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Original Article

Effects of pCO_2 on photosynthesis and respiration of tropical scleractinian corals and calcified algae

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The effects of ocean acidification (OA) on coral reefs have been studied thoroughly with a focus on the response of calcification of corals and calcified algae. However, there are still large gaps in our knowledge of the effects of OA on photosynthesis and respiration of these organisms. Comparisons among species and determination of the functional relationships between pCO_2 and either photosynthesis or respiration are difficult using previously published data, because experimental conditions typically vary widely between studies. Here, we tested the response of net photosynthesis, gross photosynthesis, dark respiration, and light-enhanced dark respiration (LEDR) of eight coral taxa and seven calcified alga taxa to six different pCO_2 levels (from 280 to 2000 μatm). Organisms were maintained during 7–10 days incubations in identical conditions of light, temperature, and pCO_2 to facilitate comparisons among species. Net photosynthesis was not affected by pCO_2 in seven of eight corals or any of the algae; gross photosynthesis did not respond to pCO_2 in six coral taxa and six algal taxa; dark respiration also was unaffected by pCO_2 in six coral and six algae; and LEDR did not respond to pCO_2 in any of the tested species. Overall, our results show that pCO_2 levels up to 2000 μatm likely will not fertilize photosynthesis or modify respiration rates of most of the main calcifiers on the back reef of Moorea, French Polynesia.

Keywords: algae, coral, ocean acidification, photosynthesis, respiration.

Introduction

Over the last decade, a growing number of studies have focused on the impact of ocean acidification (OA) on the physiology of tropical coral reef organisms. OA, which is caused by the dissolution of anthropogenic CO_2 in the oceans, leads to modifications of the carbonate chemistry of seawater that cause a decrease in pH, an increase in bicarbonate ion concentration, and a decrease in both carbonate ion concentration and calcium carbonate saturation state (Ω) (Feely *et al.*, 2004). Because corals and calcifying algae have the potential to produce large structures made of calcium carbonate, most of research on the effects of OA on coral reefs has focused on calcification of these organisms. For most species calcification likely will decline with OA, although some species appear insensitive over the range of conditions investigated (Takahashi and Kurihara 2013; Comeau *et al.* 2014). The effects of pCO_2 on respiration and photosynthesis of corals and calcifying macroalgae have received less attention than calcification, although there are reasons to expect the effects could be substantial (e.g. Anthony *et al.*, 2008).

For example, it has been suggested that aerobic respiration will increase under OA conditions to supply the increased energy demands associated with maintaining calcification in a chemical environment that is thermodynamically less favourable to mineralization (McCulloch *et al.*, 2012). However, for corals, empirical evidence shows that the effects of elevated pCO_2 on aerobic respiration are equivocal, with, for example, no effects of high pCO_2 reported on dark respiration of *Stylophora pistillata* (Reynaud *et al.*, 2003), *Acropora eurystoma* (Schneider and Erez, 2006), and *A. formosa* (Crawley *et al.*, 2010), while a decrease in respiration has been reported for massive *Porites* spp. (Edmunds, 2012), *A. millepora* (Kaniewska *et al.*, 2012), and larvae of *P. astreoides* (Albright and Langdon, 2011). In calcifying algae, there are only a few studies addressing the response of respiration to pCO_2, and in

these cases there generally is no effect of increasing pCO_2 (e.g. Semesi *et al.*, 2009; Martin *et al.*, 2013), but contrasting results such as an increase in respiration at elevated pCO_2 also have been reported, for example, on *Halimeda digitata* at CO_2 vents (Vogel *et al.*, 2015).

In addition to aerobic respiration, photosynthesis also could be affected by changes in the carbonate chemistry of seawater caused by OA, because an increase in the concentration of dissolved inorganic carbon [C_T] in seawater (mostly in the bicarbonate form) could favour photosynthesis by increasing bicarbonate and dissolved CO_2 availability (Leggat *et al.*, 1999; Mackey *et al.*, 2015). For tropical reef corals, the effect of OA on net photosynthesis has received more attention than the effects on respiration, and a recent meta-analysis based on eleven studies revealed that the mean effect size of CO_2 on coral photosynthesis was not statistically discernible from zero (Kroeker *et al.*, 2013). Among the growing number of studies that have been conducted to explore the effects of pCO_2 on coral photosynthesis contrasting results such as a net photosynthesis decreases at 1180 μatm pCO_2 (Kaniewska *et al.*, 2012), an increase at 760 μatm (Reynaud *et al.*, 2003), and no effect (Takahashi and Kurihara, 2013) have for example been found. In contrast, for calcifying reef algae, most studies have reported a reduction of photosynthesis for a decrease in pH $\leq$0.5 unit ($n = 11$ studies; Kroeker *et al.*, 2013).

To date, most experiments addressing the effects of elevated pCO_2 on respiration and photosynthesis of tropical corals and algae have been performed on only a few species, and have been conducted using experimental temperatures, irradiances, carbonate chemistry manipulations (CO_2vs.acid), and exposure times that differ greatly among studies. For example, the photosynthesis of *A. intermedia* was studied at irradiance of 700–1200 μmol quanta m^{-2} s^{-1} (Anthony *et al.*, 2008), while the photosynthesis of *S. pistillata* was determined at irradiance of 380 μmol quanta m^{-2} s^{-1} (Reynaud *et al.*, 2003). Therefore, high variation among studies in treatment conditions makes it difficult to compare results among species or to engage in synthetic analyses, and with only a few species involved in studies to date, it is challenging to evaluate the biological meaning of among species variation. Species-specific morphologies, for example, could affect the mass transfer capabilities for important metabolites associated with respiration and photosynthesis including dissolved inorganic carbon, nutrients, and oxygen (Patterson, 1992; Jokiel, 2011). Furthermore, coral species that calcify rapidly may be more sensitive to elevated pCO_2 than species that calcify slowly, potentially because rapid calcification has a greater requirement for metabolic energy to export protons from the site of calcification (Rodolfo-Metalpa *et al.*, 2010; Comeau *et al.*, 2014). Previous studies of the effects of elevated pCO_2 on the metabolism of corals and algae typically have only employed two or three pCO_2 treatments, which greatly restricts the capacity to test for curvilinear relationships between the metabolism and pCO_2.

This present research was designed to test two hypotheses: (i) photosynthesis of corals and calcifying algae is not affected by seawater pCO_2 and (ii) aerobic respiration is not affected by seawater pCO_2. To strengthen the test of the primary hypotheses, experiments were conducted with multiple species of corals and algae to investigate differences between functional groups. The investigation was performed on eight coral taxa and seven calcifying algal taxa that were maintained under six pCO_2 levels from 280 to 2000 μatm, and under identical conditions of irradiance and seawater temperature. Corals and calcifying algae were selected to represent both the common calcifiers of the back reef of Moorea, French Polynesia, and to contrast functional groups of organisms that previously differ in one aspect of their metabolism (i.e. calcification) when exposed to elevated pCO_2 (*sensu* Comeau *et al.*, 2014). Based on our earlier work showing that the response of calcification to pCO_2 differed between functional groups defined by contrasting morphologies or growth rates for both corals and algae, the same taxon were exploited in the present analysis. We contrasted the response of respiration and photosynthesis to high pCO_2 for corals assigned to branching vs. mounding functional groups, algae assigned to branching and encrusting functional groups, and both corals and algae assigned to either a fast or slow growing group as defined by empirical determinations of calcification rate (after Comeau *et al.*, 2014).

Material and methods

Sample collection and preparation

Our study combines results from two experiments conducted in Moorea, French Polynesia, in 2011 and 2012. The first experiment was performed from August to October 2011 on four corals (*Porites rus*, *Acropora pulchra*, *Pocillopora damicornis*, and *Pavona cactus*) and four algae (*Porolithon onkodes, Lithophyllum flavescens*, *Halimeda macroloba*, and *Halimeda minima*) (Comeau *et al.*, 2013a). To strengthen the diversity of calcifiers from each functional group investigated, a second experiment using an identical procedure was conducted from August to October 2012 with four different corals (massive *Porites* spp., *Psammocora profundacella*, *Porites irregularis*, and *Pocillopora verrucosa*) and three different calcified algae (*Hydrolithon reinboldii*, *Lithophyllum kotschyanum*, and *Neogoniolithon frutescens*). Each year, the experiment was separated into four consecutive trials, each lasting 2 weeks, corresponding to replications of the same experiment on different organisms/species. For each trial, individual organisms of each species were collected from mother colonies that were at least 30 m apart on the back reef on the North shore of Moorea at ~1–2 m depth. Samples of crustose coralline algae were collected by coring (3.5–4.5 cm diameter, for *P. onkodes* and *L. flavescens*); coral nubbins (3–5 cm long branches), *Halimeda* clumps (4–6 cm long thalli), and other coralline algae were hand collected. Freshly collected samples were returned to the Richard B. Gump South Pacific Research Station, where coral nubbins and coralline algae were glued with Z-Spar (A788 epoxy) to plastic bases (4 × 4 cm), and *Halimeda* clumps were attached to similar bases using nylon line.

After preparation and 2 d of recovery in a shallow tank supplied with flowing seawater, organisms were transferred for 2 weeks to an acclimation tank (Aqualogic Inc., San Diego, CA, USA) in which temperature and light were adjusted to provide conditions similar to those used during the subsequent treatments (27°C, ~700 μmol quanta m^{-2} s^{-1}). The objective of the incubation in the acclimation tank was to adjust the organisms to environmental conditions characterizing the laboratory system, thereby reducing unique effects of environmental conditions arising from the sites at which each sample was collected. New organisms were collected every 2 weeks to allow a similar 2-week acclimation period for each trial.

Incubations and CO_2 control

A detailed summary of the incubation and CO_2 control technology can be found in Comeau *et al.* (2013). In brief, 36 individuals from one coral taxon and one algal taxon were allocated randomly to six of the twelve 150-l tanks (Aqualogic) (for a final density of six coral nubbins and six algal pieces in each tank). Individuals of another

coral and algal taxon were allocated to the remaining six tanks experiencing identical incubation conditions. For each trial, the six organisms per treatment and species were used to study the response of calcification (Comeau *et al.*, 2013, 2014) and two of these organisms were selected randomly for measurements of photosynthesis and respiration after 7–10 d of incubation (see below). Organisms were maintained under six pCO_2 level during 2 weeks in an open flow system (with seawater flowing in at ~100 ml min^{-1}), then the experiment was repeated with different organisms from the same species. Water motion in the tank was provided by head pump (Rio 8HF, 2082 l h^{-1}). The six CO_2 treatments targeted six values, corresponding to preindustrial (280 μatm), present day (400 μatm), a optimistic value expected by the end of the century (~550 μatm, ~representative concentration pathway [RCP] scenario 4.5, Moss *et al.*, 2010), an average for the end of the century (~700 μatm, ~RCP scenario 6.0), a pessimistic value expected by the end of the 21st century (~1000 μatm, ~RCP scenario 8.5), and an extreme value of 2000 μatm (which creates seawater conditions close to the saturation threshold with respect to aragonite [i.e. $\Omega_{arag} \sim 1$]). Temperature in the tanks was maintained at 27°C, which was the mean *in situ* seawater temperature in September–October 2011–2012. Irradiance was provided by LED lamps (75-W, Sol LED Module, Aquaillumination) on a 12:12 h light:dark photoperiod providing ~700 μmol photons m^{-2} s^{-1} of photosynthetically active radiation (PAR, measured below the seawater surface with a 4π quantum sensor LI-193 and a LiCor LI-1400 m). This irradiance was chosen to be saturating with respect to photosynthesis for corals and algae collected from 2 m depth, where saturating irradiances for photosynthesis typically are >400 μmol photons m^{-2} s^{-1} (Chalker, 1981; Chisholm, 2003).

Carbonate chemistry

Seawater pH on the total scale (pH_T) was measured daily in each tank at 08:00 and 18:00 h, using a pH meter (Orion, 3-stars mobile mounted with a Mettler DG 115-SC pH electrode) calibrated every 2 d on the total scale using Tris–HCl buffers (supplied by A. Dickson, SIO). pH_T also was measured spectrophotometrically for all tanks once during each trial using the indicator dye m-cresol (Standard Operating Procedure 6b, Dickson *et al.*, 2007), which yielded results within ≤0.01 of pH values obtained with the pH meter. Total alkalinity (A_T) also was measured every 2 d using open cell, potentiometric titrations (automatic titrator T50, Mettler-Toledo) on 50-ml samples collected the day of the measurement. Titration of certified reference materials (A. G. Dickson batch 105 and 108) yielded A_T values within 3.5 μmol kg^{-1} of certified values. The variables A_T, pH_T, temperature, and salinity (measured by a conductivity meter YSI 3100) were used to calculate carbonate chemistry parameters using the Seacarb package (Gattuso *et al.*, 2015) running in R software (R foundation for statistical computing).

Respiration and net photosynthesis

In the middle of the 2-week incubation period (after 7–10 d), respiration and net photosynthesis were measured on four organisms per treatment and species that were randomly picked from the 12 organisms per treatment and species used during the two trials; the measurements took 4 d to complete. To determine net photosynthesis, one coral and one alga from each treatment were chosen randomly 2 h after the LED lamps switched on (i.e. at ~08:00 h, following "sunrise"), and were placed in separate 240-ml custom-made acrylic incubation chamber containing seawater from the respective incubation tanks. Changes in dissolved oxygen were monitored during ~30 min incubations with PreSens dipping oxygen optodes (PSt3) connected to Fibox 3 transmitters (Precision Sensing GmbH, Germany). The oxygen probes were calibrated every morning using a 2-point calibration in water-saturated air (100%) and anoxic seawater created by supersaturating seawater with sodium dithionite ($Na_2S_2O_4$). Temperature was held constant during incubations by circulating water in a water jacket surrounding the incubation chamber. Chambers were positioned on magnetic stir plates, and seawater flow inside the chambers was regulated with magnetic stir bar that created vigorous and turbulent water motion. A similar irradiance to that used during incubations (i.e. 700 μmol quanta m^{-2} s^{-1}) was maintained while the organisms were in the acrylic chambers by placing an LED lamp (75-W, Sol LED Module, Aquaillumination) above the chambers. On the same day that net photosynthesis was measured, and after at least 2 h of darkness (i.e. after 20:00 h), dark respiration was measured using an identical procedure to that used for photosynthesis, except the chamber was kept in darkness.

During the first year of the experiment, only dark respiration and net photosynthesis were measured. In addition to these measurements, light-enhanced dark respiration (LEDR) was determined during the second year. LEDR was assumed to be equivalent to the dark respiration measured during the first ~15 min following total darkness immediately after net photosynthesis was recorded at 700 μmol quanta m^{-2} s^{-1}. This assumption relies on the stimulatory effect of photosynthesis on respiration continuing unabated for at least 15 min following the onset of darkness following exposure to light (Kaniewska *et al.*, 2012). The stimulatory effect presumably arises from the effects on aerobic respiration of high O_2 concentrations or increased supplies of metabolic substrates translocated from the *Symbiodinium* to the cnidarian host (Stambler, 2011). Gross photosynthesis then was determined by subtracting LEDR (expressed with a negative notation indicating O_2 uptake) from net photosynthesis to provide a value that is likely to be close to the actual gross photosynthesis (Barnes and Chalker, 1990). Net and gross photosynthesis, dark respiration, and LEDR were normalized to surface area and biomass (tissue dry weight) for corals and coralline algae. Surface area was determined by the aluminium foil technique (Marsh, 1970) or image analysis (Image J, US National Institutes of Health) of planar digital images. To measure biomass, organisms were fixed in 10% formalin in seawater for 48 h then decalcified in 5% HCl for 2–4 d. The tissue tunics left after skeletal dissolution were homogenized then dried for 24–48 h at 60°C before weighing. The technique normally employed to determine surface area of complex objects [i.e. wax dipping (Stimson and Kinzie, 1991) and aluminium foil (Marsh, 1970)] could not be applied to *Halimeda*, which readily dropped segments. Biomass normalization was not used for *P. cactus* because their delicate tissues were damaged during decalcifcation.

An Akaike information criterion (AIC) approach was used to determine if linear, logarithmic, or polynomial models best described the functional relationships of dark respiration, LDER, net photosynthesis, and gross photosynthesis against pCO_2 for each species (see details in Comeau *et al.*, 2013). A linear relationship was fit to explore a "proportional effect" model for rising pCO_2 that could, for example, represent an increase in respiration associated with increasing cost of calcification; a logarithmic relationship was fit to explore a "disproportional effect" model associated, for example, with a saturation relationship against rising pCO_2, and a second-order polynomial relationship was fit to explore a "threshold

effect" model in which an optimal pCO_2 would be associated with maximization of a metabolic process.

Results

During experiments conducted in 2011 and 2012 organisms were maintained successfully in regulated conditions with pCO_2 treatments maintained at mean values of 259 ± 2, 380 ± 2, 516 ± 4, 693 ± 7, 986 ± 9, and 1971 ± 20 μatm (± SE, $n = 380$, Comeau *et al.*, 2014). These pCO_2 levels correspond to aragonite saturation states of 4.7, 3.8, 3.1, 2.6, 2.0, and 1.1 at a total alkalinity of ~2324 ± 3 μmol kg^{-1}, temperature of ~27.1°C (SE < 0.1), and salinity of ~36.1 (SE < 0.1). All corals and algae remained alive during the incubations and no bleaching was observed.

Net photosynthesis

In both corals and calcifying algae, AIC analyses justified the use of linear regressions to represent the relationships between pCO_2 and net photosynthesis normalized separately to biomass and surface area (Figures 1 and 2, Supplementary Figures S1 and S2). For corals, the rates of net photosynthesis normalized to biomass at 400 μatm pCO_2 varied from a minimum of 1.27 ± 0.09 μg O_2 h^{-1} mg^{-1} in *Psammocora profundacella* to a maximum of 4.42 ± 0.27 μg

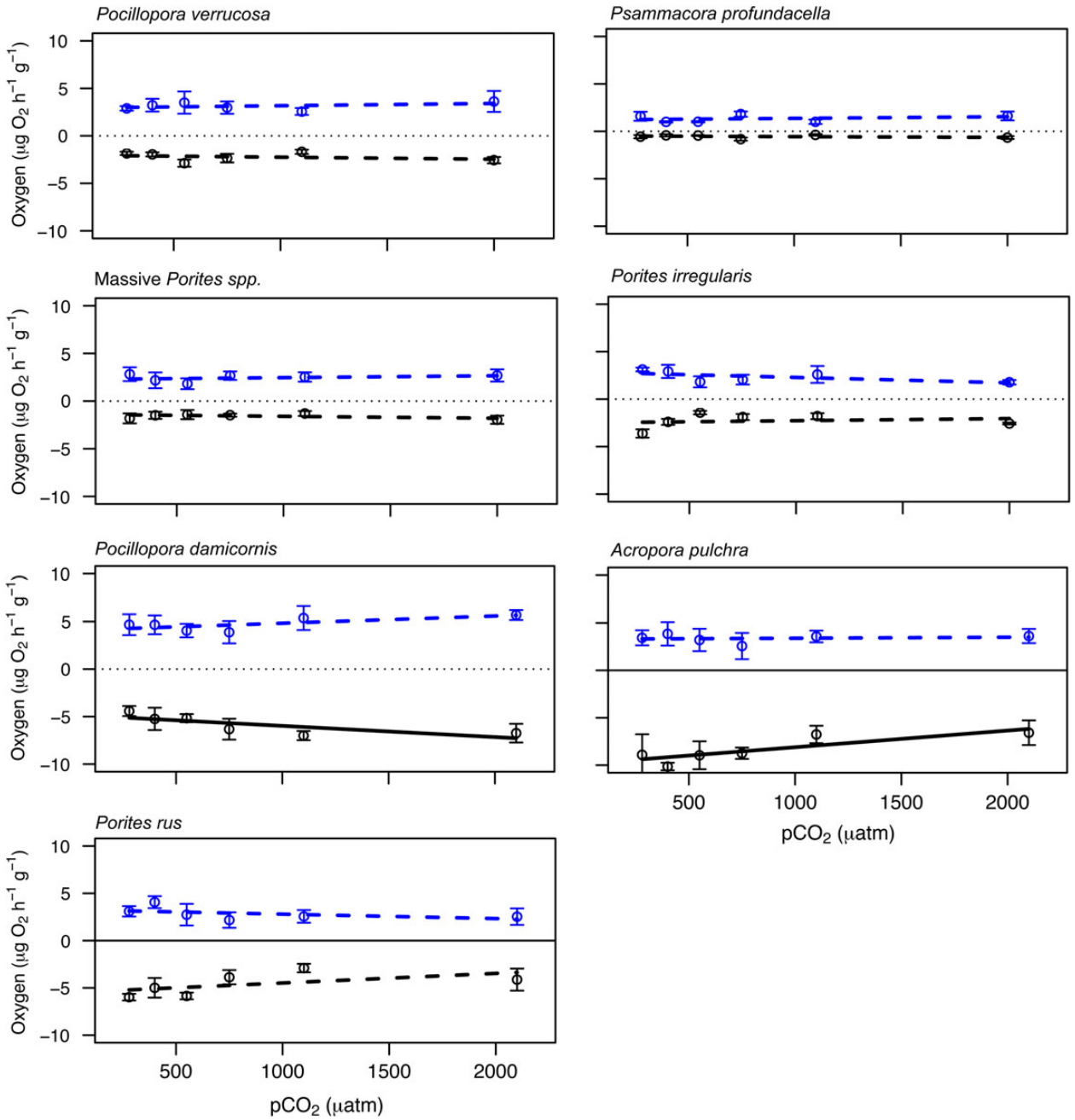

Figure 1. Biomass-normalized dark respiration (black lines) and net photosynthesis (blue lines) in corals as a function of pCO_2. The points correspond to the mean rates at each tested pCO_2 ($n = 4$) and the vertical bars show ± SE. Solid lines show a significant *p*-value for the slope and dashed lines indicate a non-significant *p*-value for the slope.

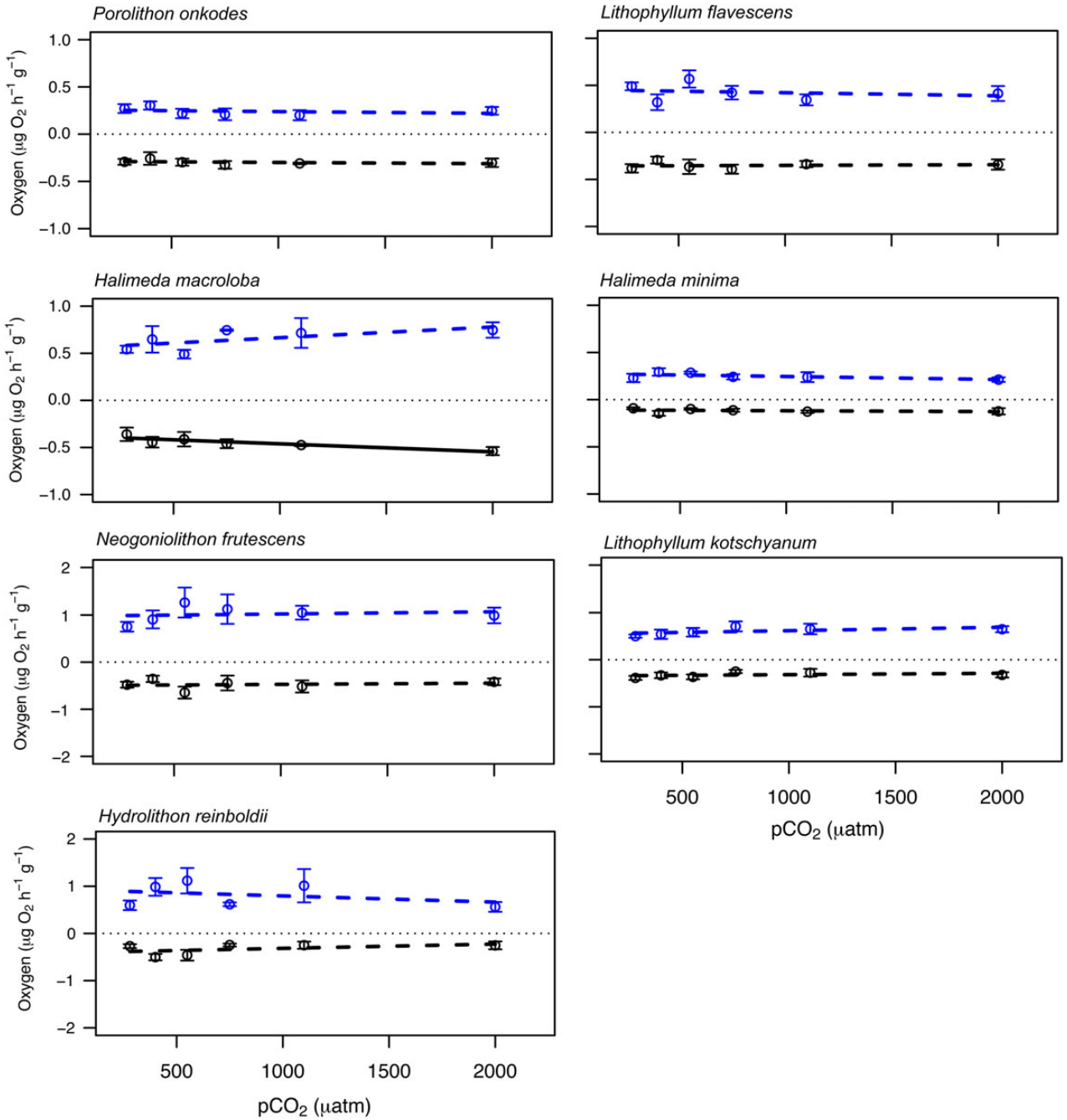

Figure 2. Biomass-normalized dark respiration (black lines) and net photosynthesis (blue lines) in algae as a function of pCO_2. The points correspond to the mean rates at each tested pCO_2 ($n = 4$) and the vertical bars show $\pm$ SE. The solid line shows a significant *p*-value for the slope and dashed lines indicate a non-significant *p*-value for the slope. This figure is available in black and white in print and in colour at *ICES Journal of Marine Science* online.

$O_2\ h^{-1}\ mg^{-1}$ in *Pocillopora damicornis* (Figure 1). No effect of pCO_2 on net photosynthesis was detected as shown by non-significant slopes for the linear regressions between this dependent variable and pCO_2 (Table 1). When normalized to surface area, the slopes of the linear regressions between net photosynthesis and pCO_2 also were not significant for all species, except *Porites irregularis* for which photosynthesis decreased with pCO_2 (Table 1, Supplementary Figure S1).

For algae, the slopes of biomass-normalized net photosynthesis against pCO_2 were not significant in the five coralline algae and the two *Halimeda* species tested (Table 1, Figure 2). Biomass-normalized net photosynthesis varied between taxa at 400 μatm pCO_2 from 0.25 μg $O_2\ h^{-1}\ mg^{-1}$ in *Porolithon onkodes* to 0.98 μg $O_2\ h^{-1}\ mg^{-1}$ in *Neogoniolithon frustescens*. Similar to biomass-normalized rates, there was no effect of pCO_2 on surface area-normalized net photosynthesis in the five species of coralline algae (Table 1, Supplementary Figure S2).

Dark respiration

The linear relationships of biomass-normalized dark respiration with pCO_2 in corals did not have significant slopes for any coral species

Table 1. Parameters of the linear equations describing the relationship between net photosynthesis and pCO_2 (μatm) for the coral and algae studied.

Species	Surface area-normalized net photosynthesis	*p*	Biomass-normalized net photosynthesis	*p*
Massive *Porites* spp.	$-1.3 \times 10^{-3} pCO_2 + 38.1$	0.797	$2.0 \times 10^{-4} pCO_2 + 2.3$	0.676
Porites irregularis	$-4.7 \times 10^{-4} pCO_2 + 22.1$	0.028	$-5.7 \times 10^{-4} pCO_2 + 2.9$	0.168
Pocillopora verrucosa	$-1.7 \times 10^{-3} pCO_2 + 26.6$	0.521	$2.4 \times 10^{-4} pCO_2 + 2.9$	0.644
Psammocora profundacella	$6.2 \times 10^{-3} pCO_2 + 45.9$	0.128	$1.7 \times 10^{-4} pCO_2 + 1.2$	0.478
Porites rus	$2.2 \times 10^{-3} pCO_2 + 26.2$	0.621	$-4.9 \times 10^{-4} pCO_2 + 3.3$	0.391
Acropora pulchra	$3.8 \times 10^{-3} pCO_2 + 11.9$	0.213	$1.2 \times 10^{-4} pCO_2 + 3.3$	0.864
Pocillopora damicornis	$1.7 \times 10^{-3} pCO_2 + 24.1$	0.718	$8.1 \times 10^{-4} pCO_2 + 4.1$	0.236
Pavona cactus	$-6.4 \times 10^{-3} pCO_2 + 24.7$	0.088	*n.d.*	
Porolithon onkodes	$-1.6 \times 10^{-3} pCO_2 + 29.2$	0.640	$-1.7 \times 10^{-5} pCO_2 + 0.26$	0.584
Lithophyllum flavescens	$-3.0 \times 10^{-3} pCO_2 + 41.2$	0.639	$-3.0 \times 10^{-5} pCO_2 + 0.45$	0.679
Neogoniolithon frutescens	$8.1 \times 10^{-4} pCO_2 + 12.0$	0.645	$5.4 \times 10^{-5} pCO_2 + 0.96$	0.735
Lithophyllum kotschyanum	$1.7 \times 10^{-3} pCO_2 + 9.8$	0.170	$7.2 \times 10^{-5} pCO_2 + 0.55$	0.254
Hydrolithon reinboldii	$-6.6 \times 10^{-4} pCO_2 + 13.9$	0.589	$-1.5 \times 10^{-4} pCO_2 + 0.94$	0.285
Halimeda macroloba	*N.d.*		$1.1 \times 10^{-5} pCO_2 + 0.56$	0.210
Halimeda minima	*N.d.*		$-3.5 \times 10^{-5} pCO_2 + 0.28$	0.203

Parameters of the relationships are given for net photosynthesis normalized by surface area (cm^2) and biomass (mg). *p*-values refer to the significance of the slope estimates in linear regressions.

Table 2. Parameters of the linear equations describing the relationship between night respiration and pCO_2 (μatm) for the coral and algae studied.

Species	Surface area-normalized dark respiration	*p*	Biomass-normalized dark respiration	*p*
Massive *Porites* spp.	$-2.7 \times 10^{-3} pCO_2 - 22.1$	0.165	$-2.0 \times 10^{-4} pCO_2 - 1.4$	0.428
Porites irregularis	$-1.1 \times 10^{-4} pCO_2 - 16.7$	0.947	$2.2 \times 10^{-4} pCO_2 - 2.5$	0.516
Pocillopora verrucosa	$4.8 \times 10^{-5} pCO_2 - 18.3$	0.973	$-2.2 \times 10^{-4} pCO_2 - 2.0$	0.392
Psammocora profundacella	$-2.2 \times 10^{-3} pCO_2 - 20.0$	0.299	$-7.7 \times 10^{-5} pCO_2 - 0.5$	0.457
Porites rus	$-3.9 \times 10^{-3} pCO_2 - 42.5$	0.253	$1.0 \times 10^{-3} pCO_2 - 5.5$	0.101
Acropora pulchra	$6.5 \times 10^{-4} pCO_2 - 36.7$	0.829	$1.8 \times 10^{-3} pCO_2 - 9.9$	0.046
Pocillopora damicornis	$-3.2 \times 10^{-3} pCO_2 - 27.9$	0.321	$-1.2 \times 10^{-3} pCO_2 - 4.8$	0.049
Pavona cactus	$2.9 \times 10^{-3} pCO_2 - 27.4$	0.062	*N.d.*	
Porolithon onkodes	$-1.1 \times 10^{-3} pCO_2 - 33.3$	0.582	$-1.2 \times 10^{-5} pCO_2 - 0.29$	0.482
Lithophyllum flavescens	$9.5 \times 10^{-4} pCO_2 - 32.5$	0.668	$7.1 \times 10^{-6} pCO_2 - 0.36$	0.799
Neogoniolithon frutescens	$1.6 \times 10^{-4} pCO_2 - 5.5$	0.770	$2.4 \times 10^{-5} pCO_2 - 0.49$	0.749
Lithophyllum kotschyanum	$2.6 \times 10^{-4} pCO_2 - 6.4$	0.730	$2.7 \times 10^{-5} pCO_2 - 0.34$	0.502
Hydrolithon reinboldii	$8.7 \times 10^{-4} pCO_2 - 6.1$	0.161	$8.9 \times 10^{-5} pCO_2 - 0.40$	0.151
Halimeda macroloba	*N.d.*		$-8.5 \times 10^{-5} pCO_2 - 0.38$	0.031
Halimeda minima	*N.d.*		$-8.4 \times 10^{-6} pCO_2 - 0.11$	0.577

Parameters of the relationships are given for night respiration normalized by surface area and biomass. *p*-values refer to the significance of the slope estimates in linear regressions.

except *Pocillopora damicornis* (1.8×10^{-3} μg O_2 h^{-1} mg^{-1} μatm pCO_2^{-1}; $p = 0.049$) and *Acropora pulchra* (-1.2×10^{-3} μg O_2 h^{-1} mg^{-1} μatm pCO_2^{-1}; $p = 0.046$) (Table 2, Figure 1). Biomass-normalized dark respiration varied among coral species, as shown at 400 μatm pCO_2 where dark respiration ranged from -0.6 ± 0.05 μg O_2 h^{-1} mg^{-1} (mean ± SE) in *Psammocora profundacella* to -9.2 ± 0.35 μg O_2 h^{-1} mg^{-1} in *A. pulchra*. When normalized to surface area, dark respiration was not affected by pCO_2 in any coral taxa (Table 2, Supplementary Figure S1).

For algae, the relationships of biomass-normalized dark respiration with pCO_2 exhibited non-significant slopes in six of seven species (Table 2, Figure 2), and for the seventh (*H. macroloba*) dark respiration increased with pCO_2 (slope = 8.5×10^{-5} μg O_2 h^{-1} mg^{-1} μatm pCO_2^{-1}; $p = 0.031$). When dark respiration was normalized to surface area, it was not affected by pCO_2 in the five coralline algae (Table 2, Supplementary Figure S2).

LEDR and gross photosynthesis

For the four corals for which LEDR was measured in 2012 (Figure 3, Supplementary Figure S3), the slopes of the relationships for LEDR (surface area and biomass normalized) against pCO_2 were not significant (Table 3). Similarly, in the three algae (*Neogoniolithon frutescens*, *Hydrolithon reinboldii*, and *Lithophyllum kotschyanum*) for which LEDR was measured, pCO_2 did not impact LEDR when normalized to surface area or biomass (Table 3, Figure 4, Supplementary Figure S4).

Discussion

Using a large range of pCO_2 values and 15 species of common reef calcifiers on the shallow reefs of Moorea, the present study shows that net photosynthesis, dark respiration, LEDR, and gross photosynthesis of corals and calcified algae are largely insensitive to pCO_2 during short-term incubations. Furthermore, the general absence of an effect of pCO_2 on the tested parameters demonstrates that the categorization by functional groups (i.e. branching vs. mounding corals) does not drive the response of photosynthesis and respiration to elevated pCO_2 in these species. These results highlight that the likelihood that the increased availability of dissolved inorganic carbon [C_T] in seawater due to OA will not increase photosynthesis in most corals and calcified algae (cf Ries *et al.*,

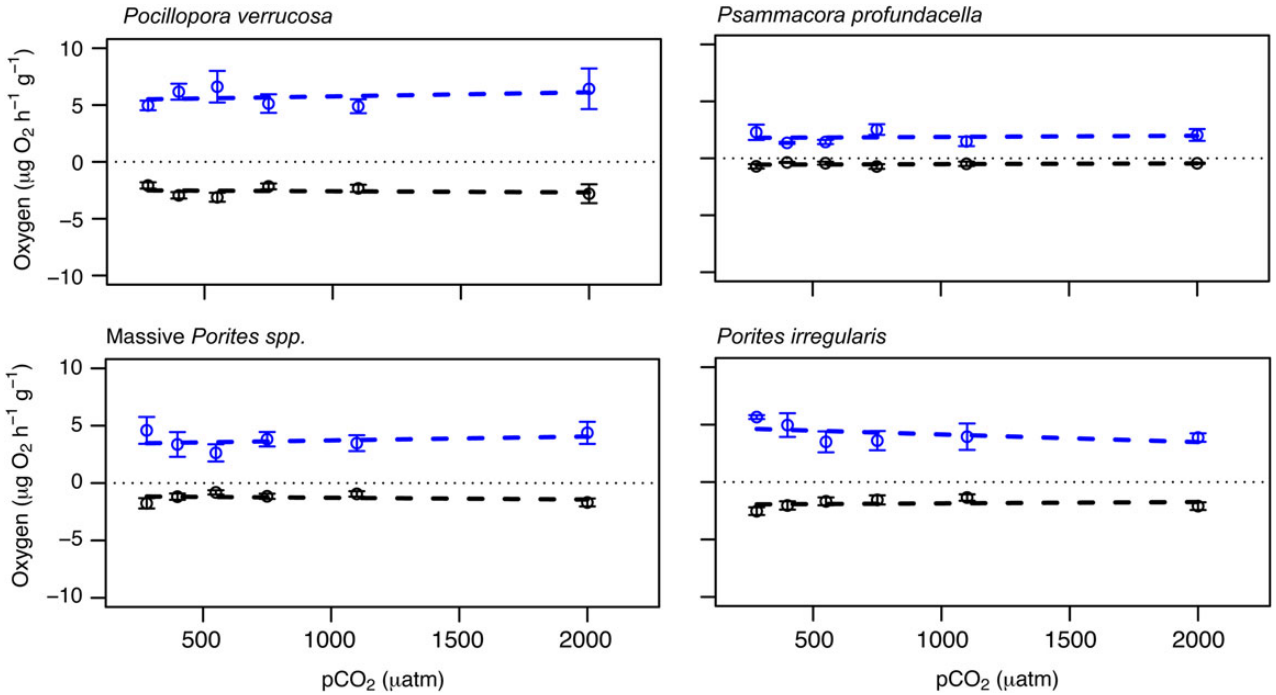

Figure 3. Biomass-normalized light respiration (black lines) and gross photosynthesis (blue lines) in corals as a function of pCO_2. The points correspond to the mean rates at each tested pCO_2 ($n = 4$) and the vertical bars show $\pm$ SE. The solid line shows a significant *p*-value for the slope and dashed lines indicate a non-significant *p*-value for the slope. This figure is available in black and white in print and in colour at *ICES Journal of Marine Science* online.

Table 3. Parameters of the linear estimates of the relationship between LEDR and pCO_2 (μatm) for the coral and algae studied.

Species	Surface area-normalized LEDR	*p*	Biomass-normalized LEDR	*p*
Massive *Porites* spp.	$-1.4 \times 10^{-3} pCO_2 - 19.9$	0.534	$1.4 \times 10^{-4} pCO_2 - 1.1$	0.582
Porites irregularis	$1.0 \times 10^{-3} pCO_2 - 15.8$	0.726	$-9.4 \times 10^{-5} pCO_2 - 2.0$	0.584
Pocillopora verrucosa	$2.6 \times 10^{-3} pCO_2 - 23.3$	0.193	$1.2 \times 10^{-4} pCO_2 - 2.5$	0.722
Psammocora profundacella	$1.4 \times 10^{-3} pCO_2 - 20.6$	0.536	$6.5 \times 10^{-5} pCO_2 - 0.57$	0.562
Neogoniolithon frutescens	$-3.1 \times 10^{-4} pCO_2 - 4.1$	0.667	$-2.0 \times 10^{-5} pCO_2 - 0.3$	0.749
Lithophyllum kotschyanum	$6.7 \times 10^{-5} pCO_2 - 5.5$	0.921	$1.4 \times 10^{-5} pCO_2 - 0.3$	0.697
Hydrolithon reinboldii	$8.8 \times 10^{-4} pCO_2 - 4.6$	0.179	$7.3 \times 10^{-5} pCO_2 - 0.3$	0.322

Parameters of the relationships are given for LEDR normalized by surface area (cm^2) and biomass (mg). *p*-values refer to the significance of the slope estimates in linear regressions.

2009). The outcome revealing that dark respiration is unaffected by elevated pCO_2 is inconsistent with the hypothesis that the metabolic costs of calcification, while rising due to the thermodynamic challenges of depositing $CaCO_3$ at low pH, is a factor limiting biogenic calcification under OA (*sensu* McCulloch *et al.*, 2012), although this result could indicate variations in allocation of energy.

Coral photosynthesis

Previous studies on the effects of elevated pCO_2 on coral photosynthesis have reported a variety of results ranging from inhibitory to stimulatory effects. A decrease of photosynthesis at high pCO_2 has been measured, for example, for *Acropora millepora* maintained under pCO_2 treatments of ~350, 685, and 1180 μatm (Kaniewska *et al.*, 2012) as well as *A. intermedia* and *Porites lobata* at ~300, 600, and 1200 μatm pCO_2 (Anthony *et al.*, 2008). In contrast, an increase of photosynthesis with pCO_2 was reported for *Stylophora pistillata* at 460 and 760 μatm (Reynaud *et al.*, 2003). Recently, a study of coral metabolism at natural CO_2 vents also has shown that photosynthesis of the coral *Porites* spp., *A. millepora*, and *P. damicornis* is enhanced at the elevated pCO_2 site (where pCO_2 reaches 862 μatm daily) compare with control conditions (Strahl *et al.*, 2015). Similarly, Inoue *et al.* (2013) found that net photosynthesis of the soft coral *Sarcophyton elegans* present at CO_2 vents is enhanced. These increases in photosynthesis measured at CO_2 vents in both hard and soft corals could be due to long-term acclimation to elevated pCO_2 in contrast to the present study that did not allow any potential acclimation. However, similarly to the present results, some studies also have reported no effect of increasing pCO_2 on coral photosynthesis, for example, for *A. digitifera* incubated at pCO_2 levels between ~340 and 2140 μatm (Takahashi and Kurihara, 2013), and on entire coral reef communities maintained at 411, 647, and 918 μatm (Leclercq *et al.*, 2002). Overall, the results from the aforementioned studies (and others) support a null effect size of pCO_2 on coral photosynthesis when evaluated in a meta-analytical framework (Kroeker *et al.*, 2013). This outcome agrees with the present results.

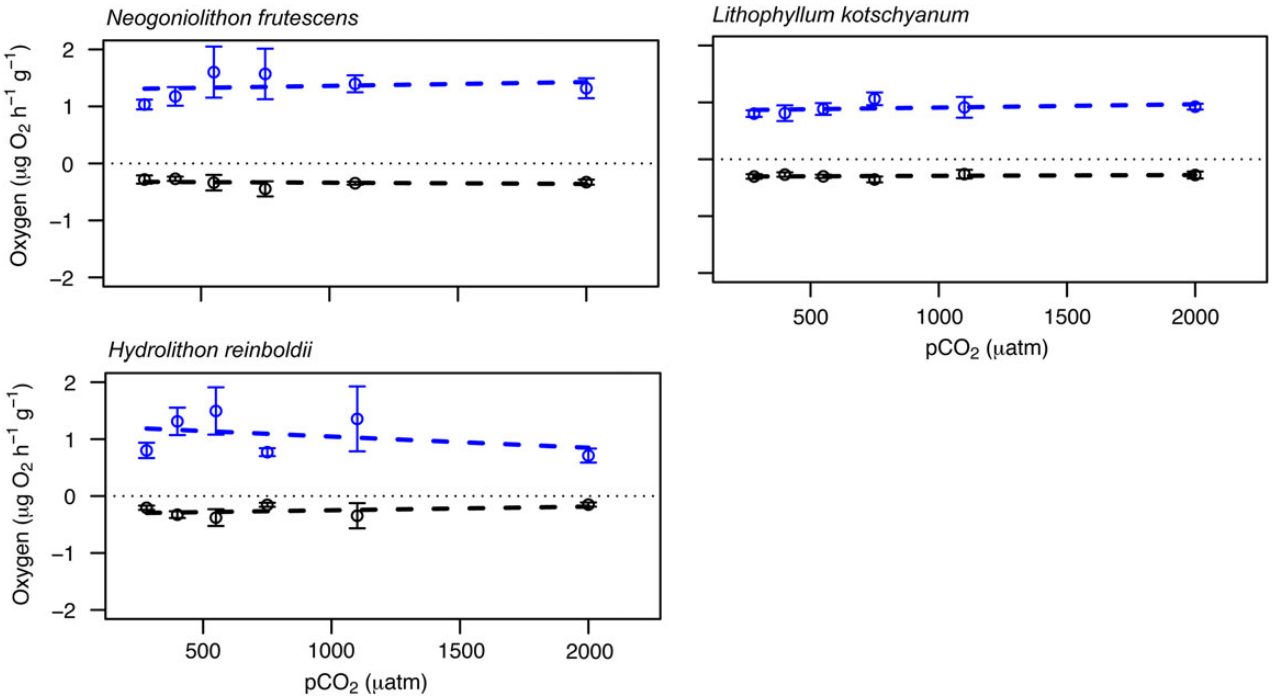

Figure 4. Biomass-normalized light respiration (black lines) and gross photosynthesis (blue lines) in algae as a function of pCO_2. The points correspond to the mean rates at each tested pCO_2 ($n = 4$) and the vertical bars show $\pm$ SE. The solid line shows a significant *p*-value for the slope and dashed lines indicate a non-significant *p*-value for the slope. This figure is available in black and white in print and in colour at *ICES Journal of Marine Science* online.

For many plants, elevated pCO_2 has a "fertilization effect" on photosynthesis, because high pCO_2 alleviates carbon limitation at the Calvin Cycle and facilitates greater rates of photosynthesis compared with control conditions of ambient pCO_2 (e.g. Kirschbaum, 1994, Norby *et al.*, 2005, Vogel *et al.*, 2015). In the present study, elevated pCO_2 and increased concentrations of bicarbonate did not affect gross or net photosynthesis of corals. There are several mechanisms by which the symbiosis between scleractinian hosts and their symbiotic algae can reduce the potential for enhancement of carbon fixation by elevated concentrations of dissolved inorganic carbon (DIC). First, the lack of a fertilization effect of high pCO_2 on coral photosynthesis also serve, by default, to suggest that carbon fixation in corals could already be maximized under ambient pCO_2. Second, *Symbiodium* in reef corals are not in direct contact with seawater, as they reside within the gastroderm of corals in an intracellular membrane-enclosed compartment (Stambler, 2011). Spatial separation from the surrounding seawater and enclosure within two animal cell membranes is likely to reduce the direct effect of modifications of seawater DIC for photosynthesis. Third, *Symbiodinium* rely on a carbon concentration mechanism (CCM) that reduces their dependence on CO_2 uptake by diffusion and allow them to utilize carbon available in the bicarbonate form (Leggat *et al.*, 1999; Mackey *et al.*, 2015). Finally, external DIC is not the only source of carbon for photosynthesis by symbiotic corals, because host respiration likely provides some of the CO_2 that is used by *Symbiodinium* for photosynthesis, although the relative importance of this source of CO_2 is uncertain (Allemand *et al.*, 1998; Furla *et al.*, 2000).

Nevertheless, despite the isolation from ambient seawater of the symbiotic *Symbiodinium* in reef corals, high pCO_2 has been reported to enhance photosynthesis in some corals (cited above), and at least two studies suggest that there is a stimulatory role of increasing bicarbonate ion concentrations in seawater for coral photosynthesis (Herfort *et al.*, 2008; Marubini *et al.*, 2008). One way to reconcile the present and previous results is to propose that corals have bicarbonate transporters (Zoccola *et al.*, 2015) that enhance the delivery of bicarbonate to the acidic symbiosome (pH = 4) surrounding each *Symbiodinium* cell, and these transports might vary in presence and expression level depending on species. Within the symbiosome, bicarbonate could then be converted into CO_2 through the effect of pH on the equilibrium reactions affecting DIC species, ultimately providing CO_2 that could be used by *Symbiodinium* for photosynthesis (Barott *et al.*, 2015). While the rate of such transport could be affected by increasing bicarbonate ion concentration, perhaps the moderate increase in bicarbonate associated with OA (i.e. $\sim$ 10% increase with a doubling of ambient pCO_2) is not sufficient to enhance photosynthesis under conditions expected by the end of the century.

Algal photosynthesis

In algae, as described above for scleractinians, there was no effect of high pCO_2 on net and gross photosynthesis in all coralline species and *Halimeda* species. Previous studies on algae have generated equivocal evidence of the effects of pCO_2 on photosynthesis. A strong decrease in net photosynthesis at elevated pCO_2 has been measured in *Porolithon onkodes* (Anthony *et al.*, 2008), but another study on the same species (Johnson *et al.*, 2014) as well as the present study did not detect an effect of high pCO_2 (to 2000 μatm). In contrast, an increase in photosynthesis has been reported in a few algal species including *Corallina officinalis* at pH 7.7 (Yildiz *et al.*, 2013) and *Halimeda cylindrica* at 660 μatm pCO_2 (Sinutok *et al.*, 2011). More complex responses than the aforementioned linear effects as a result of elevated pCO_2 have been found for the coralline alga *Lithothamnion corallioides,* in which

the response of photosynthesis to pCO_2 was curvilinear (Noisette *et al.*, 2013). In yet another example, which yielded results similar to the present study, photosynthesis was unaffected by elevated pCO_2 (to ~2000 μatm pCO_2) in the temperate algae *C. officinalis* (Cornwall *et al.*, 2012; Hofmann *et al.*, 2012) and *Arthrocardia corymbosa* (Cornwall *et al.*, 2013) (Table 4).

The diversity of responses of photosynthesis to elevated pCO_2 in macroalgae reported in the literature likely reflects species-specific effects as well as different light, exposure time, and nutrient conditions between studies (Hofmann and Bischof, 2014). While, it also has been suggested that differences in mineralized structure composition (e.g. intracellular vs. extracellular calcification and calcite vs. aragonite) could influence the response of photosynthesis to elevated pCO_2 in marine algae (Hofmann and Bischof, 2014), the present results do not support this hypothesis, as species with similar mineral structures responded to elevated pCO_2 in a different manner. Although the present study cannot address the mechanistic bases of the lack of pCO_2 effects on photosynthesis and respiration of the algal taxa studied, overall the results are consistent with two hypotheses that are not mutually exclusive. First, marine calcifying algae may possess a CCM that allows them to utilize bicarbonate as a carbon source for photosynthesis (Digby, 1977); therefore, the absence of a pCO_2 effect on photosynthesis may reflect that enough DIC is already available for this physiological process. Second, calcification and photosynthesis are tightly coupled in calcifying algae, largely through the use of internal CO_2 by photosynthesis that causes an increase in intracellular pH and creates chemical conditions favouring calcification (Borowitzka and Larkum, 1987; De Beer and Larkum, 2001). The absence of an effect of pCO_2 on algal photosynthesis could reflect the presence of large quantities of intracellular dissolved CO_2 though calcification reaction that favour CO_2 formation (Frankignoulle *et al.*, 1994), stimulating in return photosynthesis. Although reduced calcification under OA could reduce this CO_2 source, it could be compensated by the increased of seawater dissolved CO_2.

Coral respiration

For most coral taxa investigated, we did not measure an effect of pCO_2 on dark respiration. Only the dark respiration of *P. damicornis* and *A. pulchra* normalized to biomass was affected by pCO_2. These results are similar to the absence of an effect of pCO_2 on dark respiration in the corals *Stylophora pistillata* (Reynaud *et al.*, 2003), *A. eurystoma* (Schneider and Erez, 2006), and *A. digitifera* (Takahashi and Kurihara, 2013). In contrast, dark respiration of massive *Porites* spp. (Edmunds, 2012), *A. millepora* (Kaniewska *et al.*, 2012), larvae of *Porites astreoides* (Albright and Langdon, 2011), and larvae of *P. damicornis* (Rivest and Hofmann, 2014) all decreased when exposed to elevated pCO_2. For LEDR, in the four coral species for which it was measured, it was not affected by pCO_2. This is a similar outcome to the one reported for *A. millepora* incubated between 260 and 1350 μatm pCO_2 (Kaniewska *et al.*, 2012), but it contrasts with the increase in LEDR reported for *A. formosa* exposed to 380–1100 μatm pCO_2 (Crawley *et al.*, 2010).

Elevated aerobic respiration as a function of OA could result from higher cost of functioning at elevated pCO_2 caused, for example, by perturbed active cellular transport (e.g. Thomsen and Melzner, 2010), or an increasing cost of calcification (McCulloch *et al.*, 2012). In contrast, a decrease in respiration at low pH reflects a reduction in metabolic costs, and perhaps explicit metabolic depression (Pörtner *et al.*, 2004). The response of aerobic respiration to high pCO_2 in the present study was quite variable as has been reported previously (e.g. Schneider and Erez, 2006; Edmunds, 2012), notably with reduced respiration in *A. pulchra*, elevated respiration in *P. damicornis*, and null effects in all other species. A null effect on respiration could have reflected multiple pathways of metabolic responses, ranging from a simply case of no effect, to more complex cases involving energetic trade-offs among critical processes that change the allocation of energy to individual functions while conserving the overall metabolic costs (Pan *et al.*, 2015). Further work would be necessary to elucidate which pathway of allocation of energy corals use when incubated under elevated pCO_2 conditions. As it has been done in sea urchins (Pan *et al.*, 2015), such study would require characterization of the effects of pCO_2 on a large amount of physiological parameters such as calcification, protein synthesis, gene expression, and enzyme activities.

Algal respiration

Similarly to the effects of pCO_2 on dark respiration of corals, dark respiration and LEDR of most algae were not affected by pCO_2, and it was only in *H. macroloba* that a positive response of respiration to increasing pCO_2 was detected. Comparable results have been found in previous studies, for instance with no effect of pCO_2 on the respiration of *P. onkodes* (Anthony *et al.*, 2008), *C. elongata* (Egilsdottir *et al.*, 2012; Noisette *et al.*, 2013), and *C. officinalis* (Hofmann *et al.*, 2012). Non-linear responses of dark respiration as a function of variable pCO_2 have been reported in *Lithothamnion corallioides* that exhibited minimum respiration rates at 550 and 750 μatm and maximum at 380 and 1000 μatm (Noisette *et al.*, 2013). As for corals, the varying responses of algal respiration to pCO_2 reported here could be caused by a variety of mechanisms such as perturbed active cellular transport, metabolic depression, increase in the cost of calcification, or variations in allocation of energy to competing metabolic pathways.

Table 4. Parameters of the linear estimates of the relationship between gross photosynthesis and pCO_2 (μatm) for the coral and algal studied.

Species	Surface area-normalized gross photosynthesis	*p*	Biomass-normalized gross photosynthesis	*p*
Massive *Porites* spp.	$-2.7 \times 10^{-3} pCO_2 + 57.9$	0.672	$3.4 \times 10^{-4} pCO_2 + 3.4$	0.627
Porites irregularis	$-5.7 \times 10^{-3} pCO_2 + 37.9$	0.099	$-6.6 \times 10^{-4} pCO_2 + 4.8$	0.272
Pocillopora verrucosa	$-4.3 \times 10^{-3} pCO_2 + 49.9$	0.174	$3.6 \times 10^{-4} pCO_2 + 5.4$	0.631
Psammocora profundacella	$4.8 \times 10^{-3} pCO_2 + 66.5$	0.332	$1.1 \times 10^{-4} pCO_2 + 1.8$	0.750
Neogoniolithon frutescens	$1.1 \times 10^{-3} pCO_2 + 16.0$	0.617	$7.4 \times 10^{-5} pCO_2 + 1.29$	0.719
Lithophyllum kotschyanum	$1.6 \times 10^{-3} pCO_2 + 15.2$	0.319	$5.8 \times 10^{-5} pCO_2 + 0.85$	0.481
Hydrolithon reinboldii	$-1.4 \times 10^{-3} pCO_2 + 18.5$	0.374	$-2.3 \times 10^{-4} pCO_2 + 1.26$	0.289

Parameters of the relationships are given for gross photosynthesis normalized by surface area (Surface) and biomass (Biomass). Gross photosynthesis was estimated by subtracting light respiration to net photosynthesis in four corals (massive *Porites* spp., *Porites irregularis*, *Pocillopora verrucosa*, and *Psammocora* profundacella) and three algae (*Neogoniolithon frutescens*, *Lithophyllum kotschyanum*, and *Hydrolithon reinboldii*). *p*-values refer to the significance of the slope estimates in linear regressions.

Conclusion

The present study shows overall that the respiration and photosynthesis of corals and algae is relatively insensitive to increasing pCO_2 between 280 and 2000 μtam during 7–10 d incubations. The link between sensitivity to pCO_2 and respiration or photosynthesis showed different responses, because some species (*P. damicornis* and *H. macroloba*) appear to up-regulate their respiration in association with calcification (Comeau *et al.*, 2013), while other species (e.g. massive *Porites* spp.) did not. The present results suggest that the effects of elevated pCO_2 on respiration and photosynthesis are equivocal at best, and potentially trivial. While effects were detected for a few traits in some species, these effects would lead to only small absolute changes within the range of pCO_2 increases expected in the next 100 years. Against this backdrop, it would be valuable to revisit the mechanistic framework within which the effects of OA on calcified taxa are viewed, specifically to revisit the likelihood that the negative effects are modulated through perturbations in the absolute supply and demand for metabolic energy.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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