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## Invited reply

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# Response to coral reef calcification: carbonate, bicarbonate and proton flux under conditions of increasing ocean acidification

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We respond to the comment 'Coral reef calcification: carbonate, bicarbonate and proton flux under conditions of increasing ocean acidification' by Jokiel [1], who uses our data [2] to test his 'proton flux hypothesis', which accounts for the negative effect of ocean acidification (OA) on the calcification of reef corals [3]. We applaud the use of our empirical data to explore hypotheses differing from our own [2] to draw attention to possible associations between pairs of dependent variables that can provide insight into cause-and-effect relationship resulting in coral calcification being depressed by high  $p\text{CO}_2$  in seawater [4]. As described recently [5], robust data are necessary to project the impact of OA on coral reefs into the future and test hypotheses regarding the underlying mechanisms by which OA depresses calcification by coral reef calcifiers. The use of absolute units (e.g.  $\text{mg CaCO}_3 \text{d}^{-1} \text{cm}^{-2}$ ) is critical when measuring calcification in perturbation experiments in order to retain the greatest capacity for subsequent synthesis and reinterpretation, as has been accomplished by Jokiel using our data [1]. We note that this would not have been possible with calcification expressed in relative units (i.e. %), as is often the case [6].

However, regardless of the format of the data originating from studies of OA on corals and algae, careful attention must be given to the limitations of correlation, and the importance of manipulative experiments conducted with high precision. As Jokiel describes [1], correlation is not the same as cause and effect, and only manipulative experiments can untangle the relative roles of the multitude of potential drivers of calcification on the deposition of  $\text{CaCO}_3$ . Unfortunately, the strongly interdependent nature of the carbonate chemistry makes it exceptionally difficult to design appropriate experiments using clear treatments to generate unambiguous outcomes. We designed our experiment [2] to analyse the roles of carbonate ( $\text{CO}_3^{2-}$ ) and bicarbonate ( $\text{HCO}_3^-$ ) concentrations in the calcification of corals and calcified algae, and employed concentrations exceeding contemporary values on coral reefs, and even those expected to occur this century [7]. We did not conduct manipulations involving constant pH or constant DIC, and thus our data are not suited directly to test for the cause-and-effect relationship hypothesized to underlie the 'proton flux hypothesis' [3]. To test this hypothesis, it will be necessary to expose corals to combinations of constant pH (i.e. constant  $[\text{H}^+]$ ) and varying DIC, as well as constant DIC and varying pH. While such combinations will be technically challenging to accomplish, it might be possible to create them by combining fixed  $p\text{CO}_2$  treatments with additions of acid and base. For example, acid addition combined with low  $p\text{CO}_2$  could be used to maintain constant pH while decreasing DIC. The 'proton flux hypothesis' predicts that the calcification of corals would not be perturbed by these conditions (due to a constant proton concentration gradient into the coral tissue), whereas our model [2] predicts changes in calcification commensurate with the changes in  $[\text{CO}_3^{2-}]$  and  $[\text{HCO}_3^-]$ , and the extent to which light stimulates the use of  $\text{HCO}_3^-$  in calcification [2].

Contrary to the interpretation of our paper by Jokiel [1], we contend that our analysis does not challenge the paradigm of previous work indicating that the

control of coral calcification is mediated entirely by  $[\text{CO}_3^{2-}]$ . Indeed, we feel that a great strength of our work is its ability to reconcile previously contradictory results regarding the importance of  $[\text{CO}_3^{2-}]$  and  $[\text{HCO}_3^-]$  in driving coral calcification [2]. Our results from manipulative experiments show that the calcification of corals is linked to *both*  $[\text{CO}_3^{2-}]$  and  $[\text{HCO}_3^-]$ , but the use of  $[\text{HCO}_3^-]$  appears to be more important in the light than in the dark [2].

However, as pointed out by Jokiel, so far no active transporters of carbonate ions to the calcification site have been detected in corals. Nevertheless, direct connections by paracellular pathways between seawater and the calcification site have been demonstrated by exposing microcolonies of the coral *Stylophora pistillata* to the fluorochrome calcein [8]. Calcein binds to calcium, but it is a large molecule that cannot be transported through membranes; thus its presence between cells of corals that are calcifying actively demonstrates passage of calcein through paracellular pathways [8]. In addition, as we have suggested in our model describing proposed calcification mechanisms in corals, whereas  $\text{CO}_3^{2-}$  might not be directly used for calcification, it can play an important role as a proton acceptor to form  $\text{HCO}_3^-$ . Indeed, most physiological studies point to a direct use of bicarbonate ions and metabolic  $\text{CO}_2$  as main substrata for calcification in corals [9]. We note, however, that the effects of OA on calcification are exceptionally difficult to assess in corals, as in addition to the respective role of  $[\text{CO}_3^{2-}]$  and  $[\text{HCO}_3^-]$  in the calcification process, calcification is also affected by the capacity to export protons from the calcification site to the external medium [10,11].

By exploring the interactive effects of light and  $\text{pCO}_2$  on the calcification of coral recruits [12], we have obtained support for our hypothesis regarding the potential interaction between light and the DIC species driving calcification, probably through photosynthesis. Light intensities during experimental studies of the effects of OA on corals and algae clearly have not received sufficient attention, particularly since it has been known for decades that calcification in these taxa is indirectly proportional to light intensities and is greatly elevated in the light versus the dark. Both light and bicarbonate ions stimulate photosynthesis, which in return can favour calcification by (i) providing metabolic energy necessary to export protons from the calcification site, and (ii) chemically buffering the exported protons using hydroxyl ions produced during photosynthesis [13].

Since there has been no attempt to standardize light intensities in recent studies of the effects of OA on coral reef organisms, the light regime employed during experimental conditions could be a potent source of variance in

calcification results. For example, Edmunds *et al.* [5] summarized OA experiments with corals in which light intensities ranged from 11.5 to 1343  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ , and we speculate that this range is sufficiently large to include a potential threshold at which the relative importance of  $[\text{CO}_3^{2-}]$  and  $[\text{HCO}_3^-]$  shift. This possibility is supported by a recent study in which light intensity was covaried as recruits of *Pocillopora damicornis* were exposed to two  $\text{pCO}_2$  regimes to demonstrate a light-dependent shift in the response of calcification to  $\text{pCO}_2$  [12]. Calcification was unaffected by  $\text{pCO}_2$ , at low (31  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ) or high (226  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ) light levels, but was depressed at intermediate light levels (from 41 to 122  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ). Experimentally assessing the relative importance of light intensity to the responses of corals and algae to elevated  $\text{pCO}_2$  is one of the critical pieces missing to evaluate the effects of OA on coral reefs at various depths and locations. The next generation of experiments will need a greater emphasis on multifactor approaches, including ecologically relevant combination of  $\text{pCO}_2$ , temperature and light intensities to which corals will be exposed in the future. In addition, complementary physiological experiments that delve deeper into mechanisms underlying calcification than classical OA experiments also should be performed under a large range of light levels since the mechanisms of calcification, the species of DIC used and the mechanisms for protons export and buffering are impacted directly by photosynthesis.

Questions about calcification mechanisms remain at the centre of the debate over the effect of OA on coral reef organisms and communities. Several models describing the mechanisms driving calcification have been proposed [2,3,10], but experimental data to test these models and distinguish among them are still critically needed. Only carefully designed experiments coupled with new investigative techniques (e.g. confocal microscopy and vital stains) will allow for a more comprehensive understanding of these complex mechanisms and the impacts of environmental parameters, such as  $\text{pCO}_2$ , temperature, feeding and light. Without a comprehensive model of the calcification mechanism(s) in tropical corals and algae, reconciling the various biological hypotheses (preponderant role of  $[\text{HCO}_3^-]$  and  $[\text{H}^+]$ ) for the effects of OA on calcification with more geochemical hypotheses (preponderant role of  $[\text{CO}_3^{2-}]$ ) will remain challenging.

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