High pCO_2 promotes coral primary production

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Abstract

While research on ocean acidification (OA) impacts on coral reefs has focused on calcification, relatively little is known about effects on coral photosynthesis and respiration, despite these being among the most plastic metabolic processes corals may use to acclimatise to adverse conditions. Here, we present data collected between 2016 and 2018 at three natural CO_2 seeps in Papua New Guinea where we measured the metabolic flexibility (i.e., *in hospite* photosynthesis and dark respiration) of 12 coral species. Despite some species-specific variability, metabolic rates as measured by net oxygen flux tended to be higher at high pCO_2 (ca. 1200 µatm), with increases in photosynthesis exceeding those of respiration, suggesting greater productivity of *Symbiodiniacea* photosynthesis *in hospite*, and indicating the potential for metabolic flexibility that may enable these species to thrive in environments with high pCO_2. However, lab and field observations of coral mortality under high CO_2 conditions

associated with coral bleaching suggests that this metabolic subsidy does not result in coral higher resistance to extreme thermal stress. Therefore, the combined effects of OA and global warming may lead to a strong decrease in coral diversity despite the stimulating effect on coral productivity of OA alone.

Keywords: Ocean acidification; Coral reefs; Acclimatisation; Metabolic flexibility; CO_2 seeps

1. Introduction

The on-going increase in atmospheric carbon dioxide (CO_2) decreases ocean pH and modifies the carbonate chemistry of seawater, a process known as ocean acidification (OA). While OA generally leads to reduced net calcification rate for a range of marine calcifiers [1], it may also result in increased photosynthetic rates in some aquatic photoautotrophs such as seagrasses and fleshy macroalgae (e.g. [2]). For symbiotic corals, little is known about the impacts of OA on the productivity of dinoflagellates (i.e., *Symbiodiniacea*) since they are located within the host [3], complicating exchanges between the algae, the host and the external medium. Studies measuring the photosynthetic rates of the coral holobiont exposed to high pCO_2 have revealed variable effects on *Symbiodiniacea* photosynthesis *in hospite*, ranging from a 47% enhancement to total inhibition. Aerobic respiration has been suggested to increase under OA conditions to balance the increased cost of calcification [8], yet experimental manipulations have shown high variability in coral response to OA (reviewed in [4]). These responses varied between coral species [3,4,9–11] but also among *Symbiodiniacea* types [12–14], suggesting differential host carbon acquisition pathways and strain-specific tolerance to OA. Furthermore, treatment conditions and temporal scales differed among studies, with relatively few coral species tested. These discrepancies make it difficult to perform a synthetic analysis on the effect of OA on coral productivity. Here, we investigated the impact of OA on the photosynthesis and respiration rates of 12 coral species collected at volcanic CO_2 seeps in

Papua New Guinea (PNG). Volcanic CO_2 seeps provide natural analogues of future conditions [15,16] and despite some well-known limitations, they are still one of the most ecologically realistic tools for examining responses of marine organisms to OA. Following Comeau et al. [4], we hypothesized that both the photosynthetic and respiration rates of corals would not be affected by high pCO_2, that this would result in no net difference in metabolic rates, and that there would be no change in density of *Symbiodiniacea* density or chlorophyll content.

2. Material and methods

During four cruises between September 2016 and June 2018, we visited three locations with CO_2 seep sites (Fig. 1): Upa-Upasina, Dobu, and Tutum Bay. The range of ambient pCO_2 conditions and carbonate chemistry of these locations, characterized by previous studies [15,17], and monitored during our fieldwork, provide insight into the generality of OA effects in a naturally heterogeneous environment. Fragments of corals (see supplementary materials) occurring at both CO_2 seeps (high pCO_2) and nearby (0.5-1 km distant) reference reefs (ambient pCO_2) were collected from different parent colonies at each location; 12 species were collected in total, but only four were common at more than one location.

Net photosynthesis (P_n) and dark respiration (R) rates of each coral fragment was measured on board the research vessel under controlled conditions (constant at 29°C and pH_T 7.73 - 7.75 and 8.02 - 8.13) using seawater collected at their sampling sites (Table 1). A saturating light intensity of 250 ± 10 µmol photons m^{-2} s^{-1} was used during 40 min followed by a 30 min dark period. At the end of each incubation, fragments were frozen for future analyses to determine their *Symbiodiniacea* and total chlorophyll contents. Methods are described in further details in the electronic supplemental materials.

The ratio of gross photosynthesis ($P_g = P_n + R$) to R (P_g:R) was analysed first as a generalized mixed model (GLMER, see supplementary). Strong site effects varying with location, and therefore species, were further investigated using separate Wilcoxon tests to compare P_g, R, and P_g:R between reference and seep sites; the same process was used to analyse differences in *Symbiodiniacea* and chlorophyll contents. The overall effect of site upon the above combined metabolic and symbiotic responses in different suites of corals at the different locations was also analysed using a nested PERMANOVA. Multivariate effects were visualized using nMDS of centroids of species by site for each location. Univariate statistical analyses were performed in R v. 3.2.5, multivariate in PRIMER v. 6.

3. Results

(a) Gross photosynthesis, respiration and P_g:R ratio

Respiration and P_g rates covaried strongly across locations (glmer, $p < 0.001$; Table S1) and seep sites overall had higher P_g to R ratios than reference sites ($p < 0.001$; Fig. 2). There was, however, heterogeneity of response to sites between locations ($p < 0.01$) and strong species-specificity of response ($p < 0.001$).

Gross photosynthesis (P_g) of 14 of 18 sets of corals (e.g., *P. damicornis* from Tutum-Bay and from Dobu = 2 sets) were significantly higher at seep compared to those at reference sites (Wilcoxon, $p < 0.05$; Table 2), which corresponds to 11 of 12 species. No significant differences in P_g rates between seep and reference sites were found for *D. pallida* from Tutum Bay and *P. verrucosa* from Upa-Upasina, while P_g rates of *P. cylindrica* from Dobu and Upa-Upasina were lowest at the seeps ($p < 0.001$).

Respiration rates of 9 sets of corals were significantly higher at seep than at reference sites (Wilcoxon, $p < 0.05$; Table 2), which corresponds to 8 of 12 species; 6 sets were not significant, while 3 were significantly lower at seeps ($p < 0.001$).

P_g:R ratio was overall higher for corals at seeps compared to those at reference sites (Fig. 2), which was significant for 12 of 18 sets of corals (Wilcoxon, $p < 0.05$; Table 2) which corresponds to 9 of 12 species. In contrast, *D. pallida* and *P. verrucosa* both from Tutum Bay showed a higher P_g:R ratio at the reference site ($p < 0.05$). Interestingly, the two species found across locations, *P. verrucosa* and *P. cylindrica*, were significantly higher at 2 of 3 seeps and at 3 of 3 seeps respectively.

(b) Symbiodiniacea and chlorophyll contents

Symbiodiniacea content did not differ significantly between seeps and reference sites; only random effects of species were significant in the linear model. Six sets of corals showed significant pair-wise differences; 2 were lower and 4 higher at the seep sites respectively (Wilcoxon, $p < 0.05$; Table S2).

Chlorophyll content differed significantly across sites (glmer, $p < 0.001$, Table S3) but this was highly dependent upon location and species. Seven sets of corals showed no significant difference between sites (Wilcoxon, $p > 0.05$; Table S2), 7 had significantly higher chlorophyll content at seep sites ($p < 0.05$), and 4 a higher content at reference sites ($p < 0.05$).

PERMANOVA indicated significant effects of site ($p < 0.05$) and location ($p < 0.05$) upon the overall data, with coral species responding idiosyncratically to site effects depending upon location ($p < 0.001$) (Figure S1; Tables S4).

4. Discussion

This study encompassed 217 incubations with 12 coral species that endured consistently high pCO_2 in their environment. To our knowledge, this is the most exhaustive study on the long-term effect of OA conditions on the metabolic flexibility through photosynthetic and respiration changes of tropical corals. Omnibus tests using multivariate approaches suggested that overall physiological effects of exposure to acidified conditions are consistently present but idiosyncratic and species-specific in magnitude.

High pCO_2 stimulated the P_g rate of 11 of the 12 coral species, suggesting either that *Symbiodiniacea* were CO_2-limited at ambient pCO_2 or that high pCO_2 act as fertilizer [14]. Consequently, we reject our initial null hypothesis and conclude that high pCO_2 affects coral metabolism. We suggest that the variability in responses of symbiotic scleractinian corals to high pCO_2 as reported in earlier studies ([4] and references therein) might be attributed to the short duration of acclimation to high pCO_2 ranging from a few hours to several months. Indeed, our findings are consistent with previous results on soft and hard coral species that were fully acclimatised to high pCO_2 environments at CO_2 seeps [7,18]. Our study provides additional support for the notion that most symbiotic corals are able to acclimatise to high pCO_2 environments and potentially benefit from these conditions.

High coral productivity could not be attributed to changes in *Symbiodiniacea* or chlorophyll content, because symbiont content was similar between corals at seep and reference sites and differences in the chlorophyll concentration were inconsistent, with 3 different coral species containing either high or low chlorophyll concentrations at the seep sites, one species (i.e. *P. cylindrica*) containing both, while the remaining 7 were not significantly different (supplementary table S2). Moreover, a previous study found that no change occurs in *Symbiodiniacea* types at seeps [19]. Therefore, flexibility of the coral metabolism may be driven by enhanced uptake of dissolved inorganic carbon (DIC), or increased host membrane

144 diffusivity. Indeed, the identification of numerous pathways in corals that supply
145 *Symbiodiniacea* with HCO_3^- [20,21] suggests the role of this latter as the stimulating DIC
146 source for coral photosynthesis and calcification. Another potential pathway of energetic
147 stimulation on corals exposed to OA conditions is the coral-mediated dissolved organic
148 carbon (DOC) flux. As an example, an increase in the DOC retained by corals was found for
149 two species exposed for 24 days to 741 µatm pCO_2 [11]. High DOC could therefore result in
150 sustained coral energy reserves and thereby help the coral resist effects of OA.

151 The observed high P_g could also be supported by the higher respiration rates of the corals
152 from the seep sites, since this brings extra metabolic CO_2 to *Symbiodiniacea*. However, this
153 cannot be the only explanatory factor since the respiration rates were variable among species
154 and pCO_2 conditions (Table 2). Eight species had higher respiration rates at seeps, probably to
155 counter the increased cost of functioning at elevated pCO_2, such as the debated cost of
156 calcification [8,22], or perhaps simply because higher productivity might help corals to boost
157 metabolic processes such as lipid storage, reproduction, and protein synthesis. This energetic
158 benefit from CO_2 enrichment was also shown by the increased P_g:R at CO_2 seeps, suggesting
159 an increase in the energetic balance that may actually enable these species to thrive in high
160 pCO_2 [15].

161 Finally, we observed that a substantial mortality of coral reefs around Upa-Upasina had
162 occurred between January 2017 and June 2018, with most coral species found dead.
163 According to previous reports [6,15], heat induced coral bleaching has occurred frequently in
164 the area of Upa-Upasina during the last decade. Unfortunately, although we noted the massive
165 mortality, during our last cruise in 2018 dead colonies were already covered by algae and
166 were indistinguishable from previously dead ones; we were therefore unable to reliably
167 quantify the recent mortality. Although the intensity of thermal stress was unknown, these
168 observations seems to confirm what Noonan and Fabricius [6] experimentally demonstrated

during a moderate thermal stress: benefits of OA had little effect on coral survival after thermal stress because of bleaching. Therefore, the combined effects of OA and global warming may lead to a strong decrease in coral diversity despite the stimulating effect of OA alone on coral productivity [15]. With atmospheric CO_2 driving both OA and global warming, there is urgent need for research studying their interactive effects to better predict the future of coral reefs under climate change.

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Figure legends

Fig. 1. The three study locations in Ambitle and Normanby Islands.

Fig. 2. Linear regression of the gross photosynthesis (O_2 production) against dark respiration (O_2 consumption) measured on corals from reference (circle) and seep (triangle) sites. Coloured markers show the average Pg:R ratio of each species at each site and location with T, U and D referring to Tutum Bay, Upa-Upasina and Dobu, respectively.

Table 1. Mean (± SD in brackets) seawater conditions measured at the study sites (see electronic supplemental materials for details).

Location/site (no. of replicates)	**T** (ºC)	**pH$_T$**	***p*CO$_2$** µatm	**CO$_2$** µmol kg^{-1}	**HCO$_3^-$** µmol kg^{-1}	**CO$_3^{2-}$** µmol kg^{-1}	**Ω$_{arag}$**
Tutum Bay							
Reference (18)	29.09 (0.89)	8.12 (0.07)	436 (97)	11 (2)	1667 (59)	203 (24)	4.95 (0.6)
Seep (70)	28.93 (0.9)	7.75 (0.06)	1179 (166)	30 (5)	1927 (31)	99 (12)	2.41 (0.3)
Upa-Upasina							
Reference (24)	29.70 (0.46)	8.02 (0.02)	573 (25)	15 (1)	1777 (14)	175 (6)	4.28 (0.1)
Seep (87)	29.30 (0.53)	7.75 (0.05)	1197 (156)	31 (4)	1960 (30)	102 (12)	2.48 (0.3)
Dobu							
Reference (60)	28.48 (0.25)	8.13 (0.03)	443 (37)	12 (1)	1766 (29)	214 (12)	5.22 (0.3)
Seep (12)	29.38 (0.51)	7.73 (0.01)	1283 (42)	33 (1)	2043 (7)	102 (3)	2.50 (0.1)

Table 2. Mean (± SD in brackets) gross photosynthesis (P_g) and dark respiration (R), P_g:R ratio and statistical significances (Wilcoxon test) of the coral species at each location (Upa-Upasina: U, Tutum Bay: T and Dobu: D) and site (see Table 1 for the water chemistry conditions during incubation). All n = 15 with exception of instances marked '7' (e.g., U^7 where n = 7).

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Species	Loc.	Gross photosynthesis (µmol O_2 production cm^{-2} h^{-1})			Respiration (µmol O_2 consumption cm^{-2} h^{-1})			P_g:R		
		Ref.	Seep	p	Ref.	Seep	p	Ref.	Seep	p
Acropora hyacinthus	U[7]	0.52 (0.06)	0.74 (0.11)	***	0.21 (0.01)	0.23 (0.03)	n.s.	2.50 (0.32)	3.22 (0.37)	*
Acropora nana	T	1.36 (0.13)	1.65 (0.24)	***	0.64 (0.09)	0.62 (0.21)	n.s.	2.16 (0.25)	2.93 (0.87)	***
Acropora tenuis	D	0.94 (0.27)	1.47 (0.42)	***	0.56 (0.19)	0.72 (0.20)	*	1.72 (0.33)	2.07 (0.39)	**
	U	0.57 (0.5)	1.68 (0.44)	***	0.27 (0.07)	0.84 (0.23)	***	2.13 (0.45)	2.01 (0.30)	n.s.
Dipsastraea pallida	T	0.98 (0.23)	0.90 (0.2)	n.s.	0.55 (0.22)	0.80 (0.20)	*	1.95 (0.47)	1.14 (0.11)	***
Favites halicora	U[7]	0.40 (0.11)	0.58 (0.06)	*	0.26 (0.08)	0.27 (0.06)	n.s.	1.57 (0.23)	2.22 (0.36)	**
Favites pentagona	U[7]	0.27 (0.05)	0.47 (0.10)	***	0.19 (0.03)	0.26 (0.04)	**	1.41 (0.22)	1.75 (0.18)	*
Galaxea fascicularis	U[7]	0.36 (0.09)	0.59 (0.10)	**	0.19 (0.06)	0.27 (0.05)	*	1.93 (0.32)	2.21 (0.36)	n.s.
Heliopora coerulea	T	0.63 (0.23)	1.44 (0.20)	***	0.34 (0.18)	0.75 (0.22)	***	2.01 (0.49)	2.05 (0.46)	n.s.
Pocillopora damicornis	T	1.13 (0.31)	1.59 (0.44)	**	0.66 (0.23)	0.61 (0.17)	n.s.	1.77 (0.36)	2.63 (0.48)	***
Pocillopora verrucosa	D	1.51 (0.56)	2.43 (0.95)	**	1.01 (0.46)	0.95 (0.51)	n.s.	1.63 (0.37)	2.87 (0.90)	***
	T	0.92 (0.15)	1.42 (0.14)	***	0.32 (0.10)	0.63 (0.13)	***	3.13 (0.93)	2.30 (0.30)	*
	U	1.76 (0.56)	1.35 (0.55)	n.s.	1.21 (0.41)	0.60 (0.33)	***	1.47 (0.14)	2.55 (1.03)	***
Porites cylindrica	D	1.41 (0.30)	0.96 (0.35)	***	0.93 (0.22)	0.41 (0.22)	***	1.54 (0.20)	2.55 (0.72)	***
	T	0.97 (0.21)	2.08 (0.34)	***	0.47 (0.07)	0.82 (0.30)	**	2.10 (0.40)	2.75 (0.64)	*
	U	0.93 (0.25)	0.61 (0.32)	***	0.49 (0.12)	0.25 (0.15)	***	1.92 (0.32)	2.67 (0.87)	**
Seriatopora hystrix	D	1.05 (0.24)	1.29 (0.22)	*	0.50 (0.15)	0.49 (0.16)	n.s.	2.19 (0.40)	2.89 (0.95)	*
	U	1.01 (0.24)	1.56 (0.37)	***	0.28 (0.11)	0.53 (0.16)	***	4.01 (1.58)	3.10 (0.84)	n.s.

ns = not significant, * = < 0.05, ** = < 0.01, *** = < 0.001