OCEAN ACIDIFICATION AND CONTROL OF REEF CORAL CALCIFICATION BY BOUNDARY LAYER LIMITATION OF PROTON FLUX

Paul Louis Jokiel

ABSTRACT

Since the beginning of the Industrial Revolution, the concentration of atmospheric CO\(_2\) has been rising due to the burning of fossil fuels. Increased absorption of this CO\(_2\) by the oceans is lowering the seawater pH and aragonite saturation state (\(\Omega_{ar}\)). This process is known as ocean acidification (OA). Numerous studies have shown a direct correlation between declining ocean pH, declining \(\Omega_{ar}\), and declining coral growth, but the mechanism is not understood. Various experiments designed to evaluate the relative importance of pH, CO\(_3^{2-}\), \(\Omega_{ar}\), HCO\(_3^-\), aqueous CO\(_2\), total alkalinity, and total inorganic carbon (C\(_T\)) to coral calcification have led to opposing conclusions. A reanalysis of existing data suggests that the mechanism is diffusion limitation of net H\(^+\) transport through the boundary layer caused by increasing [H\(^+\)] in the water column. The resulting “proton flux hypothesis” offers an explanation for the reduction in calcification caused by OA and other phenomena associated with increasing acidification. The hypothesis states that the lowered calcification rate observed in corals under increasing conditions of OA can be attributed to higher [H\(^+\)] in the seawater with consequent decrease in the efflux of H\(^+\) through the boundary layer.

Combustion of fossil fuels continues to increase the concentration of CO\(_2\) in the atmosphere. Ocean acidification (OA) is the name given to the resulting decrease in the pH of the oceans that is caused by the uptake of anthropogenic CO\(_2\). A doubling of preindustrial levels of oceanic pCO\(_2\) is predicted to occur at some point within this century (IPCC 2001, 2007) unless we radically decrease our burning of fossil fuels. Intensive work (reviewed by Feely et al. 2009) has led to a much greater understanding of how combustion of fossil fuels leads to lowering of the aragonite saturation state (\(\Omega_{ar}\)) in the surface waters of the ocean. The \(\Omega_{ar}\) concept is widely used by physical chemists to describe the condition of seawater in relation to the mineral form of CaCO\(_3\) precipitated by reef corals. The term \(\Omega_{ar}\) is defined by:

\[
\Omega_{ar} = \frac{[\text{Ca}^{2+}][\text{CO}_3^{2-}]}{K_{sp}} \quad \text{Eq. 1}
\]

where \(K_{sp}\) is the solubility product of aragonite.

Aragonite is the mineral form of CaCO\(_3\) that is laid down by corals, so the question arose as to how the declining saturation state would impact living coral populations. Smith and Buddemeier (1992) responded to the early concern and showed that the increased CO\(_2\) would cause seawater chemistry changes that would result in reduced coral calcification rates. This observation was subsequently confirmed by laboratory studies showing that calcification rates of reef-building corals could decline by 20%–40% under twice present day pCO\(_2\) conditions (Gattuso et al. 1999, Langdon et
In the past decade, there has been a rapid increase in awareness and concern about the impact of increasing OA on corals and coral reefs (Kleypas et al. 1999, Orr et al. 2005, Hoegh-Guldberg et al. 2007, Carpenter et al. 2008, Veron 2008). Much of the discussion is centered on the relationship between coral growth and $\Omega_{ar}$ and on the rate that $\Omega_{ar}$ will change over time in the surface waters of the sea.

$[Ca^{2+}]$ in seawater is very high and shows little variation spatially and temporally. Therefore, $\Omega_{ar}$ in Equation 1 is a function of the change in $[CO_3^{2-}]$. Equation 1 shows that reduction in $[CO_3^{2-}]$ correlates with a reduction in $\Omega_{ar}$. The relationship between $\Omega_{ar}$ and calcification rate in tropical reef building corals has been demonstrated (Gattuso et al. 1999, Langdon et al. 2000, Marubini et al. 2001, 2003, Ohde and Hossain 2004, Langdon and Atkinson 2005, Schneider and Erez 2006, Jokiel et al. 2008, Cohen et al. 2009). Temperate water corals have a much lower rate of metabolism and skeletal growth, so are less limited by material flux and show less of a decrease in calcification with decreasing $\Omega_{ar}$ (see fig. 4i. in Ries et al. 2009, fig. 4 in Holcomb et al. 2010, fig. 5 in Rodolfo-Metalpa et al. 2010). Temperate water species are not exceptions to the rule that OA decreases calcification in corals, although the decrease is less dramatic.

A correlation between $\Omega_{ar}$ and calcification rate does not establish cause and effect because $\Omega_{ar}$ correlates with the various components of the seawater carbonate system. Schneider and Erez (2006) conducted laboratory experiments designed specifically to separate the effects of pH, $CO_3^{2-}$, $CO_2$ (aq), total alkalinity ($A_T$), and total inorganic carbon ($C_T$) on reef coral calcification. They concluded that calcification (both light and dark) was positively correlated with $CO_3^{2-}$ concentration, suggesting that the corals are not sensitive to pH or $C_T$, but to $CO_3^{2-}$ concentration. Schneider and Erez (2006) did not consider the role of $HCO_3^-$, which makes up the bulk of the available $C_T$ in seawater (Horne 1969).

Jury et al. (2010) conducted experiments designed to distinguish the effects of pH, $[CO_3^{2-}]$, and $[HCO_3^-]$ by conducting incubations in highly modified seawater chemistries. Carbonate parameters were manipulated to isolate the effects of each, with a total of six different chemistry regimes. The corals responded strongly to variation in $[HCO_3^-]$, but not consistently to $[CO_3^{2-}]$, $\Omega_{ar}$, or pH. Corals calcified at normal or elevated rates under low pH (7.6–7.8) when the seawater $[HCO_3^-]$ exceeded 1800 µM. Conversely, corals incubated at normal pH had low calcification rates if the $[HCO_3^-]$ was lowered. These results are complex and no clear hypothesis was presented. However, the investigators noted that $[HCO_3^-]$ appeared to be the primary factor influencing calcification. Jury et al. (2010) concluded that data from their study and others point to inconsistencies in the $\Omega_{ar}$ model, but confirm that changes in seawater chemistry do affect calcification rates.

Another recent study by de Putron et al. (2010) describes a series of experiments designed to test the relative importance of $[HCO_3^-]$ vs $[CO_3^{2-}]$ in coral calcification. They came to a conclusion contrary to that of Jury et al. (2010) in that calcification was responding to $[CO_3^{2-}]$ and not to $[HCO_3^-]$. Marubini et al. (2008) grew corals at ambient $[HCO_3^-]$ vs enriched $[HCO_3^-]$ across a range of pH values. They suggested that seawater acidification affected coral calcification by decreasing the availability of $CO_3^{2-}$. However, they confounded this explanation with other hypotheses that decrease in coral calcification could also be due to a decrease in extra- or intracellular...
pH, or perhaps to a change in the buffering capacity of the medium that would impair supply of CO$_3^{2-}$ from HCO$_3^{-}$.

Cohen and Holcomb (2010) suggested that under conditions of increasing OA, corals must expend more energy to remove H$^+$ from the calcifying space between the aboral epidermis and the skeleton in order to raise the pH of the contained seawater and convert the plentiful HCO$_3^{-}$ to CO$_3^{2-}$. At high pH, the CO$_3^{2-}$ in the calcifying fluid combines with Ca$^{2+}$ to form the CaCO$_3$ crystals of the skeleton. Past, present, and future concentrations of the materials involved in calcification are shown in Table 1. Note that [CO$_3^{2-}$] decreases while [HCO$_3^{-}$] and $C_T$ increase with increasing OA. Ries (2011) conducted a microprobe comparison of [H$^+$] in the calcifying fluid and in the surrounding seawater of the temperate coral Astrangaea poculata (Ellis and Solander, 1786). He concluded that the coral does not offset the reduction in seawater [CO$_3^{2-}$] under acidified conditions by pumping more H$^+$ from its calcifying fluid. Instead, it appears to remove fewer H$^+$ from its calcifying fluid under acidified conditions, resulting in lower [CO$_3^{2-}$] at the site of calcification and, presumably, slower rates of calcification. Calcification rates of $A$. poculata decline by > 50% as atmospheric pCO$_2$ increases from 380 ppm ($\Omega_{ar} = 3.0$) to 760 ppm ($\Omega_{ar} = 1.8$).

Note from Table 1 that CO$_3^{2-}$ is a minor part of the $C_T$, making up only a fraction (14% under preindustrial pCO$_2$ and 8.2% under twice preindustrial conditions). Although CO$_3^{2-}$ decreases slightly with increasing OA, HCO$_3^{-}$ (which is the major source of inorganic carbon) increases from 1650 to 1883 μmol kg$^{-1}$ (Table 1), and $C_T$ increases from 1922 to 2059 μmol kg$^{-1}$. Further, the majority of host intracellular $C_T$ is in the form of HCO$_3^{-}$ (Venn et al. 2009) and not CO$_3^{2-}$. Coral calcification rate increases with increasing [HCO$_3^{-}$] (e.g., Marubini and Thake 1999, Herfort et al. 2008, Marubini et al. 2008). Therefore, one view is that coral skeletal growth should increase rather than decrease with increasing OA due to increasing supply of HCO$_3^{-}$. To date, no satisfactory hypothesis has been proposed to resolve the various issues described above. The most recent review of all information concerning the mechanism of calcification in corals (Allemand et al. 2011) summarized the findings of 308 publications related to the topic and concluded that "In the context of coral, while a large body of literature was published, we have to admit that the major steps of coral calcification remain to be discovered."

There is a need for a hypothesis that can synthesize these apparently conflicting data and explain the results of the various OA investigations. As stated by de Putron et al. (2010), "Clearly, the coral calcification response to OA is variable and complex. A deeper understanding of the biomineralization mechanisms and environmental conditions underlying these variable responses is needed to support informed predictions about future OA impacts on corals and coral reefs."

Table 1. Predicted change in carbonate parameters from preindustrial to twice preindustrial conditions. Calculated values are based on alkalinity of 2300 μmol kg$^{-1}$ SW with T = 25 °C and salinity = 35 using the program CO2sys (Pierrot et al. 2006). Estimated preindustrial saturation state of the tropical ocean in 1880 for pCO$_2$ is 280 μatm (Kleypas et al. 1999).

<table>
<thead>
<tr>
<th></th>
<th>pCO$_2$ atm</th>
<th>pH</th>
<th>[H$^+$] (nmol kg$^{-1}$ SW)</th>
<th>[HCO$_3^{-}$] (μmol kg$^{-1}$ SW)</th>
<th>[CO$_3^{2-}$] (μmol kg$^{-1}$ SW)</th>
<th>$C_T$ (μmol kg$^{-1}$ SW)</th>
<th>$\Omega_{ar}$</th>
</tr>
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<tbody>
<tr>
<td>Preindustrial</td>
<td>280</td>
<td>8.16</td>
<td>6.92</td>
<td>1650</td>
<td>264</td>
<td>1922</td>
<td>4.2</td>
</tr>
<tr>
<td>Present</td>
<td>386</td>
<td>8.07</td>
<td>8.51</td>
<td>1742</td>
<td>227</td>
<td>2121</td>
<td>3.6</td>
</tr>
<tr>
<td>2× preindustrial</td>
<td>560</td>
<td>7.91</td>
<td>12.30</td>
<td>1883</td>
<td>170</td>
<td>2059</td>
<td>2.7</td>
</tr>
<tr>
<td>% change</td>
<td>+100</td>
<td>-3</td>
<td>+78</td>
<td>+14</td>
<td>-36</td>
<td>+7</td>
<td>-36</td>
</tr>
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</table>
So what causes the observed reduction in calcification under conditions of increasing OA? If all other factors are held constant, changing the [Ca\(^{2+}\)] in the surrounding seawater will change calcification rate (Marshall and Clode 2002). Likewise, changing of [HCO\(_3\)\(^{-}\)] can change the rate of calcification (Marubini and Thake 1999, Herfort et al. 2008, Marubini et al. 2008). Increasing OA will have little or no effect on [Ca\(^{2+}\)], but will slightly increase [HCO\(_3\)\(^{-}\)] by 14%. The most dramatic change will be a 78% increase in [H\(^{+}\)], suggesting that the key to understanding the role of OA in controlling calcification might involve [H\(^{+}\)] and the movement of protons out of the corallum.

**Description of the Proton Flux Hypothesis**

Most of the research directed at understanding the importance of OA on reef corals has involved incubating corals in a chamber under various seawater chemistries and measuring the resulting chemical change in the seawater. In many of these cases, only changes in calcification rate due to OA were reported. In a few key cases (most notably Schneider and Erez 2006, Marubini et al. 2003, 2008, de Putron et al. 2010, Jury et al. 2010), there was an attempt to parse out the importance of various carbonate chemistry components in order to describe the mechanisms involved in coral calcification. In all of these cases, the corallum acts as a “black box” and only net flux between the water column and the coral was actually measured. Measurements of changes within the coral tissues have been accomplished by various molecular, cellular, and tissue techniques along with isotope studies and use of microprobes. These studies are beyond the scope of the present investigation, which is focused on a synthesis of material flux measurements under conditions of increasing OA. Calcification rate in such incubation experiments is generally determined by measuring change in A\(_{\text{a}}\), or the capacity of water to neutralize [H\(^{+}\)]. Coral calcification lowers A\(_{\text{a}}\), but there is no change in A\(_{\text{a}}\) associated with photosynthetic organic carbon production (Smith and Key 1975). Stoichiometry requires that a corallum must dissipate ~1 mole of protons into the water column for every mole of CaCO\(_3\) precipitated as coral skeleton. Net rate of calcification for a corallum is ultimately limited by net proton efflux, as well as by uptake of Ca\(^{2+}\) and HCO\(_3\)\(^{-}\). HCO\(_3\)\(^{-}\), the most abundant form of C\(_{\text{a}}\) in seawater, is the primary form taken up by corals (Al-Moghrabi et al. 1996, Goiran et al. 1996, Moya et al. 2008) and Ca\(^{2+}\) is very abundant in seawater. Thus HCO\(_3\)\(^{-}\) and Ca\(^{2+}\) are reactants of the calcification process:

\[
\text{HCO}_3^- + \text{Ca}^{2+} \rightarrow \text{CaCO}_3 + \text{H}^+\]

Eq. 2

The arrow represents the “black box” calcification reaction that occurs within the corallum and may involve a number of steps (e.g., McConnaughey and Whelan 1997, Allemand et al. 1998). The products to the right of the reaction arrow are CaCO\(_3\), which precipitates out of solution, and H\(^{+}\), which is a waste product that must be removed from the corallum if the reaction is to continue. The rate of the reaction is controlled by the concentration of the reactants and by the concentration of the product H\(^{+}\). The equation shows that for a coral to continue rapid calcification, there must be continuous supply of the reactants HCO\(_3\)\(^{-}\) and Ca\(^{2+}\) to the corallum and a continuous net efflux of H\(^{+}\) into the surrounding seawater. To the extent that the minor constituent CO\(_3\)\(^{2-}\) is used as the substrate for calcification, there is no net flux
of H+ through the boundary layer (BL) because the double positive charge of the Ca^{2+} is balanced by the double negative charge of CO_{3}^{2−}.

Jokiel (1978) and others (e.g., Shashar et al. 1993, 1996, Lesser et al. 1994, Kaandorp et al. 2005) have presented evidence that diffusion gradient strength across the BL controls flux of materials involved in the metabolism of reef corals. Kühl et al. (1995) report that in the light, zooxanthellae photosynthesis of Favia sp. and Acropora sp. resulted in a build-up of O_2 in the photosynthetic tissue of up to 250% saturation and a tissue pH of up to 8.6 (i.e., 0.7 pH units above the pH value of the overlying seawater). In the dark, O_2 was depleted by the polyp and zooxanthellae respiration, and near anoxic (< 2% air saturation) conditions in the coral tissue. In darkness, the tissues had a low pH of 7.3–7.4 and consequent high [H+]’. Both O_2 and pH profiles demonstrated the presence of a 200–300 μm thick BL that separated the coral tissue from the overlying flowing seawater (Kühl et al. 1995). Microelectrode measurements (Ries 2011) revealed that pH of the internal calcifying fluid of the temperate coral A. poculata is elevated relative to external seawater pH under both control and acidified seawater conditions. The coral maintained the same ratio of external [H+]’ to internal [H+] under both treatments, yet fewer H+ were removed from its calcifying fluid under acidified conditions. This result is consistent with the hypothesis of H+ flux limitation at the BL.

The net efflux of H+ out of the corallum and into the water column is influenced by the strength of the diffusion gradient between the coral and the surrounding seawater. This gradient becomes steeper with increasing OA due to increasing [H+]’ in the water column, with a consequent decrease in calcification rate. Fick’s first law of diffusion links diffusive flux to the concentration field by stating that the flux is from areas of high concentration to areas of low concentration with a magnitude that is proportional to the concentration gradient. The efflux of waste protons from the corallum, through the BL, and into the water column will occur at a magnitude that is proportional to the concentration gradient. By this model, increasing the [H+]’ in the water column will reduce flux of protons out of the corallum. The elimination of H+ is just as important as influx of reactants from the water column. The purpose of the present study was to test this hypothesis against data from the literature.

Methods and Materials

The primary approach in the present study was to conduct a literature review and re-evaluate the relevant data from previous studies (especially Schneider and Erez 2006, Marubini et al. 2008, de Putron et al. 2010, Jury et al. 2010) in the context of the proton flux hypothesis. These previous workers produced excellent datasets. Data were extracted from reports and entered onto spreadsheets for analysis and plotting. In each case, pH was converted to [H+]’ for the analysis. Values for the various carbonate parameters were calculated using the program CO2sys (Pierrot et al. 2006) where appropriate.

Results and Discussion

Schneider and Erez (2006) report that calcification (both light and dark) was positively correlated with CO_{3}^{2−} concentration in light (R^2 = 0.55) and dark (R^2 = 0.66), suggesting that the corals are not sensitive to pH or C_t, but rather to the CO_{3}^{2−} concentration. Plotting their original results for [CO_{3}^{2−}] against [H+]’ (Fig. 1) also produces a significant correlation, so it is equally plausible that [H+]’ rather than [CO_{3}^{2−}]
determined the results of their experiments. Light and dark calcification rates measured in their study are plotted against $[\text{H}^+]$ as Figure 2. Note that in these experiments, the $[\text{H}^+]$ in various incubations ranged by over 500% from the lowest to the highest treatment, which represents a very large change in gradient strength across the BL. The most recent review of impact of OA on corals (Erez et al. 2011) cites 170 relevant papers and concludes that most of the published data indicates that the cause of reduced skeletal growth under conditions of increasing OA is the decrease in the $\text{CO}_3^{2-}$ of seawater. However, $[\text{H}^+]$ and $[\text{CO}_3^{2-}]$ are tightly correlated (Fig. 1), so coral calcification may be responsive to $[\text{H}^+]$ in addition to or instead of $[\text{CO}_3^{2-}]$. Likewise, de Putron et al. (2010) conducted a series of well defined experiments that showed coral calcification did not follow $\text{Ca}^{2+}$ or $[\text{HCO}_3^-]$, leading them to conclude that $[\text{CO}_3^{2-}]$ was the factor controlling calcification. However, $[\text{CO}_3^{2-}]$ and $[\text{H}^+]$ in their data are also correlated (Fig. 3B,D). Plotting their growth data for newly settled colonies of the corals *Favia fragum* (Esper, 1797) and *Porites astreoides* Lamarck, 1816 against $[\text{H}^+]$ is consistent with the hypothesis that coral calcification can be limited by net diffusion of $[\text{H}^+]$ through the boundary layer (Fig. 3A,C). The $[\text{H}^+]$ in this series of experiments ranged over 600%, which is a tremendous difference in gradient strength for $\text{H}^+$.

Jury et al. (2010) provide a very useful dataset based on response of coral calcification to extreme seawater chemistries that have never existed in nature and will not exist in the future ocean (Table 2). The Jury et al. (2010) study is valuable because it demonstrates the importance of reactants ($\text{Ca}^{2+}$ and $\text{C}_4$) vs products ($\text{H}^+$ and $\text{CaCO}_3$ precipitate). Treatment 1 of Jury et al. (2010; normal pH, low $\text{CO}_3^{2-}$) was characterized by normal $[\text{H}^+]$ and low $[\text{C}_4]$, with a resulting lowering of calcification rate (60% of control) due to a paucity of the reactant. Treatment 2 (normal pH, very low $\text{CO}_3^{2-}$) showed the same $[\text{H}^+]$ as the control, but much lower $[\text{C}_4]$ (43% of control) that represents a further reduced supply in the reactant $\text{HCO}_3^-$, causing a further
decrease in calcification rate (46% of control). Treatment 3 (low pH, normal CO$_3^{2-}$) and Treatment 4 (low pH, low CO$_3^{2-}$) both produced extremely high [ct] that resulted in a very high calcification rate, even though [H$^+$] was also high. Treatment 5 (very low pH, very low CO$_3^{2-}$) had a higher [ct] (108% of control), but with a much higher [H$^+$], leading to a reduction in calcification. The data in Table 2 show how changing seawater concentration of reactant C$_t$ or changing seawater concentration of the product H$^+$ can change rate of deposition of the product CaCO$_3$. However, Jury et al. (2010) were unable to demonstrate a clear quantitative pattern in their data, likely due to two causes. First, variance was introduced by using a comparison run with each coral rather than using the direct calcification data shown in their fig. 2B. Second, the extreme manipulations resulted in major changes in the distribution of carbonate species, so it was difficult to show the pattern with a single carbonate species given the complexity of the design and limited number of treatments. Corals can utilize all forms of inorganic carbon as substrate for photosynthesis and calcification, so it would be best to examine C$_t$ where radical changes in the carbonate system are involved and the number of treatments is small. When the Jury et al. (2010) data are presented as the direct growth response to the ratio of [C$_t$] to [H$^+$], a clear pattern emerges (Fig. 4). These data suggest that coral calcification is limited by the supply of the reactant inorganic carbon in relation to the water column [H$^+$] inhibitor of waste proton efflux.

Figure 2. Data from Schneider and Erez (2006) replotted as calcification vs [H$^+$]. Open triangles represent growth trials in light, solid circles represent growth trials in darkness. The relationship between calcification and [H$^+$] is significant (P < 0.001) in both light and dark treatments.
Figure 3. Data from de Putron et al. (2010) replotted as (A,C) skeletal growth in corals vs [H+] and (B,D) [CO$_3^{2-}$] vs [H+]. Open symbols are data from an acid addition experiment conducted in 2007 and closed symbols represent results of a CO$_2$ bubbling experiment conducted in 2008. The dashed line in panels B and D is the relationship between [H+] and [CO$_3^{2-}$] as calculated using the program CO2sys (Pierrot et al. 2006) based on alkalinity = 2300 µmol kg$^{-1}$ SW, temperature = 25 °C, salinity = 35. Relationship between skeletal growth and [H+] is significant (p < 0.001).
Using coral laboratory growth data and the estimated future scenarios of OA, Gattuso et al. (1999) estimated a decrease in coral CaCO₃ production of 10% between 1880 and 1990, followed by an additional 9%–30% (mid estimate: 22%) from 1990 to 2100. More recent OA estimates (Marubini et al. 2001, Anthony et al. 2008, Jokiel et al. 2008) support this generalization. Doubling of preindustrial pCO₂ level in seawater (Table 1) would lead to a 14% increase in [HCO₃⁻] and a 78% increase in [H⁺]. The last row was of Table 2 is the Gattuso et al. (1999) values of (10% + 22%, 68% of preindustrial rate as the control) to facilitate comparisons to data from the Jury et al. (2010) experiments.

Marubini et al. (2008) grew the reef coral *Stylophora pistillata* (Esper, 1797) under similar pH regimes at both ambient [HCO₃⁻] (2 mM) and enriched [HCO₃⁻] (4 mM). Doubling of preindustrial pCO₂ level in seawater (Table 1) would lead to a 14% increase in [HCO₃⁻] and a 78% increase in [H⁺]. The last row was of Table 2 is the Gattuso et al. (1999) values of (10% + 22%, 68% of preindustrial rate as the control) to facilitate comparisons to data from the Jury et al. (2010) experiments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Reactant [C₅][μmol kg⁻¹ SW] (% of control)</th>
<th>Product [H⁺][nmol kg⁻¹ SW] (% of control)</th>
<th>Product CaCO₃ (% of control)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>2094</td>
<td>–</td>
<td>8.5</td>
</tr>
<tr>
<td>1. Normal pH, low CO₃²⁻</td>
<td>1250</td>
<td>60</td>
<td>8.5</td>
</tr>
<tr>
<td>2. Normal pH, very low CO₃²⁻</td>
<td>891</td>
<td>43</td>
<td>8.5</td>
</tr>
<tr>
<td>3. Low pH, normal CO₃²⁻</td>
<td>3887</td>
<td>186</td>
<td>16.2</td>
</tr>
<tr>
<td>4. Low pH, low CO₃²⁻</td>
<td>2273</td>
<td>108</td>
<td>16.6</td>
</tr>
<tr>
<td>5. Very low pH, very low CO₃²⁻</td>
<td>2267</td>
<td>108</td>
<td>25.1</td>
</tr>
<tr>
<td>Future ocean at 2× preindustrial pCO₂ levels (from Table 1)</td>
<td>1883</td>
<td>114</td>
<td>12.3</td>
</tr>
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</table>

Results from situations where calcification increased in relation to reduced [H⁺] are consistent with the proton flux hypothesis. McConnaughey (2000) investigated the impact of increased pH on corals by evaluating the influence of fleshy macroalgae on coral metabolism. The noncalcareous alga *Chondria* sp. increased pH in static 10-hr daylight incubations, but did not change alkalinity. In other words, the fleshy alga greatly decreased the [H⁺] in the chamber seawater through photosynthesis. When corals and fleshy algae were incubated together, pH increased (and [H⁺] decreased). Calcification rates increased by 60% for the coral *Acropora* and by 130% in the coral *Montipora*, compared to corals incubated without fleshy macroalgae. An apparent
paradox in this experiment is that increased rates of photosynthesis and accelerated calcification both occurred in the face of decreasing [HCO$_3^-$] in the incubation seawater. According to the proton flux hypothesis, [H$^+$] in the surrounding seawater is a major factor influencing the metabolic response. The rapid increase in pH due to algal photosynthesis results in a substantial decrease in [H$^+$] in the surrounding seawater, which, according to the proton flux model, would accelerate outflux of H$^+$ and increase calcification rate. High pH values caused naturally by seagrass photosynthesis can enhance calcification rates of nearby calcifying macroalgae (Semesi et al. 2009a). Semesi et al. (2009b) also report that rhodoliths of the crustose coralline alga *Hydrolithon* sp. that grow in algal beds calcify more rapidly at high pH (low [H$^+$]) compared to counterparts growing outside of the beds at lower pH (high [H$^+$]). The mechanisms of calcification in reef corals and coralline algae are quite different (e.g., “trans” vs “cis” calcification of McConnaughey and Whelan 1997), but ultimately, both appear to be limited by efflux of H$^+$ through the BL.

Marubini et al. (2003) measured calcification in four phylogenetically and physiologically different species of hermatypic coral [*Acropora verweyi* Veron and Wallace, 1984, *Galaxea fascicularis* (Linnaeus, 1767), *Pavona cactus* (Forsskål, 1775), and *Turbinaria reniformis* Bernard, 1896] under “normal” (280 µmol kg$^{-1}$) and “low” (140 µmol kg$^{-1}$) carbonate-ion concentrations. The “low carbonate” treatment resulted in a significant reduction in calcification rate. According to the authors, calcification rate was affected uniformly across species (13%–18% reduction). They concluded that a decrease in [CO$_3^{2-}$] results in a decrease in the calcification rate. Application of the proton flux model to their data provides a different interpretation of their results (Fig. 6). From the point of view of proton flux, the calcification rate was not affected uniformly across species. There was a seven-fold difference in calcification rate between the different species (the y-intercept varied from 7 to 45, see Fig. 6) over the range of [H$^+$] used in the experiments. The corals with higher calcification rate showed a dramatic decline (change in slope from −0.81 to −0.11) in relation to increased [H$^+$].

Figure 4. Data from Jury et al. (2010) depicting the significant (P = 0.001) relationship for growth of the coral *Madracis auretenra* in relation to the [C$_3$]:[H$^+$] ratio.
Temperate coral species appear to follow the same pattern, but at a reduced metabolic rate. Rodolfo-Metalpa et al. (2010) report a growth rate in the temperate coral species *Cladocora caespitosa* (Linnaeus, 1767) of only 0.2–1 mg g\(^{-1}\) d\(^{-1}\). Data from Ries et al. (2009) are comparable to the Marubini et al. (2003) data and were used to calculate the relationship between growth and \([\text{H}^+]\) for the temperate coral *Oculina arbuscula* Agassiz, 1864. Incubations in both sets of experiments were conducted under similar temperature (25–26.5 °C) and water chemistry regimes, so the datasets can be compared (Fig. 6). The temperate coral fits the pattern of reduced negative slope with reduced growth rate (\(P < 0.05\)). In terms of the proton flux hypothesis, the more rapidly growing tropical corals must dissipate greater quantities of protons through the BL and thus are more vulnerable to increased \([\text{H}^+]\) in the water column.

A temperature-OA synergism with regard to calcification has been identified. Reynaud et al. (2003) grew small colonies of the reef coral *Stylophora pistillata* Esper, 1797 in a matrix of two temperature treatments (25 vs 28 °C) and two pCO\(_2\) treatments (460 vs 750 μatm) and report no statistical difference between pCO\(_2\) treatments at 25 °C, but a very large decline in calcification (~50%) at 28 °C under acidified conditions. Anlauf et al. (2011) investigated the effects of a 1 °C and a 0.20–0.25 unit pH decrease on the growth of primary polyps in the coral *Porites panamensis* Verrill, 1864. Growth in polyps was reduced marginally by acidic seawater, but the

Figure 5. Data from Marubini et al. (2008) showing change in growth of the reef coral *Stylophora pistillata* vs \([\text{H}^+]\). Triangles are growth at ambient levels [HCO\(_3^-\)] (2 mM) and circles are growth with enriched [HCO\(_3^-\)] (4 mM).
combined effect of high temperature and lowered pH caused a significant growth reduction of ~30%. Martin and Gattuso (2009) observed the same effect in the coralline alga *Lithophyllum cabiochae* (Boudouresque & Verlaque). Algae were maintained in aquaria for 1 yr at ambient or elevated temperature (+3 °C) and at ambient (~400 µatm) or elevated (~700 µatm) pcO₂. During summer, net calcification of the algae decreased by 50% when both temperature and pcO₂ were elevated, while no effect was found under elevated temperature or elevated pcO₂ alone.

The temperature-OA interaction can be explained by the proton flux model. Both temperature and pcO₂ have a direct effect on seawater carbonate chemistry (Table 3). Increasing the temperature increases [H⁺] in the water column while simultaneously reducing [HCO₃⁻]. There is an accompanying sharp decrease in [CO₂] and an increase in [HCO₃⁻], but these changes are trivial compared to the change in [HCO₃⁻]. C₇ is the sum of [HCO₃⁻], [CO₃²⁻], and [CO₂], but HCO₃⁻ is the dominant component. The ratio C₇ divided by H⁺ can be viewed as the relative availability of reactant in relation to product. Table 3 shows that increasing temperature and/or increasing pcO₂ decreases the C₇:H⁺ ratio. Therefore, removal of the waste product H⁺ from the coral-lum is slowed through the BL at higher temperature, while at the same time supply of the reactant HCO₃⁻ is reduced. Figure 7 graphically illustrates the physiological challenge that calcifying organisms will face due to increasing global temperature.
and increasing OA. However, an additional biochemical process within the coral-lum also comes into play. Coles and Jokiel (1977) showed that coral photosynthesis and respiration increase with increasing temperature, but respiration increases more rapidly. Consequently, the ratio of photosynthesis to respiration decreases with increasing temperature. Thus corals show greater demand for HCO$_3^-$ and a greater need to remove H$^+$ at higher temperatures. But corals are simultaneously faced with a decreasing CT:H$^+$ ratio (Table 3, Fig. 7). Perhaps this explains why coral skeletal growth shows a positive correlation with increasing temperature up to an “optimal temperature” (Jokiel and Coles 1977) and then declines rapidly. According to the proton flux hypothesis, corals are faced with increasing metabolic demand at higher temperature combined with decreasing flux of key metabolites, which could lead to lowered growth rates, bleaching, and increasing mortality.

The proton flux hypothesis negates a number of conceptual obstacles, and provides new insights into the importance of OA and temperature on coral reefs. The physical chemist's concept of $\Omega_{ar}$ has been essential to our understanding of global distribution and changes in the carbonate chemistry of the sea, but has no direct relevance to coral physiology. It is easy for a biologist to become focused on $\Omega_{ar}$ as an important...
independent variable related to coral calcification. According to the proton flux hypothesis, coral physiology is responding mainly to \([\text{HCO}_3^-]\) and \([\text{H}^+]\), which happen to show a correlation with \(\Omega_{ar}\). Likewise, biologists have conceptually focused on pH (which is on a log scale) rather than \([\text{H}^+]\), because we have always worked with pH and our instruments read in pH units. The important physiological parameter is \([\text{H}^+]\), while pH is defined as \(-\log[\text{H}^+]\). A pH change from 8.3 to 7.8 represents a conceptually trivial 6% change. However, when expressed in terms of \([\text{H}^+]\), this is a change of 216%. Flux of protons is controlled by the difference in \([\text{H}^+]\) across the BL, not by the difference in \(-\log[\text{H}^+]\). Some of the studies discussed previously were conducted over a range of \([\text{H}^+]\) that varied by nearly 600%. Failure to appreciate the importance of the BL in experiments is another conceptual stumbling block. Also, the preoccupation with supply of materials required for calcification rather than the need to remove waste protons further obstructed our vision. Results must be viewed in the context of both reactants and products. Temperate and tropical corals show a decrease in calcification rate due to increased \([\text{H}^+]\), but the low rate of temperate coral metabolism results in a much lower slope for the linear equation (Fig. 6).

Kaandorp et al. (2005) used simulation experiments and isotope analyses of coral skeletons to test the hypothesis that localized external gradients of \(C_T\) determine
calcification and thus the morphogenesis of branching, phototrophic corals. Their model is driven entirely by a diffusion-limited BL process and can generate coral growth patterns and morphologies that are virtually indistinguishable from three-dimensional images of the actual colonies. They concluded that carbon supply on the reactant side of Equation 2 represents the limiting factor for calcification rate, but it is equally plausible that H⁺ gradients on the product side of Equation 2 can influence or even dominate the rate of the calcification reaction. The Kaandorp et al. (2005) model is based on C₄ uptake as limited by the BL, and will show an erroneous increase in coral growth as OA increases, because C₄ will also be increasing. If the model incorporated H⁺ flux, it would produce a similar morphological output, but with a reduction rather than an increase of coral growth under acidified conditions.

The parameters Ωᵃʳ and [H⁺] do not correlate well with each other in shallow inshore systems due to the intense metabolic activity of inshore reef communities, prevalence of noncalcifying photosynthetic organisms, and input of terrigenous materials by runoff and groundwater. In a mixed inshore coral-algae community, [H⁺] can be very low during daylight hours due to high rates of photosynthesis and low rates of water exchange. The resulting low [H⁺] allows corals to calcify rapidly even when Ωᵃʳ is low. Night [H⁺] is very high due to respiration of the surrounding reef community, but this is of limited consequence because the corals are not calcifying rapidly during darkness. In the future, corals living in coastal water environments characterized by low levels of [H⁺] during daylight hours will calcify at a higher rate than their relatives growing under open ocean conditions.

In the latest comprehensive review, Erez et al. (2011) concluded that the potential mechanisms responsible for coral sensitivity to acidification are either direct input of seawater to the biomineralization site or high sensitivity of enzymes involved in calcification to pH and/or CO₂ concentrations. Thus previous hypotheses have focused on biochemical processes at or near the site of calcification. The alternative provided by the proton flux hypothesis is that calcification is ultimately limited by the physical process of proton diffusion through the BL, which is an external physical barrier not under the biological control of the coral. The model is a radical departure from previous thought, but is consistent with existing observations and warrants testing in future studies.

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