

## Research



**Cite this article:** Johnson MD, Carpenter RC. 2018 Nitrogen enrichment offsets direct negative effects of ocean acidification on a reef-building crustose coralline alga. *Biol. Lett.* **14**: 20180371.  
<http://dx.doi.org/10.1098/rsbl.2018.0371>

Received: 20 May 2018

Accepted: 20 June 2018

**Subject Areas:**

ecology, environmental science, plant science

**Keywords:**

coral reefs, eutrophication, global change, pCO<sub>2</sub>, pH, *Porolithon onkodes*

**Author for correspondence:**

Maggie D. Johnson

e-mail: [johnsonmd4@si.edu](mailto:johnsonmd4@si.edu)

Electronic supplementary material is available online at <https://dx.doi.org/10.6084/m9.figshare.c.4147190>.

## Global change biology

## Nitrogen enrichment offsets direct negative effects of ocean acidification on a reef-building crustose coralline alga

Maggie D. Johnson<sup>1,2</sup> and Robert C. Carpenter<sup>2</sup>

<sup>1</sup>Smithsonian Marine Station, Fort Pierce, FL 34949, USA

<sup>2</sup>Department of Biology, California State University, Northridge, CA 91330, USA

MDJ, 0000-0002-1319-2545

Ocean acidification (OA) and nutrient enrichment threaten the persistence of near shore ecosystems, yet little is known about their combined effects on marine organisms. Here, we show that a threefold increase in nitrogen concentrations, simulating enrichment due to coastal eutrophication or consumer excretions, offset the direct negative effects of near-future OA on calcification and photophysiology of the reef-building crustose coralline alga, *Porolithon onkodes*. Projected near-future pCO<sub>2</sub> levels (approx. 850 μatm) decreased calcification by 30% relative to ambient conditions. Conversely, nitrogen enrichment (nitrate + nitrite and ammonium) increased calcification by 90–130% in ambient and high pCO<sub>2</sub> treatments, respectively. pCO<sub>2</sub> and nitrogen enrichment interactively affected instantaneous photophysiology, with highest relative electron transport rates under high pCO<sub>2</sub> and high nitrogen. Nitrogen enrichment alone increased concentrations of the photosynthetic pigments chlorophyll *a*, phycocyanin and phycoerythrin by approximately 80–450%, regardless of pCO<sub>2</sub>. These results demonstrate that nutrient enrichment can mediate direct organismal responses to OA. In natural systems, however, such direct benefits may be counteracted by simultaneous increases in negative indirect effects, such as heightened competition. Experiments exploring the effects of multiple stressors are increasingly becoming important for improving our ability to understand the ramifications of local and global change stressors in near shore ecosystems.

## 1. Introduction

Ocean acidification (OA) has the potential to profoundly change marine ecosystems by altering biological processes such as calcification and photosynthesis. The changes in carbonate chemistry that occur with OA are predicted to impair calcification in many marine calcifiers [1], though there are taxa-specific sensitivities [1,2]. Local stressors can have similarly profound effects on benthic ecosystems. Eutrophication from agricultural run-off and sewage effluent is one of the leading causes of degradation in marine ecosystems across the globe. The associated increases in inorganic nitrogen, particularly nitrite + nitrate (NO<sub>2</sub><sup>-</sup> + NO<sub>3</sub><sup>-</sup>) and ammonium (NH<sub>4</sub><sup>+</sup>), can supply primary producers with limiting nutrients, thus stimulating photosynthesis [3] and increasing macroalgal biomass [4]. At local scales, fish and invertebrate excretions also can increase nitrogen concentrations [5,6]. Such processes of nutrient enrichment are occurring simultaneously with OA. Understanding the combined effects of these stressors is critical to determining direct organismal responses to local and global change, particularly in eutrophic environments.

Coral reefs are among the most sensitive ecosystems to OA and eutrophication [4], partly because they are built by calcifiers and are typically oligotrophic. One taxonomic group that is essential to building the reef carbonate platform is

crustose coralline algae (CCA). CCA secrete the most soluble polymorph of  $\text{CaCO}_3$  (high-Mg calcite), and are highly sensitive to seawater changes associated with OA [7,8]. The reef-building CCA, *Porolithon onkodes* (formerly *Hydrolithon onkodes*), has demonstrated lower calcification rates under near-future OA conditions [9,10]. Conversely, nutrient enrichment has stimulatory effects on algal photophysiology (electron transport rates and photosynthetic pigments) and photosynthetic rates [3]. Red algae, such as CCA, respond readily to increased availability of nitrogen because they can actively store luxury nitrogen in their accessory phycobilin pigment complexes [11]. Despite the potential synergistic effects of OA and nutrient enrichment, relatively little is known about how CCA respond to these simultaneous stressors (except see [12]). The goal of this study was to quantify the combined effects of OA and nitrogen enrichment on calcification and photophysiology of *P. onkodes*. Understanding how nutrient enrichment influences direct biological responses to OA will allow us to better predict organismal responses to global change in the framework of local habitat variability.

## 2. Material and methods

We exposed *P. onkodes* cores to a factorial combination of ambient (AC) and high  $\text{pCO}_2$  (HC) (approx. 400 and 850  $\mu\text{atm}$ ) crossed with ambient (AN) and high nitrogen (HN) concentrations for two weeks using established methods [9]. The high  $\text{pCO}_2$  treatment represents future ocean conditions projected for the end of the century [13]. The nitrogen enrichment treatment simulated elevated concentrations associated with eutrophication or local consumer excretions [4–6] (see the electronic supplementary material for details). We quantified effects of treatment on coral net calcification by assessing changes in buoyant weight. We quantified effects on photophysiology with PAM fluorometry to determine relative electron transport rates (rETR) and extractions to determine photosynthetic pigment concentrations. Treatment effects on response variables were analysed with separate linear mixed effects models using the package *lme4* in R v. 3.4.2 [14], with  $\text{pCO}_2$  and nitrogen as fixed factors and tank as a random nested factor. All methods and statistical analyses are described in detail in the electronic supplemental material. Raw data are archived at Pangaea (<https://doi.pangaea.de/10.1594/pangaea.887917>).

## 3. Results

### (a) Environmental parameters

$\text{CO}_2$  manipulation decreased mean ambient total scale pH ( $\text{pH}_T$ ) by approximately 0.20 units in the high  $\text{pCO}_2$  treatment, simulating the increase in acidity projected for the end of the century [13]. Nitrogen enrichment increased ambient nitrate + nitrite concentrations by approximately 50% and ammonium concentrations by approximately 270% (table 1), simulating elevated nutrient levels associated with coastal eutrophication [4] or localized consumer excretions [5,6] on coral reefs. There were no substantial differences in environmental parameters across replicate tanks within treatments (electronic supplementary material, tables S1 and S2).

### (b) Net calcification

There was no interactive effect of  $\text{pCO}_2$  and nitrogen enrichment on net calcification of *P. onkodes* ( $p = 0.965$ ), but there

**Table 1.** Mean ( $\pm$  s.e.) treatment parameters. Temperature, salinity, light, water flow and  $\text{pH}_T$  (total scale pH) were measured daily ( $N = 14$ ). Replicate tank values ( $N = 2$  per treatment) were averaged for each day, and then averaged to yield overall daily treatment means. Total alkalinity ( $A_T$ ) was measured every third day ( $N = 5$ ).  $\text{pCO}_2$  and  $\Omega_c$  (the saturation state of calcite) were derived from measured values of  $A_T$ , salinity, temperature and  $\text{pH}_T$  using  $\text{CO}_2\text{SYS}$  with corrections for nitrogen enrichment [15]. Water was sampled twice daily for nitrogen concentrations every 3–4 days ( $N = 4$ ). Treatment combinations were ambient  $\text{pCO}_2 \times$  ambient nitrogen (ACAN); ambient  $\text{pCO}_2 \times$  high nitrogen (ACHN), high  $\text{pCO}_2 \times$  ambient nitrogen (HCAN), high  $\text{pCO}_2 \times$  high nitrogen (HCHN).  $\text{Light}^\dagger =$  photosynthetically active radiation (PAR,  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ),  $\text{NO}_2^- + \text{NO}_3^- =$  nitrite + nitrate,  $\text{NH}_4^+ =$  ammonium.

treatment	$T$ ( $^\circ\text{C}$ )	salinity (PSU)	$\text{light}^\dagger$	flow ( $\text{ml min}^{-1}$ )	$\text{pH}_T^\ddagger$	$A_T$ ( $\mu\text{mol kg}^{-1}$ )	$\text{pCO}_2$ ( $\mu\text{atm}$ )	$\Omega_c$	$\text{NO}_2^- + \text{NO}_3^-$ ( $\mu\text{M}$ )	$\text{NH}_4^+$ ( $\mu\text{M}$ )
ACAN	$28.4 \pm 0.07$	$36.1 \pm 0.07$	$334 \pm 16$	$139 \pm 2$	$7.99 \pm 0.003$	$2342 \pm 7$	$464 \pm 4$	$5.30 \pm 0.02$	$0.66 \pm 0.02$	$6.34 \pm 0.61$
ACHN	$28.4 \pm 0.03$	$36.1 \pm 0.06$	$329 \pm 13$	$138 \pm 2$	$8.00 \pm 0.006$	$2326 \pm 4$	$454 \pm 8$	$5.32 \pm 0.05$	$0.99 \pm 0.05$	$19.08 \pm 2.27$
HCAN	$28.5 \pm 0.07$	$36.2 \pm 0.08$	$336 \pm 13$	$140 \pm 2$	$7.78 \pm 0.017$	$2338 \pm 2$	$843 \pm 34$	$3.62 \pm 0.12$	$0.77 \pm 0.09$	$6.05 \pm 0.56$
HCHN	$28.3 \pm 0.04$	$36.1 \pm 0.08$	$329 \pm 8$	$138 \pm 2$	$7.79 \pm 0.013$	$2329 \pm 5$	$800 \pm 26$	$3.66 \pm 0.09$	$0.84 \pm 0.08$	$22.45 \pm 1.55$

**Table 2.** Results of mixed model fixed effects. Significance at  $p < 0.05$  is noted in *italic*.

treatment	source	d.f.	F	<i>p</i> -value
net calcification	pCO <sub>2</sub>	1	4.582	<i>0.035</i>
	nitrogen	1	41.16	<i>&lt; 0.001</i>
	pCO <sub>2</sub> × nitrogen	1	0.002	0.965
rETR	pCO <sub>2</sub>	1	2.096	0.151
	nitrogen	1	22.46	<i>&lt; 0.001</i>
	pCO <sub>2</sub> × nitrogen	1	30.75	<i>&lt; 0.001</i>
chlorophyll <i>a</i>	pCO <sub>2</sub>	1	0.442	0.510
	nitrogen	1	15.75	<i>&lt; 0.001</i>
	pCO <sub>2</sub> × nitrogen	1	2.843	0.100
phycocyanin	pCO <sub>2</sub>	1	0.447	0.508
	nitrogen	1	21.08	<i>&lt; 0.001</i>
	pCO <sub>2</sub> × nitrogen	1	0.040	0.843
phycoerythrin	pCO <sub>2</sub>	1	0.038	0.856
	nitrogen	1	28.86	<i>0.006</i>
	pCO <sub>2</sub> × nitrogen	1	0.269	0.632

were significant negative effects of pCO<sub>2</sub> ( $p = 0.035$ ) and positive effects of nitrogen enrichment ( $p = 0.001$ ) (table 2). High pCO<sub>2</sub> alone (HCAN) decreased net calcification of *P. onkodes* by 30% relative to the ambient treatment (ACAN) with average rates ( $\pm$ s.e.) of 2.7 ( $\pm$ 0.5) and 3.9 ( $\pm$ 0.5) mg CaCO<sub>3</sub> cm<sup>-2</sup>, respectively (figure 1a). Nitrogen enrichment increased net calcification by 90% and 130% in the ACHN and HCHN treatments, respectively, relative to the respective ambient nitrogen (AN) treatments, with average rates of 7.4 ( $\pm$ 0.5) and 6.2 ( $\pm$ 0.6) mg CaCO<sub>3</sub> cm<sup>-2</sup>.

### (c) Relative electron transport rates

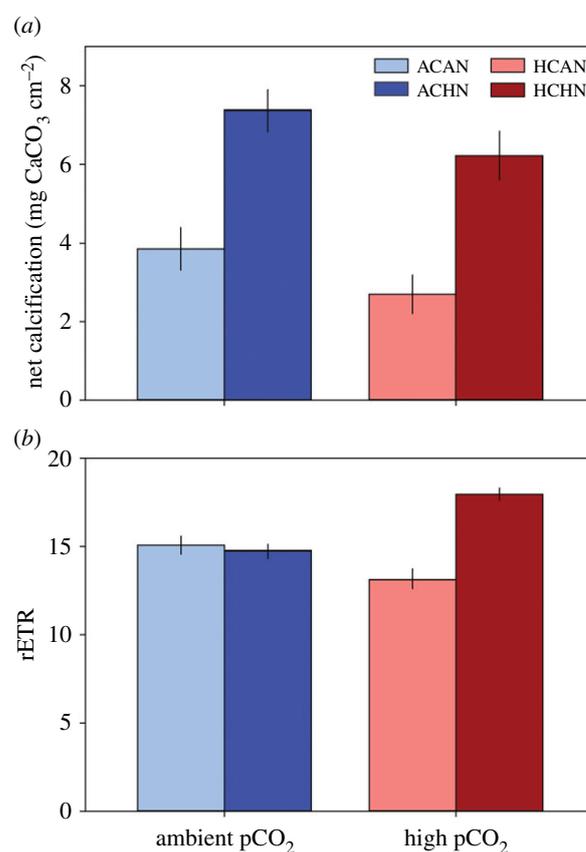
There was a significant interactive effect of pCO<sub>2</sub> and nitrogen on rETR of *P. onkodes* ( $p < 0.001$ , table 2). Nitrogen enrichment increased rETR, but only in the high pCO<sub>2</sub> treatment where it was 19–37% higher than the other three treatments (figure 1b).

### (d) Photosynthetic pigments

Nitrogen enrichment significantly increased the concentrations of chlorophyll *a* ( $p < 0.001$ ), phycocyanin ( $p < 0.001$ ) and phycoerythrin ( $p = 0.006$ ), and there was neither an effect of pCO<sub>2</sub> nor a significant interaction (table 2). Nitrogen enrichment increased chlorophyll *a* by 84–323% (figure 2a), phycocyanin by 207–379% (figure 2b) and phycoerythrin by 239–445% (figure 2c) in the ambient and high pCO<sub>2</sub> treatments relative to the respective AN treatments.

## 4. Discussion

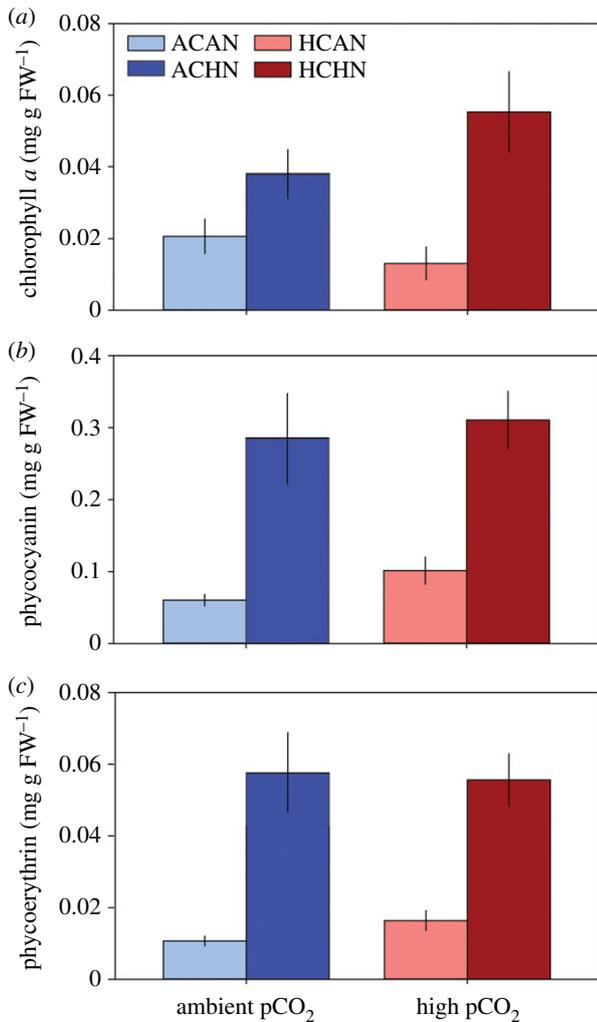
Our study demonstrates that nitrogen enrichment can mediate the direct effects of simulated OA on biological responses of a crustose coralline alga. As expected, high pCO<sub>2</sub> alone decreased coralline calcification, which agrees with established trends showing negative effects of OA on calcification



**Figure 1.** Effects of pCO<sub>2</sub> and nitrogen enrichment on *Porolithon onkodes* (a) net calcification normalized to core surface area and (b) relative electron transport rate (rETR). Blue represents ambient pCO<sub>2</sub> (AC) and red represents high pCO<sub>2</sub> (HC). Light colour represents ambient nitrogen (AN) and dark colour represents high nitrogen (HN). Values are mean  $\pm$  s.e.

of tropical CCA [8–10]. The combined effect of nitrogen enrichment significantly increased calcification rates in both ambient and high pCO<sub>2</sub> conditions. Notably, this stimulatory effect partially offset the direct negative effects of simulated OA alone on net calcification. Nitrogen enrichment and pCO<sub>2</sub> interactively affected *P. onkodes* photophysiology, with highest rETR under high nitrogen and high pCO<sub>2</sub>. Nitrogen enrichment also had a significant stimulatory effect on *P. onkodes* photosynthetic pigment content, and consistently increased chlorophyll *a*, phycocyanin and phycoerythrin content by 1–4-fold, regardless of pCO<sub>2</sub> treatment. Nitrogen enrichment increased ammonium concentrations by approximately 270%, and ambient nitrite + nitrate concentrations by up to approximately 50%. Ammonium is a biologically available form of nitrogen readily taken up by red algae [11], thus the response of *P. onkodes* to enrichment was likely driven by elevated ammonium concentrations.

The increases in photophysiology (rETR, pigments) in response to nitrogen enrichment indicates a potential for higher photosynthetic rates, and corroborates extensive literature documenting a positive relationship between nutrient availability and both algal photophysiology and photosynthesis [3]. Previous studies have similarly found stimulatory effects of nitrogen enrichment on photosystem II [16] (represented in our measurement of rETR) and on the concentrations of primary (i.e. chlorophyll *a*) and accessory (i.e. phycocyanin, phycoerythrin) photosynthetic pigments in algae [17]. Excess availability of nitrogen, and the potential for luxury nitrogen storage [11], thus may have facilitated



**Figure 2.** Effects of pCO<sub>2</sub> and nitrogen enrichment on *Porolithon onkodes* (a) chlorophyll *a*, (b) phycocyanin and (c) phycoerythrin concentrations normalized to fresh weight (FW) of extracted tissue. Blue represents ambient pCO<sub>2</sub> (AC) and red represents high pCO<sub>2</sub> (HC). Light colour represents ambient nitrogen (AN) and dark colour represents high nitrogen (HN). Values are mean ± s.e.

production of pigment molecules and stimulated photosystem and pigment activity under nitrogen enrichment. However, identifying the exact mechanisms driving the interactive effect of pCO<sub>2</sub> and nitrogen on rETR are beyond the scope of this study. Algal photosynthesis and calcification are tightly

coupled [18], thus higher rates of photosynthesis in response to nitrogen enrichment may be an underlying mechanism for the corresponding increase in *P. onkodes* net calcification.

The levels of nitrogen enrichment used in this study represent nutrient concentrations documented on coral reefs due to coastal eutrophication [4] or consumer excretions [5,6]. These results indicate that such local environmental factors may mediate the direct biological responses of some organisms to OA. For example, CCA from adjacent reef environments, such as an exposed fore reef versus a nearby fringing reef, may respond differently to OA due to organismal acclimatization to environmental parameters other than carbonate chemistry [9]. How potential direct benefits of nutrient enrichment will manifest at the community-scale are unclear, because nutrient enrichment may simultaneously increase indirect effects. Nitrogen enrichment and increased availability of dissolved CO<sub>2</sub> associated with OA can facilitate growth of macroalgae [2,4], which compete with and can overgrow coralline algae. An increase in negative indirect effects may thus counteract any direct benefit of nitrogen enrichment to coralline calcification under OA. Further, the impact of interacting global and local stressors may vary across ecosystems, as evidenced by the negative effect of high nutrients combined with high pCO<sub>2</sub> on temperate, understory corallines in Southern Australia [12]. Using a multi-stressor approach in global change experiments that incorporates local-scale variability and ecological responses will allow us to better predict the impacts of simultaneously increasing local and global stressors on near shore ecosystems.

**Ethics.** Research was conducted under a Protocole D'accueil (Scientific Research Permit) to R.C.C. from the Delegation a la Recherche de la Polynesie Francais (unnumbered).

**Data accessibility.** Data are available at Pangaea (<https://doi.pangaea.de/10.1594/pangaea.887917>).

**Authors' contributions.** Both authors conceived the study, conducted the experiment, wrote and revised the manuscript, approved the final version and agree to be held accountable for the content therein.

**Competing interests.** The authors have no competing interests to declare.

**Funding.** This research was supported by funding from the National Science Foundation to the Moorea Coral Reef Long Term Ecological Research program (OCE- 04 17412).

**Acknowledgements.** We thank VW Moriarty, C Wall and the Gump Research station staff for their assistance. This is a contribution of the Moorea Coral Reef (MCR) Long Term Ecological Research site, and is contribution no. 273 of the California State University, Northridge Marine Biology program.

## References

- Kroeker KJ, Kordas RL, Crim RN, Singh GG. 2010 Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecol. Lett.* **13**, 1419–1434. (doi:10.1111/j.1461-0248.2010.01518.x)
- Johnson MD, Price NN, Smith JE. 2014 Contrasting effects of ocean acidification on tropical fleshy and calcareous algae. *PeerJ* **2**, e411. (doi:10.7717/peerj.411)
- Geider R. 2013 *Algal photosynthesis*. Berlin, Germany: Springer.
- Szmant AM. 2002 Nutrient enrichment on coral reefs: is it a major cause of coral reef decline? *Estuaries* **25**, 743–766. (doi:10.1007/BF02804903)
- Meyer JL, Schultz ET, Helfman GS. 1983 Fish schools—an asset to corals. *Science* **220**, 1047–1049. (doi:10.1126/science.220.4601.1047)
- Williams SL, Carpenter RC. 1988 Nitrogen-limited primary productivity of coral-reef algal turfs—Potential contribution of ammonium excreted by *Diadema antillarum*. *Mar. Ecol. Prog. Ser.* **47**, 145–152. (doi:10.3354/meps047145)
- Morse JW, Andersson AJ, Mackenzie FT. 2006 Initial responses of carbonate-rich shelf sediments to rising atmospheric pCO<sub>2</sub> and 'ocean acidification. Role of high Mg-calcites. *Geochim. Cosmochim. Acta* **70**, 5814–5830. (doi:10.1016/j.gca.2006.08.017)
- McCoy SJ, Kamenos NA. 2015 Coralline algae (Rhodophyta) in a changing world: integrating ecological, physiological, and geochemical responses to global change. *J. Phycol.* **51**, 6–24. (doi:10.1111/jpy.12262)
- Johnson MD, Moriarty VW, Carpenter RC. 2014 Acclimatization of the crustose coralline alga *Porolithon onkodes* to variable pCO<sub>2</sub>. *PLoS ONE* **9**, e87678. (doi:10.1371/journal.pone.0087678)
- Johnson MD, Carpenter RC. 2012 Ocean acidification and warming decrease calcification in the crustose

- coralline alga *Hydrolithon onkodes* and increase susceptibility to grazing. *J. Exp. Mar. Biol. Ecol.* **434**, 94–101. (doi:10.1016/j.jembe.2012.08.005)
11. Ryther JH, Corwin N, Debusk TA, Williams LD. 1981 Nitrogen uptake and storage by the red alga *Gracilaria tikvahiae* (McLachlan, 1979). *Aquaculture* **26**, 107–115. (doi:10.1016/0044-8486(81)90114-9)
  12. Russell BD, Thompson JI, Falkenberg LJ, Connell SD. 2009 Synergistic effects of climate change and local stressors: CO<sub>2</sub> and nutrient-driven change in subtidal rocky habitats. *Glob. Change Biol.* **15**, 2153–2162. (doi:10.1111/j.1365-2486.2009.01886.x)
  13. IPCC. 2013 *Climate change 2013: The physical science bases. Contribution of working group 1 to the fifth assessment report of the intergovernmental panel on climate change* (ed. T Stocker et al.). Cambridge, UK: Cambridge University Press. (doi:10.1017/CBO9781107415324)
  14. Core Team R. 2016 *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
  15. Xu YY, Pierrot D, Cai WJ. 2017 Ocean carbonate system computation for anoxic waters using an updated CO<sub>2</sub>SYS program. *Mar. Chem.* **195**, 90–93. (doi:10.1016/j.marchem.2017.07.002)
  16. Barber J, Anderson JM. 2002 Photosystem II: molecular structure and function—Introduction. *Phil. Trans. R. Soc. Lond. B* **357**, 1325–1328. (doi:10.1098/rstb.2002.1153)
  17. Deboer JA. 1977 Effects of nitrogen enrichment on growth-rate and phycocolloid content in *Gracilaria* sp. and *Neogardhiella baileyi*. *J. Phycol.* **13**, 16.
  18. Borowitzka MA. 1981 Photosynthesis and calcification in the articulated coralline red algae *Amphiroa anceps* and *Amphiroa foliacea*. *Mar. Biol.* **62**, 17–23. (doi:10.1007/BF00396947)