is shown in Shick et al (1999). The two other sets of aquaria were enriched with 3 µM nitrate and maintained with or without the levels of UVR described above (N26 or UVN26 respectively). Nitrate enrichment was performed using stock solutions of 300 µM $NaNO_3$. The solutions were delivered to the aquaria with a peristaltic pump and were renewed every week. The enrichment condition chosen was similar to the nitrate concentration observed in reefs experiencing nutrient enrichment (Brodie et al., 2011; Costa et al., 2000; Govers et al., 2014; Naumann et al., 2015; Rouzé et al., 2015). The intensity of UVR provided to the two sets of 4 aquaria was recorded using an International Light ILT1400 portable radiometer equipped with two detectors: SEL033/UVA and SEL240/UVB and the coefficient of attenuation of UVR irradiance in water was taken into account. UVA and UVB values, applied during only 5 h, corresponded to a daily dose of 125 Wd^{-1} UVA and 7.5 Wd^{-1} UVB). Such doses can be considered low compared to those received by corals on shallow reefs, which can reach more than 350 Wd^{-1}

UVA and 19 Wd^{-1} UVB (Barron et al., 2009; Overmans and Agustí, 2020). In these reefs, in summer, peaks of 60 to 70 Wm^{-2} UVA and 3 Wm^{-2} UVB can occur at midday (Kaneohe Bay, Hawaii and Heron Island, Australia) (Rosic et al., 2020; Torregiani and Lesser, 2007). Our conditions, therefore, correspond to tropical corals living at 15 m depth, where UVR levels are significantly reduced.

The factorial design with UVR and nutrient levels was maintained for six weeks, before a thermal stress was applied to half of the aquaria (Fig. 1). Temperature was increased by 0.5 °C every two days until reaching 30 °C. This temperature was maintained for one week until bleaching was visually observed, and the measurements described below were performed.

2. Physiological measurements

Photosynthesis parameters

Rates of net photosynthesis (Pn) and respiration (R) were estimated on 6 nubbins per condition (from different colonies, and 3 nubbins per aquarium). For this purpose, nubbins were placed in 60 mL Plexiglass chambers filled with 0.45 µm-filtered seawater, maintained at 26 °C or 30 °C, and stirred. Each chamber was equipped with an oxygen sensor (Polymere Optical Fiber, PreSens, Regensburg, Germany) connected to an Oxy-4 (Channel fiber-optic oxygen meter, PreSens, Regensburg, Germany). Oxygen concentration was recorded for 30 min with the Oxy4v2-30fb software, in the dark for R and at 200 µmol photons.m^{-2}.s^{-1} for Pn. Two calibrations were done at 0% O_2 with nitrogen saturated seawater and at 100% O_2 with air saturated seawater. The gross photosynthesis rate was obtained by adding the absolute value of the respiration rate to the corresponding net photosynthesis rate. This obtained gross photosynthesis rate is likely to be an underestimation of the actual gross photosynthesis rate (Schrameyer et al., 2014). At the end of the measurements, nubbins were frozen for the later determination of the symbiont density, proteins and chlorophyll a and c_2 (Chl) content. Pn, Pg, and R rates were expressed as µmol O_2.h^{-1}.cm^{-2}. The surface area was obtained with the single dip wax technique (Stimson and Kinzie III, 1991).

Symbiont density, chlorophyll and proteins content

Samples used in the previous measurements were thawed and the tissues were separated from the skeleton using a Water-Pick and 10-15 mL of filtered seawater. A 100 µL sub-sample was used for the determination of the symbiont density with a Z1 Coulter Particle Counter (Beckman Coulter, US). For each sample, five technical replicates were performed. Another

5 ml sub-sample was used for the chlorophyll content analysis. For this purpose, the sub-sample was centrifuged at 5530 g for 15 min at 4 °C to separate the animal tissue (supernatant) from the dinoflagellates (pellet). Then the supernatant was discarded, and the pellet resuspended in 5 ml of acetone 100%, to extract chlorophyll in the dark at 4 °C for 24 hours. Finally, the extract was centrifuged at 5530 g for 15 minutes at 15 °C. The absorbance of the supernatant was recorded at 630 nm, 663 nm and 750 nm using a UVmc^2 Spectrophotometer (Safas, Monaco). The concentration of chlorophyll a and c_2 was then calculated using the equations by Jeffrey and Humphrey (1975). Finally, 500 µL were used for the proteins content of the total holobiont and incubated for 5 hours at 60 °C in sodium hydroxide. Measurements were done with the Bicinchoninic acid (BCA) assay kit according to Smith (1985). All measurements were normalized to the skeletal surface area of the nubbins.

3. Oxidative stress analysis

48 nubbins – 6 per conditions (from different colonies and 3 per aquaria) – were snap-frozen in liquid nitrogen and kept at -80 °C for oxidative stress analysis. For each analysis, a small coral fragment of 5-10 mm was collected from the main fragment.

Reactive oxygen species (ROS) levels

The ROS level in the tissues was quantified using the fluorescent probe 5-(and-6)-carboxy-2',7'-dichlorfluorescein diacetate (H_2DCFDA, Molecular Probes) as described by Ruiz-Leal and George (2004), with some modifications. In presence of ROS, H_2DCFDA emits fluorescence quantified by spectrofluorometry. Coral fragments were fresh collected, sonicated on ice (Frequency 70 kHz, Vibra-Cell™, Bioblock Scientific, France) in a homogenizing buffer (pH 7.75) containing Tris-HCl 100 mM, EDTA 2 mM and $MgCl_2.6H_2O$ 5 mM in MilliQ Water. After sonication, the holobiont homogenates were centrifuged at 10000 g for 10 min at 4 °C, and the protein content was quantified following the Bradford Protein Assay (Bradford, 1976). Samples were standardized to the protein concentration of 0.5 µg.µL^{-1} and 10 µL were then added in triplicates on a black 96-wells microplate with reaction buffer (pH 7.2) containing HEPES 30 mM, KCl 200 mM and MgCl2.6H2O 1 mM in MilliQ water. Finally, 10 µL of H_2-DCFDA (16 µM in ethanol) was added in each well. The fluorescence (excitation: 488 nm; emission: 525 nm) was read every 5 minutes for up to 50 minutes using a spectrofluorometer (Xenius®, SAFAS, Monaco). The area beneath the kinetic curve was considered the level of ROS in the sample. The results were expressed as fluorescence units per minute (F.U. x min).

Lipid peroxidation (LPO)

LPO was assessed following the Thiobarbituric Acid Reactive Substance protocol (TBARS method), in line with Oakes and Van Der Kraak (2003). This method quantifies damage to lipids through the reaction between malondialdehyde (MDA), a byproduct of lipid peroxidation, and thiobarbituric acid (TBA). Coral fragments were sonicated (Frequency 70 kHz, Vibra-Cell™, Bioblock Scientific, France) in a homogenizing buffer (KCl (1,15%) solution containing 35 µM butylatedhydroxytoluene (BHT)) on ice, centrifuged at 10000 g for 10 min at 4 °C, and the protein content was quantified following the Bradford Protein Assay (Bradford, 1976). Samples were standardized to the protein concentration of 0.35 $\mu g.\mu L^{-1}$ and incubated at 95 °C for 30 min in flat-bottomed Eppendorf with acetic acid solution (20%), thiobarbituric acid (TBA) solution (0.8%), SDS solution (8.1%). After cooling, 100 µl of MilliQ water and 500 µl of n-butanol were added with through vortexing. The tubes were then centrifuged at 10000 g for 10 minutes at 15 °C. Finally, 150 µl of the organic phase (supernatant) was placed, in duplicate, in 96-well black plates and the fluorescence was read at 553 nm and 515 nm each minute for up to three minutes using a spectrofluorometer (Xenius®, SAFAS, Monaco). Results were normalized considering the total protein content in the sample homogenates in each well and expressed as nmol MDA.mg $protein^{-1}$, which were calculated from a standard curve built using hydrolysed tetramethoxypropane (TMP).

Protein tyrosine nitration (PTN)

The level of 3-nitrotyrosine modified proteins in corals, a product of PTN resulting from oxidative damage to proteins by peroxynitrite, was assessed using the Nitrotyrosine ELISA Kit (ab113848, Abcam, Cambridge, UK) following manufacturer's instructions. The coral fragments were sonicated (Frequency 70 kHz, Vibra-Cell™, Bioblock Scientific) in phosphate buffered saline (PBS) (pH 7.4) on ice, centrifuged at 10,000 g for 10 minutes at 4 °C. The protein content was quantified following the Bradford Protein Assay (Bradford, 1976). Protein content of each sample was standardized to 0.6 $\mu g.\mu L^{-1}$ and incubated on ice for 20 min in extraction buffer kit solution (1:1). Standards were made with 3-Nitrotyrosine-Bovin Serum Albumin (3-NT-BSA) (reconstituted in 1 mL of 1x Incubation Buffer kit solution). Samples and standards were added on a 96-well plate coated with an antibody specific for 3-nitrotyrosine. The plate was wrapped in foil and left to incubate for two hours

at room temperature, before each well was washed twice with Wash Buffer. Liquid excess was completely removed. Then a biotin labelled anti-3-nitrotyrosine detector antibody was added to each well and left to incubate for one hour before washing twice with Wash Buffer. Each well was then incubated for one hour with HorseRadish Peroxidase (HRP)-labeled kit solution and thoroughly washed three times with Wash Buffer. Finally, the HRP Development kit solution was added in each well and the absorbance was immediately read at 600 nm every minute up to 15 minutes. Results were normalized considering the total protein content in the sample homogenates in each well and expressed as ng 3NT-BSA.mg protein^{-1}.

Non-enzymatic total antioxidant capacity (TAC)

The non-enzymatic total antioxidant capacity was assessed using the "OxiSelect Total Antioxydant Capacity (TAC) Assay Kit" (STA-360, Cell Biolabs Inc., San Diego USA) according to manufacturer's instructions. This assay measures antioxidant capacity of biomolecules via single electron transfer (SET) mechanism. The coral fragments were sonicated (Frequency 70 kHz, Vibra-Cell™, Bioblock Scientific) in PBS (pH 7.4) on ice, centrifuged at 10000 g for 10 min at 4 °C, and the protein content was quantified following the Bradford protein assay (Bradford, 1976). Samples protein content were standardized to 0.9 µg.µL^{-1}. Standards were made with a serial dilution of uric acid from 1mmol.L^{-1} to 0.00390 mmol.L^{-1}. Samples and standard were placed on a 96-well plate with reaction buffer (Kit solution buffer and PBS). The absorbance was recorded at 490 nm using a spectrofluorometer (Xenius®, SAFAS, Monaco). Samples were compared with a known uric acid standard curve, with absorbance values being proportional to the sample's total reductive capacity. Results were normalized considering the total protein content in the sample homogenates in each well and expressed as µM Copper Reducing Equivalents (CRE).mg protein^{-1}.

4. Statistical analysis

Statistical analyses were processed using the program RStudio Version 3.5.1. Data were checked for normality using the Shapiro-Wilk test and for homoskedasticity using Levene's test on the residuals from the linear model (Edmunds and Gates, 2002). When the assumptions were not met, data were transformed with a log transformation or if necessary, a Box Cox transformation. Mixed effects models were used to test the effect of the three fixed factors: "Nutrient", "UV" and "Temperature", with the random factor "Aquarium" on the

different physiological and oxidative stress parameters measured: holobiont protein biomass, dinoflagellates density, total chlorophyll a and c_2 content, net and gross photosynthesis rate per skeletal surface area and per symbiont cell, respiration rate per skeletal surface area and per symbiont cell, ROS level, lipid peroxidation and protein nitration. The Mixed Effect model showed that the effect of the random factor "Aquarium" was negligible for each parameter tested; this random factor was, therefore, removed from the models and three-way ANOVA were used with the factors: "Nutrient", "UV" and "Temperature". When the homoskedasticity assumption was not met, a White-adjusted ANOVA was run, using a heteroskedasticity-consistent covariance matrix estimator (White, 1980). Analyses were followed by a pairwise comparison test when factors effect was significant. Differences were assumed significant when $p < 0.05$.

Results

1. Effect of UVR exposure and nitrate enrichment at normal temperature (26 °C)

The result of the statistical tests are reported in the supplementary appendix in Table S1, for physiological parameters, Table S2, S3 for photosynthesis parameters normalized to skeletal surface area and to symbiont cell and Table S4, for oxidative stress parameters.

At 26 °C, no significant effect of UVR exposure and/or nitrate enrichment was observed on the tissue parameters (Fig. 2a, b, c) compared to the control (C26). No significant change was observed on the respiration rates per skeletal surface area (ANOVA, $p > 0.91$, Fig. 3c) or per symbiont cell (ANOVA, $p > 0.27$, Fig. S1c). However, there was a 80% decrease in net photosynthesis, in the N26 condition compared to C26 condition, when photosynthesis was expressed both per skeletal surface area (pairwise comparison, $p < 0.004$, Fig. 3a) or symbiont cell (pairwise comparison, $p < 0.0095$, Fig. S1a). However, net photosynthesis increased by 60% in the combined nitrate + UVR (UVN26), compared to the N26 condition (pairwise comparison - per surface: $p < 0.003$, Fig. 3a - per symbiont: $p < 0.0004$, Fig. S1a). Gross photosynthesis rates per skeletal surface area, also, increased under UVR exposure (ANOVA, $p < 0.006$, Fig. 3b). Finally, the non-enzymatic total antioxidant capacity (TAC) significantly increased in all UVR condition (ANOVA, $p < 0.0001$, Fig. 4a) as well as the protein nitration in the UVN26 compared to N26 condition (pairwise comparison, $p < 0.004$, Fig. 4b).

2. Effect of UVR exposure and nitrate enrichment under thermal stress (30 °C)

The results of the statistical tests are reported in the supplementary appendix in Table S1, for physiological parameters, Table S2, S3 for photosynthesis parameters normalized per skeletal surface area and per symbiont cell and Table S4, for oxidative stress parameters. The linear models (ANOVA) indicate that thermal stress had both independent and combined effects (with UVR exposure and nutrient enrichment) on coral physiology.

Compared to the same conditions at 26°C, thermal stress alone drove a significant decrease in symbiont density in all conditions (ANOVA, $p < 0.0001$, Fig. 2b). It also induced, an increase in Pg (ANOVA, $p < 0.0001$, Fig. S1b), in respiration rates per symbiont (ANOVA, $p < 0.0001$, Fig. S1c), and an increase in ROS levels (ANOVA, $p < 0.03$, Fig. 4c), and in lipid peroxidation damages (ANOVA, $p < 0.00011$, Fig. 4d). In addition, temperature interacted with nitrate enrichment, since the Pn rates per symbiont (White-adjust ANOVA, $p < 0.04$)

were lower in the N30 condition compared to the N26 condition (pairwise comparison, $p < 0.0004$, Fig. S1a).

Temperature also interacted with UVR exposure for the protein nitration level, which is higher in the UV30 than in UV26 conditions (pairwise comparison, $p < 0.02$, Fig. 4b, T. S4b).

UVR exposure increased the antioxidant capacity of corals in all conditions (ANOVA, $p < 0.0001$, Fig. 4a). In addition, at high temperature (30°C), UVR exposure led to an increased the protein biomass in coral tissue (pairwise comparison, C30/UV30 - N30/UVN30, $p < 0.047$, Fig. 2a) and in total chlorophyll a and c_2 content (ANOVA, $p < 0.045$, Fig. 2c) compared to corals non exposed to UVR. It also increased the Pg normalized to skeletal surface area (ANOVA, $p < 0.006$, Fig. 3b) and symbiont cell (pairwise comparison, C30/UV30 - N30/UVN30, $p < 0.024$, Fig. S1b). Finally, the level of protein nitration in the UV30 was significantly higher than in the C30 condition (pairwise comparison, $p < 0.035$, Fig. 4b). The interaction between nitrate and UVR exposure impacted ROS levels in coral tissue (ANOVA, $p < 0.012$), which significantly increased in the UVN30 compared to N30 (pairwise comparison, $p < 0.0013$, Fig. 4c).

Discussion

Reef ecosystems are under increasing pressure from interacting multiple stressors (e.g. rising temperatures, and excessive irradiance and nutrient levels). Stressors can have synergistic, antagonistic or additive effects on coral physiology, but these interactions are, however, not well understood (Chumun et al., 2013; Higuchi et al., 2015). By quantifying the combined effects of UVR exposure, nitrate enrichment and thermal stress on the physiology and the oxidative stress response of the widespread scleractinian coral *Pocillopora damicornis*, this study brings a deeper understanding of the response of the coral symbioses to multiple environmental changes. Specifically, our results demonstrate that low UVR intensities (and low doses) can mitigate the negative effects of thermal stress by enhancing the antioxidant capacity of coral colonies. In addition, low UVR intensities can prevent the negative effects of nitrate on symbiont photosynthesis, most likely, by regulating the complex links between carbon and nitrogen metabolism.

1. Nitrate enrichment without UVR exposure impaired symbiont photosynthesis

Nitrate enrichment in absence of UVR exposure induced a significant decrease in net photosynthesis (normalized to surface area or symbiont cell) of *P. damicornis*. Nitrate-induced decrease in carbon fixation or photosynthetic efficiency has already been observed in several coral species (Chumun et al., 2013; Courtial et al., 2017; Ezzat et al., 2015; Nordemar et al., 2003). This was coupled with a decline in the amount of photosynthates transferred from the symbionts to the host tissue (Ezzat et al., 2015). A bleaching with nitrate enrichment has sometimes been observed (Burkepile et al., 2019) and could explain the lower rates of photosynthesis observed here with the nitrate-enriched corals. However, this is unlikely as no significant reduction in symbiont density and/or chlorophyll a and c_2 content occurred. The deleterious effects of nitrate on coral physiology may also be due to an imbalanced nitrogen to phosphorus ratio (N:P ratio). In this case, symbiont growth is promoted by an increase in nitrogen availability, but the lack of phosphorus weakens the lipid membranes of the symbionts, especially during thermal stress (Ezzat et al., 2016; Rosset et al., 2017; Wiedenmann et al., 2013). Although this might happen under an important phosphorus deficiency, here, phosphorus concentrations were around 0.2 µM and symbiont growth was not enhanced by nitrogen supplementation. In addition, corals under UVR exposure and nitrate enrichment (UVN26) did not decrease their photosynthetic rates. All together, these

observations suggest that, the N:P ratio was not imbalanced in our study and has thus not driven the decrease in photosynthesis observed in both the N26 and N30 conditions.

The photosynthesis impairment under nitrate enrichment is likely due to a competition, between carbon and nitrogen assimilation for ATP and reductants generated by photosynthetic electron transport (Nunes-Nesi et al., 2010). Conversion of one nitrate molecule into ammonium indeed requires ATP and consumes one NADPH and six reduced ferredoxins. Therefore, in plants, 25% of the ATP and NADPH produced from light energy by the electron transfer complex is used for nitrate assimilation, and only 75% remains available for carbon fixation (Bloom, 2015). In plants, when light is limited, or when nitrogen metabolism is not paired with carbon metabolism, nitrate assimilation and carbon dioxide fixation will directly compete for both ATP and reductants generated by the photosynthetic electron transport (Nunes-Nesi et al., 2010). In this case, nitrate can cause a reduction in carbon assimilation rates. It can also disrupt the C:N balance and alter essential metabolites (Saiz-Fernández et al., 2017). In turn, shortage of carbon skeletons may delay the assimilation of nitrate into amino acids (Bloom, 2015). This can cause an oxidative stress condition, by an accumulation of nitrite inside the coral tissue. Although in this experiment there was no significant increase in ROS under nitrate enrichment, there was a slight increase in LPO, a proxy for cellular damage (Gutteridge, 1995). This suggests that corals might have experienced a slight damage from nitrate exposure, possibly through the generation of nitric oxide (Lundberg et al., 2008). In addition, TAC values decreased in the N30 treatment compared to control (C26), further indicating an oxidative imbalance. Overall, competition between carbon and nitrogen for both ATP and reductants is the most plausible explanations for the decrease in photosynthesis observed in the coral nubbins (Fig. 5). Any increase in carbon fixation might therefore reduce the nitrate effect on photosynthesis, as observed with corals exposed to low doses of UVR (see below).

2. Low doses of UVR are beneficial to corals, both under thermal stress or nitrate enrichment

In shallow reefs, corals receive high levels of UVR. However, they synthesize UV-absorbing compounds such as mycosporine-like amino acids (MAAS) which partially protect them from UVR (Shick et al. 1999). Some of these MAAs can also act as antioxidant compounds (Rosic and Dove, 2011; Yakovleva et al., 2004). In this experiment, exposure of *P. damicornis* to UVR increased TAC in coral tissue at 30 °C, which likely helped avoid an accumulation of ROS and, thus, a potential damage to biomolecules (as shown by LPO). On

the contrary, corals which were not exposed to UVR, experienced decreased TAC level at 30°C and significant bleaching. Alternatively, UVR might have also protected corals from photoinhibition during exposure to high temperature and light. It has indeed been shown that UVR trigger photoreceptors that control extraction-contraction of coral polyps (Ben-Zvi et al., 2019). Contraction of the polyps under UVR may have prevented photodamage and bleaching in the thermal stressed corals.

In this experiment, UVR exposure also significantly enhanced photosynthesis at elevated temperature notably through an increased chlorophyll level in symbiont cells. A similar effect had previously been observed, with UVA, on the photosynthetic performances of either free algae (Gao et al., 2007; Gao and Xu, 2008; Xu and Gao, 2009) or of Symbiodiniacea in symbiosis with the larvae of *Pocillopora damicornis* and *Seriatopora calendrum* (Zhou et al., 2017, 2016). Such positive effect of UVA on photosynthesis might be due to the fact that the activity of the carbonic anhydrase (which transports inorganic carbon used for photosynthesis) is enhanced by UVA radiation. Enhanced photosynthesis was even recorded in free algae when exposed to UVB (Chen et al., 2020), which were shown to protect them against photoinhibition (Hanelt et al., 2006). The positive effect of UVR on coral photosynthesis contradicts the usual observation of increased bleaching under UVR exposure and thermal stress (Lesser and Farrell, 2004). However, bleaching is mainly observed under high UVR doses. On the contrary, the daily dose of UVR used in this study is lower, and corresponds to that received by coral colonies around 15 m depth (Barron et al., 2009; Overmans and Agustí, 2020; Rosic et al., 2020). Taken all together, these observations suggest that the effect of UVR on coral physiology is dose-dependent and that low levels of UVR may enhance coral photosynthesis by increasing cellular chlorophyll content or enhancing the carbonic anhydrase activity.

In addition to its photoprotective role at a high temperature, low doses of UVR protects symbionts against the negative effect nitrate has on photosynthesis. Indeed, the impairment of coral photosynthesis under nitrate enrichment was not observed when corals received UVR. Many studies have shown the effect of nutrient enrichment or depletion on the UV-induced decrease in photosynthesis (Rojo et al., 2019). However, this is the first time the contrary is observed - the alleviation of nitrate-induced decrease in photosynthesis by UVR. In macroalgae, activities of both nitrate reductase (involved in nitrate assimilation) and carbonic anhydrases (involved in carbon fixation) are stimulated by exposure to UVR (Kumar et al., 1996; Viñegla et al., 2006). They show a peak in the evening, whereas the peak is delayed

without UVR. In addition, Figueroa & Vinegla (2001) observed in two marine algae that UVR acts as an environmental signal involved in the control of carbon and nitrogen cycles, and regulates feedback processes that control N assimilation as a function of carbon content. Therefore, in corals, UVR may have prevented the nitrate-induced inhibition of photosynthesis by regulating the complex links between carbon and nitrogen metabolism (Fig. 5). In addition, since UVR promotes the synthesis of MAAs, rich in nitrogen (Korbee et al., 2005; Peinado et al., 2004; Shick et al., 2005; Zheng and Gao, 2009), it might have also stimulated the reduction of nitrate into ammonium and its subsequent incorporation into MAAs.

In conclusion, this multifactorial study shows that light conditions, and specifically UVR levels may influence the sensitivity of coral species to thermal stress and nutrient pollution. We, indeed, demonstrated that UVR plays an important role in mitigating the effects of thermal stress and nitrate enrichment on coral photosynthesis. However, since UVR can also be detrimental to coral's photosynthesis under high doses, future studies should aim at investigating the dose-effect response of corals to UVR exposure. Overall, our results indicate that UVR may be a critical factor, which not only affects the distribution of corals, but also their response to environmental stress.

References

Aminot, A., Kérouel, R., Coverly, S.C., 2009. Nutrients in seawater using segmented flow analysis, in: Practical Guidelines for the Analysis of Seawater. CRC Press Taylor & Francis Group, Boca Raton, Florida, USA, pp. 143–178.

Aslam, M., Huffaker, R.C., Rains, D.W., Rao, K.P., 1979. Influence of Light and Ambient Carbon Dioxide Concentration on Nitrate Assimilation by Intact Barley Seedlings. Plant Physiology. https://doi.org/10.1104/pp.63.6.1205

Barron, M.G., Vivian, D.N., Yee, S.H., Santavy, D.L., 2009. Methods to estimate solar radiation dosimetry in coral reefs using remote sensed, modeled, and in situ data. Environmental monitoring and assessment 151, 445–455.

Ben-Zvi, O., Eyal, G., Loya, Y., 2019. Response of fluorescence morphs of the mesophotic coral Euphyllia paradivisa to ultra-violet radiation. Scientific reports 9, 1–9.

Bloom, A.J., 2015. Photorespiration and nitrate assimilation: a major intersection between plant carbon and nitrogen. Photosynthesis Research 123, 117–128. https://doi.org/10.1007/s11120-014-0056-y

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Brodie, J.E., Devlin, M., Haynes, D., Waterhouse, J., 2011. Assessment of the eutrophication status of the Great Barrier Reef lagoon (Australia). Biogeochemistry 106, 281–302. https://doi.org/10.1007/s10533-010-9542-2

Burkepile, D.E., Shantz, A.A., Adam, T.C., Munsterman, K.S., Speare, K.E., Ladd, M.C., Rice, M.M., Ezzat, L., McIlroy, S., Wong, J.C., 2019. Nitrogen identity drives differential impacts of nutrients on coral bleaching and mortality. Ecosystems 1–14. https://doi.org/10.1007/s10021-019-00433-2

Charpy, L., 2001. Phosphorus supply for atoll biological productivity. Coral reefs 20, 357–360. https://doi.org/10.1007/s00338-001-0182-9

Chen, Z., Jiang, H.-B., Gao, K., Qiu, B.-S., 2020. Acclimation to low ultraviolet- B radiation increases photosystem I abundance and cyclic electron transfer with enhanced photosynthesis and growth in the cyanobacterium Nostoc sphaeroides. Environmental Microbiology 22, 183–197. https://doi.org/10.1111/1462-2920.14836

Chumun, P.K., Casareto, B.E., Higuchi, T., Irikawa, A., Bhagooli, R., Ishikawa, Y., Suzuki, Y., 2013. High nitrate levels exacerbate thermal photo-physiological stress of zooxanthellae in the reef-building coral Pocillopora damicornis. Eco-Engineering 25, 75–83.

Costa, O.S., Leão, Z.M. de A.N., Nimmo, M., Attrill, M.J., 2000. Nutrification impacts on coral reefs from northern Bahia, Brazil, in: Island, Ocean and Deep-Sea Biology. Presented at the 34th European Marine Biology Symposium, Springer, Ponta Delgada, Portugal, pp. 307–315. https://doi.org/10.1007/978-94-017-1982-7_28

Couch, C.S., Burns, J.H.R., Liu, G., Steward, K., Gutlay, T.N., Kenyon, J., Eakin, C.M., Kosaki, R.K., 2017. Mass coral bleaching due to unprecedented marine heatwave in Papahānaumokuākea Marine National Monument (Northwestern Hawaiian Islands). PLoS One 12, e0185121–e0185121. https://doi.org/10.1371/journal.pone.0185121

Courtial, L., Roberty, S., Shick, J.M., Houlbrèque, F., Ferrier- Pagès, C., 2017. Interactive effects of ultraviolet radiation and thermal stress on two reef- building corals. Limnology and Oceanography 62, 1000–1013. https://doi.org/10.1002/lno.10481

Crossland, C.J., Hatcher, B.G., Smith, S.V., 1991. Role of coral reefs in global ocean production. Coral Reefs 10, 55–64. https://doi.org/10.1007/BF00571824

Downs, C.A., McDougall, K.E., Woodley, C.M., Fauth, J.E., Richmond, R.H., Kushmaro, A., Gibb, S.W., Loya, Y., Ostrander, G.K., Kramarsky-Winter, E., 2013. Heat-stress and light-stress induce different cellular pathologies in the symbiotic dinoflagellate during coral bleaching. PLoS One 8. https://doi.org/10.1371/journal.pone.0077173

Eakin, C.M., Sweatman, H.P.A., Brainard, R.E., 2019. The 2014–2017 global-scale coral bleaching event: insights and impacts. Coral Reefs 38, 539–545. https://doi.org/10.1007/s00338-019-01844-2

509 Edmunds, P., Gates, R., 2002. Normalizing physiological data for scleractinian corals. Coral Reefs 21,
510 193–197. https://doi.org/10.1007/s00338-002-0214-0
511 Ellis, J.I., Jamil, T., Anlauf, H., Coker, D.J., Curdia, J., Hewitt, J., Jones, B.H., Krokos, G., Kürten,
512 B., Hariprasad, D., 2019. Multiple stressor effects on coral reef ecosystems. Global change
513 biology 25, 4131–4146. https://doi.org/10.1111/gcb.14819
514 Ezzat, L., Maguer, J.-F., Grover, R., Ferrier-Pagès, C., 2016. Limited phosphorus availability is the
515 Achilles heel of tropical reef corals in a warming ocean. Scientific Reports 6, 31768.
516 https://doi.org/10.1038/srep31768
517 Ezzat, L., Maguer, J.-F., Grover, R., Ferrier-Pagès, C., 2015. New insights into carbon acquisition and
518 exchanges within the coral–dinoflagellate symbiosis under NH4+ and NO3− supply. Proc. R.
519 Soc. B 282, 20150610. https://doi.org/10.1098/rspb.2015.0610
520 Fabricius, K.E., 2011. Factors determining the resilience of coral reefs to eutrophication: a review and
521 conceptual model, in: Coral Reefs: An Ecosystem in Transition. Springer, pp. 493–505.
522 Fabricius, K.E., 2005. Effects of terrestrial runoff on the ecology of corals and coral reefs: review and
523 synthesis. Marine Pollution Bulletin 50, 125–146.
524 https://doi.org/10.1016/j.marpolbul.2004.11.028
525 Fabricius, K.E., Cséke, S., Humphrey, C., De'ath, G., 2013. Does trophic status enhance or reduce the
526 thermal tolerance of scleractinian corals? A review, experiment and conceptual framework.
527 PloS one 8. https://doi.org/10.1371/journal.pone.0054399
528 Figueroa, F.L., Viñegla, B., 2001. Effects of solar UV radiation on photosynthesis and enzyme
529 activities (carbonic anhydrase and nitrate reductase) in marine macroalgae from southern
530 Spain. Rev. Chil. Hist. Nat 74, 237–249.
531 Freeman, B.A., Crapo, J.D., 1982. Biology of disease: free radicals and tissue injury. Laboratory
532 investigation; a journal of technical methods and pathology 47, 412–426.
533 Gao, K., Wu, Y., Li, G., Wu, H., Villafañe, V.E., Helbling, E.W., 2007. Solar UV radiation drives
534 CO2 fixation in marine phytoplankton: A double-edged sword. Plant Physiol. 144, 54.
535 https://doi.org/10.1104/pp.107.098491
536 Gao, K., Xu, J., 2008. Effects of solar UV radiation on diurnal photosynthetic performance and
537 growth of Gracilaria lemaneiformis (Rhodophyta). European Journal of Phycology 43, 297–
538 307. https://doi.org/10.1080/09670260801986837
539 Godinot, C., Ferrier-Pagès, C., Grover, R., 2009. Control of phosphate uptake by zooxanthellae and
540 host cells in the scleractinian coral Stylophora pistillata. Limnology and Oceanography 54,
541 1627–1633. https://doi.org/10.4319/lo.2009.54.5.1627
542 Govers, L.L., Lamers, L.P., Bouma, T.J., de Brouwer, J.H., van Katwijk, M.M., 2014. Eutrophication
543 threatens Caribbean seagrasses–An example from Curaçao and Bonaire. Marine pollution
544 bulletin 89, 481–486. https://doi.org/10.1016/j.marpolbul.2014.09.003
545 Gutteridge, J.M., 1995. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clinical
546 chemistry 41, 1819–1828. https://doi.org/10.1093/clinchem/41.12.1819
547 Halac, S.R., Villafañe, V.E., Helbling, E.W., 2010. Temperature benefits the photosynthetic
548 performance of the diatoms Chaetoceros gracilis and Thalassiosira weissflogii when exposed
549 to UVR. Journal of Photochemistry and Photobiology B: Biology 101, 196–205.
550 https://doi.org/10.1016/j.jphotobiol.2010.07.003
551 Hanelt, D., Hawes, I., Rae, R., 2006. Reduction of UV-B radiation causes an enhancement of
552 photoinhibition in high light stressed aquatic plants from New Zealand lakes. Journal of
553 Photochemistry and Photobiology B: Biology 84, 89–102.
554 https://doi.org/10.1016/j.jphotobiol.2006.01.013
555 Higuchi, T., Yuyama, I., Nakamura, T., 2015. The combined effects of nitrate with high temperature
556 and high light intensity on coral bleaching and antioxidant enzyme activities. Regional
557 Studies in Marine Science 2, 27–31. https://doi.org/10.1016/j.rsma.2015.08.012
558 Hughes, T.P., Kerry, J.T., Álvarez-Noriega, M., Álvarez-Romero, J.G., Anderson, K.D., Baird, A.H.,
559 Babcock, R.C., Beger, M., Bellwood, D.R., Berkelmans, R., 2017. Global warming and
560 recurrent mass bleaching of corals. Nature 543, 373. https://doi.org/10.1038/nature21707
561 Jeffrey, S. t, Humphrey, G.F., 1975. New spectrophotometric equations for determining chlorophylls
562 a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochemie und physiologie
563 der pflanzen 167, 191–194. https://doi.org/10.1016/S0015-3796(17)30778-3

564 Kahng, S.E., Akkaynak, D., Shlesinger, T., Hochberg, E.J., Wiedenmann, J., Tamir, R., Tchernov, D.,
565 2019. Light, temperature, photosynthesis, heterotrophy, and the lower depth limits of
566 mesophotic coral ecosystems, in: Mesophotic Coral Ecosystems. Springer, pp. 801–828.
567 Kinsey, D.W., Davies, P.J., 1979. Effects of elevated nitrogen and phosphorus on coral reef growth 1.
568 Limnology and oceanography 24, 935–940. https://doi.org/10.4319/lo.1979.24.5.0935
569 Korbee, N., Huovinen, P., Figueroa, F.L., Aguilera, J., Karsten, U., 2005. Availability of ammonium
570 influences photosynthesis and the accumulation of mycosporine-like amino acids in two
571 Porphyra species (Bangiales, Rhodophyta). Marine Biology 146, 645–654.
572 https://doi.org/10.1007/s00227-004-1484-6
573 Krueger, T., Becker, S., Pontasch, S., Dove, S., Hoegh- Guldberg, O., Leggat, W., Fisher, P.L., Davy,
574 S.K., 2014. Antioxidant plasticity and thermal sensitivity in four types of S ymbiodinium sp.
575 Journal of phycology 50, 1035–1047. https://doi.org/10.1111/jpy.12232
576 Krueger, T., Hawkins, T.D., Becker, S., Pontasch, S., Dove, S., Hoegh-Guldberg, O., Leggat, W.,
577 Fisher, P.L., Davy, S.K., 2015. Differential coral bleaching-Contrasting the activity and
578 response of enzymatic antioxidants in symbiotic partners under thermal stress. Comparative
579 Biochemistry and Physiology Part A: Molecular & Integrative Physiology 190, 15–25.
580 https://doi.org/10.1016/j.cbpa.2015.08.012
581 Kumar, A., Sinha, R.P., Häder, D.-P., 1996. Effect of UV-B on enzymes of nitrogen metabolism in
582 the cyanobacterium Nostoc calcicola. Journal of Plant Physiology 148, 86–91.
583 https://doi.org/10.1016/S0176-1617(96)80298-7
584 LaJeunesse, T.C., Parkinson, J.E., Gabrielson, P.W., Jeong, H.J., Reimer, J.D., Voolstra, C.R., Santos,
585 S.R., 2018. Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of
586 coral endosymbionts. Current Biology 28, 2570-2580. e6.
587 https://doi.org/10.1016/j.cub.2018.07.008
588 Lesser, M.P., 2006. Oxidative stress in marine environments: biochemistry and physiological ecology.
589 Annual Review of Physiology 68, 253–278.
590 https://doi.org/10.1146/annurev.physiol.68.040104.110001
591 Lesser, M.P., Farrell, J.H., 2004. Exposure to solar radiation increases damage to both host tissues and
592 algal symbionts of corals during thermal stress. Coral reefs 23, 367–377.
593 https://doi.org/10.1007/s00338-004-0392-z
594 Liñán- Cabello, M.A., Flores- Ramírez, L.A., Zenteno- Savin, T., Olguín- Monroy, N.O.,
595 Sosa- Avalos, R., Patiño- Barragan, M., Olivos- Ortiz, A., 2010. Seasonal changes of
596 antioxidant and oxidative parameters in the coral Pocillopora capitata on the Pacific coast of
597 Mexico. Marine Ecology 31, 407–417. https://doi.org/10.1111/j.1439-0485.2009.00349.x
598 Lomas, M.W., Lipschultz, F., 2006. Forming the primary nitrite maximum: nitrifiers or
599 phytoplankton? Limnology and Oceanography 51, 2453–2467.
600 https://doi.org/10.4319/lo.2006.51.5.2453
601 Lundberg, J.O., Weitzberg, E., Gladwin, M.T., 2008. The nitrate–nitrite–nitric oxide pathway in
602 physiology and therapeutics. Nature reviews Drug discovery 7, 156–167.
603 https://doi.org/10.1038/nrd2466
604 Marangoni, L., Ferrier-Pagès, C., Rottier, C., Bianchini, A., Grover, R., 2020. Unravelling the
605 different causes of nitrate and ammonium effects on coral bleaching. Scientific Reports 10, 1–
606 14. https://doi.org/10.1038/s41598-020-68916-0
607 McCauley, M., Banaszak, A.T., Goulet, T.L., 2018. Species traits dictate seasonal-dependent
608 responses of octocoral–algal symbioses to elevated temperature and ultraviolet radiation.
609 Coral Reefs 37, 901–917. https://doi.org/10.1007/s00338-018-1716-8
610 Meeder, E., Mackey, K.R., Paytan, A., Shaked, Y., Iluz, D., Stambler, N., Rivlin, T., Post, A.F.,
611 Lazar, B., 2012. Nitrite dynamics in the open ocean clues from seasonal and diurnal
612 variations. Marine Ecology Progress Series 453, 11–26. https://doi.org/10.3354/meps09525
613 Moberg, F., Folke, C., 1999. Ecological goods and services of coral reef ecosystems. Ecological
614 economics 29, 215–233. https://doi.org/10.1016/S0921-8009(99)00009-9
615 Moniczewski, A., Gawlik, M., Smaga, I., Niedzielska, E., Krzek, J., Przegaliński, E., Pera, J., Filip,
616 M., 2015. Oxidative stress as an etiological factor and a potential treatment target of
617 psychiatric disorders. Part 1. Chemical aspects and biological sources of oxidative stress in

618 the brain. Pharmacological Reports 67, 560–568.
619 https://doi.org/10.1016/j.pharep.2014.12.014
620 Muller-Parker, G., D'elia, C.F., Cook, C.B., 2015. Interactions between corals and their symbiotic
621 algae, in: Coral Reefs in the Anthropocene. Springer, pp. 99–116.
622 Muscatine, L., Falkowski, P.G., Porter, J.W., Dubinsky, Z., 1984. Fate of photosynthetic fixed carbon
623 in light-and shade-adapted colonies of the symbiotic coral Stylophora pistillata. Proceedings
624 of the Royal Society of London. Series B. Biological Sciences 222, 181–202.
625 https://doi.org/10.1098/rspb.1984.0058
626 Naumann, M.S., Bednarz, V.N., Ferse, S.C., Niggl, W., Wild, C., 2015. Monitoring of coastal coral
627 reefs near Dahab (Gulf of Aqaba, Red Sea) indicates local eutrophication as potential cause
628 for change in benthic communities. Environmental monitoring and assessment 187, 44.
629 https://doi.org/10.1007/s10661-014-4257-9
630 Nordemar, I., Nyström, M., Dizon, R., 2003. Effects of elevated seawater temperature and nitrate
631 enrichment on the branching coral Porites cylindrica in the absence of particulate food.
632 Marine Biology 142, 669–677. https://doi.org/10.1007/s00227-002-0989-0
633 Nunes-Nesi, A., Fernie, A.R., Stitt, M., 2010. Metabolic and signaling aspects underpinning the
634 regulation of plant carbon nitrogen interactions. Molecular plant 3, 973–996.
635 https://doi.org/10.1093/mp/ssq049
636 Overmans, S., Agustí, S., 2020. Unraveling the Seasonality of UV Exposure in Reef Waters of a
637 Rapidly Warming (Sub-) tropical Sea. Frontiers in Marine Science 7, 111.
638 https://doi.org/10.3389/fmars.2020.00111
639 Peinado, N.K., Abdala Díaz, R.T., Figueroa, F.L., Helbling, E.W., 2004. Ammonium and
640 UVradiations stimulate the accumulation of mycosporine-like amino acids in Pophyra
641 columbiana (Rhodophyta) from Patagonia, Argentia. Journal of Phycology 40, 248–259.
642 https://doi.org/10.1046/j.1529-8817.2004.03013.x
643 Rädecker, N., Pogoreutz, C., Voolstra, C.R., Wiedenmann, J., Wild, C., 2015. Nitrogen cycling in
644 corals: the key to understanding holobiont functioning? Trends in microbiology 23, 490–497.
645 https://doi.org/10.1016/j.tim.2015.03.008
646 Rahav, O., Dubinsky, Z., Achituv, Y., Falkowski, P.G., 1989. Ammonium metabolism in the
647 zooxanthellate coral, Stylophora pistillata. Proceedings of the Royal Society of London. B.
648 Biological Sciences 236, 325–337.
649 Richardson, L.E., Middleton, J.F., James, N.P., Kyser, T.K., Opdyke, B.N., 2020. Upwelling
650 characteristics and nutrient enrichment of the Kangaroo Island upwelling region, South
651 Australia. Continental Shelf Research 104111. https://doi.org/10.1016/j.csr.2020.104111
652 Rojo, C., Puche, E., Rodrigo, M.A., 2019. The antagonistic effect of UV radiation on warming or
653 nitrate enrichment depends on ecotypes of freshwater macroalgae (Charophytes). Journal of
654 phycology 55, 714–729. https://doi.org/10.1111/jpy.12859
655 Rosic, N., Rémond, C., Mello-Athayde, M.A., 2020. Differential impact of heat stress on reef-
656 building corals under different light conditions. Marine Environmental Research 104947.
657 https://doi.org/10.1016/j.marenvres.2020.104947
658 Rosic, N.N., Dove, S., 2011. Mycosporine-like amino acids from coral dinoflagellates. Appl. Environ.
659 Microbiol. 77, 8478–8486. https://doi.org/10.1128/AEM.05870-11
660 Rosset, S., Wiedenmann, J., Reed, A.J., D'Angelo, C., 2017. Phosphate deficiency promotes coral
661 bleaching and is reflected by the ultrastructure of symbiotic dinoflagellates. Marine Pollution
662 Bulletin 118, 180–187. https://doi.org/10.1016/j.marpolbul.2017.02.044
663 Rouzé, H., Lecellier, G., Langlade, M.-J., Planes, S., Berteaux-Lecellier, V., 2015. Fringing reefs
664 exposed to different levels of eutrophication and sedimentation can support similar benthic
665 communities. Marine pollution bulletin 92, 212–221.
666 https://doi.org/10.1016/j.marpolbul.2014.12.016
667 Ruiz-Leal, M., George, S., 2004. An in vitro procedure for evaluation of early stage oxidative stress in
668 an established fish cell line applied to investigation of PHAH and pesticide toxicity. Marine
669 environmental research 58, 631–635. https://doi.org/10.1016/j.marenvres.2004.03.054
670 Saiz-Fernández, I., De Diego, N., Brzobohatý, B., Muñoz-Rueda, A., Lacuesta, M., 2017. The
671 imbalance between C and N metabolism during high nitrate supply inhibits photosynthesis

672 and overall growth in maize (Zea mays L.). Plant Physiology and Biochemistry 120, 213–
673 222. https://doi.org/10.1016/j.plaphy.2017.10.006
674 Schlöder, C., D'Croz, L., 2004. Responses of massive and branching coral species to the combined
675 effects of water temperature and nitrate enrichment. Journal of Experimental Marine Biology
676 and Ecology 313, 255–268. https://doi.org/10.1016/j.jembe.2004.08.012
677 Schrameyer, V., Wangpraseurt, D., Hill, R., Kühl, M., Larkum, A.W., Ralph, P.J., 2014. Light
678 respiratory processes and gross photosynthesis in two scleractinian corals. PLoS One 9,
679 e110814.
680 Serrano, X.M., Miller, M.W., Hendee, J.C., Jensen, B.A., Gapayao, J.Z., Pasparakis, C., Grosell, M.,
681 Baker, A.C., 2018. Effects of thermal stress and nitrate enrichment on the larval performance
682 of two Caribbean reef corals. Coral Reefs 37, 173–182. https://doi.org/10.1007/s00338-017-
683 1645-y
684 Shick, J.M., Ferrier-Pagès, C., Grover, R., Allemand, D., 2005. Effects of starvation, ammonium
685 concentration, and photosynthesis on the UV-dependent accumulation of mycosporine-like
686 amino acids (MAAs) in the coral Stylophora pistillata. Marine Ecology Progress Series 295,
687 135–156. https://doi.org/10.3354/meps295135
688 Shick, J.M., Romaine-Lioud, S., Romaine-Lioud, S., Ferrier-Pagès, C., Gattuso, J.-P., 1999.
689 Ultraviolet- B radiation stimulates shikimate pathway- dependent accumulation of
690 mycosporine- like amino acids in the coral Stylophora pistillata despite decreases in its
691 population of symbiotic dinoflagellates. Limnology and Oceanography 44, 1667–1682.
692 Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, Md., Fujimoto,
693 E.K., Goeke, N.M., Olson, B.J., Klenk, D.C., 1985. Measurement of protein using
694 bicinchoninic acid. Analytical biochemistry 150, 76–85. https://doi.org/10.1016/0003-
695 2697(85)90442-7
696 Stimson, J., Kinzie III, R.A., 1991. The temporal pattern and rate of release of zooxanthellae from the
697 reef coral Pocillopora damicornis (Linnaeus) under nitrogen-enrichment and control
698 conditions. Journal of Experimental Marine Biology and Ecology 153, 63–74.
699 https://doi.org/10.1016/S0022-0981(05)80006-1
700 Suggett, D.J., Smith, D.J., 2020. Coral bleaching patterns are the outcome of complex biological and
701 environmental networking. Global Change Biology 26, 68–79.
702 https://doi.org/10.1111/gcb.14871
703 Tedetti, M., Sempéré, R., 2006. Penetration of ultraviolet radiation in the marine environment. A
704 review. Photochemistry and photobiology 82, 389–397. https://doi.org/10.1562/2005-11-09-
705 IR-733
706 Torregiani, J.H., Lesser, M.P., 2007. The effects of short-term exposures to ultraviolet radiation in the
707 Hawaiian coral Montipora verrucosa. Journal of experimental marine biology and ecology
708 340, 194–203. https://doi.org/10.1016/j.jembe.2006.09.004
709 Tremblay, P., Grover, R., Maguer, J.-F., Hoogenboom, M., Ferrier-Pagès, C., 2014. Carbon
710 translocation from symbiont to host depends on irradiance and food availability in the tropical
711 coral Stylophora pistillata. Coral Reefs 33, 1–13. https://doi.org/10.1007/s00338-013-1100-7
712 Viñegla, B., Segovia, M., Figueroa, F.L., 2006. Effect of artificial UV radiation on carbon and
713 nitrogen metabolism in the macroalgae Fucus spiralis L. and Ulva olivascens Dangeard.
714 Hydrobiologia 560, 31–42. https://doi.org/10.1007/s10750-005-1097-1
715 Weis, V.M., 2008. Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of
716 symbiosis. Journal of Experimental Biology 211, 3059–3066.
717 https://doi.org/10.1242/jeb.009597
718 White, H., 1980. A heteroskedasticity-consistent covariance matrix estimator and a direct test for
719 heteroskedasticity. Econometrica: journal of the Econometric Society 817–838.
720 https://doi.org/10.2307/1912934
721 Wiedenmann, J., D'Angelo, C., Smith, E.G., Hunt, A.N., Legiret, F.-E., Postle, A.D., Achterberg,
722 E.P., 2013. Nutrient enrichment can increase the susceptibility of reef corals to bleaching.
723 Nature Climate Change 3, 160. https://doi.org/10.1038/nclimate1661
724 Wilkerson, F.P., Trench, R.K., 1986. Uptake of dissolved inorganic nitrogen by the symbiotic clam
725 Tridacna gigas and the coral Acropora sp. Marine Biology 93, 237–246.
726 https://doi.org/10.1007/BF00508261

Xu, Z., Gao, K., 2009. Impacts of UV radiation on growth and photosynthetic carbon acquisition in Gracilaria lemaneiformis (Rhodophyta) under phosphorus-limited and replete conditions. Functional Plant Biology 36, 1057–1064. https://doi.org/10.1071/FP09092

Yakovleva, I., Bhagooli, R., Takemura, A., Hidaka, M., 2004. Differential susceptibility to oxidative stress of two scleractinian corals: antioxidant functioning of mycosporine-glycine. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 139, 721–730. https://doi.org/10.1016/j.cbpc.2004.08.016

Zheng, Y., Gao, K., 2009. Impacts of solar UV radiation on the photosynthesis, growth, and UV-absorbing compounds in Gracilaria lemaneiformis (Rhodophyta) growm at different nitrate concentrations. Journal of Phycology 45, 314–323. https://doi.org/10.1111/j.1529-8817.2009.00654.x

Zhou, J., Fan, T.-Y., Beardall, J., Gao, K., 2016. Incident ultraviolet irradiances influence physiology, development and settlement of larva in the coral Pocillopora damicornis. Photochemistry and photobiology 92, 293–300. https://doi.org/10.1111/php.12567

Zhou, J., Huang, H., Beardall, J., Gao, K., 2017. Effect of UV radiation on the expulsion of Symbiodinium from the coral Pocillopora damicornis. Journal of Photochemistry and Photobiology B: Biology 166, 12–17. https://doi.org/10.1016/j.jphotobiol.2016.11.003

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Authors' contributions

Alice Blanckaert: Conceptualization, Methodology, Writing.
Laura F. B. Marangoni: Methodology, Writing.
Cécile Rottier: Methodology.
Renaud Grover and Christine Ferrier-Pagès: Conceptualization, Funding acquisition, Writing

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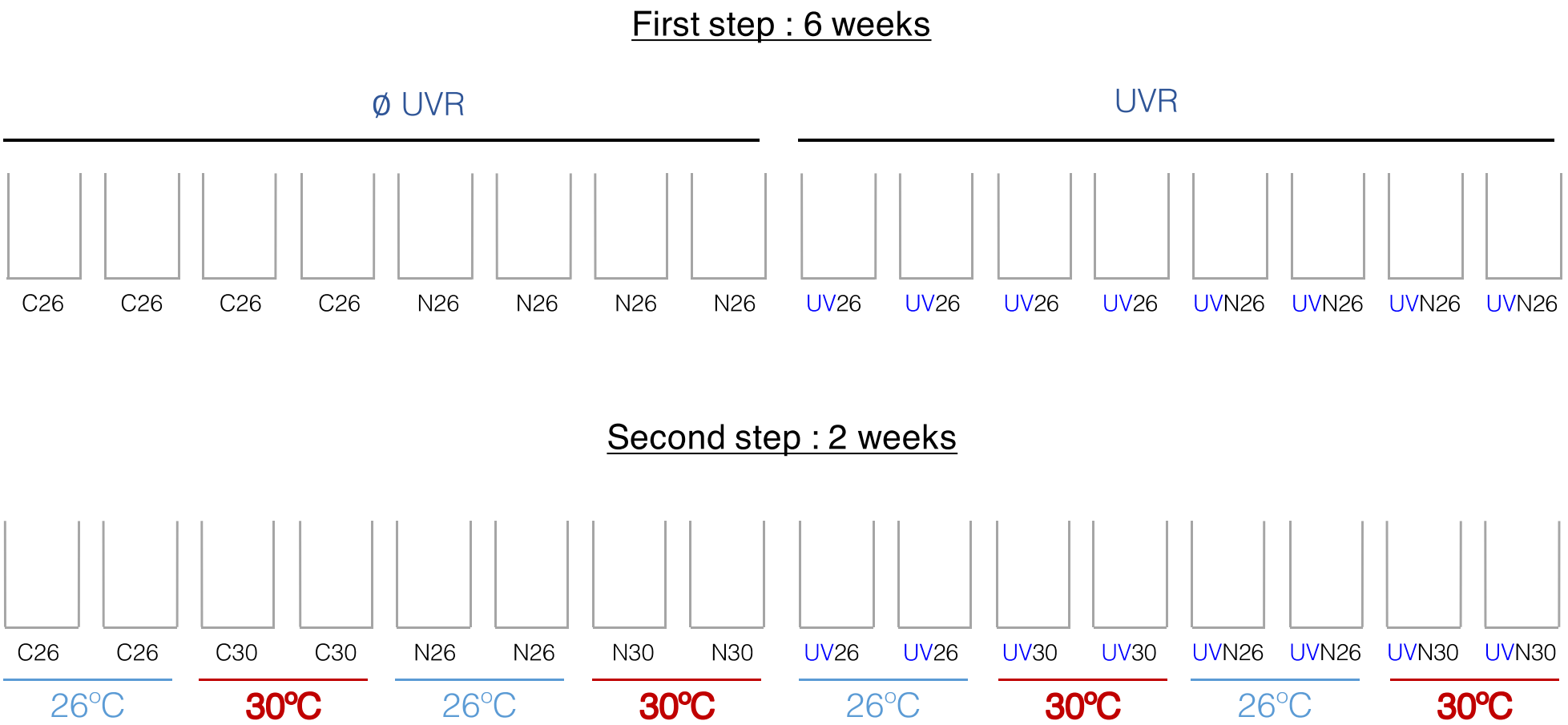
Conflict of interest

The authors declare that they have no conflict of interest.

763 **Figure 1**



764

764 **Figure 2**
765

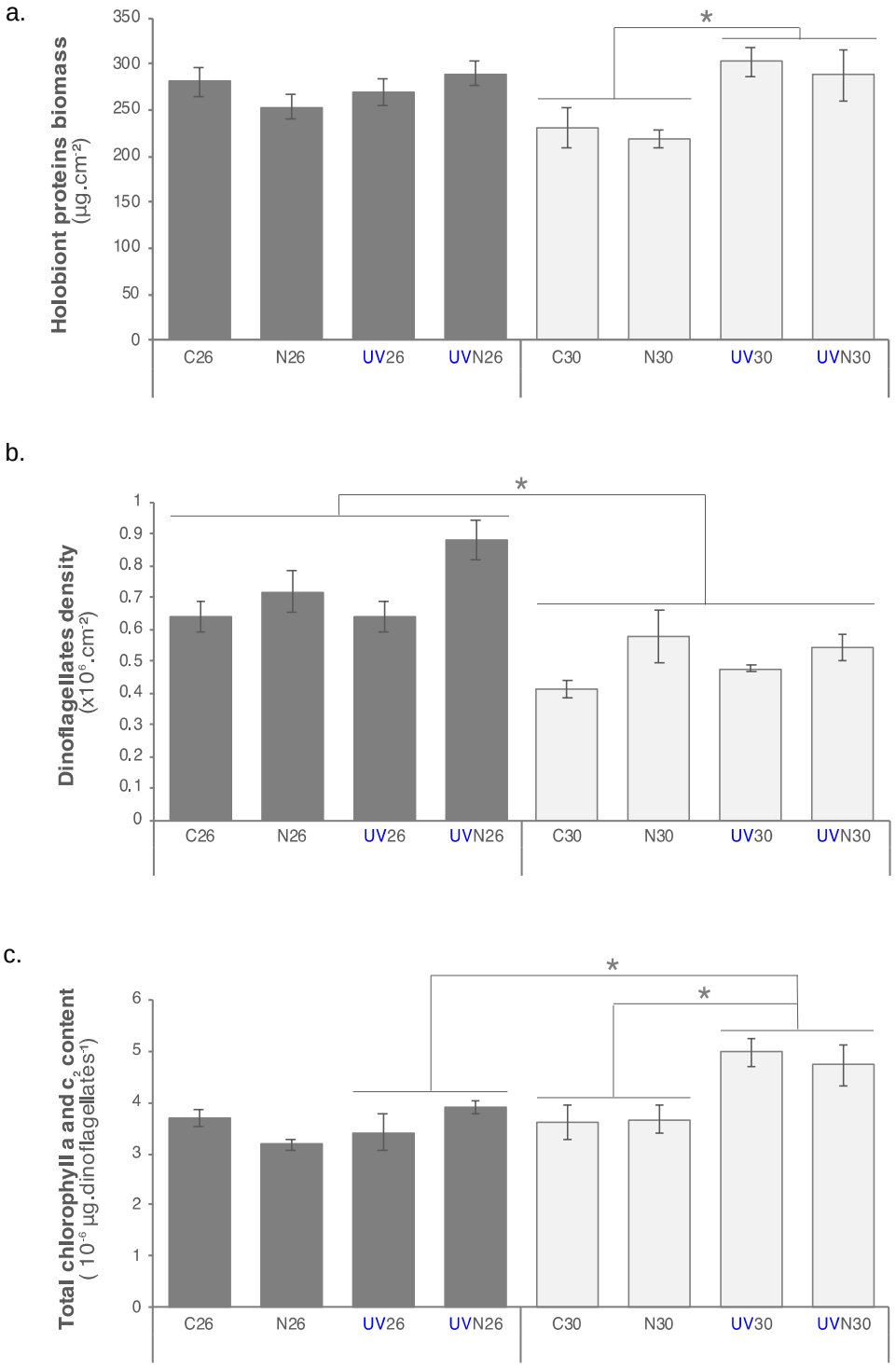
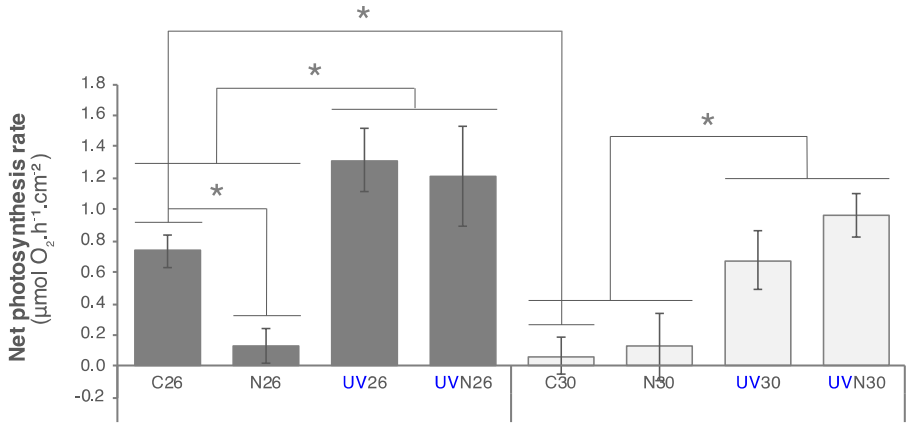
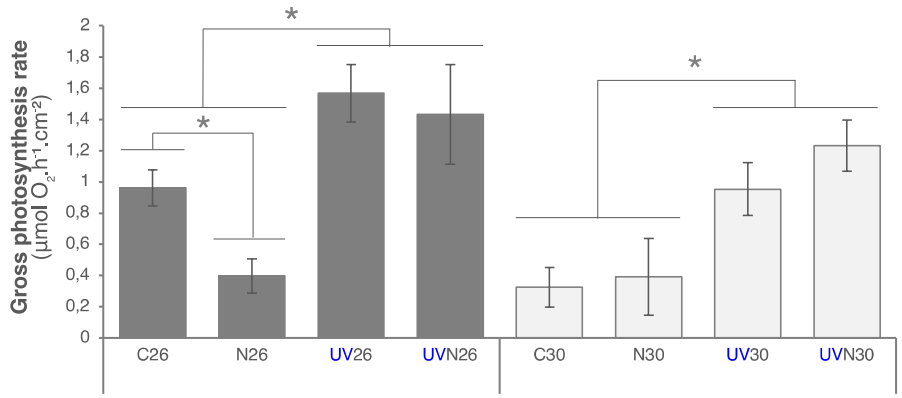


Figure 3

a.


Net photosynthesis rate
(µmol O2.h-1.cm-2)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
*
*
*
*
C26
N26
UV26
UVN26
C30
N30
UV30
UVN30

b.


Gross photosynthesis rate
(µmol O2.h-1.cm-2)
2
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
*
*
*
C26
N26
UV26
UVN26
C30
N30
UV30
UVN30

c.

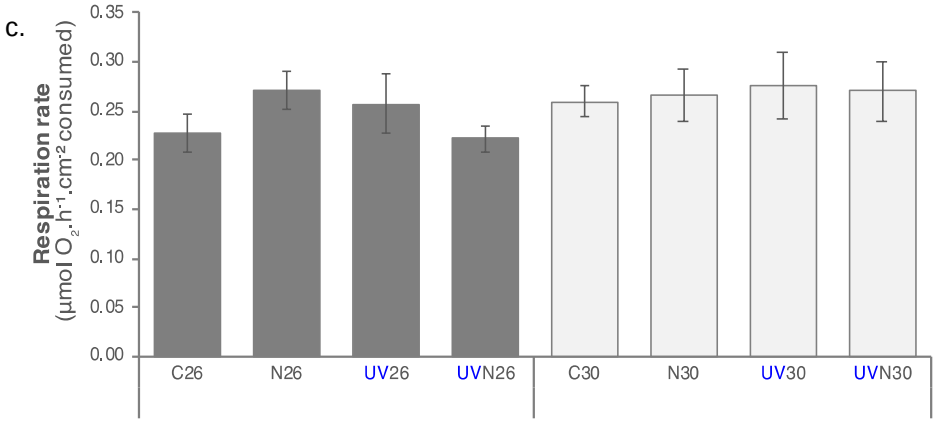

Respiration rate
(µmol O2.h-1.cm-2 consumed)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
C26
N26
UV26
UVN26
C30
N30
UV30
UVN30

Figure 4

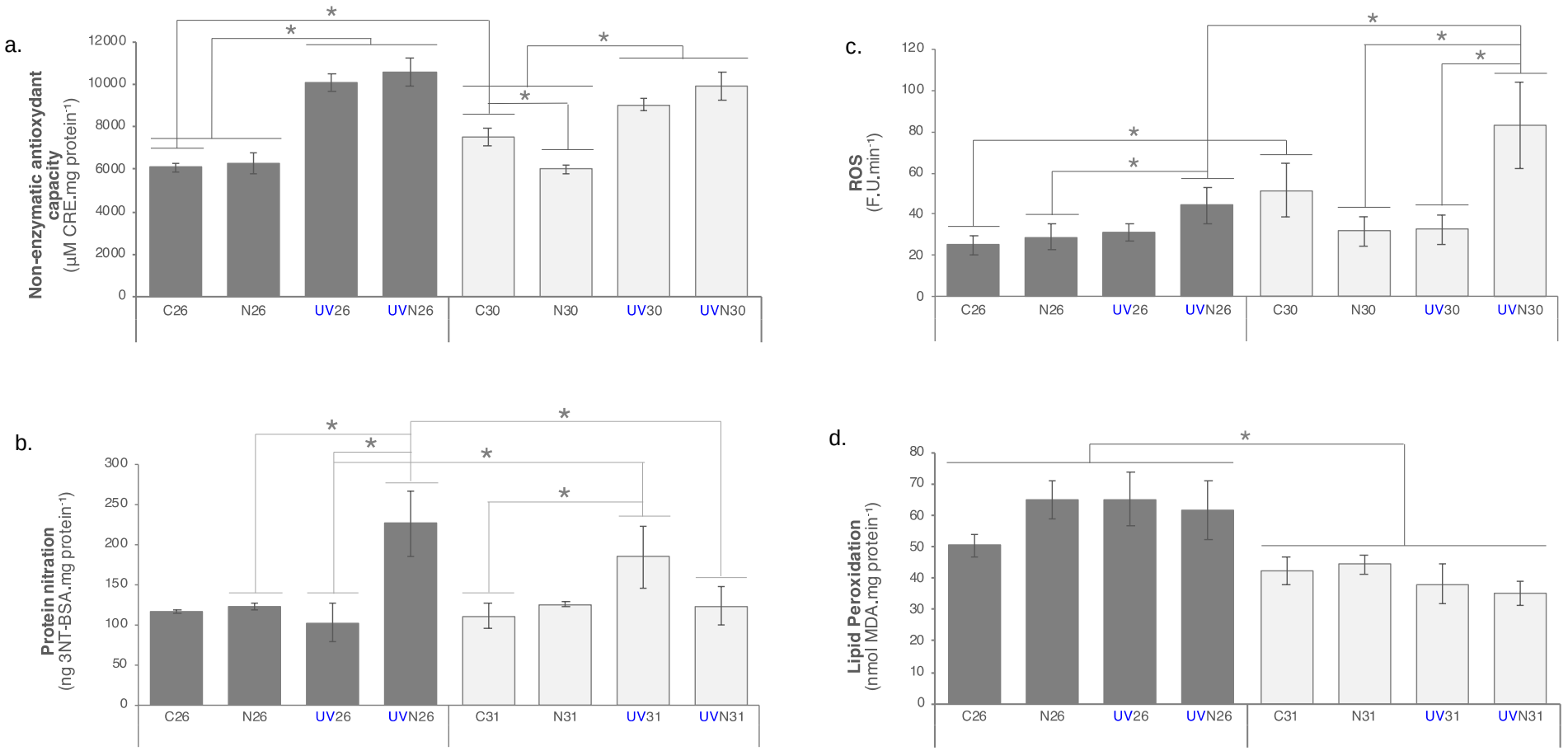
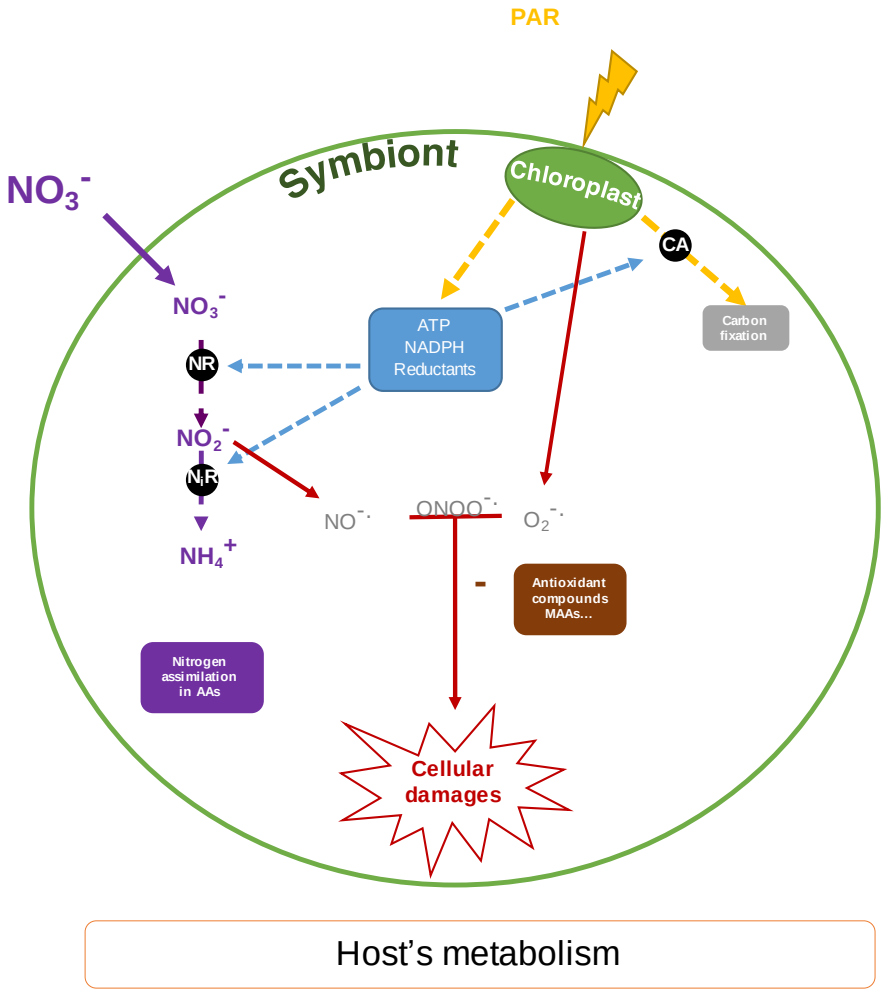
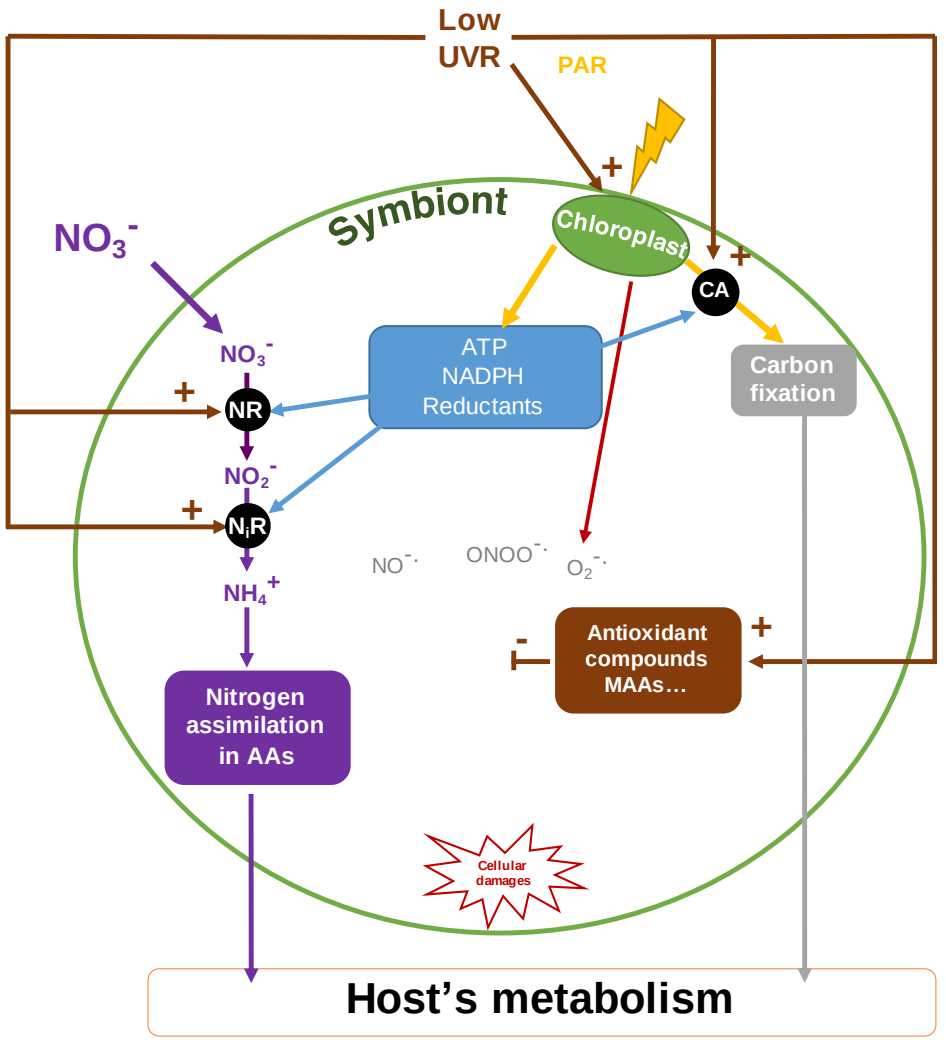

a.
Non-enzymatic antioxydant capacity
(µM CRE.mg protein-1)
12000
10000
8000
6000
4000
2000
0
C26
N26
UV26
UVN26
C30
N30
UV30
UVN30
c.
ROS
(F.U.min-1)
120
100
80
60
40
20
0
b.
Protein nitration
(ng 3NT-BSA.mg protein-1)
300
250
200
150
100
50
0
C31
N31
UV31
UVN31
d.
Lipid Peroxidation
(nmol MDA.mg protein-1)
70
60
50
40
30
10

Figure 5



Nitrate enrichment
No UVR exposure



Nitrate enrichment
UVR exposure

Figure legends

Figure 1

Cross-factorial experimental design.

16 aquaria were used with 10 nubbins per aquaria. All tanks were supplied with continuous fresh seawater (0.5 µM nitrate) and kept under PAR irradiance of 200 ± 10 µmol photons.m^{-2}.s^{-1}. Corals were not fed.

During six weeks, 8 aquaria were kept under UVR exposure (25 Wm^{-2} UVA and 1.5 Wm^{-2} UVB) and 8 aquaria without UVR exposure. Out of the 8 aquaria, 4 were kept in control seawater and 4 were enriched with nitrate (3 µM nitrate). Then, for 2 weeks, for each nitrate (N)-UVR condition 2 aquaria were kept at 26 ºC and 2 aquaria were raised to 30 ºC.

The experimental conditions are the following: **C26-C30**: control corals maintained at 26°C or 30°C without nitrate enrichment and UVR exposure, **N26-N30**: corals maintained under nitrate enrichment (3 µM nitrate) at 26 ºC or 30°C, **UV26-UV30**: corals exposed to UVR (25 Wm^{-2} UVA and 1.5 Wm^{-2} UVB) at 26 ºC or 30°C, **UVN26-UVN30**: corals maintained under UVR exposure and nitrate enrichment (3 µM nitrate) at 26 ºC or 30°C.

Figure 2

Effects of temperature, UVR and nitrate levels on the physiological parameters of the coral *Pocillopora damicornis*.

The barplots represents proteins biomass normalized by surface area (µg.cm^{-2}) (a.), dinoflagellates density normalized by surface area (10^6.cm^{-2}) (b.) and total chlorophyll a and c_2 content normalized by dinoflagellate cell (10^{-6} µg.dinoflagellates^{-1}) (c.), under different nitrate (N)-UVR conditions. Dark grey bars represent corals maintained at 26°C (26) while light grey bars are corals maintained at 30°C (30) - **C26-C30**: control corals maintained at 26°C or 30°C without nitrate enrichment and UVR exposure, **N26-N30**: corals maintained under nitrate enrichment (3 µM nitrate) at 26 ºC or 30°C, **UV26-UV30**: corals exposed to UVR (25 Wm^{-2} UVA and 1.5 Wm^{-2} UVB) at 26 ºC or 30°C, **UVN26-UVN30**: corals maintained under UVR exposure and nitrate enrichment (3 µM nitrate) at 26 ºC or 30°C.

Data represent mean and standard error of 6 replicates. Stars represent significantly different values (p value < 0.05).

Figure 3

Effects of temperature, UVR and nitrate levels on the oxygen fluxes (per skeletal surface area) measured for the coral *Pocillopora damicornis* maintained under different environmental conditions.

The barplots represent net (a.) and gross (b.) photosynthesis rates and respiration (c.) rates (µmol O_2.h^{-1}.cm^{-2}) normalized per surface area under different nitrate (N)-UVR conditions. Dark grey bars represent corals maintained at 26°C (26) while light grey bars are corals maintained at 30°C (30) - **C26-C30**: control corals maintained at 26°C or 30°C without nitrate enrichment and UVR exposure, **N26-N30**: corals maintained under nitrate enrichment (3 µM nitrate) at 26 ºC or 30°C, **UV26-UV30**: corals exposed to UVR (25 Wm^{-2} UVA and 1.5 Wm^{-2} UVB) at 26 ºC or 30°C, **UVN26-UVN30**: corals maintained under UVR exposure and nitrate enrichment (3 µM nitrate) at 26 ºC or 30°C. Data represent mean and standard error of 6 replicates. Stars represent significantly different values (p value < 0.05).

Figure 4

Effects of temperature, UVR and nitrate levels on the oxidative stress paramaters of the coral holobiont *Pocillopora damicornis*

The barplots represent the non-enzymatic antioxidant capacity (µM CRE.mg protein^{-1}) (a.), protein nitration level (ng3NT-BSA.mg protein^{-1}) (b.), reactive oxygen species levels (F.U.min^{-1}) (c.) and lipid peroxidation level (nmol MDA.mg protein^{-1}) (d.) under different nitrate (N)-UVR conditions.

Dark grey bars represent corals maintained at 26°C (26) while light grey bars are corals maintained at 30°C (30) - **C26-C30**: control corals maintained at 26°C or 30°C without nitrate enrichment and UVR exposure, **N26-N30**: corals maintained under nitrate enrichment (3 μM nitrate) at 26 ºC or 30°C, **UV26-UV30**: corals exposed to UVR (25 Wm^{-2} UVA and 1.5 Wm^{-2} UVB) at 26 ºC or 30°C, **UVN26-UVN30**: corals maintained under UVR exposure and nitrate enrichment (3 μM nitrate) at 26 ºC or 30°C.Data represent mean and standard error of six replicates. Stars represent significantly different values (p value < 0.05).

Figure 5

Summary of the main results obtained with nitrate-enriched corals with or without exposure to ultraviolet radiation.

The figure briefly summarizes the results obtained on the effect of nitrate enrichment on the dinoflagellate symbionts of the scleractinian coral *Pocillopora damicornis* in absence of ultraviolet radiation (**UVR**) (left panel) and with exposure to low UVR levels (right panel). Dashed arrows represents reduced processes while plain arrows (and increased text-box size) represent UVR-enhanced processes.

PAR: photosynthetically active radiation; **NR** and **N_iR**: nitrate and nitrite reductase respectively; **NO_3^-**, **NO_2^-** and **NH_4^+**: nitrate, nitrite and ammonium respectively, **$NO^{-\bullet}$** and **$ONOO^-$** $^\bullet$ are the reactive species of nitrogen: nitric oxide and peroxynitrite; **$O2^{-\bullet}$** is a reactive species of oxygen: superoxide anion; **CA:** carbonic anhydrase. **MAAs** and **AAs**: Mycosporine-like amino acids and amino acids respectively; **ATP** and **NADPH**: Adenosine triphosphate and Nicotinamide adenine dinucleotide phosphate

Carbon fixation and the reduction of nitrate into ammonium require ATP and other energetic molecules produced by photosynthesis. Low UVR exposure enhances photosynthesis and the production of energetic molecules (especially at 30°C), by increasing chlorophyll content. It is also known to stimulate the signaling pathway of the NR, N_iR and CA, enhancing their activity. Therefore, nitrate reduction and carbon assimilation are enhanced in presence of low UVR levels. Finally, UVR stimulates the synthesis of antioxidant compounds such as MAAs, which offer a protection against oxidative stress.