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RESEARCH ARTICLE

Interactive effects of oxygen, carbon dioxide and flow on photosynthesis and respiration in the scleractinian coral *Galaxea fascicularis*

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ABSTRACT

Rates of dark respiration and net photosynthesis were measured for six replicate clonal fragments of the stony coral *Galaxea fascicularis* (Linnaeus 1767), which were incubated under 12 different combinations of dissolved oxygen (20%, 100% and 150% saturation), dissolved carbon dioxide (9.5 and 19.1 µmol l^{-1}) and water flow (1–1.6 versus 4–13 cm s^{-1}) in a repeated measures design. Dark respiration was enhanced by increased flow and increased oxygen saturation in an interactive way, which relates to improved oxygen influx into the coral tissue. Oxygen saturation did not influence net photosynthesis: neither hypoxia nor hyperoxia affected net photosynthesis, irrespective of flow and pH, which suggests that hyperoxia does not induce high rates of photorespiration in this coral. Flow and pH had a synergistic effect on net photosynthesis: at high flow, a decrease in pH stimulated net photosynthesis by 14%. These results indicate that for this individual of *G. fascicularis*, increased uptake of carbon dioxide rather than increased efflux of oxygen explains the beneficial effect of water flow on photosynthesis. Rates of net photosynthesis measured in this study are among the highest ever recorded for scleractinian corals and confirm a strong scope for growth.

KEY WORDS: Coral, Oxygen, Flow, Carbon dioxide, Photosynthesis, Respiration

INTRODUCTION

The symbiosis between scleractinian corals and phototrophic dinoflagellates (zooxanthellae) has been studied intensively (reviewed in Furla et al., 2005; Weis, 2008; Osinga et al., 2012). Factors reported to influence the efficiency of photosynthetic processes inside corals include light (reviewed in Osinga et al., 2012), temperature (reviewed in Smith et al., 2005), nutrients (reviewed in Osinga et al., 2011) and gas exchange (e.g. Dennison and Barnes, 1988; Finelli et al., 2006). Gas exchange involves both diffusion of the primary substrate for photosynthesis, carbon dioxide, into the coral and diffusion of photosynthetically produced oxygen out of the coral tissue.

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The role of gas exchange in coral photosynthesis has been studied mainly in relation to water flow around the coral. Water flow influences the rate of diffusion of dissolved gases from the surrounding water into the coral tissue and vice versa (Patterson et al., 1991). Increased water flow reduces the width of a thin, stagnant water layer directly adjacent to the coral surface termed the diffusive boundary layer (DBL) (Vogel, 1994). The width of the DBL directly affects the rate of diffusion. In addition, diffusion rates depend on the steepness of the gas concentration gradient as described in Fick's first law of diffusion (Patterson et al., 1991):

$$J = -D\,\frac{\delta C}{\delta x}, \qquad (1)$$

where J is the diffusive flux (mol m^{-2} s^{-1}), D is the gas-specific diffusion constant (m^2 s^{-1}), C is the concentration of the gas (mol m^{-3}) and x is the diffusion distance (m).

Several authors have reported positive effects of water flow on photosynthesis in corals (Dennison and Barnes, 1988; Lesser et al., 1994; Nakamura et al., 2005; Finelli et al., 2006; Mass et al., 2010; Schutter et al., 2011). In a flume experiment with *Galaxea fascicularis*, Schutter et al. (2011) found an instantaneous increase in photosynthesis upon an increase in flow velocity from 5 to 20 cm s^{-1}, which supports the idea that the effects of flow on photosynthesis are related to an improved exchange of dissolved gases. However, it remains unclear whether this flow effect relates to a higher influx of carbon dioxide into the coral tissue (Dennison and Barnes, 1988; Lesser et al., 1994) or to a higher efflux of oxygen out of the coral tissue (Nakamura et al., 2005; Finelli et al., 2006; Mass et al., 2010; Schutter et al., 2011). Mass et al. (2010) demonstrated that the onset of flow led to a decrease of oxygen supersaturation from 500% to 200% inside illuminated tissue of the scleractinian coral *Favia veroni*. This decrease in internal oxygen was accompanied by an increase in the rate of net photosynthesis. Mass et al. (2010) concluded that a flow-induced, enhanced efflux of oxygen out of the coral tissue increases the efficiency of photosynthetic carbon fixation by reducing the rate of photorespiration. Photorespiration is a reaction in which oxygen instead of carbon dioxide binds to RuBisCO, the initial carbon dioxide-fixing enzyme in the Calvin–Benson–Bassham (CBB) cycle (Peterhansel et al., 2010). RuBisCO then converts oxygen and ribulose bisphosphate into 2-phosphoglycerate (2PG), instead of converting carbon dioxide and ribulose bisphosphate into 3-phosphoglycerate (3PG). Although 3PG can be regenerated from 2PG, this process requires several reaction steps, which consume more metabolic energy than regenerated 3PG can return after its uptake in the CBB cycle (Peterhansel et al., 2010). Hence, photorespiration decreases the efficiency of the photosynthesis process.

So far, only RuBisCO type II, which is the type of RuBisCO that is most sensitive to photorespiration, has been found in

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zooxanthellae (Rowan et al., 1996), which supports the view that flow modulates photosynthesis mainly through a reduction in photorespiration. Notwithstanding this, Smith et al. (2005) stated that photorespiration in corals is not likely to play a major role because of the presence of an efficient carbon-concentrating mechanism (CCM) in *Symbiodinium* (Leggat et al., 1999).

The contradicting views on the effects of dissolved oxygen on photosynthesis and photorespiration in corals and, consequently, on the mechanism that underlies flow-enhanced photosynthesis prompted us to address the following research questions. (1) How does the ambient oxygen concentration in seawater affect photosynthesis in corals? (2) Does water flow stimulate photosynthesis in corals through stimulating the uptake of carbon dioxide or through reducing the negative effects of oxygen? To answer these questions, we studied the effect of flow on net photosynthesis and dark respiration in the stony coral *Galaxea fascicularis* (Linnaeus 1767) under three different levels of dissolved oxygen (hypoxia, 20% saturation; normoxia, 100% saturation; and hyperoxia, 150% saturation) and two different values for dissolved carbon dioxide (9.5 and 19.1 µmol l^{-1}), as reflected by two corresponding values for pH (8.1 and 7.84). The applied values for pH and oxygen saturation are within the range experienced by corals in nature: natural diel fluctuations in pH (8.7–7.8) and oxygen saturation (27–247%) have been reported for reef flats and tropical lagoons (Ohde and Van Woesik, 1999). Our results suggest that in *G. fascicularis*, flow stimulates photosynthesis through a more efficient uptake of carbon dioxide rather than through a decrease in photorespiration, whereas respiration is stimulated by flow through an increased uptake of oxygen.

MATERIALS AND METHODS

Corals

Fragments of a captive-bred specimen of the encrusting coral *G. fascicularis* (originally obtained under CITES no. 52139) were provided by Burgers' Zoo BV (Arnhem, The Netherlands). All experiments were conducted at Wageningen University (Wageningen, The Netherlands). No approval from an ethics committee was required as scleractinian corals are exempted from legislation concerning the use of animals for scientific purposes in the European Union (Directive 2010/63/EU). Six genetically identical fragments of *G. fascicularis* were obtained from one parent colony. The fragments were glued to PVC plates of 25 cm^2, and were allowed to recover from fragmentation for a period of 3 weeks before the start of the experiments. The coral fragments were held in an aquarium of 356 l, wherein flow velocity, temperature and salinity were maintained at constant values (flow velocity: 10–20 cm s^{-1}, temperature 25°C, salinity 35.0 g l^{-1}). Corals were subjected to a 12 h:12 h light:dark cycle at a quantum irradiance (E) of 280 µmol quanta m^{-2} s^{-1}. Alkalinity and calcium concentrations were measured 5 times a week and were adjusted when necessary by adding $NaHCO_3$ and $CaCl_2{\cdot}H_2O$, respectively. Total alkalinity was maintained at the current natural oceanic value of 2.4 mequiv. l^{-1} and calcium at 400 mg l^{-1}. Corals were fed 30 ml of a concentrated suspension of freshly hatched *Artemia* nauplii (~3000 nauplii ml^{-1}) on a daily basis. Artificial seawater was made with enriched-blend, phosphate- and nitrate-free Reef Crystals (Aquarium Systems, Sarrebourg, France) and was aerated a few days before use.

Prior to the incubations, the projected surface area of the coral fragments was determined according to Wijgerde et al. (2012a) from top-view pictures of the fragments using the software Image Tool for Windows.

Experimental design, water chemistry and metabolic measurements

Interactive effects of flow, CO_2 and O_2 on net photosynthesis (P_n) and dark respiration (R_d) were studied by subjecting corals to different combinations of these parameters in a 2×2×3 factorial design for repeated measures. Rates of P_n and R_d were determined in 1500 cm^3 Perspex cylindrical incubation chambers using an oxygen evolution technique as described in Schutter et al. (2008). Temperature in the chambers was maintained at 26°C by pumping freshwater of 26.5–27°C (maintained with a TC20 Aquarium Cooler, Teco, Ravenna, Italy) through a water jacket surrounding the cylindrical chamber.

Flow was created in the incubation chambers by a magnetic stirrer, using two different-sized stirring bars to obtain two distinct flow regimes: high flow velocity and low flow velocity. The actual range of velocities within each flow regime was determined by video recordings of neutrally buoyant particles that were moving over the experimental coral fragments perpendicular to the camera. The flow velocity of at least five particles per flow regime was determined by dividing the travelled distance of the particle by the time needed to travel that distance. For the low flow regime, flow velocity within a chamber varied between 1 and 1.6 cm s^{-1}, whereas for the high flow regime, flow velocity within a chamber varied between 4 and 13 cm s^{-1}.

Two values for dissolved CO_2 concentration were applied: 9.5 and 19.1 µmol l^{-1}. The former is representative of current atmospheric and oceanic conditions; the latter represents a doubling of the current atmospheric concentration. Three oxygen saturation levels were applied, 20%, 100% and 150%, which were obtained by flushing seawater with either N_2 gas or pure O_2 gas.

Following Riebesell et al. (2010), it was calculated that at prefixed, oceanic values for total alkalinity (2.4 mequiv. l^{-1}), salinity (35‰) and temperature (26°C; representative for coral reefs), the desired levels of dissolved CO_2 of 9.5 and 19.1 µmol l^{-1} correspond to pH values of 8.10 and 7.84, respectively. We applied these pH values to experimentally control the concentration of dissolved CO_2 by measurement of pH. Hence, the two values for pH (8.10 and 7.84) will be used in the subsequent text to indicate the two experimental CO_2 treatments.

Before the start of each incubation, water from the aquarium in which the corals were maintained was specifically prepared for that particular treatment in a 10 l^{-1} bucket. First, the seawater was titrated with a 1 mol l^{-1} solution of HCl to acquire a pH of 8.10. Subsequently, total alkalinity was measured and $NaHCO_3$ was added to restore the alkalinity to the current oceanic value of 2.4 mequiv. l^{-1}. The water was then further prepared by addition of CO_2, O_2 and/or N_2 to achieve the particular combination of dissolved gases needed for each treatment, and afterwards the prepared water was carefully poured into the incubation cells.

During preparation of the water, the O_2 saturation was monitored by measuring the dissolved O_2 concentration with an optical oxygen probe (LDO101, HQ 40d multi, Hach-Lange GmbH, Düsseldorf, Germany; calibrated prior to use in water-saturated air) and the CO_2 concentration was measured indirectly by measuring pH in the water sample. All measurements of seawater pH were done with a pH probe (Hach) that was calibrated using two NBS pH calibration fluids (WTW, Weilheim, Germany) representing a pH of 7.0 and a pH of 10.0, which were supplemented with 25.4 g l^{-1} NaCl (or 10 g l^{-1} Na^+), to approximate the ionic strength of seawater at a

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salinity of 35 g l^{-1} (corresponding to 10 g l^{-1} Na^+; Wijgerde et al., 2014).

Corals were placed in the incubation chambers immediately after filling the chambers with the customised seawater, after which the chambers were sealed airtight by tightening the lid. Two corals were measured at the same time, in parallel with one control (incubation cell only containing seawater) to correct for background photosynthesis/respiration or possible chemical water composition changes caused by the addition of the gases. Control values were subtracted from incubations with corals.

To avoid light limitation effects, P_n was measured at an E of 500 µmol quanta m^{-2} s^{-1}, which is considered to be saturating for this clone of *G. fascicularis* (Schutter et al., 2008). Aquablue Spezial T5 fluorescent bulbs (ATI, Hamm, Germany) were used to ensure a good light spectrum. E was measured with a Li-1400 light meter (Li-Cor, Lincoln, NE, USA). P_n and R_d were assessed by measuring the change in dissolved O_2 concentration in the incubation cells by optical oxygen probes (Hach) that had been calibrated prior to use in water-saturated air (see above). O_2 concentration was measured every minute for a period of 80 min. After an acclimation period of 10 min, a subsequent period of 30 min was applied to determine P_n. Then, the lights were turned off and the incubation cells were darkened to measure R_d. R_d was assessed by measuring the dissolved oxygen concentration for 30 min after a second acclimation period of 10 min. Throughout the incubations, oxygen saturation levels never deviated more than 5% from the initial values. These small deviations did not notably affect P_n or R_d, as regression coefficients for the observed increases or decreases in oxygen over time were always above 0.99.

In total, 72 measurements were performed using six replicate corals, which were subjected to each of the 12 treatments. The sequence of the experiments was randomised using Microsoft Excel. By using such a repeated measures design, variability due to differences in size and shape of the experimental colonies could be minimised.

An additional experiment was performed in which a more extreme O_2 saturation was chosen to further elucidate the effects of O_2 on P_n. The same six experimental colonies of *G. fascicularis* were subjected to both 100% O_2 saturation and 280% O_2 supersaturation, which resembles the daily maximum of 247% reported by Ohde and Van Woesik (1999) for Indo-Pacific reef flats. Other experimental conditions were identical to those of the previously described experiment.

Data analysis

Data were analysed in SPSS Statistics version 19 (IBM, Armonk, NY, USA). Data were checked for normality (Shapiro–Wilk's test) and sphericity (Mauchly's test; only applied to data regarding different oxygen saturation levels) to test whether the assumptions for ANOVA were met. All data for R_d and P_n were normally distributed based on the skewness and kurtosis of residual histograms. The assumption of sphericity was met for oxygen saturation (P>0.050). A three-way factorial ANOVA for repeated measures was applied to analyse both data series. Significant interactive effects were followed up by simple contrasts, to determine the effect of a given factor at various level combinations of the remaining two factors. The supersaturation control experiment was analysed by a paired t-test. P<0.05 was considered statistically significant.

RESULTS

Average rates of R_d and P_n under the 12 experimental treatments are presented in Fig. 1. Table 1 shows the corresponding results of the three-way ANOVA. Data for R_d are graphically presented as negative oxygen production (i.e. oxygen consumption). Throughout the text, absolute values will be used to express rates of R_d.

Values for R_d ranged between 0.050 and 0.087 mg O_2 cm^{-2} h^{-1}). There was a significant main effect of flow velocity on dark respiration (Table 1). No significant main effect of pH on dark respiration was observed. Oxygen saturation, in contrast, did have a significant main effect on R_d rates (Table 1). A significant interaction was found between the effects of oxygen and flow velocity on R_d in *G. fascicularis* (Table 1). Under hypoxic (20% O_2) conditions, flow velocity had a strong and significant effect on R_d (42% increase, P=0.008 at low pH and 43% increase, P=0.014 at high pH). Under normoxic conditions (100% O_2), flow also significantly enhanced R_d (16% increase, P=0.004 at low pH and 30% increase, P=0.035 at high pH). The flow effect was not significant under hyperoxic (150% O_2, P=0.388 and P=0.874, respectively) conditions. No three-way interaction was found (Table 1).

Values for P_n in *G. fascicularis* (Fig. 1) ranged between 0.178 and 0.256 mg O_2 cm^{-2} h^{-1}, the rates of P_n being 2–5 times higher than the rates of R_d. Flow and pH exerted a significant main and interactive effect on P_n (Table 1). At high flow, there was a significant effect of pH on P_n (14% increase at pH 7.84; P=0.026), whereas the pH effect was not significant at the low flow velocity (P=0.113). Conversely, the effect of flow was significant (P=0.001) at pH 7.84, but not at pH 8.10 (P=0.066). The interaction is graphically depicted in Fig. 2. In contrast, no main effect of oxygen saturation on P_n was found and no interactive effect of oxygen with the other factors (flow, pH) could be observed (Table 1). Furthermore, in the supersaturation control experiment, the additional oxygen (280% supersaturation) did not significantly affect the rate of P_n (paired t-test, P>0.05), the rates being 0.17±0.0191 and 0.174±0.026 mg O_2 cm^{-2} h^{-1} (means±s.d.) for 100% and 280% saturation, respectively.

DISCUSSION

Effect of O_2 on photosynthesis

Our data show that photosynthesis in this particular genotype of *G. fascicularis* is not affected by ambient oxygen levels. Changes in

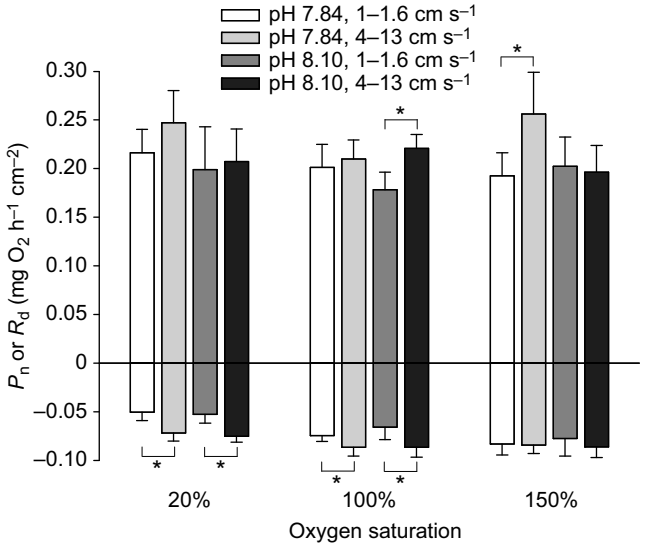

Fig. 1. Rates of net photosynthesis (P_n, positive values) and dark respiration (R_d, negative values) of *Galaxea fascicularis* under 12 different incubation regimes with varying levels of dissolved oxygen, pH and water flow. The experiment was performed once with six replicate coral colonies, each colony being subjected to all experimental treatments (n=6; repeated measures). Data are means+s.d. *Simple contrasts with P<0.05.

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Table 1. Results of a three-way factorial ANOVA for repeated measures on data for dark respiration and net photosynthesis under the 12 experimental treatments

	Dark respiration				Net photosynthesis			
Factor	*F*	d.f.	Error	*P*	*F*	d.f.	Error	*P*
Oxygen	24.968	2	10	0.000*	1.210	2	10	0.338
Flow	31.043	1	5	0.003*	22.184	1	5	0.005*
pH	0.828	1	5	0.404	7.474	1	5	0.041*
Oxygen×flow	5.554	2	10	0.024*	0.409	2	10	0.675
Oxygen×pH	1.378	2	10	0.296	1.324	2	10	0.309
pH×flow	1.525	1	5	0.272	14.525	1	5	0.012*
Oxygen×flow×pH	0.214	2	10	0.811	3.912	2	10	0.056

Asterisks indicate significance.

oxygen saturation (both hypoxia and hyperoxia, up to 280% supersaturation) did not influence the P_n output of *G. fascicularis* under saturating light. As we will outline below, this result implies that photorespiration is not of quantitative importance in this genotype of *G. fascicularis*. A conceptual picture (Fig. 3) illustrates how we deduced this conclusion from our results.

Oxygen produced inside the coral tissue through photosynthesis (gross oxygen production) has three potential sinks: (1) release into the external environment via diffusion (oxygen evolution, also termed P_n); (2) respiration, a process referred to as light respiration under illuminated conditions and dark respiration in darkness; (3) photorespiration. Our results show that P_n in *G. fascicularis* is not affected by ambient oxygen. Hence, an increase in light respiration and photorespiration under high ambient oxygen, as found in *Favia veroni* (Mass et al., 2010), would only be possible if gross photosynthesis increased as well (Fig. 3B, scenario 1). An increase in gross photosynthesis under high oxygen is not likely, as photosynthetic oxygen production is not limited by oxygen. In contrast, very high oxygen levels (>800% supersaturation) may even decrease the photochemical yield of the zooxanthellae (Finelli et al., 2006), thus leading to a lower gross productivity. Therefore, it seems reasonable to assume that gross photosynthesis was not affected by ambient oxygen, which excludes scenario 1 in Fig. 3. With both gross and net photosynthesis remaining unchanged, it is not very likely that light respiration and photorespiration changed under the influence of ambient oxygen. Both processes would in that case have to show an opposite response (scenarios 2 and 3, Fig. 3C,D). We conclude from this analysis that photorespiration is not quantitatively important in *G. fascicularis* (scenario 4, Fig. 3E), as otherwise we should have observed a decrease in P_n at high ambient oxygen.

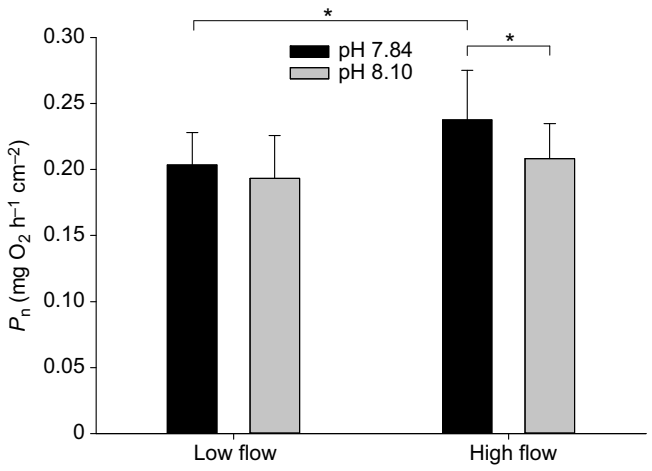

Fig. 2. Interactive effect of flow and pH on the rate of P_n of *G. fascicularis*. The experimental data are the same as presented in Fig. 1; data for different oxygen levels were pooled to visualise the significant interaction (2-way ANOVA for repeated measures, P=0.012) between flow and pH. Data are means+s.d. *Simple contrasts with P<0.05.

This conclusion is in agreement with work by Muscatine (1980), who found no effect of oxygen on photosynthesis by zooxanthellae *in hospite*. Zooxanthellae *in hospite* may not be very susceptible to photorespiration because of efficient CCMs that operate within the coral–zooxanthellae symbiosis (Smith et al., 2005). Notwithstanding this, *ex hospite* photosynthesis of zooxanthellae isolated from *Pocillopora damicornis* was very sensitive to oxygen (Downton et al., 1976), which suggests that the CCM may be largely host related. Indeed, recent work by Tansik et al. (2015) showed that in several coral species, the dissolved inorganic carbon (DIC) influx into the coral tissue is host controlled. These corals exhibit external carbonic anhydrase activity (i.e. bound to the outer cell membrane of the coral ectoderm cells) that mediates the influx of carbon dioxide into the coral tissue. The *G. fascicularis–Symbiodinium* symbiosis has both a carbonic anhydrase-driven CCM and transport pathways for HCO_3^- (Al-Moghrabi et al., 1996; Goiran et al., 1996), thus enabling an efficient supply of DIC to the site of photosynthesis.

The contrast between our results and the study by Mass et al. (2010) shows that effects of oxygen on coral photosynthesis are species specific. Sensitivity to high oxygen levels may depend on the effectiveness of the CCM in the coral. In contrast to *Favia veroni* (Mass et al., 2010), *G. fascicularis* apparently has a highly efficient CCM, making photosynthesis in this species rather insensitive to changes in ambient oxygen.

Interestingly, oxygen supersaturation does affect calcification in this genotype of *G. fascicularis*. A 20–33% decline in light calcification rates was found when colonies of this genotype were exposed to oxygen saturation levels of 150% and higher (Wijgerde et al., 2012a). The authors attributed this decline to the formation of reactive oxygen species (superoxides) under high oxygen levels. The differential response of photosynthesis (this study) and calcification (Wijgerde et al., 2012a) to high oxygen may relate to the spatial separation of the two processes. Calcification occurs in the ectodermal layer, whereas photosynthesis takes place within zooxanthellae located in the endodermal layers of the coral (Jokiel, 2011). Endodermal layers have been shown to exhibit higher superoxide dismutase (SOD) activity (Richier et al., 2003). SOD can counteract the toxic effects of superoxides. The difference in sensitivity to oxygen between calcification and photosynthesis might thus be explained by a difference in defence possibilities against superoxide toxicity between ectodermal and endodermal cells.

Effects of CO_2 and flow on photosynthesis

In contrast to dissolved oxygen, the supply of additional CO_2 into the seawater did stimulate P_n in *G. fascicularis*, an effect that was

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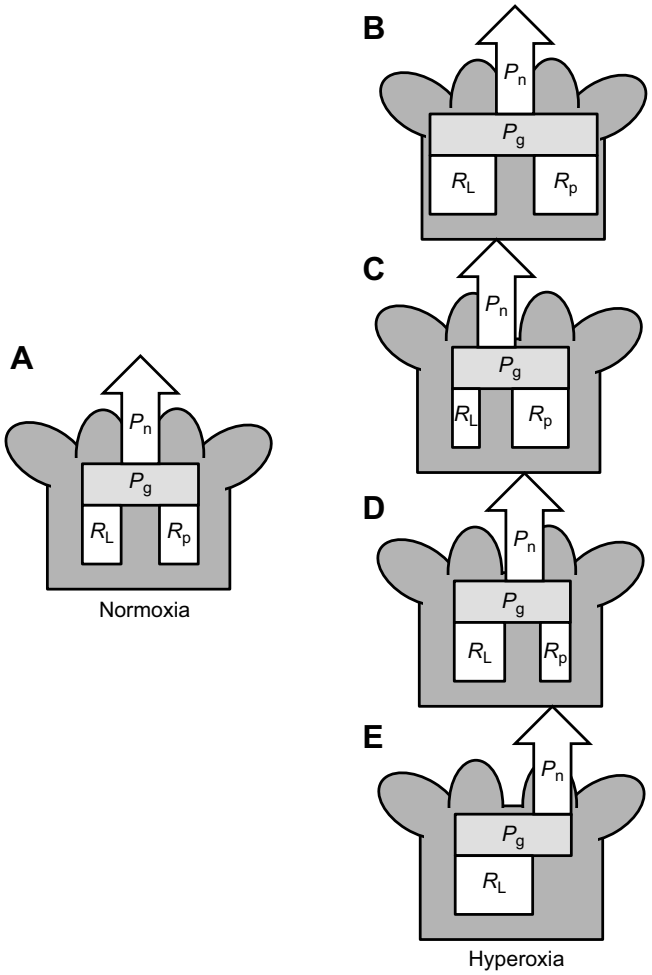

Fig. 3. Hypothetical scenarios to explain the observed lack of effect of an increased ambient oxygen concentration on P_n in *G. fascicularis*. Gross photosynthesis (P_g) is represented by a grey box, P_n is represented by a white box arrow, whereas light respiration (R_L) and photorespiration (R_p) are represented by white boxes. The width of the boxes represents the rate of the corresponding processes inside the coral polyp. As there was no effect of ambient oxygen on P_n, the width of the corresponding box arrow is the same in all scenarios. (A) Reference scenario under normoxia (100% oxygen saturation), assuming an equal contribution of R_L and R_p to oxygen consumption inside the coral. (B) Hyperoxia (scenario 1). This scenario represents an increase in P_g compared with the reference scenario, with proportional increases in R_L and R_p, thus leaving P_n unchanged. (C) Hyperoxia (scenario 2). A shift in the levels of R_L and R_p occurs without a concurrent change in either P_g or P_n. (D) Hyperoxia (scenario 3). A reversed shift in the levels of R_L and R_p occurs without a concurrent change in either P_g or P_n. (E) Hyperoxia (scenario 4). P_g and P_n remain unchanged and R_p is absent. Scenario 1 is not likely, as it can be argued that P_g will not increase under elevated ambient oxygen levels. An exactly proportional increase/decrease in R_L and R_p or vice versa (scenarios 2 and 3) is also unlikely, leaving scenario 4 (absence of R_p) as the most likely situation in *G. fascicularis*.

significant only under the high flow regime of 4–13 cm s^{-1}. This finding suggests that the often-reported positive effect of flow on coral photosynthesis is, in the case of *G. fascicularis*, due to a flow-induced increase in the uptake of CO_2 rather than an increased efflux of oxygen out of the coral tissue.

Increased influx of DIC under high flow has previously been suggested to stimulate photosynthesis in the symbiotic coral species *Acropora formosa* (Dennison and Barnes, 1988). These authors based their suggestion upon an observed increase in photosynthesis under stirred conditions versus non-stirred conditions. As no effect of stirring was observed at the compensation point (i.e. no net gas exchange), the observed effect was ascribed to diffusion limitation. Dennison and Barnes (1988) concluded that diffusion limitation of CO_2 rather than inhibition by accumulation of oxygen was responsible for the flow effect on photosynthesis. Their conclusion was based on a preceding study (Crossland and Barnes, 1977) on a related coral species, in which no photosynthetic response to varying oxygen levels had been found. Our results provide direct evidence for this limiting role of CO_2 diffusion, as we studied the effects of CO_2 and oxygen simultaneously on the same species.

Nevertheless, our results contradict with other literature. According to Tansik et al. (2015), coral photosynthesis is not likely to be DIC limited because of the highly efficient external carbonic anhydrase system. This assumption is in line with several other studies (e.g. Reynaud et al., 2003; Schneider and Erez, 2006; Marubini et al., 2008) in which no beneficial effects of P_{CO_2} on coral photosynthesis were found.

The apparent contrast with our observations probably relates to the specific combination of abiotic parameters that was applied. In our study, light availability was more than twofold higher than in the study by Tansik et al. (2015), at a level that was saturating for photosynthesis (see Schutter et al., 2008), which may thus have invoked DIC limitation. The other studies (Reynaud et al., 2003; Schneider and Erez, 2006; Marubini et al., 2008) used higher irradiance levels than Tansik et al. (2015), but those studies do not provide details on the flow conditions that were applied in the experiments. As flow can augment the P_{CO_2} effect as observed in this study, incubations performed under low flow conditions may not show detectable effects of P_{CO_2} on photosynthesis. Additionally, the studies by Marubini et al. (2008) and Reynaud et al. (2003) lasted for periods of 1 and 5 weeks, respectively, whereas the current experiment investigated the acute effects (30 min exposure) of CO_2. Possibly, there is an initial effect of CO_2 that is later countered by adaptation of the coral. For example, coral may adapt to a higher P_{CO_2} by lowering carbonic anhydrase activity (Lesser et al., 1994), leading to a lower efficiency of the CCM.

There is one earlier study that describes effects of dissolved inorganic carbon on P_n in *G. fascicularis* (Goiran et al., 1996), in which an optimal pH of 8.8 was reported for P_n. Our study disagrees with this result for reasons that remain to be investigated.

We conclude that our study represented a situation in which the influx of DIC limited coral photosynthesis. DIC limitation of photosynthesis may occur in shallow water where light is saturating (Osinga et al., 2011). Our results demonstrate that under such conditions, high flow and increased levels of carbon dioxide can alleviate DIC limitation and stimulate coral photosynthesis.

R_d

Changes in P_{CO_2} and corresponding changes in pH did not change rates of R_d in *G. fascicularis*. Apparently, a decrease in ocean pH to 7.84 does not quantitatively affect catabolism in this coral.

Flow and ambient oxygen saturation level both stimulated R_d of *G. fascicularis*, in an interactive way. The positive effect of flow on R_d found here is in line with earlier research (Patterson et al., 1991; Sebens et al., 2003), which showed that flow enhances R_d in corals within the range of flow velocities applied in our study (1–13 cm s^{-1}). The significant positive main effect of oxygen saturation on R_d supports the often-proposed theory (e.g. Chalker and Taylor, 1975; Rinkevich and Loya, 1984; Ip et al., 1991; Colombo-Pallotta et al., 2010; Wijgerde et al., 2012a) that dark

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Table 2. Net photosynthesis (P_n) and corresponding levels of quantum irradiance (E) reported in the literature compared with the results of this study

Species	P_n (nmol O_2 cm^{-2} min^{-1})	E (nmol quanta cm^{-2} min^{-1})	Reference
Galaxea fascicularis	130	3000	This study
	80	3000	Schutter et al., 2008
	60	1560	Schutter et al., 2008
	20	480	Schutter et al., 2008
	10	540	Schutter et al., 2010
	44.5	3360	Schutter et al., 2011
Montastrea annularis	21	3000	Patterson et al., 1991
Stylophora pistillata	3.3	2100	Houlbrèque et al., 2004
Acropora eurystoma	8.3	2100	Schneider and Erez, 2006
Montipora monasteriata	33	600	Anthony and Hoegh-Guldberg, 2003
	33	900	Anthony and Hoegh-Guldberg, 2003
	33	1200	Anthony and Hoegh-Guldberg, 2003
Stylophora pistillata	20	Variable–high	Mass et al., 2007
Coral assemblage	62	Variable–high	Langdon and Atkinson, 2005

Values of P_n are expressed in nmol O_2 cm^{-2} s^{-1} to enable direct comparison with the available amount of light quanta (E/P_n gives the number of quanta available per molecule oxygen that is eluted).

calcification in corals is limited by a lack of metabolic energy due to R_d being oxygen limited. Both flow and ambient oxygen saturation influence the availability of oxygen inside the tissue by affecting diffusion rates. The effect of flow was significant under hypoxic and normoxic conditions, but not under hyperoxic conditions. This indicates that R_d is no longer diffusion limited under such a high availability of dissolved oxygen. An earlier study on the effect of flow on R_d in *G. fascicularis* under normoxic conditions showed that flow rates above 10 cm s^{-1} did not further promote R_d (Schutter et al., 2010), indicating a similar boundary to the relief of diffusion limitation by flow.

Productivity and scope for growth

The rates of P_n measured in this study are among the highest ever reported in scleractinian corals (Table 2). In addition, light utilisation was very efficient. At the highest rate of P_n measured in this study, 23 quanta were used per molecule of eluted oxygen, an efficiency only exceeded by shade-adapted cave corals (Anthony and Hoegh-Guldberg, 2003). It should be noted here that our corals were grown at an E of 200 µmol quanta m^{-2} s^{-1} and incubated at an E of 500 µmol quanta m^{-2} s^{-1}. Hence, one might argue that the observed rates of P_n reflect a stress response. However, the values for P_n measured in this study correspond well to values within photosynthesis:irradiance (P:I) curves determined earlier for the same clone of *G. fascicularis* (Schutter et al., 2008). The shape and values of those earlier P:I curves are in good agreement with P:I curves measured in the field for several coral species, thus indicating that the values reported here reflect normal photosynthetic responses to the light levels that were applied.

Under all treatments in this study, photosynthetic oxygen production by *G. fascicularis* was more than 2 times higher than its consumption of oxygen in darkness. This indicates that there was a surplus of photosynthetically derived organic carbon available for *G. fascicularis* and its zooxanthellae under all experimental circumstances. Such a positive carbon balance implies that an organism has scope for growth (SfG). SfG is defined as the amount of organic carbon (acquired autotrophically and/or heterotrophically) that is available to an organism after subtracting the daily respiratory demand and excretory losses (Anthony and Fabricius, 2000). Subtracting daily R_d from daily P_n gives SfG′, the amount of photosynthetically derived organic carbon that is available to the coral holobiont for growth and excretion. As an example, we calculated SfG′ for the experimental treatment with the highest P_n (0.256 mg O_2 cm^{-2} h^{-1}). This value was measured at 150% O_2 saturation, 4–13 cm s^{-1} and pH 7.84. A corresponding value for R_d of 0.084 mg O_2 cm^{-2} h^{-1} was measured under these conditions. Taking into account the 12 h:12 h light:dark cycle that was applied in our study, and assuming a molar ratio of 1:1 for the number of carbon molecules fixed per molecule of oxygen eluted, daily P_n could be estimated at 1.15 mg C cm^{-2} and daily R_d at 0.38 mg C cm^{-2}. These values yield a SfG′ of 0.77 mg C cm^{-2}. For comparison, a SfG′ of 0.13 mg C cm^{-2} day^{-1} was calculated for *Porites cylindrica* and 0.06 mg C cm^{-2} day^{-1} for *Goniastrea retiformis* (Anthony and Fabricius, 2000). Those values are an order of magnitude lower than the values found for *G. fascicularis* in the current study. Hence, we conclude that under the experimental circumstances applied, *G. fascicularis* and its zooxanthellae had a high surplus of carbon available for processes such as somatic growth, reproduction and repair of tissue damage. Indeed, growth rates reported for *G. fascicularis* are very high, specific growth rates sometimes exceeding 0.020 day^{-1} for young colonies (Wijgerde et al., 2012b). These high initial growth rates were obtained at low levels of E of 40–60 µmol quanta m^{-2} s^{-1}, which confirms that *G. fascicularis* is very efficient in collecting light. Similarly, Schutter et al. (2008) reported an initial specific growth rate for *G. fascicularis* of 0.025 day^{-1} at an E value of 90 µmol quanta m^{-2} s^{-1}. In addition, they found that *G. fascicularis* was still capable of growing with a specific growth rate of 0.006 day^{-1} at a level of E as low as 39 µmol quanta m^{-2} s^{-1}.

Conclusions

The results obtained in this study show that *G. fascicularis* has a high net productivity that is insensitive to the ambient level of dissolved oxygen. Photorespiration is not likely to play a quantitatively important role in this coral. The high productivity of *G. fascicularis* leads to a saturated light respiration and a positive SfG. Under saturating light, photosynthesis in *G. fascicularis* may become DIC limited, as shown by the positive, interactive effects of flow and pH on net photosynthesis.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: R.O., M.D.-H., J.A.J.V.; Methodology: R.O., M.D.-H., T.W.; Validation: M.D.-H.; Formal analysis: R.O., M.D.-H., T.W., J.A.J.V.; Investigation: R.O., M.D.-H., T.W.; Resources: R.O.; Writing - original draft: R.O., M.D.-H.; Writing - review & editing: R.O., M.D.-H., T.W., J.A.J.V.; Visualization: R.O., T.W.; Supervision: R.O., T.W., J.A.J.V.; Project administration: R.O.; Funding acquisition: R.O.

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References

Al-Moghrabi, S., Goiran, C., Allemand, D., Speziale, N. and Jaubert, J. (1996). Inorganic carbon uptake for photosynthesis by symbiotic coral/dinoflagellate associations. II. Mechanisms for bicarbonate uptake. *J. Exp. Mar. Biol. Ecol.* **199**, 227-248.

Anthony, K. R. N. and Fabricius, K. E. (2000). Shifting roles of heterotrophy and autotrophy in coral energetics under varying turbidity. *J. Exp. Mar. Biol. Ecol.* **252**, 221-253.

Anthony, K. R. N. and Hoegh-Guldberg, O. (2003). Variation in coral photosynthesis, respiration and growth characteristics in contrasting light microhabitats: an analogue to plants in forest gaps and understoreys? *Funct. Ecol.* **17**, 246-259.

Chalker, B. E. and Taylor, D. L. (1975). Light-enhanced calcification, and the role of oxidative phosphorylation in calcification of the coral Acropora cervicornis. *Proc. R. Soc. B* **190**, 323-331.

Colombo-Pallotta, M. F., Rodríguez-Romain, A. and Iglesias-Prieto, R. (2010). Calcification in bleached and unbleached Montastraea faveolata: evaluating the role of oxygen and glycerol. *Coral Reefs* **29**, 899-907.

Crossland, C. J. and Barnes, D. J. (1977). Gas-exchange studies with the staghorn coral *Acropora acuminata* and its zooxanthellae. *Mar. Biol.* **40**, 185-194.

Dennison, W. C. and Barnes, D. J. (1988). Effect of water motion on coral photosynthesis and calcification. *J. Exp. Mar. Biol. Ecol.* **115**, 67-77.

Downton, W. J. S., Bishop, D. G., Larkum, A. W. D. and Osmond, C. B. (1976). Oxygen inhibition of photosynthetic oxygen evolution in marine plants. *Aust. J. Plant Physiol.* **3**, 73-79.

Finelli, C. M., Helmuth, B. S. T., Pentcheff, N. D. and Wethey, D. S. (2006). Water flow influences oxygen transport and photosynthetic efficiency in corals. *Coral Reefs* **25**, 47-57.

Furla, P., Allemand, D., Shick, J. M., Ferrier-Pagés, C., Richier, S., Plantivaux, A., Merle, P.-L. and Tambutté, S. (2005). The symbiotic anthozoan: a physiological chimera between alga and animal. *Integr. Comp. Biol.* **45**: 595-604.

Goiran, C., Al-Moghrabi, S., Allemand, D. and Jaubert, J. (1996). Inorganic carbon uptake for photosynthesis by the symbiotic coral/dinoflagellate association I. Photosynthetic performances of symbionts and dependence on sea water bicarbonate. *J. Exp. Mar. Biol. Ecol.* **199**, 207-225.

Houlbrèque, F., Tambutté, E., Allemand, D. and Ferrier-Pagès, C. (2004). Interactions between zooplankton feeding, photosynthesis and skeletal growth in the scleractinian coral Stylophora pistillata. *J. Exp. Biol.* **207**, 1461-1469.

Ip, Y. K., Lim, A. L. L. and Lim, R. W. L. (1991). Some properties of calciumactivated adenosine triphosphatase from the hermatypic coral Galaxea fascicularis. *Mar. Biol.* **111**, 191-197.

Jokiel, P. L. (2011). The reef coral two compartment proton flux model: A new approach relating tissue-level physiological processes to gross corallum morphology. *J. Exp. Mar. Biol. Ecol.* **409**, 1-12.

Langdon, C. and Atkinson, M. J. (2005). Effect of elevated pCO2 on photosynthesis and calcification of corals and interactions with seasonal change in temperature/irradiance and nutrient enrichment. *J. Geophys. Res.* **110.**

Leggat, W., Badger, M. R. and Yellowlees, D. (1999). Evidence for an inorganic carbon-concentrating mechanism in the symbiotic dinoflagellate *Symbiodinum sp. Plant Physiol.* **121**, 1247-1255.

Lesser, M. P., Weis, V. M., Patterson, M. R. and Jokiel, P. L. (1994). Effects of morphology and water motion on carbon delivery and productivity in the reef coral, *Pocillopora damicornis* (Linnaeus): Diffusion barriers, inorganic carbon limitation, and biochemical plasticity. *J. Exp. Mar. Biol. Ecol.* **178**, 153-179.

Marubini, F., Ferrier-Pagès, C., Furla, P. and Allemand, D. (2008). Coral calcification responds to seawater acidification: a working hypothesis towards a physiological mechanism. *Coral Reefs* **27**, 491-499.

Mass, T., Einbinder, S., Brokovich, E., Shashar, N., Vago, R., Erez, J. and Dubinsky, Z. (2007). Photoacclimation of Stylophora pistillata to light extremes: metabolism and calcification. *Mar. Ecol. Prog. Ser.* **334**, 93-102.

Mass, T., Genin, A., Shavit, U., Grinstein, M. and Tchernov, D. (2010). Flow enhances photosynthesis in marine benthic autotrophs by increasing the efflux of oxygen from the organism to the water. *Proc. Nat. Acad. Sci.* **107**, 2527-2531.

Muscatine, L. (1980). *Productivity of zooxanthellae.* In *Primary Productivity in the Sea* (ed. P. G. Falkowski), pp. 381-402. New York: Plenum Publishing Corporation.

Nakamura, T., Van Woesik, R. and Yamasaki, H. (2005). Photoinhibition of photosynthesis is reduced by water flow in the reef-building coral Acropora digitifera. *Mar. Ecol. Prog. Ser.* **301**, 109-118.

Ohde, S. and Van Woesik, R. (1999). Carbon dioxide flux and metabolic processes of a coral reef, Okinawa. *Bull. Mar. Sci.* **65**, 559-576.

Osinga, R., Schutter, M., Griffioen, B., Wijffels, R. H., Verreth, J. A. J., Shafir, S., Henard, S., Taruffi, M., Gili, C. and Lavorano, S. (2011). The biology and economics of coral growth. *Mar. Biotechnol.* **13**, 658-671.

Osinga, R., Iglesias-Prieto, R. and Enríquez, S. (2012). Measuring photosynthesis in symbiotic invertebrates: a review of methodologies, rates and processes. In *Applied Photosynthesis* (ed. M. Najafpour), pp. 229-256. Rijeka, Croatia: Intech, Open Access Publishers.

Patterson, M. R., Sebens, K. P. and Olson, R. R. (1991). In situ measurements of flow effects on primary production and dark respiration in reef corals. *Limnol. Oceanogr.* **36**, 936-948.

Peterhansel, C., Horst, I., Niessen, M., Blume, C., Kebeish, R., Kürkcüoglu, S. and Kreuzaler, F. (2010). Photorespiration. *Arabidopsis Book* **8**, e0130.

Reynaud, S., Leclercq, N., Romaine-Lioud, S., Ferrier-Pagés, C., Jaubert, J. and Gattuso, J.-P. (2003). Interacting effects of CO2 partial pressure and temperature on photosynthesis and calcification in a scleractinian coral. *Global Change Biol.* **9**, 1660-1668.

Richier, S., Merle, P.-L., Furla, P., Pigozzi, D., Sola, F. and Allemand, D. (2003). Characterization of superoxide dismutases in anoxia- and hyperoxia-tolerant symbiotic cnidarians." *Biochim. Biophys. Acta* **1621**, 84-91.

Riebesell, U., Fabry, V. J., Hansson, L. and Gattuso, J.-P. (ed.) (2010). *Guide to Best Practices for Ocean Acidification Research and Data Reporting*, 260 pp. Luxembourg: Publications Office of the European Union.

Rinkevich, B. and Loya, Y. (1984). Does light enhance calcification in hermatypic corals? *Mar. Biol.* **80**, 1-6.

Rowan, R., Whitney, S. M., Fowler, A. and Yellowlees, D. (1996). Rubisco in marine symbiotic dinoflagellates: form II enzymes in eukaryotic encoded in a multigene family. *Plant Cell* **8**, 539-553.

Schneider, K. and Erez, J. (2006). The effect of carbonate chemistry on calcification and photosynthesis in the hermatypic coral Acropora eurystoma. *Limnol. Oceanogr.* **51**, 1284-1293.

Schutter, M., Van Velthoven, B., Janse, M., Osinga, R., Janssen, M., Wijffels, R. and Verreth, J. A. J. (2008). The effect of irradiance on long-term skeletal growth and net photosynthesis in *Galaxea fascicularis* under four light conditions. *J. Exp. Mar. Biol. Ecol.* **367**, 75-80.

Schutter, M., Crocker, J., Paijmans, A., Janse, M., Osinga, R., Verreth, A. J. and Wijffels, R. H. (2010). The effect of different flow regimes on the growth and metabolic rates of the scleractinian coral Galaxea fascicularis. *Coral Reefs* **29**, 737-748.

Schutter, M., Kranenbarg, S., Wijffels, R. H., Verreth, J. and Osinga, R. (2011). Modification of light utilization for skeletal growth by water flow in the scleractinian coral Galaxea fascicularis. *Mar. Biol.* **158**, 769-777.

Sebens, K. P., Helmuth, B., Carrington, E. and Agius, B. (2003). Effects of water flow on growth and energetics of the scleractinian coral Agaricia tenuifolia in Belize. *Coral Reefs* **22**, 35-47.

Smith, D. J., Suggett, D. J. and Baker, N. R. (2005). Is photoinhibition of zooxanthellae photosynthesis the primary cause of thermal bleaching in corals? *Global Change Biol.* **11**, 1-11.

Tansik, A. L., Fitt, W. K. and Hopkinson, B. M. (2015). External carbonic anhydrase in three Caribbean corals: quantification of activity and role in CO_2 uptake. *Coral Reefs* **34**, 703-713.

Vogel, S. (1994). *Life in Moving Fluids: The Physical Biology of Flow*, 2nd edn, 467 pp. Princeton: Princeton University Press.

Weis, V. M. (2008). Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. *J. Exp. Biol.* **221**, 3059-3066.

Wijgerde, T., Jurriaans, S., Hoofd, M., Verreth, J. A. J. and Osinga, R. (2012a). Oxygen and heterotrophy affect calcification of the scleractinian coral galaxea fascicularis. *PLoS ONE* **7**, e52702.

Wijgerde, T., Henkemans, P. and Osinga, R. (2012b). Effects of irradiance and light spectrum on growth of the scleractinian coral *Galaxea fascicularis* — applicability of LEP and LED lighting to coral aquaculture. *Aquaculture* **344-349**, 188-193.

Wijgerde, T., Silva, C. I. F., Scherders, V., Van Bleijswijk, J. and Osinga, R. (2014). Coral calcification under daily oxygen saturation and pH dynamics reveals the dominant role of oxygen. *Biology Open* **3**, 489-493.