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Light Respiratory Processes and Gross Photosynthesis in Two Scleractinian Corals

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Abstract

The light dependency of respiratory activity of two scleractinian corals was examined using O_2 microsensors and CO_2 exchange measurements. Light respiration increased strongly but asymptotically with elevated irradiance in both species. Light respiration in *Pocillopora damicornis* was higher than in *Pavona decussata* under low irradiance, indicating species-specific differences in light-dependent metabolic processes. Overall, the coral *P. decussata* exhibited higher CO_2 uptake rates than *P. damicornis* over the experimental irradiance range. *P. decussata* also harboured twice as many algal symbionts and higher total protein biomass compared to *P. damicornis*, possibly resulting in self-shading of the symbionts and/or changes in host tissue specific light distribution. Differences in light respiration and CO_2 availability could be due to host-specific characteristics that modulate the symbiont microenvironment, its photosynthesis, and hence the overall performance of the coral holobiont.

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Introduction

The success of scleractinian corals in oligotrophic tropical waters is based on the endosymbiosis between the coral host and single-celled microalgae, i.e., dinoflagellates in the genus *Symbiodinium* that reside within the host's endodermal cells. The algal symbionts translocate up to 95% of their photosynthetically fixed carbon (C) to the coral host under optimal conditions [1], whilst the algal symbionts receive nutrients and shelter from the host [2,3]. There is considerable genotypic variation within the *Symbiodinium* genus [4] that can modulate the stress resilience of the holobiont [5].

The dark reactions of photosynthesis fix CO_2 into organic carbon using the enzyme Ribulose-1,5-bisphosphate-carboxylase/oxygenase (RuBisCO). *Symbiodinium* contains a prokaryotic-type II RuBisCO, which has a low affinity for CO_2 [6–9]. High concentrations of CO_2 are therefore necessary to promote carbon assimilation and to meet the hosts' energetic demand for symbiont-derived photosynthates [10–12]. Holobiont respiration may present an additional internal CO_2 source contributing to the complex carbon exchange and transfer system within corals. Chlororespiration, involving plastoquinone (PQ) oxidation with O_2 and a terminal oxidase (PTOX) [13] can be active within the chloroplasts of *Symbiodinium*. Furthermore, calcification occurring in the calicodermis of the coral [14] and host mitochondrial respiration can further contribute to the internal CO_2 supply in the holobiont [15,16].

Coral host respiration is just one source of inorganic carbon for symbiont photosynthesis [17–19]; external inorganic carbon sources such as seawater are also utilised. However, the supply of inorganic carbon via passive diffusion from the surrounding seawater and host tissue is restricted by several factors: 1) the generally low CO_2 content of seawater, 2) the presence of a diffusive boundary layer, and 3) the presence of multiple membranes of the host tissue surrounding the endodermal *Symbiodinium* cells, which need to be traversed. Both, coral host and symbionts employ a range of carbon concentrating mechanisms (CCMs) [20–24] to enhance the carbon supply from the external medium and thus increase CO_2 availability to the *Symbiodinium* chloroplasts [25] as well as for calcification purposes [26].

The rate of photosynthesis by the symbionts and therefore their carbon demand is closely correlated with photon irradiance [27], and may become carbon limited under high irradiance [28]. As the delivery of carbon to the algal symbionts is controlled by the activity of CCMs (of coral host as well as algal symbionts), as well

as host respiration [19], the host metabolism can thus have a strong impact on symbiont photosynthesis, e.g., by supplying sufficient inorganic carbon under high irradiance. While demands on the host-supplied carbon shift with irradiance, e.g., due to extra demand in light-enhanced calcification [29], there are only few experimental investigations of such responses in the literature [26,30]. We investigated if respiratory-dependent processes in the coral would follow a typical asymptotic rise with increasing irradiance, as it is known for photosynthetic processes.

Photosynthesis and calcification require carbon as substrate [31,32]; photosynthesis is directly dependent on light and coral calcification is known to be light-enhanced [33,34]. Indeed, there is a close interplay of internal utilization of metabolically derived carbon for both processes. Carbonic anhydrase enzymes catalyse the reaction $CO_2+H_2O \leftrightarrow HCO_3^-+H^+$, and therefore generate substrate for the calcification reaction ($CO_2+H_2O+Ca^{++} \leftrightarrow CaCO_3+2H^+$), as well as for photosynthesis: $CO_2+H_2O \leftrightarrow CH_2O+O_2$ [35,36].

The exchange of respiratory gases (O_2 and CO_2) in photosynthetic symbioses is difficult to study in the light because respiratory O_2 uptake is masked by the O_2 production from photosynthesis. At low irradiance, where symbiont photosynthesis is lower than respiratory activity in the coral, i.e., below the irradiance compensation point net O_2 uptake and CO_2 release can be measured [37]. To measure these gas exchange patterns in corals is challenging, as several discrete 'compartments' of respiration operate in parallel and in close proximity, and therefore there is a close coupling between autotrophic and heterotrophic processes [38].

Enhanced post-illumination dark respiration (EPIR), which is the respiratory activity measured just after transition from light to darkness, has been used to support assumptions about light-driven respiratory processes in corals [16,34]. However, in the absence of light there is no production of reducing agents due to the absence of photosynthetic light reactions, so that EPIR likely underestimates light respiration. To quantify respiration in the light, O_2 microsensors can be used to quantify gross photosynthesis rates ($GP_{O2\ micro}$) in corals independent of respiration [14,39,40]. In conjunction with flux calculations of the net photosynthetic rate ($Pnet_{O2\ micro}$) from measured steady-state O_2 concentration profiles, microsensor measurements allow for the determination of respiration rates in the light [41].

In this study, we present the first direct measurements of light respiration in corals as a function of irradiance. We combine O_2 microsensor measurements with detailed CO_2 exchange measurements to assess the relationship between CO_2 exchange and symbiont gross photosynthesis rates in two scleractinian corals, *Pocillopora damicornis* (Linnaeus, 1758) and *Pavona decussata* (Dana, 1846), that are known to harbour the same *Symbiodinium* subclade (C1) [42]. The light dependency of external carbon uptake and respiratory activity was also examined, to see if respiratory processes followed an asymptotic rise with irradiance similar to photosynthetic processes.

Materials and Methods

Coral collection and preparation

Specimens of *Pocillopora damicornis* (Pocilloporidae) and *Pavona decussata* (Agariciidae) were collected from Heron Island reef flat (23° 26′ 60 S, 151° 55′ 0 E) (Great Barrier Reef Marine Park Authority collection permit G09/30854.1) and maintained for up to 2 months at the University of Technology Sydney. The coral *P. damicornis* is finely branched and highly sensitive to environmental factors that cause bleaching, while *P. decussata* is foliaceous (plate-like) and tolerant to environmental factors that cause bleaching [43]. After fragmentation of coral colonies, a number of similar sized pieces (average surface area: 28.6 ± 11.3 cm^2 and 23.5 ± 7.2 cm^2 for *P. damicornis* and *P. decussata*, respectively; mean ±s.e.m.; n = 3–4) were fixed with non-toxic epoxy (AquaKnead, Selleys, Australia) to sample holders. Corals were kept at 26 ± 1°C under irradiance of ~40 µmol photons m^{-2} s^{-1} (12 h: 12 h, light: dark cycle) in aquaria with recirculating artificial seawater (ASW; Aquasonic, Australia; salinity of 33 and a carbonate content of 140 ppm).

Experimental setup

We used a novel instrumental array, a photobioreactor (PBR) (Gademann Instruments GmbH, Effeltrich, Germany), combining two metabolic gas exchange measuring techniques (O_2 exchange and CO_2 exchange). Only CO_2 measurements are presented in this study. The setup consisted of a closed, continuously stirred thermostated chamber with a known volume of seawater containing a coral sample and an overlaying headspace [44]. The CO_2 content in the overlying headspace of the chamber was measured on a calibrated infrared gas analyser (IRGA; MGA3000, ANRI instruments, Ferntree Gully, Victoria, Australia) with a 1 s sampling frequency. The sample chamber had a vertically mounted 'warm white' LED panel (NS2L123BT, Nichia, Japan) with 96 single-spot LEDs capable of applying up to 1500 µmol photons m^{-2} s^{-1} at the sample surface.

Dissolved CO_2, as well as incident irradiance and temperature were measured for each specimen held in the PBR chamber. During PBR operation, the gas-phase effervesced through the liquid-phase to equilibrate dissolved CO_2. CO_2 concentration changes within the headspace of the PBR chamber were estimated according to Henry's gas law, which states that at a constant temperature and pressure the gas content between gas- and liquid-phase will move into a steady-state equilibrium. Measured CO_2 concentrations (ppm) in the headspace were therefore used to calculate molar changes of CO_2. The molar volume of CO_2 (Vn) in the seawater was determined as follows:

$$Vn = (R_{co2} \times T) \div P \quad (1)$$

where R_{CO2} is the specific CO_2 gas constant 188.9 m^3 Pa K^{-1} mol^{-1}, T is the incubating temperature 26°C (299.15 K), and P is the ambient atmospheric pressure at sea level 1000 Pa [45]. In the measurement setup, Vn = 56.5 m^3 mol^{-1}. By dividing Vn with the molar mass of CO_2 (44.01 g mol^{-1}) the molar volume of CO_2 per 1 ppm was then determined to be M = 1.3 mg m^3. The measured CO_2 concentrations in units of ppm could thus be converted to metric units and further into molar flux rates considering molar mass, the time of incubation, the volume of the gas-phase of the PBR, as well as the coral surface area. CO_2 exchange was expressed as nmol CO_2 cm^{-2}s^{-1}.

Experimental protocol

At the beginning of the experiment, each coral specimen was incubated for ~20 min in the PBR to account for the establishment of equilibrium between gas- and liquid- phase.

Photosynthesis–irradiance (P–E) curve measurements for *P. damicornis* (n = 4) and *P. decussata* (n = 3) began with a dark incubation to determine dark CO_2 respiration rates followed by subsequent illumination using 9 photon irradiance levels (10, 20, 40, 78, 210, 360, 560, 780 and 1100 µmol-photons-m-2-s-1). Each illumination period lasted for 20 min and was followed by a 20 min dark incubation period. Incubation times were chosen to

account for equilibration of gas- and liquid-phase. Gas exchange readings were taken from the last 5 min of each incubation interval.

Net CO_2 uptake, measured during the light in the PBR, as well as respiratory CO_2 production, measured during the dark in the PBR, were used to estimate gross CO_2 exchange ($G_{CO2\ PBR}$). For an overview of parameters see Table 1.

Oxygen microsensor measurements

We used O_2 microsensors to quantify gross and net photosynthesis under a set of increasing photon irradiance levels. Corals were placed in an acrylic flow-through chamber (flow velocity ~1 cm s^{-1}) [46] with aerated, artificial seawater (see above). Samples were illuminated vertically using a fiber-optic tungsten-halogen lamp equipped with a heat filter and a collimating lens (KL-2500, Schott GmbH, Germany). The O_2 microsensors were mounted on a PC-controlled motorized micromanipulator for automatic profiling (Pyro-Science GmbH, Germany) at an angle of 20° relative to the vertical incident light. A detailed description of the microsensor setup can be found in Wangpraseurt et al. [46]. Microscale O_2 measurements were performed with Clark-type O_2 microsensors (tip size: 25 µm; stirring sensitivity: <1%, 90% response time: <0.5 s; Unisense A/S, Aarhus, Denmark). Given that this high-precision technique requires more measuring time, only six photon irradiance levels (0, 40, 80, 210, 550, 1100 µmol photons $m^{-2}s^{-1}$) were applied for 20 min each (matching irradiance levels used in the PBR). At each irradiance, net and gross photosynthesis rates were determined by measuring steady-state O_2 concentration profiles and O_2 concentration dynamics under light-dark shifts, respectively [39,40]. The O_2 concentration profiles were measured from the coral surface upwards into the water column in vertical steps of 40 µm. Light-dark shifts were conducted from the coral surface down to the coral skeleton, which covered a distance of ~80 µm for both species. The position of the sensor on the skeleton surface was identified as a slight bending of the microsensor. For each fragment, three locations at least 2 cm apart were randomly chosen and measurements were averaged. Measurements were exclusively conducted on the coenosarc (tissue connecting polyps) to minimize the influence of tissue movement [39].

Net O_2 exchange fluxes were calculated from the measured steady-state concentration profiles using Fick's first law of diffusion with a molecular diffusion coefficient for O_2 of 2.241×10^{-5} cm^2 s^{-1} (25°C and salinity 33) [47]. Area-specific gross photosynthesis rates ($GP_{O2\ micro}$) were obtained by dividing the measurements of volume-specific GP with the thickness of the tissue, i.e. 80 µm (see above). The light respiration rate ($R_{light\ O2\ micro}$) was then calculated by subtracting the area-specific $GP_{O2\ micro}$ and net photosynthesis rate ($Pnet_{O2\ micro}$):

$$R_{light\ O2\ micro} = GP_{O2\ micro} - Pnet_{O2\ micro} \quad (2)$$

Biometric measures

Following gas exchange measurements, coral specimens were snap frozen in liquid N_2 for subsequent determinations of algal symbiont density, chlorophyll concentration and protein content. Once removed from the liquid N_2, corals were transferred to a 100 mL Erlenmeyer flask and kept on ice, with 15 mL of homogenization buffer (4°C) consisting 1 mM phenylmethylsulfonyl fluoride (protease inhibitor) in 0.2 µm-filtered seawater (FSW). The flask was sealed with Parafilm and shaken for 10 min by hand in a circular motion, allowing the coral tissue to be torn off the skeleton. The resulting liquid was homogenized on ice (Ultra-Turrax, Ika, Rawang, Malaysia) for 30 s. The homogenate was centrifuged at 700 g for 5 min at 4°C and the resulting pellet of *Symbiodinium* was retained for algal cell density counts and for chlorophyll concentration analyses (see below). The supernatant contained coral tissue remains, of which 2 mL were sampled for protein content determination using the Bradford assay, with bovine serum albumin standards [48]. Protein assay absorbance was measured at 595 nm with a 96-well plate reader (Bio Rad Bench Mark Plus spectrophotometer, Hercules, California, USA) and analysed using the Microplate Manager Software (Bio Rad, Hercules, California, USA).

The *Symbiodinium* pellet was re-suspended in 4 mL of FSW and subsamples were taken for algal symbiont counts according to Edmunds and Gates [49]. The algal suspension was again centrifuged at 1789 g and the pellet re-suspended in 3 mL of 90% acetone and incubated for 24 h at 4°C to extract pigments. Chlorophyll *a* and c_2 concentrations were measured using a spectrophotometer (Cary UV-VIS, Agilent Technologies, Australia) using absorbance readings according to Ritchie [50]. The coral skeleton surface area was determined using the single-dip paraffin wax technique [51].

Statistical analyses

Differences in biometric parameters between the two coral species were analysed using Student's t-test (t; $\alpha = 0.05$). Differences in respiratory rates were determined by using univariate one-way and two-way analysis of variance (ANOVA; F; $\alpha = 0.05$). ANOVA assumptions for normal distribution and homogeneity of variance were tested using Shapiro Wilk and Levene's tests, respectively. Tukey's honest significant difference (HSD) test (t;

Table 1. Overview of abbreviations and definition of gas exchange parameters from analyses with the photobioreactor (PBR) and from microsensor measurements.

Abbreviation	Parameter	Definition
$GP_{O2\ micro}$	*In hospite* gross O_2 production	Measured using microsensor within the coral tissue as a direct measure
$Pnet_{O2\ micro}$	Net photosynthetic O_2 production	Measured using microsensor above the coral tissue e.g. including O_2 uptake processes
$R_{light\ O2\ micro}$	Light O_2 respiration	Measured using microsensor measurements; determined through calculation of net and gross O_2 production
$R_{dark\ O2\ micro}$	Steady-state O_2 dark respiration	Measured using microsensor within the coral tissue as a direct measure after sufficient dark incubation; respiratory O_2 consumption
$G_{CO2\ PBR}$	Gross CO_2 exchange	Measured with the PBR; determined as the sum of net and respiratory CO_2 exchange

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$\alpha = 0.05$) was used for post-hoc comparison of means to identify differences at 95% confidence interval. All statistical analyses were carried out using Statistica 10 (Statsoft Inc., Tulsa, OK, USA).

Results

Biometric measures

The two coral species differed in protein content, total chlorophyll (Chl; Chl $a+c_2$) concentration, as well as algal symbiont cell density (Table 2). The coral *P. decussata* displayed a significantly higher protein concentration than *P. damicornis* ($t(6) = 3.925$, $p = 0.026$). Further, *P. decussata* contained more than twice the total Chl concentration (mg cm^{-2}) ($t(6) = 3.83$, $p = 0.009$) and harboured significantly higher algal cell densities than *P. damicornis* ($t(6) = 2.73$, $p = 0.034$). However, *Symbiodinium* in both species contained similar amounts of total Chl $cell^{-1}$.

Gross CO_2 exchange

For *P. damicornis*, gross CO_2 uptake from the seawater declined up to an irradiance of 78 μmol photons m^{-2} s^{-1} (one-way ANOVA, $F(8, 27) = 2.90$, $p = 0.018$; Fig. 1 A), and then increased slightly up to an irradiance of 560 μmol photons m^{-2} s^{-1} followed by a small but significant decline at irradiances of 780 and 1100 μmol photons m^{-2} s^{-1} (Tukey HSD, $p<0.05$; Fig. 1 A, also see Table S1). In contrast, gross CO_2 uptake of *P. decussata* showed no decline in the first phase of illumination (10 and 20 μmol photons m^{-2} s^{-1}). Gross CO_2 uptake increased from an irradiance of 40 up to 78 μmol photons m^{-2} s^{-1} and then remained steady besides a dip at an irradiance of 560 μmol photons m^{-2} s^{-1} (Fig. 1 B). The metabolic activity differed most significantly between the two species under low irradiance (pooled CO_2 rates for irradiances of 10–40 μmol photons m^{-2} s^{-1}; $t(22) = 3.54$, $p<0.001$; Figs. 1 A and B, also see Table S1).

Microsensor measurements of gross photosynthesis, $GP_{O2\ micro}$ in *P. damicornis* revealed maximum rates of 0.502 ± 0.017 nmol O_2 cm^{-2} s^{-1} at irradiances of 210 μmol photons m^{-2} s^{-1} (one-way ANOVA, $F(4,5) = 115.06$, $p<0.001$; Tukey HSD; $p<0.05$; Fig. 1 A, also see Table S1). In *P. decussata*, $GP_{O2\ micro}$ increased more gradually reaching a maximum at an irradiance of 560 μmol photons m^{-2} s^{-1} (one-way ANOVA, $F(4,5) = 8.9182$, $p = 0.017$; Tukey HSD, $p<0.05$) with an average $GP_{O2\ micro}$ rate of 0.527 ± 0.020 nmol O_2 cm^{-2} s^{-1} (Fig. 1 B). In both species we did not detect down-regulation of $GP_{O2\ micro}$ at above saturating irradiance levels (i.e. up to 1100 μmol photons m^{-2} s^{-1}).

Respiration

Light respiration ($R_{light\ O2\ micro}$) increased with increasing irradiance (Fig. 2), with a maximum $R_{light\ O2\ micro}$ of ~0.5 nmol O_2 cm^{-2} s^{-1} for both coral species (one-way ANOVA, $F(5,6) = 10.26$; $p = 0.007$ for *P. decussata* and $F(5) = 101.08$; $p<0.001$ for *P. damicornis*). However, $R_{light\ O2\ micro}$ increased more rapidly with irradiance in *P. damicornis* than in *P. decussata* (Fig. 2, also see Table S1).

A comparison of $R_{light\ O2\ micro}$ with $R_{dark\ O2\ micro}$ revealed a strong light response at photon irradiances >210 μmol photons m^{-2} s^{-1} in both species (data not displayed). Where the increase in light-driven respiration rates compared to dark respiration rates was greater in *P. damicornis* than it was found for *P. decussata*. For example, at 210 μmol photons m^{-2} s^{-1} light respiration increased 25 times in *P. damicornis* but only 11 times in *P. decussata*.

The ratio of $R_{light\ O2\ micro}$ to microsensor derived gross photosynthesis ($GP_{O2\ micro}$) differed between the two species. The maximum $R_{light\ O2\ micro}$ constituted ~97% of $GP_{O2\ micro}$ in *P. damicornis*, while it only accounted for ~88% in *P. decussata*.

Discussion

This is the first study reporting an integrated approach measuring coral light respiration and gross photosynthesis with O_2 microsensors and CO_2 gas exchange techniques across a range of irradiance. The two main finding of this study are that i) light-saturated (at 210 μmol photons m^{-2} s^{-1}) respiration rates ($R_{light\ O2\ micro}$) were multiple times higher than steady-state dark respiration rates ($R_{dark\ O2\ micro}$) (11 times for *P. decussata* and 25 times for *P. damicornis*, and ii) *P. damicornis* and *P. decussata* differ in their photophysiological function despite likely harbouring the same symbiont subclade C1 [42] (see Fig. 3 for a conceptual diagram of the main findings).

Sufficient supply of CO_2 to the algal symbionts is of paramount importance for the functioning of a coral symbiosis [18,52,53], where an increased supply enhances photosynthesis [31]. Gross photosynthesis rates ($GP_{O2\ micro}$) were similar for both coral species across the applied irradiance levels. However, gross CO_2 uptake rates, as well as algal symbiont density were generally higher in *P. decussata* (Fig. 1 B). These results raise the question as to why a coral with twice as many symbionts and greater CO_2 uptake (*P. decussata*) did not show a greater photosynthetic productivity. The coral *P. decussata* had a much greater protein biomass than the coral *P. damicornis* and the algal symbionts would have been more densely packed within the coral tissue. Self-shading of the algal symbionts [54], as well as species-specific differences in light propagation within the host tissue [46,55] could explain our findings for *P. decussata*. A model of how canopy-understory development can influence the photosynthesis-irradiance (P-I) relationship has previously been introduced [56]. Here we could expand that model to introduce the light respiratory activity as well as carbon uptake in relation to how canopy-

Table 2. Biometric measures of the hard corals *Pocillopora damicornis* and *Pavona decussata,* displaying total protein content (mg cm^{-2}), total chlorophyll per area (Chl $a+c_2$) (mg cm^{-2}), algal cell densities (cells cm^{-2}) and total Chl per cell (pg $cell^{-1}$) (n = 4; mean ±s.e.m.).

	Pocillopora damicornis	*Pavona decussate*
Total protein (mg cm^{-2})	1.19 ± 0.22	2.31 ± 0.36*
Chl (mg cm^{-2})	0.003 ± 0.001	0.007 ± 0.001*
Algal cell densities (cells cm^{-2})	$5.32\times10^5\pm1.91\times10^5$	$16.7\times10^5\pm2.00\times10^5$*
Total Chl (pg $cell^{-1}$)	9.735 ± 1.509	9.666 ± 2.463

*Significantly different values are indicated with an asterisk.

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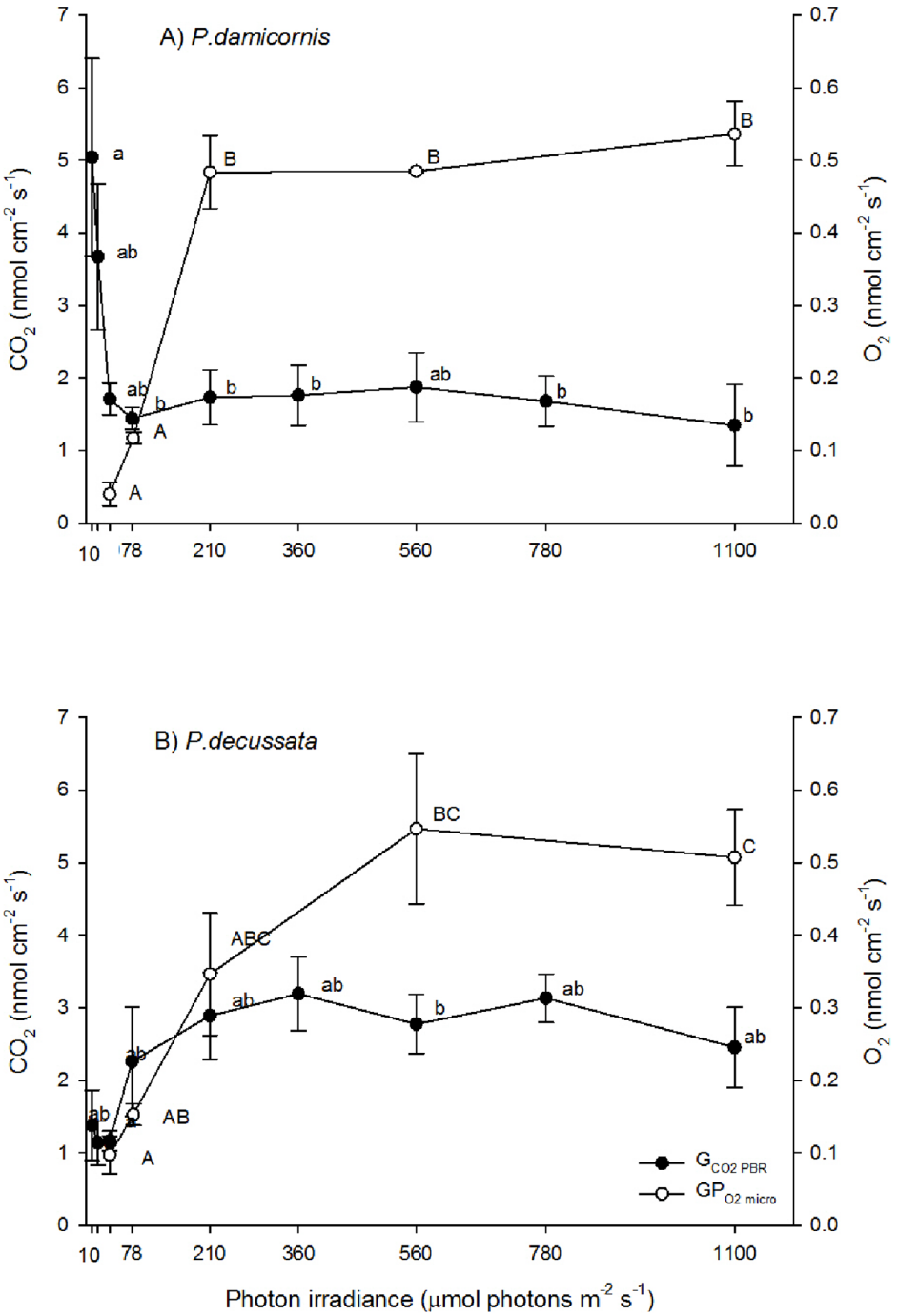

Figure 1. Variation in gas exchange measurements with irradiance. The graphs display gross CO_2 exchange ($G_{CO2\ PBR}$; black circles; CO_2 nmol cm^{-2} s^{-1}) and microsensor derived gross photosynthetic O_2 production ($GP_{O2\ micro}$; open circles; O_2 nmol cm^{-2} s^{-1}) of the hard coral species *Pocillopora damicornis* (A) and *Pavona decussata* (B) as a function of nine irradiances (mean ±s.e.m.; $G_{CO2\ PBR}$: n = 4 and $GP_{O2\ micro}$: n = 2); Tukey honest significant difference test results are indicated for $G_{CO2\ PBR}$ (lower case letters) and $GP_{O2\ micro}$ (capitals) ($p<0.05$).
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understory influences the P-I relationship in the two corals examined here (see Fig. 3).

Light respiration in *P. damicornis* reached its maximum at a lower irradiance than in *P. decussata* and exceeded dark respiration (Fig. 2). A higher proportion of $GP_{O2\ micro}$ was therefore contributed by light respiration in *P. damicornis* than in *P. decussata*. Our results suggest therefore that species-specific light-driven respiratory processes are active within the two coral species.

Light-driven respiration is often coupled to calcification in the calicodermis [14,29,33,36,57] and it seems possible that the calcification process accounts for a large fraction of the light respiration. For calcification to take place, O_2 and photosynthate are necessary so that the coral host can liberate adenosine-triphospate (ATP) for the calcifying process [58,59]. The

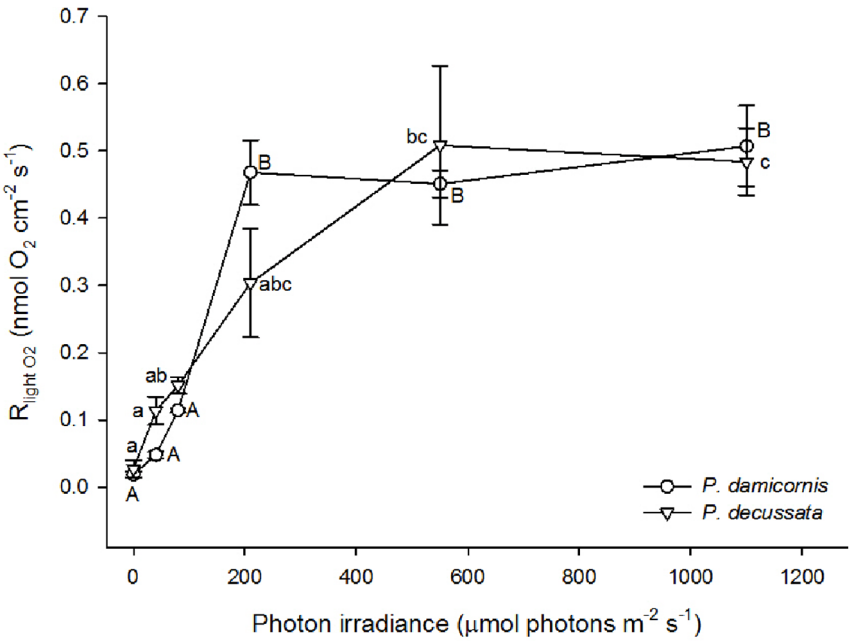

Figure 2. Light respiration ($R_{light\ O2\ micro}$) of the hard coral species *Pocillopora damicornis* (clear circle) and *Pavona decussata* (clear triangle) are displayed as a function of 6 irradiances (mean ±s.e.m.; n = 2). Tukey honest significance difference test results are indicated, where capital letters are describing groupings for *P. damicornis* and lower case letters groupings of *P. decussata* ($p<0.05$).
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hyperbolic increase in light respiration for both species, up to the maximum measured photon irradiance (1100 µmol photons m^{-2} s^{-1}; Fig. 2) suggests that host respiration is closely coupled to release of photosynthates from zooxanthellae. However, recent attempts to investigate calcification and light respiration rates in corals, using an indirect measuring technique, found that light respiration increased the most in zooxanthellae as opposed to the coral host [60]. Given these results, it seems more likely that metabolic activity supporting calcification, e.g., *Symbiodinium*'s photosynthetic reaction and carbon fixation, are responsible for most of the increase in light respiration. Calcification itself is a positive feedback mechanism for *Symbiodinium* photosynthesis, as CO_2 is being produced during skeleton accretion [29]. Both species showed steady and light-independent gross CO_2 uptake rates at >78 µmol photons m^{-2} s^{-1}, where calcification could then fuel the photosynthetic activity through internal carbon

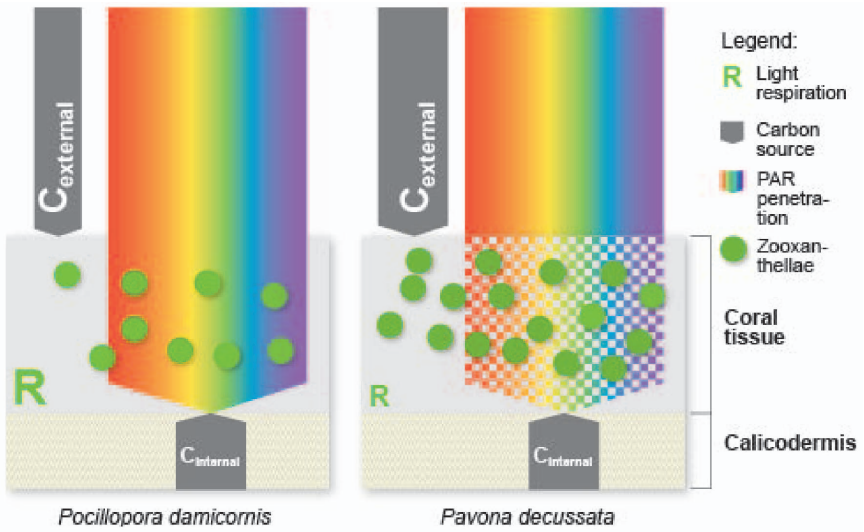

Figure 3. Conceptual model of light and carbon availability, in the two hard coral species, *Pocillopora damicornis* and *Pavona decussata* in moderate light (~100 µmol photons m^{-2} s^{-1}). The schematic diagram of a coral shows the coral tissue containing algal symbionts (green circles), which lies above the calicoblastic layer. Photosynthetic active radiation (PAR) (rainbow arrow) penetrates the coral tissue. In *P. decussata* a higher density of symbionts reduced light availability compared to *P. damicornis*. Dissolved inorganic carbon (grey arrows; quantity is relative to arrow thickness) can originate from internal sources such as the calicoblastic layer or from the external environment, where *P. decussata* draws stronger on the external carbon uptake. Light respiration (R) (strength indicated through size), was greater in *P. damicornis* than in *P. decussata*.
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release. However, the recently proposed 'proton flux hypothesis' [36], where the shedding of protons generated during the calcification process is proposed to result in a lag of CO_2 uptake could also explain our results. Whether light respiration is simply controlled by the availability and source of carbon substrates or other metabolic controls remains to be investigated.

In both corals, *P. damicornis* and *P. decussata*, light-saturated respiration rates ($R_{light\ O2\ micro}$) at 210 µmol photons $m^{-2}\ s^{-1}$ were similar. Light stimulated respiration in *P. damicornis* increased to a greater degree than that in *P. decussata* (25 versus 11 times). Light-saturated respiration rates in both species reached an asymptotic value of 5 nmol $cm^{-2}\ s^{-1}$ at photon irradiances > 210 µmol photons $m^{-2}\ s^{-1}$ (Fig. 2). The strong increase of respiration rates during the light as compared to steady-state dark respiration rates are most likely due to the low-light acclimation of the experimental corals (40 µmol photons $m^{-2}\ s^{-1}$). Dark respiration rates are generally dependent upon pre-experimental incubation irradiances [61,62]. Under low light adaptation steady-state dark respiration rates are low but once exposed to light, the metabolic activity increases and so do light respiration rates and other oxygen uptake processes. The magnitude of this increase is independent on the pre-experimental incubation irradiance [62].

Photoacclimation is a process of morphological (here in terms of coral host) and physiological adjustments of a phototrophic organism towards growth irradiances. Pigmentation (coral host pigmentation [63] and light harvesting pigments such as accessory pigments and chlorophyll [64]), as well as photochemical quenching capacity (xanthophyll pool [65,66]) can be increased and decreased in abundance and concentrations. During high light exposure these adjustments help acclimatization in the phototroph only to some extend, and as a result, high light stress results in the accumulation of reactive oxygen species [67], the stimulation of alternative electron transport systems [68,69], often consuming oxygen, and of photorepair mechanisms [70,71]. The cost of all these processes results in low net photosynthesis [62], due to increased respiration and other oxygen uptake [39,72]. The light source in the experiments of this study excluded the naturally occurring ultraviolet radiation, which corals experience in the field and which is a major cause of photodamage [73,74]. Translating our findings to corals in the field, the increase of oxygen uptake rates on going from dark to light (or from low to high light) might therefore not be as great as found in this study; however, once photorepair processes are entrained the actual oxygen uptake rates might be just as high or even higher.

Pronounced stimulation of respiration in light has been reported for the coral species *Galaxea fascicularis*, where light respiration was ~12 times higher than dark respiration under an irradiance of 140 µmol photons $m^{-2}\ s^{-1}$ [14]. Kühl et al. [39] observed values of light respiration to be ~6 times higher than during dark respiration in *Favia* sp. under an irradiance of 350 µmol photons $m^{-2}\ s^{-1}$. Here light respiration accounted for 77% of the gross photosynthetic O_2 production. The differing increase of respiration rates from dark to light between the reporting studies and our results are probably due to species differences and differential pre-experimental and experimental irradiances. In our study light respiration accounted for 88% of gross photosynthetic O_2 production in *P. decussata* and 97% of gross photosynthetic O_2 production in *P. damicornis* at 210 µmol photons $m^{-2}\ s^{-1}$. Maximum gross photosynthetic O_2 production were on average ~0.53 nmol $O_2\ cm^{-2}\ s^{-1}$ for both coral species (Fig. 1) and were of a similar magnitude to other microsensor measurements of gross photosynthesis rates in corals [75].

Light dependent increase in O_2 consumption through respiratory processes has been discussed previously [68]. Tchernov et al. [68] concluded that ongoing activity of the MAP cycle could be accounted for by the increased O_2 uptake with increasing photon irradiance. Indeed, various light-driven O_2 consuming processes, such as photorespiration [76,77] and the MAP cycle [68,78,79] could also be involved in the high level of light respiration observed here. However, the activity of the MAP cycle does not result in net O_2 concentration changes [78]; it therefore cannot be measured in O_2 exchange measurements with microsensors [80]. Hence, we conclude that the only other process to explain the light respiration results apart from light-stimulated mitochondrial O_2 uptake is photorespiration, involving oxygenase activity of RuBisCO [81]. However, further investigations are needed to verify and describe these processes.

Conclusions

Light-saturated respiration rates ($R_{light\ O2\ micro}$) were similar in both corals and multiple times higher than steady-state dark respiration rates ($R_{dark\ O2\ micro}$). This is interpreted as the activity of light-driven metabolic pathways that increase with increasing irradiance. The light respiration rates show, that differential CO_2 uptake rates of the two species examined could indicate that carbon availability influences the metabolic processes of the holobiont. Although both coral hosts are known to harbour the same *Symbiodinium* subclade C1 [42], it seems that they experience different host-specific microenvironmental conditions (see Figure 3).

Supporting Information

Table S1 Gas exchange rates measured as a function of irradiance for *Pocillopora damicornis* and *Pavona decussata*. Following gas exchange rates are presented: $GP_{O2\ micro}$ – *In hospite* gross O_2 production (microsensor based), $Pnet_{O2\ micro}$ – net photosynthetic O_2 production (microsensor based), $R_{light\ O2\ micro}$ – light O_2 respiration (microsensor based), $G_{CO2\ PBR}$ – Gross CO_2 exchange for 6 light intensities.
(DOCX)

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Author Contributions

N/A. Conceived and designed the experiments: VS AWDL DW MK PJR. Performed the experiments: VS DW AWDL. Analyzed the data: VS DW AWDL. Contributed reagents/materials/analysis tools: VS DW MK PJR. Wrote the paper: VS DW RH MK AWDL PJR.

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