Author for correspondence:
S. Comeau
e-mail: steve.comeau@csun.edu

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Coral reef calcifiers buffer their response to ocean acidification using both bicarbonate and carbonate

S. Comeau, R. C. Carpenter and P. J. Edmunds

Department of Biology, California State University, 18111 Nordhoff Street, Northridge, CA 91330-8303, USA

Central to evaluating the effects of ocean acidification (OA) on coral reefs is understanding how calcification is affected by the dissolution of CO_2 in sea water, which causes declines in carbonate ion concentration $[CO_3^{2-}]$ and increases in bicarbonate ion concentration $[HCO_3^-]$. To address this topic, we manipulated $[CO_3^{2-}]$ and $[HCO_3^-]$ to test the effects on calcification of the coral *Porites rus* and the alga *Hydrolithon onkodes*, measured from the start to the end of a 15-day incubation, as well as in the day and night. $[CO_3^{2-}]$ played a significant role in light and dark calcification of *P. rus*, whereas $[HCO_3^-]$ mainly affected calcification in the light. Both $[CO_3^{2-}]$ and $[HCO_3^-]$ had a significant effect on the calcification of *H. onkodes*, but the strongest relationship was found with $[CO_3^{2-}]$. Our results show that the negative effect of declining $[CO_3^{2-}]$ on the calcification of corals and algae can be partly mitigated by the use of HCO_3^- for calcification and perhaps photosynthesis. These results add empirical support to two conceptual models that can form a template for further research to account for the calcification response of corals and crustose coralline algae to OA.

1. Introduction

The calcium carbonate framework produced by coral reefs extends over large areas and hosts the highest known marine biodiversity [1]. In addition to the emblematic scleractinian corals, calcifying taxa such as crustose coralline algae (CCA) play key roles in the function of reefs by cementing components of the substratum together and providing cues for coral settlement [2]. Over the past century, however, coral reefs have been impacted by a diversity of disturbances [3] and now are threatened by ocean acidification (OA). This phenomenon is caused by the dissolution of atmospheric CO_2 in sea water, which reduces pH, depresses carbonate ion concentration $[CO_3^{2-}]$ and increases bicarbonate ion concentration $[HCO_3^-]$ and aqueous CO_2 [4]. As a result, most experimental studies of the effects of OA on the calcification of marine taxa have reported negative effects [5].

The threats of OA are well publicized for coral reefs [6], where there is concern that this ecosystem might cease to form calcified structures within 100 years [7]. This trend places corals and CCA at the centre of the debate regarding the magnitude of the effects of OA on tropical reefs, but a poor understanding of the calcification mechanisms involved has impaired progress in this debate. Even the fundamental question regarding the source of inorganic carbon favoured for mineralization by corals and algae remains unanswered [8,9]. Evaluating the roles of CO_3^{2-} and HCO_3^- in supplying dissolved inorganic carbon (DIC) for calcification by corals and CCA poses a significant challenge to progress in this field of investigation, notably in terms of describing calcification mechanisms upon which further studies of OA can be based, and determining the response of tropical reefs to OA. Critically, if calcifying taxa can use HCO_3^- directly to support the DIC needs of calcification [10] or indirectly by converting HCO_3^- to CO_3^{2-} at the calcification site [11], there is the potential for OA to stimulate calcification in contrast to the frequently described trend of calcification impairment through declines in CO_3^{2-}. This possibility has important implications, not least of which is the capacity to reconcile inconsistent results originating from experiments in which corals have been exposed

to OA. Results of experiments simulating the effects of OA on calcifying taxa vary greatly, with a few revealing positive [10,12] or null effects [13] of rising partial pressure of CO_2 (pCO_2), and many revealing negative effects including as much as an 85 per cent reduction in calcification when pCO_2 doubles [14]. Here, we present results from a manipulative experiment designed to test the roles of HCO_3^- and CO_3^{2-} on calcification of the coral *Porites rus* and the CCA *Hydrolithon onkodes* in the light and dark. Based on our results and existing theory [8,15], we synthesize conceptual models of calcification mechanisms for corals and CCA that can account for the differential effects of OA on these organisms and provide a framework for future mechanistic studies of calcification.

2. Material and methods

(a) Mesocosm and carbonate chemistry manipulation
This study was conducted during July 2011 in Moorea, French Polynesia, using branches (4 cm long) of *P. rus* and cores (4 cm diameter) of *H. onkodes* collected from the back reef (2–3 m depth). Specimens were transported to the Richard B. Gump South Pacific Research Station and glued (Z-Spar A788 epoxy) to 5×5 cm plastic racks to create nubbins ($n = 108$ per taxon). Before experimental incubations began, nubbins were maintained for 3 days in a sea-table with a high flow of sea water freshly pumped from Cook's Bay to allow for recovery.

Six nubbins of coral and six cores of CCA were placed in each of nine, 150 l tanks in a 12-tank mesocosm filled with unfiltered sea water pumped from Cook's Bay. Temperature in the tanks was maintained at $27.3 \pm 0.3°C$ (mean $\pm$ s.d., $n = 252$), and tanks were illuminated with LED lamps (75 W, Sol LED Module, Aquaillumination) on a 12 L : 12 D cycle providing approximately 700 ± 75 µmol photons m^{-2} s^{-1} of photosynthetically active radiation (measured below the sea water surface with a 4π quantum sensor LI-193 and a LiCor LI-1400 m). Water motion in the tanks was created with pumps (Rio 8HF, 2080 l h^{-1}).

Carbonate chemistry was manipulated using combinations of CO_2-equilibrated air, 1 M HCl (Thermo Fisher Scientific, Fair Lawn, NJ, USA), 1 M NaOH (Thermo Fisher Scientific), and Na_2CO_3 (EMD, Gibbstown, NJ, USA; electronic supplementary material, table S1). CO_2 treatments were created by bubbling ambient air, CO_2-enriched air or CO_2-depleted air into the tanks. CO_2-enriched air was created with a solenoid-controlled gas regulation system (model A352, Qubit Systems) that mixed pure CO_2 and ambient air at the desired values. CO_2-depleted air was obtained by scrubbing CO_2 from ambient air passing through a soda lime column. The flow of air and CO_2-manipulated air was delivered continuously in each tank and adjusted independently by needle valves, with adjustments conducted twice daily following pH measurements. The manual adjustments of the needle valves were used to ensure that the actual tank pH (see below) was close to target values. Half of the sea water in each tank was changed every second day with fresh sea water pre-equilibrated at the targeted CO_2 and DIC values. pH of the sea water was monitored twice daily, and sea water samples collected for total alkalinity (A_T) measurements (see §2b). The experiment ran for two weeks, and was conducted in two trials that began 2 days apart, with each using new nubbins of coral and cores of CCA.

(b) Carbonate chemistry
Sea water pH was measured twice daily (08.00 and 18.00 h) in each tank, using a pH meter (Orion, 3-stars mobile) or an automatic titrator (T50, Mettler-Toledo) calibrated every 2–3 days on the total scale using Tris/HCl buffers (Dickson, San Diego,

CA, USA) with a salinity of 34.5. A_T of the sea water in the tanks was measured every 2 days following water changes, using single samples drawn from each tank in glass-stoppered bottles (250 ml). Samples were analysed for A_T within 1 day using open cell, potentiometric titration and an automatic titrator (T50, Mettler-Toledo). Titrations were conducted on 50 ml samples at approximately 24°C, and A_T calculated as described by Dickson *et al.* [16]. Titrations of certified reference materials provided by A. G. Dickson (batch 105) yielded A_T values within 4.0 µmol kg^{-1} of the nominal value (s.d. = 4.1 µmol kg^{-1}; $n = 12$). Salinity in the experimental tanks was measured every 2 days using a conductivity meter (YSI 3100). A_T, pH$_T$, temperature and salinity were used to calculate [HCO_3^-] and [CO_3^{2-}] using the seacarb package [17] running in R software (R Foundation for Statistical Computing).

(c) Mean calcification
Calcification was measured using two techniques: buoyant weight [18] was used to evaluate the importance of [HCO_3^-] and [CO_3^{2-}] to calcification over two weeks, and the alkalinity anomaly technique [19] was used to differentiate short-term (approx. 1 h) calcification in the light and dark in response to variable [HCO_3^-] and [CO_3^{2-}]. Buoyant weight (± 1 mg) was recorded before and after the 15 days of incubation, and the difference between the two was converted to dry weight using an aragonite density of 2.93 g cm^{-3} for *P. rus*, and a calcite density of 2.71 g cm^{-3} for *H. onkodes*. Calcification was normalized to surface area estimated using aluminium foil for *P. rus*, and by digital photography and image analysis (IMAGEJ, NIH US Department of Health and Human Services) for *H. onkodes*.

(d) Light versus dark calcification
Light and dark calcification were estimated, using the alkalinity anomaly technique. One coral and one piece of CCA from each treatment were chosen randomly at 12.00 h on days 5 and 7 of the incubation, and placed in separate 500 ml glass beakers containing 320 ml of sea water from the respective incubation tanks. Incubations lasted 1 h at conditions that were the same as those in the 150 l incubation tanks (27°C, light intensity approx. 700 µmol photons m^{-2} s^{-1}). Small pumps (115 l h^{-1}) circulated sea water in the beakers. To monitor pCO_2 and ensure that [HCO_3^-] and [CO_3^{2-}] remain constant throughout the incubation, pH was controlled using a pH meter (Orion, 3-stars mobile) every 20 min during the incubation and adjusted when it deviated approximately 0.05 from the respective treatments by addition of pure CO_2 or CO_2-free air. As the incubations lasted 1 h, we assumed that the variation in A_T (measured before and at the end of the incubation) was owing to calcification/dissolution, and used the stoichiometric relation of two moles A_T being removed for each one mole of $CaCO_3$ precipitated to calculate calcification [19]. On the same day (days 5 and 7 of the incubation) and in darkness 2–5 h after sunset at 17.30 h, calcification in the dark was measured using an identical procedure.

(e) Statistical analyses
For the analysis of calcification by buoyant weight, a three-way model I ANOVA was used to test the effects of trial, [CO_3^{2-}] and [HCO_3^-] (see the electronic supplementary material, table S2). While the significance of all effects was evaluated against a preset α of 0.05, a more conservative criterion of $p > 0.25$ [20] was used as a basis to drop the trial effect from the statistical model. Trial was not significant for *P. rus* ($p > 0.25$) [20] and it therefore was dropped from the model and the analysis repeated as a two-way model I ANOVA (see the electronic supplementary material, table S3). The trial effect was significant for *H. onkodes* ($p < 0.001$), but the directions of the
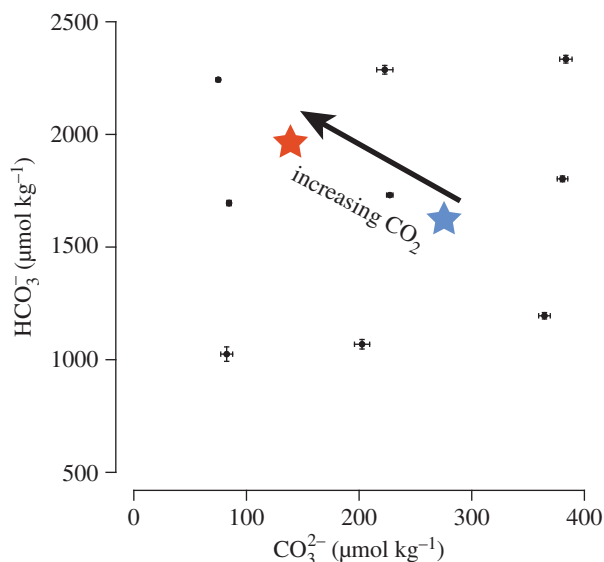
Figure 1. Mean carbonate $[CO_3^{2-}]$ and bicarbonate $[HCO_3^-]$ ion concentrations in nine treatments over two, two-week incubations. The horizontal and vertical error bars represent the s.e.m. of the $[CO_3^{2-}]$ and $[HCO_3^-]$, respectively ($n = 45$); the central point corresponds to the control (present-day values). The red star represents $[CO_3^{2-}]$ and $[HCO_3^-]$ expected in 2100 (27°C, pCO$_2$ approx. 800 μ atm, $A_T = 2300$ μmol kg^{-1}), and the blue star is an estimate of pre-industrial $[CO_3^{2-}]$ and $[HCO_3^-]$ (27°C, pCO$_2$ approx. 280 μatm, $A_T = 2300$ μmol kg^{-1}).

treatment effects were similar in both trials and only the magnitude differed. For this reason, we also dropped the trial effect for *H. onkodes* and repeated the analysis as a two-way model I ANOVA (see the electronic supplementary material, table S3).

For the analysis of calcification by the alkalinity anomaly method, a three-way model I ANOVA was used to test independently in the light and dark the effect of trial, $[CO_3^{2-}]$ and $[HCO_3^-]$ (see the electronic supplementary material, tables S4 and S5). An effect of trial was detected for *H. onkodes* dark calcification ($p = 0.156$) but was considered not biologically meaningful, because the direction of the effect was similar between trials.

All analyses were conducted separately by taxon, and the assumptions of normality and equality of variance were evaluated through graphical analyses of residuals. All analyses were performed, using the software R. Data were deposited in the Biological and Chemical Oceanography Data System (http://osprey.bco-dmo.org/project.cfm?flag=view&id=265&sortby=project).

3. Results

(a) Carbonate chemistry manipulations

Our approach to manipulating the carbonate chemistry of treatment sea water yielded precise and replicable results creating nine treatments (see figure 1 and electronic supplementary material, table S6). The treatments extended the range of conditions used commonly in OA perturbation experiments (i.e. from pre-industrial pCO$_2$ to the conditions expected by the end of the current century; figure 1).

(b) Mean calcification

For *P. rus*, calcification was associated positively with $[CO_3^{2-}]$ ($p < 0.001$), but not $[HCO_3^-]$ ($p = 0.368$), and was affected by the statistical interaction between $[CO_3^{2-}]$ and $[HCO_3^-]$ ($p = 0.009$; electronic supplementary material, table S3;

figure 2*a*). The maximum rate of calcification (2.07 ± 0.21 mg CaCO$_3$ d^{-1} cm^{-2}) occurred at high concentrations of both $[CO_3^{2-}]$ and $[HCO_3^-]$, and the lowest (0.66 ± 0.14 mg CaCO$_3$ d^{-1} cm^{-2}) under low $[CO_3^{2-}]$ and $[HCO_3^-]$ (figure 2*a*). *Porites rus* deposited CaCO$_3$ under conditions in which external inorganic carbon was reduced (i.e. low $[CO_3^{2-}]$ and $[HCO_3^-]$).

For *H. onkodes*, calcification was affected by $[CO_3^{2-}]$ ($p = 0.014$) and $[HCO_3^-]$ ($p = 0.019$), but not by the interaction between the two ($p = 0.161$; electronic supplementary material, table S3; figure 2). Similar to *P. rus*, mean calcification of *H. onkodes* was highest (2.13 ± 0.25 mg CaCO$_3$ d^{-1} cm^{-2}) when both $[CO_3^{2-}]$ and $[HCO_3^-]$ were elevated, and lowest (−0.18 ± 0.49 mg CaCO$_3$ d^{-1} cm^{-2}) when both $[CO_3^{2-}]$ and $[HCO_3^-]$ were depressed (figure 2*b*). In contrast to *P. rus*, however, net dissolution occurred under the lowest concentrations of $[CO_3^{2-}]$ and $[HCO_3^-]$, demonstrating that dissolution exceeded precipitation of CaCO$_3$.

(c) Light versus dark calcification

In both *P. rus* and *H. onkodes*, calcification was higher in the light than in the dark. In contrast to calcification over both light and dark cycles as measured by changes in buoyant weight, calcification in the light for *P. rus* was affected by $[CO_3^{2-}]$ ($p < 0.001$) and $[HCO_3^-]$ ($p < 0.001$; figure 2*c*; electronic supplementary material, table S4). In the dark, calcification was affected strongly by $[CO_3^{2-}]$ ($p < 0.001$), but not by $[HCO_3^-]$ ($p = 0.078$; figure 2*e*; electronic supplementary material, table S4).

For *H. onkodes*, light and dark calcification were affected by $[CO_3^{2-}]$ ($p < 0.001$) and $[HCO_3^-]$ ($p < 0.001$; figure 2*d*,*f* and electronic supplementary material, table S5) in a pattern similar to that recorded for calcification over the whole experiment. Calcification in the light and dark was similar in high $[CO_3^{2-}]$, whereas dark calcification was more negatively affected than light calcification by decreasing $[CO_3^{2-}]$. In the low $[CO_3^{2-}]$ treatments, net calcification became negative with dissolution exceeding calcification.

4. Discussion

(a) Empirical data

The results from our analysis contrast to previous findings [21–25] as we show that both $[HCO_3^-]$ and $[CO_3^{2-}]$ positively affect calcification in a tropical coral during a two-week incubation. Critically however, our experiment yields results that can reconcile these differences through mechanisms that are experimentally testable. Some previous studies suggest that coral calcification is governed by $[HCO_3^-]$ and not $[CO_3^{2-}]$ [21,22], but these earlier studies were limited to an analysis of calcification in the light, as measured using the alkalinity anomaly technique [21], or did not distinguish the differential effects on calcification of $[HCO_3^-]$ and $[CO_3^{2-}]$, two parameters of DIC chemistry in sea water that covary [22]. The conclusions of these studies are partly in agreement with our results, because $[HCO_3^-]$ played an important role in coral calcification in the light. By contrast, most previous experimental studies [23–25], and the review articles [14,26], suggest that coral calcification is controlled by the saturation state of aragonite (Ω_a), and the concentration of CO$_3^{2-}$ from which it is derived. We propose that dependency

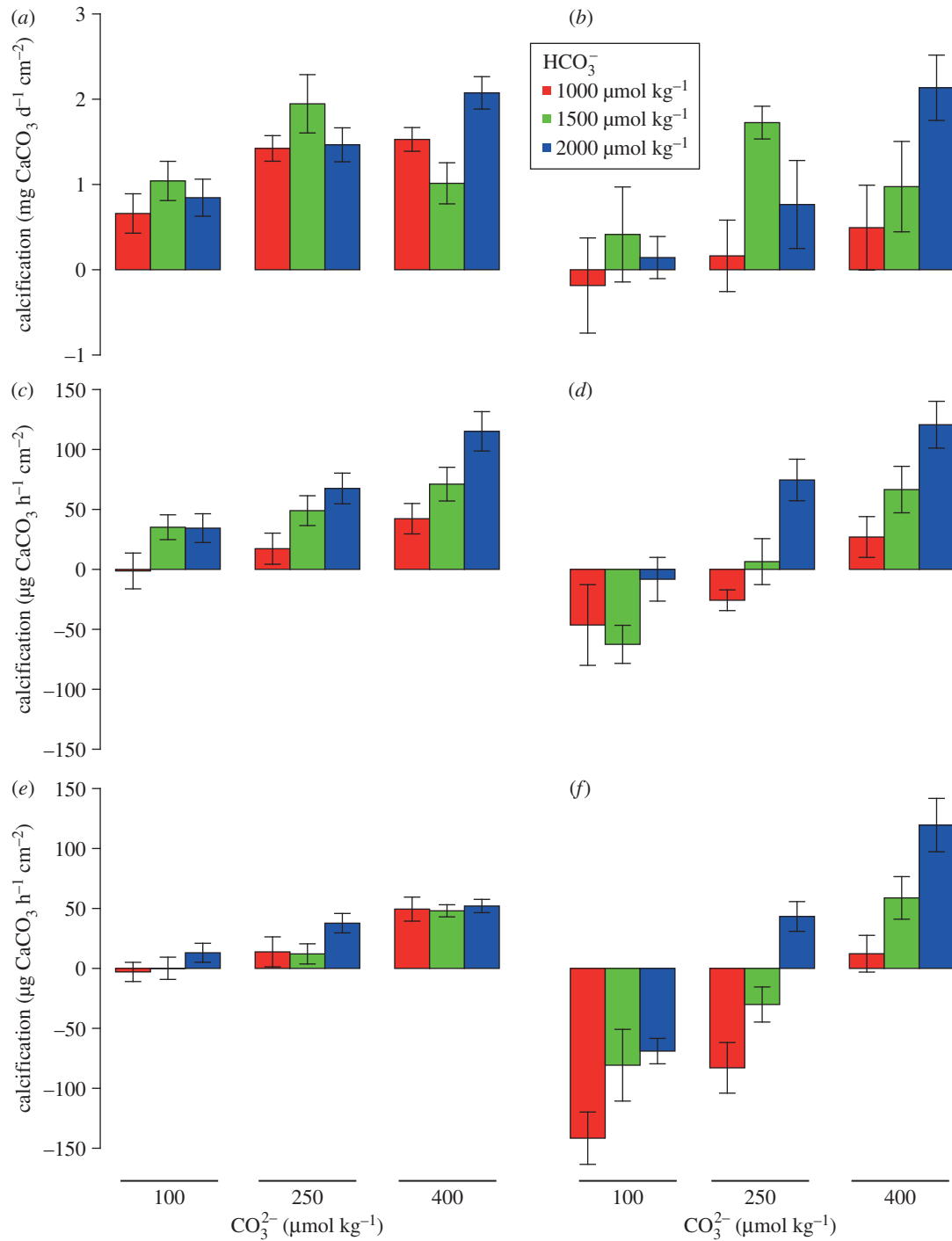**Figure 2.** Calcification rates of *Porites rus* (left column) and *Hydrolithon onkodes* (right column) as a function of $[CO_3^{2-}]$ and $[HCO_3^-]$. (*a,b*) Net calcification measured by buoyant weight over two, two-week incubations; (*c,d*) net calcification in the light measured by the alkalinity anomaly technique; and (*e,f*) calcification in the dark measured by the alkalinity anomaly technique. Each group of bars corresponds to three $[CO_3^{2-}]$ (approx. 100, 250 and 400 μmol kg^{-1}); colours of bars are dependent on $[HCO_3^-]$ (red approx. 1000, green approx. 1500, and blue approx. 2200 μmol kg^{-1}). All values are mean $\pm$ s.e.m. ($n = 12$ for buoyant weight and $n = 4$ for the light and dark calcification).

on Ω_a and $[CO_3^{2-}]$ is also in agreement with our study, because we demonstrate that mean calcification of *P. rus* was related to $[CO_3^{2-}]$. It is important to note however that our pCO$_2$ treatments would also have resulted in slight changes in aqueous CO$_2$ that ranges from 3 to 56 μmol kg^{-1} (see the electronic supplementary material, table S6). It is possible that this influenced the experimental outcome (e.g. the functional relationships between calcification and both CO_3^{2-} and HCO_3^-), as it is suggested to do with photosynthetic carbon fixation in the coccolithophore *Emiliania huxleyi* [27]. While we cannot exclude this possibility in our system, we suspect that aqueous CO$_2$ did not

play an important role in affecting calcification, because elevated rates of calcification were measured in the high $[CO_3^{2-}]$ treatment where aqueous CO$_2$ was low (between 3 and 11 μmol kg^{-1}; electronic supplementary material, table S6). To rigorously test for an effect of aqueous CO$_2$ on the calcification of corals and CCA, an experimental design differing from the one used here would be required. The separation of HCO_3^- and CO$_{2aq}$ could notably be obtained by increasing pCO$_2$ to increase aqueous CO$_2$ and by adding HCl to maintain $[HCO_3^-]$ low. However, such manipulation of the carbonate chemistry would induce working at very low pH, far from realistic values.
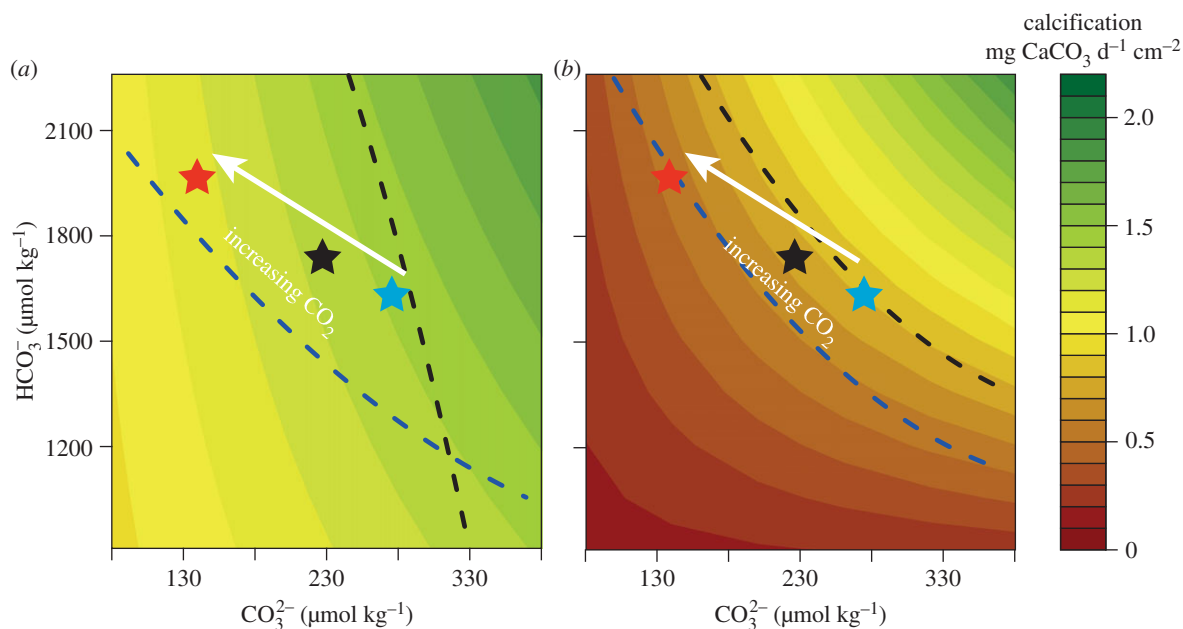
Figure 3. Estimated calcification of (*a*) *Porites rus* and (*b*) *Hydrolithon onkodes* as a function of past, present, and future [CO_3^{2-}] and [HCO_3^-]; the colour of the background represents rates of calcification. The black star shows the position of the present [CO_3^{2-}] and [HCO_3^-]; blue and red stars are predicted estimates of these concentrations in the years 1800 and 2100, respectively. The dashed lines represent the theoretical [CO_3^{2-}] and [HCO_3^-] necessary to sustain a calcification rate at a level equivalent to the control in the dark (black line) and light (blue line).

As our results indicate that the use of HCO_3^- by corals for calcification is linked to light intensity, it is intriguing to speculate that this phenomenon is related functionally to other light-dependent process, notably light-enhanced calcification [28] and photosynthesis. Photosynthesis in corals has long been hypothesized to be coupled directly with calcification [24], and can (under some conditions) be stimulated through HCO_3^- additions [22]. Based on these observations as well as our results, we hypothesize that there is a light-dependent increase in the importance of [HCO_3^-] for coral calcification, and further, that saturation of this relationship is reached at a high irradiance, when the maximum photosynthetic rate is reached.

In CCA, calcification has received less attention than in corals. The few studies investigating the response of CCA to modified carbonate chemistry conclude that calcification declines when [CO_3^{2-}] diminishes [29–31] or displays a parabolic response to decreasing [CO_3^{2-}] [11]. By contrast, the response of photosynthesis of CCA to [HCO_3^-] is controversial [30,32]. It is possible however that both calcification and photosynthesis in CCA presently are limited by DIC [11,31], which is consistent with our study where calcification was dependent on both [HCO_3^-] and [CO_3^{2-}] in the light and dark. Critically, DIC limitation suggests CCA may be somewhat more resistant to the effects of OA than other organisms because they would be able to use increases in [HCO_3^-] caused by the dissolution of CO_2 in sea water. For CCA, the magnitude of this effect presumably overcomes the negative implications of depositing an unusually soluble form of $CaCO_3$ (i.e. high-magnesium calcite). Nevertheless, our results, as well as previous studies [33–35, but see 11], suggest that although coralline algae can use HCO_3^- to calcify, it is not sufficient to compensate for the decrease in [CO_3^{2-}] that occurs with OA. As a result, net calcification is nullified or becomes negative as a result of dissolution under low [CO_3^{2-}] [35].

Our results have the potential to explain a portion of the high variance in responses described in experiments in which the calcification of corals and CCA has been measured under OA conditions [6,14]. The disparate results in previous experiments may originate, in part, from the choice of taxa for study that vary in their capacity to support calcification through the use of [HCO_3^-] and/or [CO_3^{2-}], which is mediated by their capacity to tightly control pH at the calcification site [15]. Calcifying taxa such as *P. rus* and *H. onkodes* that are able to use HCO_3^- for calcification may be less affected by OA than taxa that are more dependent on CO_3^{2-}. To test this hypothesis, we performed a linear interpolation of our results to estimate the mean calcification rate of both *P. rus* and *H. onkodes* under the range of [HCO_3^-] and [CO_3^{2-}] used during this experiment to evaluate the potential for increases in [HCO_3^-] from OA to compensate for the decrease in [CO_3^{2-}] (figure 3). The results of this interpolation suggest that in the light, corals can sustain present-day calcification if the decrease in [CO_3^{2-}] is compensated for by an increase in [HCO_3^-] (figure 3*a*, blue dashed line). However, to maintain present-day rates of light calcification through to the end of the current century, the necessary increase in [HCO_3^-] (approx. 15%) is slightly greater than the effect anticipated (approx. 10%) from OA modelled under the representative concentration pathway scenario 6.0. By contrast, increasing [HCO_3^-] when [CO_3^{2-}] is decreased is not sufficient to maintain dark calcification at present-day rates (figure 3*a*, black dashed line). Unlike *P. rus*, for *H. onkodes* when [CO_3^{2-}] decreases owing to rising pCO_2, light and dark calcification can be maintained at the present-day value only when there is an increase in [HCO_3^-] beyond that caused simply by the effects of OA on the equilibrium reactions controlling DIC in sea water (figure 3*b*). Our hypothesis suggests that the negative effect of declining [CO_3^{2-}] on the calcification of corals and calcified algae can be partially mitigated by the use of HCO_3^- to support calcification, particularly in the light.
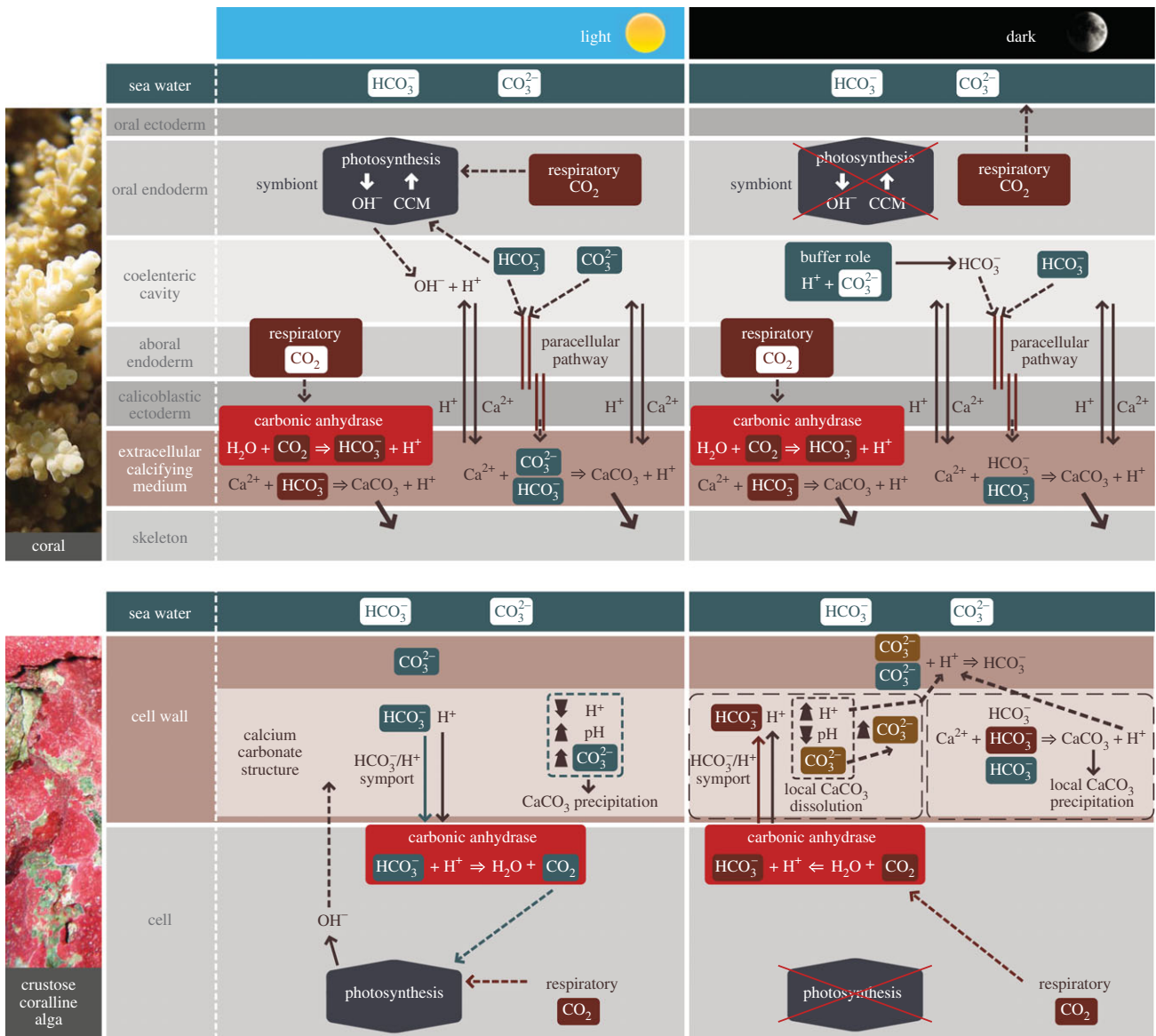
Figure 4. Conceptual models of calcification mechanisms in coral and CCA in the light and dark. The first and second rows are respectively schematic of a cross section of coral tissue and a cross section of a CCA cell. The ions with a green background correspond to ions originating from the external environment; the ions with a brown background are from internal carbon (i.e. respiratory CO_2). Dashed arrows correspond to diffusion and solid arrows to active transport. For corals, under light conditions, increasing $[CO_3^{2-}]$ has a stimulatory effect on calcification, and increasing $[HCO_3^-]$ has a stimulatory effect on calcification as well as photosynthesis and calcification. In dark conditions, $[CO_3^{2-}]$ play a central role by buffering the H^+ released in the coelenteric cavity that allows maintaining a relatively high pH at the calcification site. In CCA, under light conditions, $[CO_3^{2-}]$ has a direct stimulatory effect on calcification, whereas $[HCO_3^-]$ has a stimulatory effect on photosynthesis, which in turn stimulates calcification indirectly. In the dark, both $[HCO_3^-]$ and $[CO_3^{2-}]$ are involved in calcification, but $[CO_3^{2-}]$ plays a central role by buffering the excess of H^+. A detailed description of the mechanisms is presented in §4.

(b) Conceptual models of calcification

Understanding the mechanism(s) of calcification in corals and algae is critical to assess the impact of global climate change on reefs. Building from our results and previous theoretical work [8,15], we synthesize two conceptual models to describe the mechanisms of calcification in the light and dark in corals and CCA (figure 4).

For corals in the light, that HCO_3^- is involved in calcification and photosynthesis, whereas CO_3^{2-} is used only in calcification. Inorganic carbon at the calcification site is supplied by HCO_3^- and CO_3^{2-} possibly delivered from the external environment by paracellular pathways [36], and by the transformation of metabolic CO_2 into HCO_3^-, which can be catalysed by the enzyme carbonic anhydrase. The transformation of HCO_3^- into CO_2 for photosynthesis results in the production of hydroxyl ions (OH^-) [37],

which chemically buffer protons (H^+) released in the gastrovascular cavity by H^+/Ca^{2+} ion exchangers that help transport Ca^{2+} to, and remove H^+ from, the extracellular calcifying medium [38]. Because of the exchanges of these ions, potentially promoted by metabolic energy derived from photosynthetically fixed carbon, corals are able to maintain a high pH within the extracellular calcifying medium beneath the calicoblastic ectoderm (pH = 8.6–10) [15,39,40]. This high pH increases Ω_a favouring the precipitation of calcium carbonate in the presence of an organic matrix, which is believed to play a role as a template modulating calcium carbonate precipitation [41]. In this model, increasing $[CO_3^{2-}]$ has a stimulatory effect on calcification, and increasing $[HCO_3^-]$ has a stimulatory effect on calcification as well as photosynthesis, which in turn stimulates calcification.

In the dark, because OH^- ions from photosynthesis are absent, CO_3^{2-} buffers H^+ and is transformed in the gastrovascular cavity mostly into HCO_3^-, which then is transferred to the extracellular calcification medium. In this model, relatively high pH at the calcification site (pH approx. 8.4) [40] and the flux of protons from the extracellular calcifying medium to the gastrovascular cavity normally is favoured by the buffering capacity of CO_3^{2-}. However, when $[CO_3^{2-}]$ decreases, as under OA conditions, the transfer of H^+ away from the extracellular calcifying medium is reduced, causing H^+ to accumulate in the extracellular calcifying medium. As a result, pH as well as Ω_a is depressed, thereby slowing rates of precipitation of $CaCO_3$.

Calcification in CCA differs from corals as it takes place within the cell wall of the thallus [42]. In the light, HCO_3^- and H^+ are transported into the cell by HCO_3^-/H^+ symports [42], where they are transformed into CO_2 and H_2O by carbonic anhydrase. The supply of CO_2 for photosynthesis is provided by the HCO_3^- transformed into CO_2, and by the direct use of respiratory CO_2 (rather than HCO_3^- by *Symbiodinium*) [42]. Photosynthesis induces the releases of OH^- that diffuse into the cell wall where they cause a further increase in pH favouring increased $[CO_3^{2-}]$ and increased Ω_c that facilitate precipitation of $CaCO_3$. In this model, increasing $[CO_3^{2-}]$ has a direct stimulatory effect on calcification, whereas increasing $[HCO_3^-]$ has a stimulatory effect on photosynthesis, which in turn stimulates calcification indirectly.

We hypothesize for CCA in the dark that carbonic anhydrase catalyses the hydration of respiratory CO_2 to HCO_3^- and H^+ that are transferred from within the cell to the cell wall, where H^+ are buffered by CO_3^{2-} to form HCO_3^-. Inorganic carbon is supplied in the alkaline region of the cell wall from three sources: (i) the transformation of respiratory CO_2 into HCO_3^-, (ii) HCO_3^- from external sea water, and (iii) from the ions released by dissolution of the skeleton. As in the light, both $[HCO_3^-]$ and $[CO_3^{2-}]$ are involved in dark calcification, but $[CO_3^{2-}]$ plays a central role by buffering the release of H^+ and limiting $CaCO_3$ dissolution.

Together, our results suggest that the response of tropical coral reef communities to OA might be less dramatic than predicted previously [7]. However, it is important to note that our results originate in experiments involving 15-day incubation of organisms under experimental $[HCO_3^-]$ and $[CO_3^{2-}]$, and we do not know the extent to which these results would be replicated over ecologically relevant durations that arguably should be months to years. The capacity of research facilities in the tropics to conduct such experiments currently is inadequate; for example of the 33 perturbation experiments compiled by Erez *et al*. [14], 21 lasted less than or equal to two weeks and 12 lasted more than two weeks. There is some evidence that at least corals might be able to acclimatize to longer exposures to high pCO_2 [43], which might be caused by modulating the ability to use HCO_3^-. Furthermore, it is possible that the capacity to use HCO_3^- is accentuated during longer-term exposure as appears to be the case in the cold water coral *Lophelia pertusa*. In this coral, calcification was reduced by OA during short incubations (8 days) but was unaffected during long-term incubations (six months) [12]. Further, our study lends support to the emerging consensus that the response of calcified taxa to OA will be heterogeneous [44,45] and perhaps dependent on the capacity of organisms to use HCO_3^- to calcify. Nevertheless, despite the ability of corals and CCA to use HCO_3^- in the light, the decrease in dark calcification and/or dissolution under dark or low light conditions undoubtedly will lead to an overall reduction in the calcification of coral reefs in a future characterized by a more acidic ocean.

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