Effect of feeding and thermal stress on photosynthesis, respiration and the carbon budget of the scleractinian coral *Pocillopora damicornis*

Niclas Heidelberg Lyndby^{1,2*}, Jacob Boiesen Holm¹, Daniel Wangpraseurt^{1,3,4}, Renaud Grover⁵, Cécile Rottier⁵, Michael Kühl^{1,6}, Christine Ferrier-Pagès⁵

¹Marine Biological Section, Department of Biology, University of Copenhagen, Helsingør 3000, Denmark

²Laboratory for Biological Geochemistry, School of Architecture, Civil and Environmental Engineering (ENAC), Ecole Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland

³Scripps institution of Oceanography, University of California, San Diego, USA

⁴Department of Chemistry, University of Cambridge, Cambridge UK

⁵Centre Scientifique de Monaco, 8 quai Antoine 1er, Monaco, MC 98000

⁶Climate Change Cluster, University of Technology Sydney. Ultimo, NSW 2007, Australia

Abstract

Studying carbon dynamics in the coral holobiont provides essential knowledge of nutritional strategies and is thus central to understanding coral ecophysiology. In this study, the first aim was to investigate the effect of daily feeding and thermal stress on oxygen (O₂) rates measured at polyp-scale with microsenors and at whole fragment scale using incubation methods. The second aim was to assess the carbon budget of the symbiotic association using H¹³CO₃, under the different conditions. Micro- and macro-scale measurements revealed enhanced O₂ evolution rates for fed compared to unfed corals. However, gross O₂ production in fed corals was increased at high temperature on a macroscale but not on a microscale basis, likely due to a heterogeneous distribution of photosynthesis over the coral surface. Starved corals always exhibited reduced photosynthetic activity at high temperature, which suggests that the nutritional status of the coral host is a key limiting factor for coral productivity under thermal stress. Quantification of photosynthate translocation and carbon budgets showed very low incorporation rates, for both symbionts and host (0.03 – 0.6 µg C cm⁻² h⁻¹) equivalent to only 0.008 – 0.6 %, of the photosynthetically fixed carbon for *P. damicornis*, in all treatments. Carbon loss (via respiration and/or mucus release) was about 41 – 47 % and 52 – 76% of the fixed carbon for starved and fed corals, respectively. Such high loss of translocated carbon suggests that *P. damicornis* is nitrogen and/or phosphorus limited. Heterotrophy might thus cover a larger portion of the nutritional demand for *P. damicornis* than previously assumed. Our results suggest that active feeding plays a fundamental role in metabolic dynamics and bleaching susceptibility of corals.

Keywords: coral bleaching, photobiology, ¹³C, carbon dynamics, heterotrophy, autotrophy

Introduction

Coral reefs in tropical to subtropical waters are primarily founded by calcifying (scleractinian) corals that are fueled by an endosymbiotic relationship between the cnidarian host and their photosynthesizing dinoflagellates, commonly referred to as zooxanthellae, belonging to the genus *Symbiodinium*. This mutual association enables corals to thrive in oligotrophic waters by translocation of photosynthates from the symbiont to the animal host (Muscantine *et al.*, 1981; Tremblay *et al.*, 2012). In return, the zooxanthellae receive shelter and inorganic nutrients via waste products from the coral host metabolism (Muscantine & Cernichiari, 1969).

It is generally accepted that symbiont photosynthesis covers up to 95% of the daily energy demand of the animal host (Muscantine *et al.*, 1984), especially in shallow waters where photosynthetically active radiation (PAR) is not limiting. However, corals that inhabit deeper and/or turbid waters, are far more dependent on active feeding, as photosynthesis becomes light limited (Falkowski *et al.*, 1984; Palardy *et al.*, 2008). Studies have shown that corals utilize heterotrophy for supplementary carbon and nutrient acquisition, even in light saturated environments, thus implying an advantageous effect from active feeding (Yonge & Nicholls, 1931; Wellington, 1982; Sebens *et al.*, 1996; Houlbrèque *et al.*, 2004). Furthermore, the animal host relies on heterotrophy for obtaining nitrogen, phosphorus and other essential nutrients, that are not acquired through autotrophy (Houlbrèque & Ferrier-Pagès, 2009).

Heterotrophy becomes particularly important during bleaching events, as corals rely on zooxanthellae to sustain their daily metabolic energy demand and the loss of symbionts induce coral starvation and can subsequently lead to coral death (Brown, 1997). In addition, coral bleaching can lead to an 'optical feedback loop' (Enríquez *et al.*, 2005), where symbiont loss promotes increased tissue light penetration

55 and skeletal backscattering, which subsequently leads to enhanced light absorption by the remaining
 56 zooxanthellae (Enríquez *et al.*, 2005; Wangpraseurt *et al.*, 2017). This can lead to additional photodamage
 57 and production of reactive oxygen species (ROS), which can further accelerate the bleaching process (Weis
 58 2008; Lesser 1996). In such conditions, heterotrophy increases resilience to bleaching by storage of long
 59 lasting energy deposits (Hughes & Grottoli, 2013), and sustains basic coral host metabolism and the
 60 recruitment and re-colonization of zooxanthellae when more favorable conditions are present (Grottoli
 61 *et al.*, 2006; Rodrigues & Grottoli, 2007; Hughes *et al.*, 2010). Therefore, investigations of the proportional
 62 input from heterotrophy compared to autotrophy during coral bleaching reveal a potentially very high (up
 63 to 100%) contribution of heterotrophically acquired carbon to daily animal respiration (CHAR) in certain
 64 species (Grottoli *et al.*, 2006; Courtial *et al.*, 2017). This implies the existence of species-specific adaptations
 65 among corals to utilize active feeding as a primary nutrient source under stressed conditions.

66 Many other factors interact with heterotrophy to mitigate coral bleaching. For example, corals can receive
 67 an enhanced transfer of photosynthates by the remaining symbionts (Tremblay *et al.*, 2016), or decrease
 68 their metabolism (as assessed by the decrease in respiration rates and carbon release) during stress. These
 69 parameters can be assessed by making the carbon budget of corals, through the use of ¹³C-isotope tracer
 70 experiments (Tremblay *et al.*, 2012), and O₂ fluxes. The combination of the two techniques allows the
 71 quantification of the autotrophic carbon fixation rates, of the fraction stored in either symbionts or host
 72 tissue, or lost through respiration and mucus release, and yield valuable insight on the current health and
 73 productivity state of the coral holobiont. One limiting step in these fluxes is the right determination of the
 74 O₂ fluxes, or in other word, the right determination of the respiration and gross photosynthesis rates.
 75 Most measurements indeed tend to focus on a single spatial scale (e.g. whole colony scale logging), and
 76 assume a homogenous distribution across the surface. However, O₂ produced by symbiont

photosynthesis is often used immediately for symbiont and coral respiration. This intimate association between autotrophic and heterotrophic processes makes the quantitative and spatial separation of respiration and photosynthesis difficult (Kühl *et al.*, 1995, 1996). A method for measuring gross photosynthesis independent of respiration is available based on the use of O₂ microelectrodes (Revsbech & Jørgensen, 1983). In addition, gross photosynthesis is dependent on the light reaching the symbionts (Wangpraseurt *et al.*, 2015), but the fate and turnover rate of the incident irradiance reaching the coral surface (radiative energy budget), is still poorly known. Establishing a radiative energy budget allows the determination of the energy lost to the system by reflection or absorbed in the coral tissue where it can either be dissipated as heat or used for photosynthesis (Brodersen *et al.*, 2014; Lichtenberg, Brodersen & Kühl, 2017; Lyndby *et al.*, submitted).

In this present study, the main aim is to investigate O₂ rates at different spatial scales by employing a combination of microsensor and incubation techniques to examine the effect of daily feeding and thermal stress on coral bleaching susceptibility. Furthermore, we establish the first carbon and radiative energy budgets on *P. damicornis*, a frequently used model species in coral ecophysiology. This part of the study will focus on the implication of thermal stress and feeding on the carbon budget, whereas an accompanying paper (Lyndby *et al.*, submitted) will focus on the radiative energy budget of *P. damicornis* under same conditions.

Materials and methods

Pocillopora damicornis corals cultured at the Centre Scientifique de Monaco (CSM), were prepared by cutting eight mother colonies into ~120 fragments of 2-3 cm in diameter, and hung from nylon threads in the tanks. All corals were kept under white light (250W metal halide lamps), at a downwelling irradiance

(400-700 nm) of 200-250 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, illuminated for 12 hours a day. Eight tanks (water renewal rate of 10 L h⁻¹, 25°C, salinity of 35) were set up two months in advance of the bleaching experiment as to randomly divide the corals into two groups of four tanks each with either fed or unfed corals. Fed corals were fed once a day with ~4000 *Artemia* nauplii per coral fragment, four times per week, and unfed corals were kept unfed throughout the study, including the two months prior to measurements.

About 20 fragments were kept in each control tank, while three thermal stress tanks contained a total of 40 fragments for each feeding treatment. At the beginning of the study, all eight tanks started at 25°C. The tanks used for thermal stress were then gradually ramped up to 30°C over a period of five days, increasing 1°C per day. Thermally stressed corals were kept at 30°C for an additional two to three days before starting measurements. Corals measured on after the two-three days of 30°C thermal stress are denoted as time point 1 (T₁ shortened). After an additional five days of thermal stress, measurements were conducted again, denoted as time point 2 (T₂). Measurements on control corals were done in between the waiting period for T₁ and T₂, and are denoted time point 0 (T₀).

Experimental setup and approach

Corals were placed in a black acrylic flow chamber for all microsensor measurements. The flow chamber was supplied with seawater (25°C, salinity 35) from a heated water reservoir (10 L) at a flow rate of ~0.25 cm s⁻¹ through the chamber. A motorized micromanipulator (MU-1, PyroScience, Germany) was attached to a heavy-duty stand to facilitate positioning of microsensors on fragments in a 45° angle relative to the vertically incident light. A digital microscope (Dino-Lite Edge AM7515MZTL, AnMo Electronics Corporation, Taiwan) was used to carefully position the sensor tip on the coral tissue surface (See figure 1e-f in accompanying paper by Lyndby *et al.*, submitted). A tungsten halogen lamp (KL-2500 LCD, Schott, Germany), fitted with a fiber light guide and collimating lens, was used to illuminate the flow chamber

with white light (See Supplementary Figure 1) vertically from above. A calibrated spectroradiometer (MSC15, GigaHertz-Optik, Germany) was used to quantify the absolute downwelling photon irradiance at different lamp settings (80, 167, 250, 480, 970, and 2400 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), and in addition storing the radiometric energy spectra in $\text{W m}^{-2} \text{nm}^{-1}$. The intensity was adjusted via the aperture of the lamp without spectral distortion. The setups were covered with black cloth during measurements. For each coral used in this study, microsensor measurements were done on three randomly chosen polyps located on the branch tips (See dotted circles in figure 1a-b in accompanying paper by Lyndby *et al.*, submitted).

Microsensor measurements

Gross photosynthesis was measured with Clark-type O_2 microelectrodes with a tip diameter of $\sim 25 \mu\text{m}$, a low stirring sensitivity ($< 5\%$), and a fast response time ($< 0.5 \text{ s}$; OX-25 fast, Unisense, Denmark). The microelectrodes were connected to a pA meter (Unisense, Denmark), and were linearly calibrated from readings in 100% air saturated seawater and anoxic water (by addition of Na_2SO_3) at experimental temperature. The percentage of O_2 air saturation was converted to $\mu\text{mol L}^{-1}$ using gas tables for the O_2 solubility in air-saturated water as a function of temperature and salinity (www.unisense.dk). For gross photosynthesis estimates, data were recorded on a strip-chart recorder (BD25, Kipp&Zonen, Netherlands) connected to the picoammeter. Gross photosynthesis was estimated using the light-dark shift method as described in detail by Revsbech & Jørgensen (1983). O_2 measurements performed at the coral tissue surface were regarded as representative of the entire tissue volume of *P. damicornis* given that the average tissue was $\sim 200 \mu\text{m}$ thick, which approximates the spatial resolution of the light-dark shift method during the 2-3 second period of darkening.

For measurements of holobiont dark respiration, coral fragments were dark adapted for 15 min before measuring O_2 concentration profiles across the diffusive boundary layer (DBL) between the mixed

overlying water and the coral tissue surface. Measurements started at the tissue surface (depth = 0 μm) and the microsensor tip was moved upwards in steps of 50 μm (10 s rest time per measuring position) until reaching the ambient water with a constant O_2 concentration.

Subsequently, net photosynthesis vs. irradiance curves were measured under 7 increasing levels of incident photon irradiance ($E_d(\text{PAR}) = 0, 80, 167, 250, 480, 970$, and $2400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). Each coral fragment was incubated for 10 min at each irradiance level, ensuring steady-state conditions. A total of 4 replicate fragments were measured per treatment and sample times using 3 randomly selected coenosarc and polyp tissue areas, i.e. a total of 24 measurements per treatment. Towards the end of the bleaching treatment, some fragments showed no O_2 production on the coenosarc and we thus limited the replicate measurements for those fragments.

Local net-photosynthesis and dark-respiration rates were determined as the net O_2 flux, J ($\text{nmol O}_2 \text{ cm}^{-2} \text{s}^{-1}$), as calculated from the measured steady-state O_2 profiles using Fick's first law of diffusion:

Eq. 1:
$$J = D_0 \frac{\partial C}{\partial z}$$

where D_0 is the molecular diffusion coefficient of O_2 in seawater at experimental salinity and temperature ($D_0 = 2.2327 \cdot 10^{-5} \text{ cm}^2 \text{s}^{-1}$ at 25°C and $S=35$, and $D_0 = 2.5521 \cdot 10^{-5} \text{ cm}^2 \text{s}^{-1}$ at 30°C and $S=35$; Broecker & Peng, 1974), $\delta C/\delta z$ is the slope of the linear part of the O_2 concentration gradient in the DBL, defined by the change in O_2 concentration (δC) over a specific depth interval (δz).

Gas exchange measurements

Additional measurements of respiration and photosynthesis were made by measuring the net O_2 gas exchange of coral fragments (Hoogenboom *et al.*, 2010) under photon irradiance levels of 0, 50, 100, 200 and $400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. For this, coral fragments (3-5 for each treatment) were positioned upright

in small incubation chambers (50 mL) and the fragments were illuminated from the side, with a 250W metal halide lamp. To ensure even illumination of the entire coral fragment, aluminium foil was placed behind the incubation chamber, reflecting the incident beam and illuminating the otherwise shaded areas of the coral fragment. The photon scalar irradiance in the incubation chamber was measured for defined lamp settings using a spherical mini quantum sensor (sphere diameter of 3.7 mm, US-SQS/L, Walz GmbH, Germany) attached to a light meter (LI-190, Li-Cor, Lincoln, NE, USA).

The temperature in the experimental chambers was kept at 25°C for control and at 30°C for heat-treated fragments and the chambers were constantly stirred with a magnet. The O₂ concentration was logged over time with an O₂-optode (O₂ MiniOptode, Unisense A/S, Aarhus, Denmark) connected to a computer with Oxy-4 software (4-channel fiber-optic O₂ meter, PreSens, Regensburg, Germany). O₂ optodes were linearly calibrated from measurements in air saturated water (bubbled with air) and anoxic water (flushed with N₂) according to the manufacturers recommendations.

Dark holobiont respiration was determined as the O₂ depletion after 15 min dark adaptation and light respiration as the initial post-illumination O₂ depletion after each experimental irradiance level. Net photosynthesis (P_n) was determined as the O₂ concentration increase after 15 min illumination at the given photon irradiance similar to light respiration measurements. The O₂ depletion and production curves were fitted with linear regression and rates of respiration and net-photosynthesis were calculated as:

$$\text{Eq. 2: } P_n \text{ and } R = \left(\left(\left(\frac{\left(\frac{\Delta \% C_{solO_2}}{\Delta t} \right)}{100\%} \right) \cdot \frac{P_{chamber}}{P_{atmospheric}} \right) \cdot 60 \text{ min} \right) \cdot \frac{V_{chamber}}{1000 \text{ ml}}$$

THERMAL STRESS AND CARBON BUDGET IN *P. DAMICORNIS*

10

182 $\Delta\%C/\Delta t$ is the change in % O_2 over time ($\% \text{ min}^{-1}$), sol_{O_2} is the O_2 solubility ($\mu\text{mol L}^{-1}$) for the given
 183 temperature and salinity ($sol_{O_2} = 211.3 \mu\text{mol/L}$ at 25°C and $S=35$, and $sol_{O_2} = 194.6 \mu\text{mol/L}$ at 30°C and
 184 $S=35$), $P_{\text{chamber}}/P_{\text{atmospheric}}$ is the relative difference in partial pressure (hPa) between atmospheric air and
 185 the water mass in the incubation chamber, V_{chamber} is the volume of the chamber (mL), and P_n and R are
 186 rates of net- O_2 production and O_2 consumption, respectively ($\mu\text{mol } O_2 \text{ h}^{-1}$). Gross photosynthetic (P_g) rates
 187 (in $\mu\text{mol } O_2 \text{ h}^{-1}$) were then estimated by adding R to P_n .

188 Besides gas exchange measurements on intact coral fragments, we also measured O_2 gas exchange on
 189 freshly isolated symbionts from 3 previously measured fragments. The symbionts were extracted from 3
 190 fragments per treatment and time point, by first removing the coral tissue using an airbrush with 35 mL
 191 filtered seawater (FSW; $0.45 \mu\text{m}$). Subsequent homogenization of tissue with a Potter tissue grinder, and
 192 transfer of the tissue slurry to a 50 mL Falcon® tube that was centrifuged for 10 min at $850g$. After removal
 193 of the supernatant the remaining symbiont pellet was resuspended in FSW ($0.45 \mu\text{m}$).

194 The symbiont density was quantified in a 100 μL sub-sample using a Z1 Coulter Particle Counter (Beckman
 195 Coulter). The O_2 fluxes were estimated using similar techniques as whole fragment measurements
 196 described previously, and were converted to carbon units according to Anthony & Fabricius (2000).

197 Eq. 3:
$$P_c = P_g \cdot \left(\frac{12}{PQ} \right)$$

198 Eq. 4:
$$R_c = R \cdot (12 \cdot RQ)$$

199 Where P_c is the total amount of inorganic C fixed through photosynthesis, P_g is the O_2 gross production
 200 calculated from P_n and R in $\mu\text{mol } O_2 \text{ cm}^{-2} \text{ h}^{-1}$; 12 is the mass of a carbon atom, PQ is the photosynthetic
 201 quotient, here assumed to be 1.1 mol O_2 :mol C (Muscantine *et al.*, 1981), R_c is the rate of consumed

THERMAL STRESS AND CARBON BUDGET IN *P. DAMICORNIS*

11

202 inorganic C ($\mu\text{g C m}^{-2} \text{ h}^{-1}$) by respiration R , and RQ is the respiratory quotient, here assumed - equal to 0.8
203 mol C:mol O_2 (Muscatine *et al.*, 1981).

204 H^{13}CO_3 tracer incubations

205 Corals used for stable isotope incubations with H^{13}CO_3 were mounted in beakers incubated with FSW
206 (controls) or ^{13}C -enriched FSW (treatments) for pulse periods of 4 hours (Tremblay *et al.*, 2012). The
207 treated fragments were exposed to a solution of 0.6 mM $\text{NaH}^{13}\text{CO}_3$ (98% ^{13}C ; #372382, Sigma-Aldrich, St
208 Louis, MO, USA), mixed with 200 mL FSW (Tremblay *et al.*, 2012). The beakers were left open and the
209 solutions were constantly stirred by a magnet bar to avoid re-use of respired ^{13}C .

210 The beakers with fragments of *P. damicornis* (3-5 for each treatment) were kept at a constant temperature
211 of 25°C for control and 30°C for heat-stressed corals by a water bath under thermal regulation, and were
212 illuminated with a constant incident photon irradiance of 200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. During these
213 incubations, O_2 production and consumption were not recorded, but the rates of carbon fixation and
214 respiration were derived from the above mentioned O_2 gas exchange measurements at 200 $\mu\text{mol photons}$
215 $\text{m}^{-2} \text{ s}^{-1}$. For quantification of ^{13}C enrichment in coral fragments, the tissue was detached from the skeleton
216 by an air brush with 10 mL FSW. The tissue slurry was homogenized using a Potter tissue grinder and the
217 animal and symbiont fractions were then separated by centrifugation (1328g for 5 min). To avoid tissue
218 contamination, the symbionts were rinsed several times in FSW and checked under a microscope. Tissue
219 and symbionts were separated, frozen in liquid N_2 and freeze dried for later analysis. The ^{13}C enrichment
220 and carbon content in the animal tissue and symbionts were quantified with a mass spectrometer (Delta
221 Plus, Thermo Fisher Scientific, Bremen, Germany) coupled via a type III interface with a C/N analyzer (Flash
222 EA, Thermo Fisher Scientific), and compared with $^{13}\text{C}/^{12}\text{C}$ in the control fragments (Tremblay *et al.*, 2012).

Carbon budget calculations

Incorporation rates in symbionts and host (ρ ; defined as the rate of incorporated C per area and time, i.e., $\mu\text{g C cm}^{-2} \text{ h}^{-1}$) were calculated according to Tremblay *et al.* (2012), by the following equations:

Eq. 5:
$$\rho_S = \frac{(C_{\text{meas}} - C_{\text{nat}}) \cdot M_{\text{sample}} \cdot M_C}{(C_{\text{inc}} - C_{\text{meas}}) \cdot t_{\text{pulse}} \cdot S}$$

Eq. 6:
$$\rho_H = \frac{(C_{\text{meas}} - C_{\text{nat}}) \cdot M_{\text{sample}} \cdot M_C}{(C_{\text{inc}} - C_{\text{meas}}) \cdot t_{\text{pulse}} \cdot S}$$

Eq. 7:
$$\rho_{\text{TOC}} = T_S - R_H - \rho_H$$

where ρ refers to the carbon incorporation rate ($\mu\text{g C cm}^{-2} \text{ h}^{-1}$) into the symbionts (ρ_S , Eq. 5) and coral host (ρ_H , Eq. 6), and ρ_{TOC} is calculated as the released C from the host ($\mu\text{g C cm}^{-2} \text{ h}^{-1}$).

T_S is the translocation rate from symbiont to host, C_{meas} and C_{nat} are the percentage of ^{13}C measured in our experimentally treated samples (symbionts and host tissue) and the control samples, respectively, M_{sample} is the mass of the freeze-dried sample (mg), M_C is the mass of carbon per milligram of symbiont or host tissue ($\mu\text{g mg}^{-1}$) or the release of C from the coral holobiont (μg). S denotes the surface area (in cm^2) of the coral fragment as quantified by the wax dipping technique (Veal *et al.*, 2010), t_{pulse} is the incubation time (hours) of the fragments in control and enriched incubation medium, and C_{inc} is the percent ^{13}C of the DIC in the medium. We used seawater that contained $2 \mu\text{M HCO}_3^-$ with a natural ^{13}C abundance of 1.1285% and added $0.6 \mu\text{M HCO}_3^-$ with a ^{13}C abundance of 98%. C_{inc} was then determined by the following equation:

Eq. 8:
$$C_{\text{inc}} = \frac{(2 \cdot 1.1285) + (0.6 \cdot 98)}{(2 + 0.6)}$$

Based on the previous determined incorporation rates, respiration rates and the amount of fixed inorganic C going into the symbionts, the amount of C translocation from the symbionts to the coral host was calculated as the net remaining C after symbiont respiration and incorporation:

Eq. 9:
$$T_S = P_C - \rho_S - R_S$$

See Figure 3 for schematic overview of the carbon budget and its pathways.

Statistical analysis

Photosynthetic rates and carbon fluxes were tested for homogeneity of variance using a two-way *F*-test and for significant differences using a two sample Student's *t*-test (for equal variances) or Welch's *t*-test (for unequal variances). Statistical tests were performed in OriginPro 9.1 (Origin, USA).

Results

Photosynthesis vs. irradiance curves

O₂ production was enhanced in fed vs unfed *Pocillopora damicornis* corals for polyp-scale microsensor (microscale) and whole fragment incubation (macroscale) measurements (Figure1). Fed corals from time point T₀ and T₁ (Figure 1a, c) exhibited higher gross photosynthesis for both measuring techniques with small variations ($\pm 0.1\text{--}0.4 \mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$) compared to unfed corals that showed a more profound variation between the techniques ($\pm 0.5\text{--}0.8 \mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$; Figure 1b, d). The effect of thermal ramping on fed corals led to a 20% increase in O₂ production from T₀ to T₁. However, on a longer term (T₂), we found a significant effect of increased temperature at the macroscale, leading to a 2.2-fold enhancement in O₂ production at T₂ (4.1 ± 0.75 vs $1.3 \pm 0.2 \mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$) as compared to T₀ (Student's *t*-test: $p < 0.001$; Figure 1c). The O₂ production at microscale was on the contrary reduced by $0.6 \pm 0.3 \mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$

261 $^2 \text{ h}^{-1}$ from T_1 to T_2 (Figure 1a), which deviates from the observed gross photosynthesis on a macroscale at
 262 T_2 (Figure 1a, c). O_2 production was reduced for unfed corals on both micro- and macroscale.
 263 Gas-exchange measurements of areal net photosynthesis showed onset of net O_2 production in fed corals
 264 at a photon irradiance $>100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for both micro- and macroscale measurements (Figure
 265 2a, c). In contrast to fed corals, unfed corals only exhibited net O_2 production at irradiances $>150 \mu\text{mol}$
 266 $\text{photons m}^{-2} \text{ s}^{-1}$ (Figure 2b, d). The combination of temperature increase and starvation led to a slightly
 267 higher compensation irradiance (i.e. the irradiance above which net O_2 production is observed) at 250
 268 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ at T_0 , and $400 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ at T_1 on a microscale, and $150 \mu\text{mol photons m}^{-2}$
 269 s^{-1} at T_0 - T_1 on a macroscale (Figure 2b, d). We found enhanced light respiration with microscale
 270 measurements for both fed and unfed corals (Figure 2a, b), as compared with macroscale measurements.
 271 Dark holobiont respiration was similar for all treatments except for fed corals at T_2 (Figure 2c), which
 272 showed a 3-fold increase in O_2 consumption compared to fed corals at T_0 (0.6 ± 0.05 vs $2.4 \pm 0.25 \mu\text{mol}$
 273 $\text{O}_2 \text{ cm}^{-2} \text{ h}^{-1}$).

274 Cell specific O_2 flux for zooxanthellae

275 Cell specific rates of gross photosynthesis for intact corals showed an enhanced O_2 production in fed corals
 276 compared to unfed corals at both normal and increased temperatures (Figure4). Under increased
 277 temperature, we found a significant enhancement of cell-specific photosynthetic rates, with up to 3-fold
 278 increase in O_2 production in fed corals at T_2 with a photon irradiance of 200 and $400 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$
 279 1 (Figure 4a) compared to fed corals at T_0 (Student's t -test: $p < 0.001$; Welch's t -test: $p = 0.042$,
 280 respectively). A similar trend was present for unfed corals as O_2 production increased towards higher
 281 irradiance under heat-stress.

THERMAL STRESS AND CARBON BUDGET IN *P. DAMICORNIS*

15

282 Freshly isolated symbionts also showed enhanced cell specific gross photosynthesis rates with feeding.
 283 They, however, showed no significant change upon increased temperature, except fed T_2 at 50 μmol
 284 photons $\text{m}^{-2} \text{s}^{-1}$ (Figure 4b) showing a clear enhanced O_2 production relative to the control (T_0)
 285 measurements ($1 \times 10^{-7} \pm 1.7 \times 10^{-8}$ vs $3.5 \times 10^{-7} \pm 6.5 \times 10^{-8}$ $\mu\text{mol O}_2 \text{ cell}^{-1} \text{ h}^{-1}$, respectively; mean $\pm$ SEM).
 286 However, we found increased O_2 production in fed corals compared to unfed corals for both isolated
 287 symbionts, and symbionts *in vivo* (Student's *t*-test: $p < 0.001$; isolated symbionts from fed corals compared
 288 to unfed corals at T_0).

289 Carbon budget of entire coral fragments (macroscale)

290 Carbon flux was strongly influenced by both the feeding status and heat stress of *P. damicornis*. Gross
 291 production rates were enhanced under increased temperature and daily feeding (Figure 1c, d).
 292 Respiration rates (R_s) of symbionts in control corals (T_0) showed a similar rate of respired carbon for both
 293 fed and unfed corals before induced heat stress (~ 14 -14.2 %; Table 1), and during heat stress (T_{1-2} ; 5.3-6.9
 294 %; Table 1).

295 Host respiration rates (R_H in % fixed carbon) were ~ 28.8 % higher for fed corals compared to unfed corals
 296 at T_0 (Table 1). Heat stress enhanced host respiration for fed corals at T_{1-2} by about 1.5-fold compared to
 297 fed corals at T_0 (Table 1; Welch's *t*-test: $p = 0.009$; fed corals $T_{0 \rightarrow 1}$ and $T_{0 \rightarrow 2}$). Carbon translocation rates
 298 (T_s) from symbiont to host showed a slight increase during heat stress for all treatments (85.8 ± 0.1 vs
 299 93.7 ± 0.5 %; Table 1; T_{1-2} compared to T_0). The carbon incorporation rates were low for both symbionts
 300 (ρ_s) and hosts (ρ_H) in all treatments as compared to other assessed coral species (Tremblay *et al.*, 2016),
 301 and incorporation accounted for only 0.03-0.6 % of the fixed carbon (Table 1). A high excretion of carbon

was calculated, reaching a release of between ~50-75 % of the initially fixed carbon in all treatments (Table 1).

Discussion

Implications of measuring O₂ production at different spatial scales

The O₂ production and consumption in *P. damicornis* were assessed with methods operating on two different spatial scales, i.e., polyp scale (mm resolution, microscale) and fragment scale (cm resolution, macroscale; Figure 1 and 2). Micro- and macro-scale measurements revealed enhanced O₂ evolution rates for fed corals compared to unfed corals (Figure 1 and 2). However, gross O₂ production on a macroscale was increased at high temperature (Figure 1c, d and 2c, d) compared to gross O₂ production on a microscale, especially at T₁ → T₂ where rates were 2.4-fold enhanced on a macroscale (Figure 1c). Our microscale measurements were restricted to the polyp region, at a defined angle near the apical tips of the coral branch, which could potentially underestimate the productivity of polyp areas that were not assessed with microsensor measurements. The difference between micro- and macroscale measurements for fed corals at T₂ (Figure 1a, c) could thus be that photosynthesis was heterogeneously distributed over the coral surface (Al-Horani *et al.*, 2005). Scalar irradiance on bare skeletons of *P. damicornis* (Supplementary figure 5) revealed that light exposure near the base of the coral branch was increased by about 100 % compared to the apical branch tip. Previous studies on spatial distribution of photosynthesis across the tissue surface revealed that the majority of photosynthetic activity is located around the tissue covering the corallite septa, with up to one order of magnitude increased photosynthesis compared to adjacent coenosarc tissue (Al-Horani *et al.*, 2005). These findings emphasize the potential underestimation of the photosynthetic activity of the polyp regions near the base of the coral branch

(Supplementary figure 5). The O₂ production observed for macroscale measurements of fed corals at T₂ may thus be additionally affected by the backscattering properties in polyp areas around the base of the coral branch, as fed corals at T₂ had reduced cell density compared to controls (T₀; figure 3a in Lyndby *et al.*, submitted), which cause an enhanced radiative exposure of the symbionts residing in the deeper tissue layers leading to an increased O₂ production.

Cell specific rates of O₂ production (Figure 4a) were similar to areal estimates measured on intact coral fragments during the course of the experiment (Figure 4 vs Figure 1). Chlorophyll *a*+*c*₂ and cell density per area decreased with increased temperature (Figure 3b in Lyndby *et al.*, submitted), while chlorophyll *a*+*c*₂ per cell values remained more or less constant (Figure 3c in Lyndby *et al.*, submitted). Fewer symbionts within the coral tissue lead to reduced algal self-shading (Lesser *et al.*, 2000; Terán *et al.*, 2010; Wangpraseurt *et al.*, 2012), increasing the light exposure per cell and thus higher cell specific photosynthetic rates (Enríquez *et al.*, 2005; Wangpraseurt *et al.*, 2017).

Gross O₂ production rates of isolated zooxanthellae cells (Figure 4b), were about one order of magnitude lower than the measurements performed *in vivo* (Figure 4a). This suggests that the symbionts benefit from the animal tissue e.g. through optimized light exposure and potentially other host-affected factors such as pH, nutrients or spectral filtering by host pigments, leading to enhanced *in vivo* photosynthetic efficiency (Enríquez *et al.*, 2005). Even though both *in vivo* and *in vitro* measurements were performed under identical levels of incident downwelling irradiance, the actual scalar irradiance will differ between the two measurements because of light scattering in the coral tissue and on the skeletal surface (Wangpraseurt *et al.*, 2012, 2014). However, inorganic nutrient supply from the coral host (e.g. NH₄⁺) can also contribute to the observed enhancement of photosynthetic efficiency *in vivo* (Borell & Bischof, 2008).

344 O₂ dynamics during heat stress

345 The O₂ dynamics of *Pocillopora damicornis* measured on a whole fragment scale (macroscale) and polyp
346 scale (microscale) agree with previous studies from other coral species showing a 35-60% increase in
347 chlorophyll per area and an increased cell density in fed corals (Muscatine *et al.*, 1989; Fitt & Cook, 2001;
348 Ferrier-Pagès *et al.*, 2003). In contrast, the lack of host feeding in unfed corals could explain the lower cell
349 density for both whole fragment scale and polyp scale, as sufficient nutrients to produce e.g. antioxidants
350 to cope with ROS accumulation are not met, causing ingestion or expulsion of excess zooxanthellae (Figure
351 1c-d, Figure 2c-d; Figure 3 in Lyndby *et al.*, submitted).

352 Earlier studies on thermally stressed *P. damicornis* revealed increased feeding rates of up to 127 %
353 compared to fed controls (Courtial *et al.*, 2017), implying an increased demand of nitrogen to withstand
354 stress factors by synthesizing protective compounds (e.g. antioxidants and heat-stress proteins) to avoid
355 oxidative stress (Downs *et al.*, 2002).

356 Our results suggest that the nutritional input from active feeding enables the coral host to maintain a
357 higher photosynthetic activity as compared to unfed corals, and we speculate that feeding enhances
358 essential nutrients (e.g. nitrogen) for synthesis of antioxidants and heat-stress proteins to better prevent
359 damage from reactive oxygen species (ROS; Demmig-Adams & Adams, 2002; Fitt *et al.*, 2009).

360

361 Autotrophic carbon fluxes and mass balanced results of photosynthates and translocation

362 The C incorporation rates, i.e., the rate of inorganic carbon assimilated per area and time ($\mu\text{g C cm}^{-2} \text{ h}^{-1}$)
363 of the initially fixed inorganic carbon, were surprisingly low for both symbionts and the animal host in all
364 treatments (Table 1). In contrast, previous studies found several orders of magnitude higher incorporation

THERMAL STRESS AND CARBON BUDGET IN *P. DAMICORNIS*

19

365 rates for the branching coral *Stylophora pistillata*, which belongs to the same family as *P. damicornis*
 366 (Pocilloporidae; Tremblay *et al.*, 2012). The majority of autotrophically acquired carbon is used for daily
 367 household metabolism, and colonies growing in high light assimilate a very small amount of
 368 photosynthates, due to lack of nitrogen (Falkowski *et al.*, 1984). This might explain the very high excretion
 369 of translocated carbon in *P. damicornis* observed in all our treatments (Table 1). A high amount of fixed
 370 carbon could surpass the metabolic demand and would therefore be excreted from the coral host, as
 371 sufficient supplies of nutrients are available from heterotrophy for fed corals (Fed T₀₋₂; Table 1). Studies
 372 on energy and carbon dynamics in *Pocillopora eydouxi* (Davies, 1984) show a similar high release of
 373 photosynthates (about 50 % of fixed carbon) from coral to ambient water, which correlates well with our
 374 results of *P. damicornis* (Fed T₁₋₂; Table 1).

375 This further supports the idea that the growth of *P. damicornis* is limited by the absence of organic
 376 nitrogen, despite the availability of autotrophic carbon (Davies, 1984). Excess carbon can also maintain a
 377 food source for surface associated microbes (Bourne & Munn, 2005; Thompson *et al.*, 2015) that may
 378 confer benefits to the coral holobionts, e.g. in terms of better resilience to heat stress (Rosenberg *et al.*,
 379 2007) or nutrient supply via fixed nitrogen from diazotrophs being translocated to the coral host (Bednarz
 380 *et al.*, 2017).

381 The host respiration for unfed corals compared to fed corals at T₀ was 10.2 % and 38.9 % of the initially
 382 fixed carbon, respectively (Fed and unfed T₀; Table 1). This could indicate a host stress response in unfed
 383 corals due to lack of organic nutrients, which are readily available for fed corals (Ferrier-Pagès *et al.*, 2003).
 384 Additionally, studies on host response to thermal stress revealed a higher production of super oxide
 385 dismutase and heat stress proteins (Fitt *et al.*, 2009), which will naturally increase the host metabolism,

386 and correlates well with our observation of an increased host respiration in both fed and unfed corals at
387 T_{1-2} (Table 1). We also see a clear reduction in the P:R ratio during heat stress for unfed corals from 2.56
388 at T_0 down to 1.88 at T_1 (Table 1), in line with previous assessments of thermally stressed *P. damicornis*
389 (Coles & Jokiel, 1977).

390 The translocation rates of the fixed carbon going from the symbionts to the animal host seem to deviate
391 from previously assessed corals from the Pocilloporidae family. Whereas the translocation rate usually
392 decrease during heat stress, it remained high for *P. damicornis* throughout the treatments (Tremblay *et*
393 *al.*, 2012). Therefore, contrary to other coral species whose zooxanthellae keep more autotrophic carbon
394 for their own needs and growth during thermal stress, symbionts of *P. damicornis* in this experiment
395 always translocated more than 85 % of the acquired carbon. This may be due to the lack of other nutrients,
396 which prevent an efficient use of the photosynthesized carbon.

397 Conclusion

398 By applying multiple methods to determine carbon translocation and photosynthetic efficiency in fed and
399 unfed specimens of the scleractinian coral *Pocillopora damicornis* during heat-stress, we found that
400 carbon budgets showed low incorporation rates and high release rates of autotrophically fixed carbon
401 compared to closely related coral species, which emphasizes the benefits of heterotrophically acquired
402 nutrients for *P. damicornis*. Heterotrophy might thus cover a larger portion of the nutritional demand for
403 *P. damicornis* than previously assumed, and underlines how active feeding plays a fundamental role in
404 metabolic dynamics and bleaching susceptibility.

Acknowledgement

We thank the staff at the Scientific Center of Monaco for their excellent assistance with experimental procedures and labor intensive general maintenance and care of the corals for the duration of this study. The study was funded by grants from the Carlsberg Foundation (DW), a Sapere Aude Advanced grant from the Independent Research Fund Denmark (MK), and the Scientific Center of Monaco (part of the RTPi Nutress).

Reference list

- Al-Horani FA *et al.* (2005) Spatial distribution of calcification and photosynthesis in the scleractinian coral *Galaxea fascicularis*. *Coral Reefs*, 24(1), pp. 173–180. doi: 10.1007/s00338-004-0461-3.
- Anthony KRN & Fabricius KE (2000) Shifting roles of heterotrophy and autotrophy in coral energetics under varying turbidity. *Journal of Experimental Marine Biology and Ecology*, 252(2), pp. 221–253. doi: 10.1016/S0022-0981(00)00237-9.
- Bednarz VN *et al.* (2017) The Assimilation of Diazotroph-Derived Nitrogen by Scleractinian Corals Depends on Their Metabolic Status. *American Society for Microbiology*, 8(1), pp. 1–14.
- Borell EM & Bischof K (2008) Feeding sustains photosynthetic quantum yield of a scleractinian coral during thermal stress. *Oecologia*, 157(4), pp. 593–601. doi: 10.1007/s00442-008-1102-2.
- Bourne DG & Munn CB (2005) Diversity of bacteria associated with the coral *Pocillopora damicornis* from the Great Barrier Reef. *Environmental Microbiology*, 7(8), pp. 1162–1174. doi: 10.1111/j.1462-2920.2005.00793.x.
- Brodersen KE *et al.* (2014) Radiative energy budget reveals high photosynthetic efficiency in symbiont-bearing corals. *Journal of The Royal Society Interface*, 11. Available at: <http://rsif.royalsocietypublishing.org/content/11/93/20130997.short> (Accessed: 28 October 2014).
- Broecker WS & Peng T-H (1974) Gas exchange rates between air and sea. *Tellus*, 26(1–2), pp. 21–35.
- Brown BE (1997) Coral bleaching: causes and consequences. *Coral Reefs*, 16(0), pp. S129–S138. doi: 10.1007/s003380050249.
- Coles SL & Jokiel PL (1977) Effects of temperature on photosynthesis and respiration in hermatypic corals. *Marine Biology*, 43(3), pp. 209–216. doi: 10.1007/BF00402313.
- Courtial L *et al.* (2017) Interactive effects of ultraviolet radiation and thermal stress on two reef-building corals. *Limnology and Oceanography*. doi: 10.1002/lno.10481.
- Davies PS (1984) The role of zooxanthellae in the nutritional energy requirements of *Pocillopora eydouxi*. *Coral Reefs*, 2(4), pp. 181–186. doi: 10.1007/BF00263571.
- Demmig-Adams B & Adams WW (2002) Antioxidants in photosynthesis and human nutrition. *Science* (New York, N.Y.), 298(5601), pp. 2149–53. doi: 10.1126/science.1078002.
- Downs CA *et al.* (2002) Oxidative stress and seasonal coral bleaching. *Free Radical Biology and Medicine*, 33(4), pp. 533–543.
- Enríquez S, Méndez ER & Iglesias-Prieto R (2005) Multiple scattering on coral skeletons enhances light absorption by symbiotic algae. *Limnology and Oceanography*, 50(4), pp. 1025–1032. doi: 10.4319/lo.2005.50.4.1025.
- Falkowski PG *et al.* (1984) Light and the bioenergetics of a symbiotic coral. *BioScience*, 34(11), pp. 705–709. doi: 10.2307/1309663.

- 446 Ferrier-Pagès C *et al.* (2003) Effect of natural zooplankton feeding on the tissue and skeletal growth of the
447 scleractinian coral *Stylophora pistillata*. *Coral Reefs*, 22(3), pp. 229–240. doi: 10.1007/s00338-003-0312-
448 7.
- 449 Fitt WK *et al.* (2009) Response of two species of Indo-Pacific corals, *Porites cylindrica* and *Stylophora*
450 *pistillata*, to short-term thermal stress: The host does matter in determining the tolerance of corals to
451 bleaching. *Journal of Experimental Marine Biology and Ecology*, Elsevier B.V., 373(2), pp. 102–110. doi:
452 10.1016/j.jembe.2009.03.011.
- 453 Fitt WK & Cook CB (2001) The effects of feeding or addition of dissolved inorganic nutrients in maintaining
454 the symbiosis between dinoflagellates and a tropical marine cnidarian. *Marine Biology*, 139(3), pp. 507–
455 517. doi: 10.1007/s002270100598.
- 456 Grottoli AG, Rodrigues LJ & Palardy JE (2006) Heterotrophic plasticity and resilience in bleached corals.
457 *Nature*, 440(7088), pp. 1186–1189. doi: 10.1038/nature04565.
- 458 Hoogenboom M, Beraud E & Ferrier-Pagès C (2010) Relationship between symbiont density and
459 photosynthetic carbon acquisition in the temperate coral *Cladocora caespitosa*. *Coral Reefs*, 29(1), pp.
460 21–29. doi: 10.1007/s00338-009-0558-9.
- 461 Houlbrèque F *et al.* (2004) Importance of a micro-diet for scleractinian corals. *Marine Ecology Progress*
462 *Series*, 282, pp. 151–160. doi: 10.3354/meps282151.
- 463 Houlbrèque F & Ferrier-Pagès C (2009) Heterotrophy in tropical scleractinian corals. *Biological Reviews*,
464 84(1), pp. 1–17. doi: 10.1111/j.1469-185X.2008.00058.x.
- 465 Hughes AD *et al.* (2010) Acquisition and assimilation of carbon in non-bleached and bleached corals.
466 *Marine Ecology Progress Series*, 420(2), pp. 91–101. doi: 10.3354/meps08866.
- 467 Hughes AD & Grottoli AG (2013) Heterotrophic compensation: A possible mechanism for resilience of
468 coral reefs to global warming or a sign of prolonged stress? *PLoS ONE*, 8(11), pp. 1–10. doi:
469 10.1371/journal.pone.0081172.
- 470 Kühl M *et al.* (1995) Microenvironment and photosynthesis of zooxanthellae in scleractinian corals studied
471 with microsensors for O₂, pH and light. *Marine ecology progress series*, 117(Muscantine 1990), pp. 159–
472 172. Available at: http://www.mbl.ku.dk/MKuhl/pages/PDF/Kuhl_et_al_1995.pdf (Accessed: 28 October
473 2014).
- 474 Kühl M *et al.* (1996) Microenvironmental control of photosynthesis and photosynthesis-coupled
475 respiration in an epilithic cyanobacterial biofilm. *Journal of Phycology*, 32(5), pp. 799–812. doi:
476 10.1111/j.0022-3646.1996.00799.x.
- 477 Lesser MP (1996) Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit
478 photosynthesis in symbiotic dinoflagellates. *Limnology and Oceanography*, 41(2), pp. 271–283. doi:
479 10.4319/lo.1996.41.2.0271.
- 480 Lesser MP *et al.* (2000) Light absorption and utilization by colonies of the congeneric *Hermatypic* corals
481 *Montastraea faveolata* and *Montastraea cavernosa*. *Limnology and Oceanography*, 45(1), pp. 76–86. doi:
482 10.4319/lo.2000.45.1.0076.

THERMAL STRESS AND CARBON BUDGET IN P. DAMICORNIS

24

- 483 Lichtenberg M, Brodersen KE & Kühl M (2017) Radiative Energy Budgets of Phototrophic Surface-
484 Associated Microbial Communities and their Photosynthetic Efficiency Under Diffuse and Collimated
485 Light. *Frontiers in microbiology*, 8, p. 452. doi: 10.3389/fmicb.2017.00452.
- 486 Lyndby NH *et al.* (no date) Effects of feeding on the bleaching response of the scleractinian coral
487 *Pocillopora damicornis*: Light and radiative energy budgets. *Marine Ecology Progress Series*.
- 488 Muscatine L *et al.* (1984) Fate of photosynthetic fixed carbon in light- and shade-adapted colonies of the
489 symbiotic coral *Stylophora pistillata*. *Proc. R. Soc. Lond. B*, 222, pp. 181–202. doi: 10.1098/rspa.1974.0120.
- 490 Muscatine L *et al.* (1989) The Effect of External Nutrient Resources on the Population Dynamics of
491 Zooxanthellae in a Reef Coral. *Proceedings of the Royal Society B: Biological Sciences*, 236(1284), pp. 311–
492 324. doi: 10.1098/rspb.1989.0025.
- 493 Muscatine L & Cernichiaro E (1969) Assimilation of Photosynthetic Products of Zooxanthellae by a Reef
494 Coral. *Biol Bull Mar Biol Lab*, 137(3), pp. 506–523.
- 495 Muscatine L, McCloskey LR & Marian RE (1981) Estimating the daily contribution of carbon from
496 zooxanthellae to coral animal respiration. *Limnology and Oceanography*, 26(4), pp. 601–611. doi:
497 10.4319/lo.1981.26.4.0601.
- 498 Palardy JE, Rodrigues LJ & Grottoli AG (2008) The importance of zooplankton to the daily metabolic carbon
499 requirements of healthy and bleached corals at two depths. *Journal of Experimental Marine Biology and*
500 *Ecology*, Elsevier B.V., 367(2), pp. 180–188. doi: 10.1016/j.jembe.2008.09.015.
- 501 Revsbech NP & Jørgensen BB (1983) Photosynthesis of benthic microflora measured with high spatial
502 resolution by the oxygen microprofile method: Capabilities and limitations of the method. *Limnology and*
503 *Oceanography*, 28(4), pp. 749–756. doi: 10.4319/lo.1983.28.4.0749.
- 504 Rodrigues LJ & Grottoli AG (2007) Energy reserves and metabolism as indicators of coral recovery from
505 bleaching. *Limnology and Oceanography*, 52(5), pp. 1874–1882. doi: 10.4319/lo.2007.52.5.1874.
- 506 Rosenberg E *et al.* (2007) The role of microorganisms in coral health, disease and evolution. *Nature*
507 *Reviews Microbiology*, 5(5), pp. 355–362. doi: 10.1038/nrmicro1635.
- 508 Sebens KP *et al.* (1996) Zooplankton capture by two scleractinian corals, *Madracis mirabilis* and
509 *Montastrea cavernosa*, in a field enclosure. *Marine Biology*, 127(2), pp. 303–317. doi:
510 10.1007/BF00942116.
- 511 Terán E *et al.* (2010) Multiple light scattering and absorption in reef-building corals. *Applied optics*, 49(27),
512 pp. 5032–42.
- 513 Thompson JR *et al.* (2015) Microbes in the coral holobiont: partners through evolution, development, and
514 ecological interactions. *Frontiers in cellular and infection microbiology*, 4(January), p. 176. doi:
515 10.3389/fcimb.2014.00176.
- 516 Tremblay P *et al.* (2012) Autotrophic carbon budget in coral tissue: a new ¹³C-based model of
517 photosynthate translocation. *The Journal of experimental biology*, 215(Pt 8), pp. 1384–93. doi:
518 10.1242/jeb.065201.

THERMAL STRESS AND CARBON BUDGET IN *P. DAMICORNIS*

25

- 519 Tremblay P *et al.* (2016) Heterotrophy promotes the re-establishment of photosynthate translocation in
520 a symbiotic coral after heat stress. Nature Publishing Group, Nature Publishing Group, 6, p. 38112. doi:
521 10.1038/srep38112.
- 522 Veal CJ *et al.* (2010) Shallow-water wave lensing in coral reefs: a physical and biological case study. The
523 Journal of experimental biology, 213(24), pp. 4304–4312. doi: 10.1242/jeb.044941.
- 524 Wangpraseurt D *et al.* (2012) Light gradients and optical microniches in coral tissues. Frontiers in
525 microbiology, 3(August), p. 316. doi: 10.3389/fmicb.2012.00316.
- 526 Wangpraseurt D *et al.* (2014) Lateral light transfer ensures efficient resource distribution in symbiont-
527 bearing corals. The Journal of experimental biology, 217(Pt 4), pp. 489–98. doi: 10.1242/jeb.091116.
- 528 Wangpraseurt D *et al.* (2015) Light microenvironment and single-cell gradients of carbon fixation in tissues
529 of symbiont-bearing corals. The ISME Journal, pp. 1–5. doi: 10.1038/ismej.2015.133.
- 530 Wangpraseurt D *et al.* (2017) In vivo Microscale Measurements of Light and Photosynthesis during Coral
531 Bleaching: Evidence for the Optical Feedback Loop ? Frontiers in microbiology, 8(January), pp. 0–12. doi:
532 10.3389/fmicb.2017.00059.
- 533 Weis VM (2008) Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis.
534 Journal of Experimental Biology, 211(19), pp. 3059–3066. doi: 10.1242/jeb.009597.
- 535 Wellington GM (1982) An experimental analysis of the effects of light and zooplankton on coral zonation.
536 Oecologia, 52(3), pp. 311–320. doi: 10.1007/BF00367953.
- 537 Yonge CM & Nicholls AG (1931) Studies on the physiology of corals. IV. The structure, distribution and
538 physiology of the zooxanthellae. Scientific reports of The Great Barrier Reef Expedition I., pp. 135–176.
539

Figures and Tables

Figure 1 | Gross O₂ production in *Pocillopora damicornis* as a function of increasing downwelling photon irradiance as measured with O₂ microsensors on a polyp scale (a-b) and with O₂ gas exchange measurements of whole coral fragments in incubation chambers (c-d). Measurements were performed

THERMAL STRESS AND CARBON BUDGET IN *P. DAMICORNIS*

27

on daily fed corals (a, c) and unfed corals (b, d) for each of three temperature treatments; T_0 (control, solid lines), T_1 (3 days at 30°C; dashed lines) and T_2 (8 days at 30°C; dotted lines). Symbols with error bars represent mean $\pm$ SEM ($n = 5-12$).

549

THERMAL STRESS AND CARBON BUDGET IN *P. DAMICORNIS*

28

550 Figure 2 | Net O₂ production and consumption in *Pocillopora damicornis* as a function of increasing
 551 downwelling photon irradiance measured with O₂ microsensors on a polyp scale (a-b) and with O₂ gas
 552 exchange measurements of whole fragments in incubation chambers (c-d). Measurements were
 553 performed on daily fed corals (a, c) and unfed corals (b, d) for each of three temperature treatments; T₀
 554 (control, solid lines), T₁ (3 days at 30°C; dashed lines) and T₂ (8 days at 30°C; dotted lines). Symbols with
 555 error bars represent means ± SEM (n = 12).

556

557

558

559

560

561

562

563

564

565

566

567

THERMAL STRESS AND CARBON BUDGET IN *P. DAMICORNIS*

29

Table 1 | Mass balance of photosynthate translocation and carbon budget for fed and unfed *Pocillopora damicornis* as a function of time under heat stress. Incorporation rates of carbon are based on ^{13}C -isotope incubation experiments using a pulse period of 4 hours. See Figure 3 for a schematic overview of the carbon budget and abbreviations used. Data represent means $\pm$ SEM ($n = 3-5$) and values in brackets have the unit $\mu\text{g C cm}^{-2} \text{ h}^{-1}$.

Parameter	Fed T_0	Fed T_1	Fed T_2	Unfed T_0	Unfed T_1
P_c	100 % (11.1 $\pm$ 1.9)	100 % (17.5 $\pm$ 6.6)	100 % (32.6 $\pm$ 6.5)	100 % (3.36 $\pm$ 2.1)	100 % (8.77 $\pm$ 2)
T_s	85.9 $\pm$ 0.02 % (9.5 $\pm$ 0.002)	93.5 $\pm$ 0.02 % (16.3 $\pm$ 0.003)	93.1 $\pm$ 0.008 % (30.3 $\pm$ 0.003)	85.7 $\pm$ 0.05 % (2.88 $\pm$ 0.002)	94.6 $\pm$ 0.02 % (8.30 $\pm$ 0.002)
ρ_s	0.11 $\pm$ 0.02 % (0.012 $\pm$ 0.002)	0.13 $\pm$ 0.02 % (0.022 $\pm$ 0.003)	0.034 $\pm$ 0.008 % (0.011 $\pm$ 0.003)	0.11 $\pm$ 0.04 % (0.04 $\pm$ 0.002)	0.054 $\pm$ 0.02 % (0.005 $\pm$ 0.002)
ρ_H	0.6 $\pm$ 0.05 % (0.067 $\pm$ 0.05)	0.147 $\pm$ 0.02 % (0.026 $\pm$ 0.003)	0.112 $\pm$ 0.02 % (0.036 $\pm$ 0.006)	0.50 $\pm$ 0.05 % (0.017 $\pm$ 0.002)	0.21 $\pm$ 0.05 % (0.018 $\pm$ 0.004)
R_s	14 $\pm$ 7.1 % (1.54 $\pm$ 0.78)	6.4 $\pm$ 0.6 % (1.12 $\pm$ 0.07)	6.91 $\pm$ 2.3 % (2.25 $\pm$ 0.76)	14.2 $\pm$ 24 % (0.48 $\pm$ 0.8)	5.3 $\pm$ 0.8 % (0.46 $\pm$ 0.07)
R_H	10.1 $\pm$ 2 % (1.12 $\pm$ 0.3)	41.3 $\pm$ 2 % (7.22 $\pm$ 0.3)	35.7 $\pm$ 5 % (11.6 $\pm$ 1.6)	38.9 $\pm$ 3 % (1.31 $\pm$ 0.1)	53.234 $\pm$ 1 % (4.668 $\pm$ 0.1)
ρ_{TOC}	75.8 $\pm$ 0.01 % (8.32 $\pm$ 0.005)	52.1 $\pm$ 0.01 % (9.08 $\pm$ 0.005)	57.4 $\pm$ 0.01 % (18.7 $\pm$ 0.008)	46.6 $\pm$ 0.05 % (1.56 $\pm$ 0.003)	41.3 $\pm$ 0.02 % (3.61 $\pm$ 0.002)

Figure 3 | Schematic overview of photosynthate translocation and the carbon budget overall. P_c is the total amount of inorganic C fixed through photosynthesis, hence 100 % fixed for all treatments and time points (see Table 1 for detailed data). T_s is the C translocated from symbiont to coral host, ρ is the C incorporated into the symbionts (ρ_s) and coral host (ρ_H). R is the C used for respiration by symbionts (R_s) and by the coral host (R_H). Finally, ρ_{TOC} is the total amount of C lost from the holobiont as organic carbon.

THERMAL STRESS AND CARBON BUDGET IN *P. DAMICORNIS*

30

Figure 4 | Cell specific gross photosynthetic O₂ production rates as a function of incident photon irradiance measured on intact fragments of fed and unfed *Pocillopora damicornis* (*in vivo*, panel a) and freshly isolated zooxanthellae from *P. damicornis* (*in vitro*, panel b) at increasing exposure time to heat stress. Blue columns represent measurements on fed corals, and red columns measurements on unfed corals. Data are means $\pm$ SEM (n= 3-5).