High pCO_2 promotes coral primary production

Biscéré T.[1*], Zampighi M.[1], Lorrain A.[2], Jurriaans S.[3], Foggo A.[4], Houlbrèque F.[1], Rodolfo-Metalpa R.[1*]

[1] ENTROPIE IRD - Université de La Réunion - CNRS, Nouméa 98848, New Caledonia
[2] Univ Brest, CNRS, IRD, Ifremer, LEMAR, F-29280 Plouzané, France
[3] College of Science and Engineering, James Cook University, Townsville, QLD, Australia
[4] Marine Biology and Ecology Research Centre, School of Biological and Marine Sciences, Plymouth University, Drake Circus, Plymouth PL4 8AA, UK

Supplementary Material and methods

Site description and sampling

This study was conducted in Papua New Guinea during four cruises on board of the R/V Alis or the diving boat Chertan in various sessions from 2016-2018. Coral metabolism was studied at three locations with CO_2 seeps: Upa-Upasina Reef and Dobu Island (Normanby Island, Milne Bay Province; [1]), and Tutum Bay (Ambitle Island, New Ireland Province; [2]) (Fig. 1). The seawater carbonate chemistry of the two sites located in Milne Bay were characterized by studies following the pioneering work of Fabricius and colleagues [1], whereas the seawater carbonate chemistry of Tutum Bay was only recently described [3]. Across the three locations, the CO_2 seeps were shallow (1-8 m depth) with similar gas composition and seawater pH fluctuations [3]. At each location, we defined an area near the CO_2 seep (hereafter, seep site) at 2-4 m depth with an average pH_T of 7.7-7.8 measured using SeaFETs (SeaFET V2, Sea-Bird Scientific, Bellevue, WA 98005, USA), and a reference area (hereafter, reference site) at similar depth with an average pH_T of 8.0-8.1. This allowed us to select coral species living at similar depth but with contrasting pH environments. The distances between seep and reference sites were 500 m in Upa-Upasina and Dobu (same sites used by Fabricius et al 2011) and within one km in Tutum Bay (Pichler et al 2019). These two

studies already reported the temperature data collected at the study sites we used and no differences were found.

Coral species and collection

Species were selected if they were found at seep and reference sites within each location. We chose only species that we could identify with certainty based on visual characteristics. Twelve coral species were used: *Acropora hyacinthus*, *Acropora nana*, *Acropora tenuis*, *Dipsastraea pallida*, *Favites halicora*, *Favites pentagona*, *Galaxea fascicularis*, *Heliopora coerulea*, *Pocillopora damicornis*, *Pocillopora verrucosa*, *Porites cylindrica* and *Seriatopora hystrix* (Table 2). Of these species, *P. cylindrica* and *P. verrucosa* were common across all locations, and *A. tenuis* and *S. hystrix* were common at Upa-Upasina and Dobu.

Coral fragments (3-5 cm) were collected by SCUBA diving using a plier at the seep and reference sites (15 fragments per species for 4 species at Upa-Upasina, 4 at Dobu, 6 at Tutum Bay but only 7 fragments for other 4 species at Upa-Upasina). Fragments of each coral species were collected from both seeps and reference sites and they were measured during the same incubation. Each fragment was collected from a different parent colony and transported in individual zip-lock bag in a cooler containing seawater freshly collected. At the end of each diving > 20L seawater was collected in jerrycans and quickly transported (within 15 min from collection) together with the fragments using a dinghy. The distance between the site of collection and the vessel was < 1000 m. Onboard the vessel, seawater was immediately measured for temperature and pH (see below). Fragments were allowed to recover prior to the start of the incubations for 2 h in two aquarium of 20L supplied with seawater from the sites of collection (seeps and reference) mixed using a submersible pump (Aquarium system, micro-jet MC 320, Mentor, OH, USA). Irradiance was controlled at 200 µmol m^{-2} s^{-1} using LED Mitras LX 6100 (GHL, Germany). Water temperature was controlled at 29°C using a cooler system (Resun CL-600, China) to remain within 0.5 °C of *in situ* temperature.

Photosynthetic and dark respiration rates

After recovery, small glass chambers (115 mL, n = 7 to 15 per site/treatment) were immersed into the aquaria and one fragment transferred into each chamber. Three control chambers per site/treatment pH (seeps and reference) were left without corals and used as a proxy for microbial or plankton activity. Chambers were sealed underwater using plastic transparent film and rubber band. Care was taken to avoid any air getting trapped inside the chamber. In each chambers a stirring bar ensured seawater circulation. They were then distributed in a water bath. The water bath contained two 15-place magnetic stirring plates (Telesystem 15, Thermo Scientific), one heather (Visitherm 300W, Aquarium systems) and two submersible water pumps (MiniJet, Aquarium Systems) that homogenized the water temperature at 29°C ± 0.5°C, which represents the averaged *in situ* temperature (Table 1). Water bath was connected to a cooler (Resun CL-600, China) that circulated the water.

Corals were first incubated in the light during 40 min under saturating light of ~250 ± 22 µmol photons m^{-2} s^{-1} provided by LED Mitras LX 6100 (GHL, Germany) and verified with Li–Cor 4π spherical underwater quantum sensor (LI–193SA). Oxygen production (i.e. coral net photosynthesis) was measured at the beginning of the incubation. Temperature and O_2 were measured for each coral, immediately before to seal the chambers. At the end of the incubation the temperature probe was immersed in the chamber by piercing the film and the O_2 content was measured by positioning the probe outside the chamber in front of the O_2 spot (see below). The film was removed, seawater partially discharged and the chambers containing the corals were immersed in the water baths originally used and kept at the same temperature. Darkness was created by placing black-out material over the water baths and 30 min of transition time was respected to allow the coral respiration rates to stabilise to the dark conditions. Then the O_2 was measured and each chamber was sealed again and incubated but

in the dark. After the light incubation, Oxygen consumption (i.e., dark respiration) was measured during 30 min in darkness.

To assess dissolved oxygen concentration and water temperature inside each chamber a Fibox 4 transmitters (PreSens, Germany) [4] was used. Each chamber was equipped with an O_2 sensor spot to allow an optical measure. Preliminary tests showed that the duration and volume of the incubation chamber were suitable to detect a maximum 30% increase or decrease of the initial oxygen concentration. At the end of the dark incubations, the water volume of the chamber was measured using two 50 ml measuring cylinders. Coral fragments were frozen at -20°C for further analysis of the chlorophyll and symbiont content and skeletal surface area. After each cruise, fragments were transported to the laboratory in Nouméa (New-Caledonia) by plane on dry ice, or on board R/V Alis at -20°C.

Seawater carbonate chemistry during incubations

The *in situ* seawater that was used to seal the chambers was immediately measured after collection to characterize the carbonate chemistry at the seep and reference sites. Temperature was measured using a temperature sensor connected to Fibox 4 oxygen meter (PreSens, Germany); Salinity using YSI MPS 556 probe (YSI, USA); pH_T using a pH meter with a glass electrode (Methler 826 pH mobile) calibrated with Tris reference solutions ([5]; batch T28). In addition, total alkalinity (A_T) was measured at the start of the incubations performed for Tutum Bay and Upa-Upasina (A_T = 2211 and 2172 μmol kg^{-1}, n = 27 and 36, respectively) and derived from [1] for Dobu (A_T = 2293 μmol kg^{-1}). It was calculated from the Gran function applied to pH variations from 4.2 to 3.0 as mEq L^{-1} from the slope of the HCl volume versus pH curve. Readability was verified by titrations of A_T standards provided by A.G. Dickson (batch 155). Seawater carbonate parameters (pCO_2, CO_3^{2-}, HCO_3^-) and

aragonite saturation state (Ω_{arag}) were then calculated from pH_T, A_T, temperature and mean salinity using the free-access CO_2SYS package [6].

Symbiont and chlorophyll content

Coral tissue was removed from the skeleton using an air pick in 20 mL of filtered seawater and homogenised with a Potter tissue grinder. The tissue slurry was divided in two portions, 10 mL was taken to determine the symbiont content and 10 mL to measure the chlorophyll content. Cells were counted by light microscopy using a Neubauer's cell. Each sample was counted five times of which an average symbiont content per sample was calculated.

To determine the chlorophyll content, 10 mL of the tissue slurry was centrifuged at 3000 g during 10 min to isolate the symbiont (pellet) from the host tissue (supernatant). Symbionts were re-suspended into 10 mL of 100% acetone to extract chlorophyll *a* and c_2 during 24 h in darkness at 4°C. The extracts were then centrifuged at 10,000 g for 15 min and the absorbances were read at 630, 663, 750 nm using a spectrophotometer (Evolution 201, Thermo Fisher Scientific, USA). Chlorophyll concentrations were computed according to the spectrometric equations of [7]. Chlorophyll *a* and c_2 were added together to obtain the chlorophyll content per sample. Data were normalized by surface area. After removing the coral tissue, coral skeletons were dried in an oven at 55°C during one night. Coral surface area of each fragment was measured using the single wax-dipping method described in [8].

Data analysis

The change in oxygen concentration between the start and end of the incubation (ΔO_2) was calculated for each chamber. An average ΔO_2 was taken of the three control chambers (i.e.,

without the coral) and subtracted from ΔO_2 of each coral incubation. Net photosynthesis (P_n) and dark respiration (R) rates of each fragment were then calculated as follows:

$$P_N \; and \; R \; (t) = \frac{\left[\frac{(\Delta O_2) \cdot V}{I_t \cdot SA}\right]}{1000} \qquad \text{[Eq. 1]}$$

where V is seawater volume (L) of the incubation chamber, I_t is incubation time, and SA is coral surface area (cm^2). Gross photosynthesis (P_g) rates were calculated as P_n + R.

Univariate data were analysed using the statistical software R version 3.2.5 (R Core Team, 2018). The ratio of gross photosynthesis ($P_g = P_n + R$) to R (P_g:R) was analysed first as a generalized mixed model (R package lme4, [9]) with R as a continuous covariate of Pg, site (2 levels) and location (3 levels) as fixed factors, and species (12 levels) as a random factor nested within location. Site effects upon Pg:R were visualised by plotting separate linear regressions using coefficients from the GLMER. Strong site effects varying with location and hence species were further investigated using separate Wilcoxon tests to compare Pg, R, and Pg:R between reference and seeps sites for species by location combinations. The same approach was used to analyse *Symbiodineacea* density and chlorophyll content, with the omission of the continuous covariate, R. Model validation was carried out by inspection of residuals; accordingly Pg:R was modelled using a Gamma error structure the other variables were analysed using Gaussian errors. To further unpick the effects of site on the responses of different combinations of species and location, Wilcoxon Rank Sum tests were performed on Seep v Reference comparisons for all 5 variables: Pg, R, Pg:R, *Symbiodiniacea* density and chlorophyll content.

The overall effect of site upon the combined metabolic and symbiotic responses (Pg, R, *Symbiodineacea* density and chlorophyll content) in different suites of corals at the different locations was also analysed using a nested PERMANOVA based upon a Euclidean distance matrix calculated from normalised raw data with location and site as fixed factors and coral

species as a random factor nested within location (levels as above). 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.

Supplementary Table S1. Results of Gamma family general linear mixed model examining the Pg:R relationship. Fixed factor *p* values derive from type III analysis of deviance with Wald chi-squared statistic, the random factor *p* value from change in log-likelihood on term deletion.

	Chi-sq	Df	p
Species (Loc)	180.320	2	< 0.0001
R	70.364	1	< 0.0001
Site	14.311	1	< 0.001
Loc	9.962	2	< 0.01
R:site	3.061	1	0.080
R:loc	9.108	2	< 0.05
Site:loc	6.448	2	< 0.05
R:site:loc	12.553	2	< 0.01

Supplementary Table S2. Mean (± SD) *Symbiodiniacea* (10^6 cells cm^{-2}) and chlorophyll content (µg chl cm^{-2}) of twelve coral species found at three sites (Upa-Upasina; U, Tutum Bay: T, and Dobu: D) and incubated to *in situ* conditions measured at seep (i.e. high pCO_2) and reference (Ref.) (i.e. ambient pCO_2) sites. *P*-values show statistical differences (Wilcoxon test) between seep and reference site.

Species	Loc.	*Symbiodiniacea*			Chlorophyll		
		Ref.	Seep	p	Ref.	Seep	p
Acropora hyacinthus	U	1.236 (0.352)	1.005 (0.285)	ns	9.53 (3.11)	7.97 (2.73)	ns
Acropora nana	T	1.733 (0.636)	1.530 (0.478)	ns	13.59 (3.79)	17.92 (7.36)	ns
Acropora tenuis	D	1.738 (0.666)	1.598 (1.038)	ns	9.22 (3.98)	19.09 (11.40)	*
	U	1.626 (0.498)	1.377 (0.503)	ns	10.90 (2.18)	18.70 (6.20)	**
Dipsastraea pallida	T	2.847 (0.715)	2.216 (0.407)	*	36.19 (6.49)	38.54 (9.42)	ns
Favites halicora	U	1.347 (0.620)	1.084 (0.278)	ns	17.58 (7.00)	6.66 (1.62)	**
Favites pentagona	U	1.181 (0.516)	1.158 (0.406)	ns	9.17 (4.45)	6.84 (2.35)	ns
Galaxea fascicularis	U	1.871 (0.716)	1.941 (0.386)	ns	18.10 (7.94)	13.85 (4.29)	ns
Heliopora coerulea	T	2.571 (0.485)	1.254 (0.796)	*	23.70 (4.63)	10.51 (4.35)	***
Pocillopora damicornis	T	2.000 (1.122)	2.531 (0.692)	ns	10.24 (5.58)	15.33 (4.67)	**
Pocillopora verrucosa	D	1.768 (0.697)	2.559 (0.661)	*	8.30 (4.57)	27.17 (10.75)	***
	T	2.346 (0.953)	2.881 (1.090)	ns	24.17 (7.55)	28.50 (6.47)	ns
	U	4.093 (0.101)	2.116 (0.347)	**	4.92 (2.68)	14.11 (2.52)	***
Porites cylindrica	D	2.932 (0.839)	2.519 (0.887)	ns	12.38 (5.51)	23.91 (10.13)	***
	T	4.090 (1.020)	4.594 (0.792)	ns	51.53 (12.41)	75.15 (10.48)	***
	U	2.195 (0.424)	1.649 (0.294)	*	18.37 (6.56)	4.15 (2.23)	***
Seriatopora hystrix	D	1.856 (0.491)	2.592 (0.550)	*	8.81 (3.94)	17.89 (11.53)	ns
	U	2.299 (0.556)	1.854 (0.647)	ns	18.44 (6.13)	10.17 (5.13)	***

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

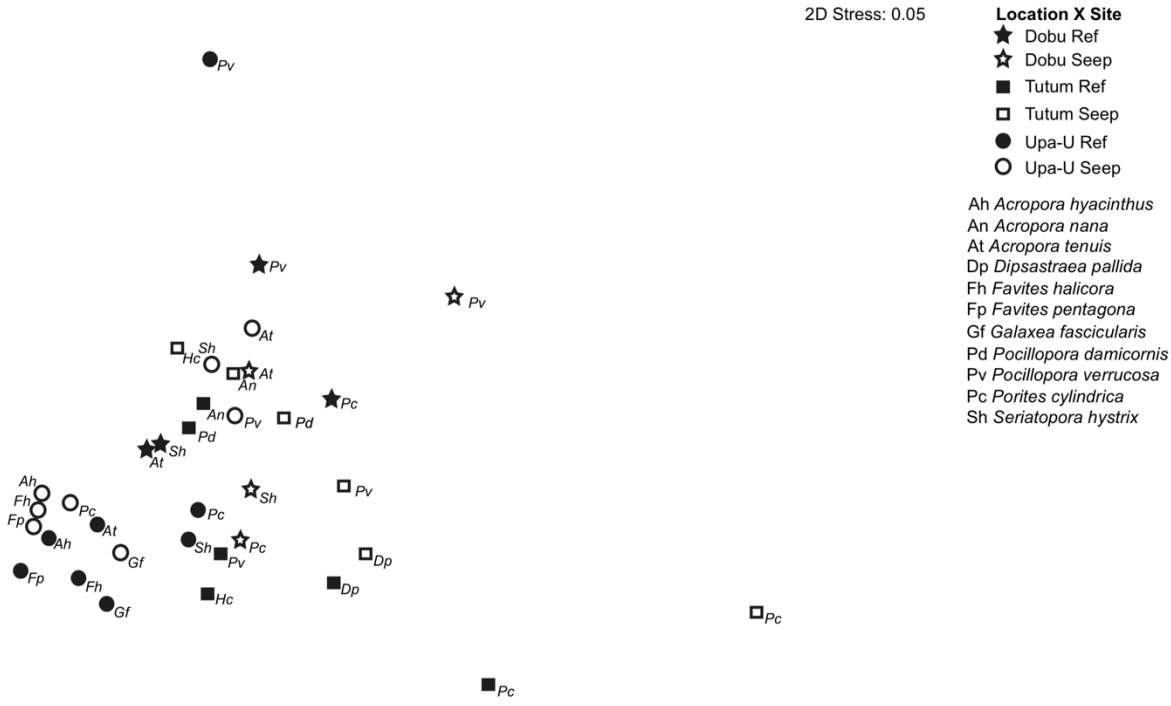
Supplementary Table S3. Results of linear mixed model examining the effects of location, site and species upon chlorophyll content. Fixed factor *p* values derive from type III analysis of deviance with Wald chi-squared statistic, random factor *p* value from change in log-likelihood on term deletion.

	Chi-sq	Df	p
Species (Loc)	289.590	2	< 0.0001
Site	51.026	1	< 0.0001
Loc	1.460	2	0.4819
Site:loc	46.927	2	< 0.0001

Supplementary Table S4. Results of nested PERMANOVA examining the effects of location, site and species upon metabolic/symbiotic parameters *in toto*.

Source	df	MS	Pseudo-F	P(perm)
Site	1	37.745	3.2616	0.0397
Location	2	118.3	3.2977	0.0102
Species (Location)	15	35.48	23.858	0.0001
Site x Location	2	14.961	1.2987	0.2853
Site x Species (Location)	15	11.404	7.6687	0.0001
Res	324	1.4871		

Supplementary Figure S1. Non-metric multidimensional scaling (nMDS) plot displaying site-related shift in relative centroid positions of the physiological/metabolic responses of each coral species across locations.



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