

Contribution to Special Issue: 'Towards a Broader Perspective on Ocean Acidification Research' Food for Thought

The Omega myth: what really drives lower calcification rates in an acidifying ocean

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The literature on ocean acidification (OA) contains a prevalent misconception that reduced organismal calcification rates in an acidifying ocean are driven by a reduction in carbonate ion (CO_3^{2-}) substrate availability (e.g. Omega or Ω). However, recent research in diverse organisms suggests that a reduction in seawater pH (i.e. increasing proton concentrations, $[\text{H}^+]$) is the most likely driver of reduced calcification rates in these organisms. OA leads to higher $[\text{H}^+]$ in seawater which alters the proton gradient between internal cellular reservoirs and external bulk seawater, making it difficult for organisms to maintain pH homeostasis. Biologically mediated calcification is a complex process, so it is unlikely that simple CO_3^{2-} substrate limitation is responsible for the observed decreases in calcification rates under OA conditions. Despite these inherent complexities, current predictions concerning the fate of calcifying organisms in an acidifying ocean have relied on the relationship between calcification rates and Ω . To more accurately predict how OA will affect the calcification of marine organisms, and consequently the global carbon cycle, we need to further elucidate the mechanisms driving observed decreases in calcification under acidified conditions.

Keywords: calcification, coral reef, ocean acidification, Omega, phytoplankton, saturation state.

Ocean acidification (OA) refers to the unprecedented reduction in seawater pH caused by anthropogenic CO_2 inputs (Hönisch *et al.*, 2012). OA is expected to reduce the ability of marine organisms such as corals, coccolithophores, foraminifera, and molluscs to secrete calcium carbonate (CaCO_3) skeletons, a process known as calcification (see Table 1) (Chan and Connolly, 2012; Kroeker *et al.*, 2013). As CO_2 dissolves into seawater, it lowers the pH and shifts the carbonate equilibria, decreasing the carbonate ion concentration ($[\text{CO}_3^{2-}]$). This lowers a chemical property of seawater known as the calcium carbonate saturation state or Ω . Seawater Ω is a function of CO_3^{2-} and calcium ion concentrations ($[\text{Ca}^{2+}]$) as follows

$$\Omega = \frac{[\text{Ca}^{2+}][\text{CO}_3^{2-}]}{K_{\text{sp}}}, \quad (1)$$

where K_{sp} is the solubility product of a specific CaCO_3 mineral phase (e.g. aragonite or calcite) at a specified temperature, salinity, and

pressure (Zeebe and Wolf-Gladrow, 2001). Therefore, the thermodynamics of inorganic CaCO_3 precipitation and dissolution can largely be described by seawater Ω , with precipitation occurring at $\Omega > 1$ and dissolution at $\Omega < 1$ (Morse and Arvidson, 2002).



From an OA perspective, Ω is mainly controlled by changing $[\text{CO}_3^{2-}]$, which is lowered as anthropogenic CO_2 dissolves into seawater, while $[\text{Ca}^{2+}]$ remains unaffected.

Having the thermodynamic principles of inorganic CaCO_3 precipitation in mind, early OA work investigated the calcification rates of organisms such as corals in seawater of varying Ω by manipulating bulk seawater $[\text{CO}_3^{2-}]$ and $[\text{Ca}^{2+}]$ (e.g. Gattuso *et al.*, 1998; Langdon *et al.*, 2000). Experimental evidence showed a positive correlation between calcification and Ω , which led to the idea that seawater $[\text{CO}_3^{2-}]$ could drive calcification rates. However, to determine

Table 1. Definition of terms referring to calcification and inorganic CaCO₃ precipitation/dissolution in the OA literature.

Term	Definition
Calcification or gross calcification	Refers to the biologically controlled process of CaCO ₃ production, often occurs isolated from bulk seawater in media called the calcifying fluid
CaCO ₃ precipitation	Refers to the inorganic formation of CaCO ₃ minerals from a super saturated solution
CaCO ₃ dissolution	Refers to the inorganic dissolution of CaCO ₃ minerals in an under saturated solution, sometimes decalcification is used synonymously
Net calcification	The net effect of gross calcification and dissolution, usually refers to individual organisms
Net community calcification or net ecosystem calcification	The net effect of gross calcification and dissolution in an entire ecosystem

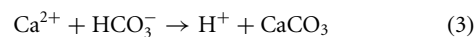
how OA affects calcification it becomes critical to differentiate between the inorganic precipitation and dissolution of CaCO₃ (which is thermodynamically constrained by seawater Ω) and biologically mediated calcification (see Table 1). In fact, it has been suggested that inorganic CaCO₃ dissolution may be more of a threat to marine calcifying organisms and calcareous ecosystems than decreasing calcification rates under OA (Andersson *et al.*, 2009; Roleda *et al.*, 2012; Eyre *et al.*, 2014). However, many OA studies have not been able to isolate the effects of seawater Ω on organismal calcification rates in the absence of dissolution (i.e. gross calcification) because CaCO₃ is exposed to bulk seawater as either sediment or skeletal material. Therefore, any dissolution of exposed CaCO₃ could produce or enhance the observed relationships between Ω and net calcification. On the other hand, gross calcification is under biological control and mediated by organic tissue that separates the calcifying surface from overlying seawater. Therefore calcification occurs in a media (i.e. the calcifying fluid) that has significantly different [CO₃²⁻] than the bulk seawater. However, despite the complexities inherent to biological mediated calcification, much of the current OA literature presents the problem of reduced calcification under OA scenarios as an issue of simple CO₃²⁻ substrate availability [e.g. Equation (2)] (Hendriks *et al.*, 2015).

Recent work has demonstrated that corals can actively control carbonate chemistry at the site of calcification (Venn *et al.*, 2011; McCulloch *et al.*, 2012), which brings into question the mechanistic understanding of how external seawater Ω could influence organismal calcification rates. Currently, lines of research in different organisms and ecosystems are beginning to reach the same conclusion, that external seawater Ω (i.e. [CO₃²⁻]) is not what drives changes in calcification rates. Here we show that these insights have been developed in two vastly different systems; scleractinian corals and open ocean phytoplankton known as coccolithophores.

The ability to modify carbonate chemistry at the site of calcification can produce internal conditions that are more thermodynamically favourable for inorganic CaCO₃ precipitation than in the surrounding seawater (i.e. a greater Ω). It has been shown that corals transport seawater to the site of calcification, however, once it is part of the calcifying fluid the chemistry of that seawater is actively modified (Gagnon *et al.*, 2012). Coccolithophores passively regulate cytosolic pH through voltage-gated H⁺ channels; however, they actively regulate carbonate chemistry in their calcifying vesicles (Mackinder *et al.*, 2010). Marine organisms have also been shown to modulate calcification through the use of organic molecules that can both stimulate or inhibit specific crystal lattice structures (Marsh, 1994). Therefore, in order for the external seawater chemistry to affect calcification rates, it must somehow affect that organism's ability to modulate its internal carbonate chemistry or produce organic compounds that mediate precipitation. Two lines of

physiological evidence suggest that bulk seawater Ω does not control the Ω in the calcifying fluid, and thus, is not the major factor controlling organismal calcification rates.

1. While CO₃²⁻ is the substrate for inorganic CaCO₃ precipitation, no CO₃²⁻ transporter has been described in corals (Goiran *et al.*, 1996) or coccolithophores (Mackinder *et al.*, 2010). However, there is ample evidence that bicarbonate (HCO₃⁻) is actively transported into the calcifying fluid of corals (Goiran *et al.*, 1996; Moya *et al.*, 2008; Jury *et al.*, 2010) and coccolithophores (Berry *et al.*, 2002; Herfort *et al.*, 2002, Rost *et al.* 2003). Therefore, once HCO₃⁻ is transported into the calcifying fluid and CO₃²⁻ is combined with Ca²⁺ during calcification, H⁺ ions build-up (Figure 1);



Since there is ~9 times the [HCO₃⁻] as [CO₃²⁻] in seawater, and OA increases [HCO₃⁻], it is unlikely that HCO₃⁻ substrate limitation under decreasing seawater pH is a problem for calcification. However, increasing seawater [H⁺] could be quite problematic.

2. As H⁺ ions build-up in the calcifying fluid the pH is lowered and the carbonate system shifts away from CO₃²⁻, thus lowering Ω . Therefore, to maintain conditions favourable to inorganic CaCO₃ precipitation within the calcifying fluid, the organism must passively or actively remove H⁺ ions through membrane channels/transporters (Allemand *et al.*, 2011; Taylor *et al.*, 2012). As the oceans absorb CO₂ and seawater H⁺ concentrations increase, the electrochemical gradient between coral tissue and cytosolic fluids will decrease, making it more difficult to maintain high Ω in the calcifying fluid. This is the underlying concept behind the proton flux limitation model of calcification.

The conceptual framework of the proton flux limitation model has been elucidated in both corals (Jokiel, 2011a, b) and coccolithophores (Suffrian *et al.*, 2011; Bach *et al.*, 2013) (Figure 1). Once protons are transported into the tissue or cytosolic fluid from the calcifying fluid they must be removed to the water column to maintain intracellular pH, which becomes more energetically demanding as the proton concentration of seawater increases. If proton flux limitation at the boundary layer between the organism and seawater is the limiting factor for calcification, then increased energy production should lead to higher calcification rates by allowing more H⁺ ions to be actively pumped out of the calcifying fluid. This has been demonstrated in two distinct pathways in corals: (i) more light (and increases in photosynthesis) results in increased calcification rates (Marubini *et al.*, 2001; Al-Horani *et al.*, 2003) and (ii) feeding the coral with

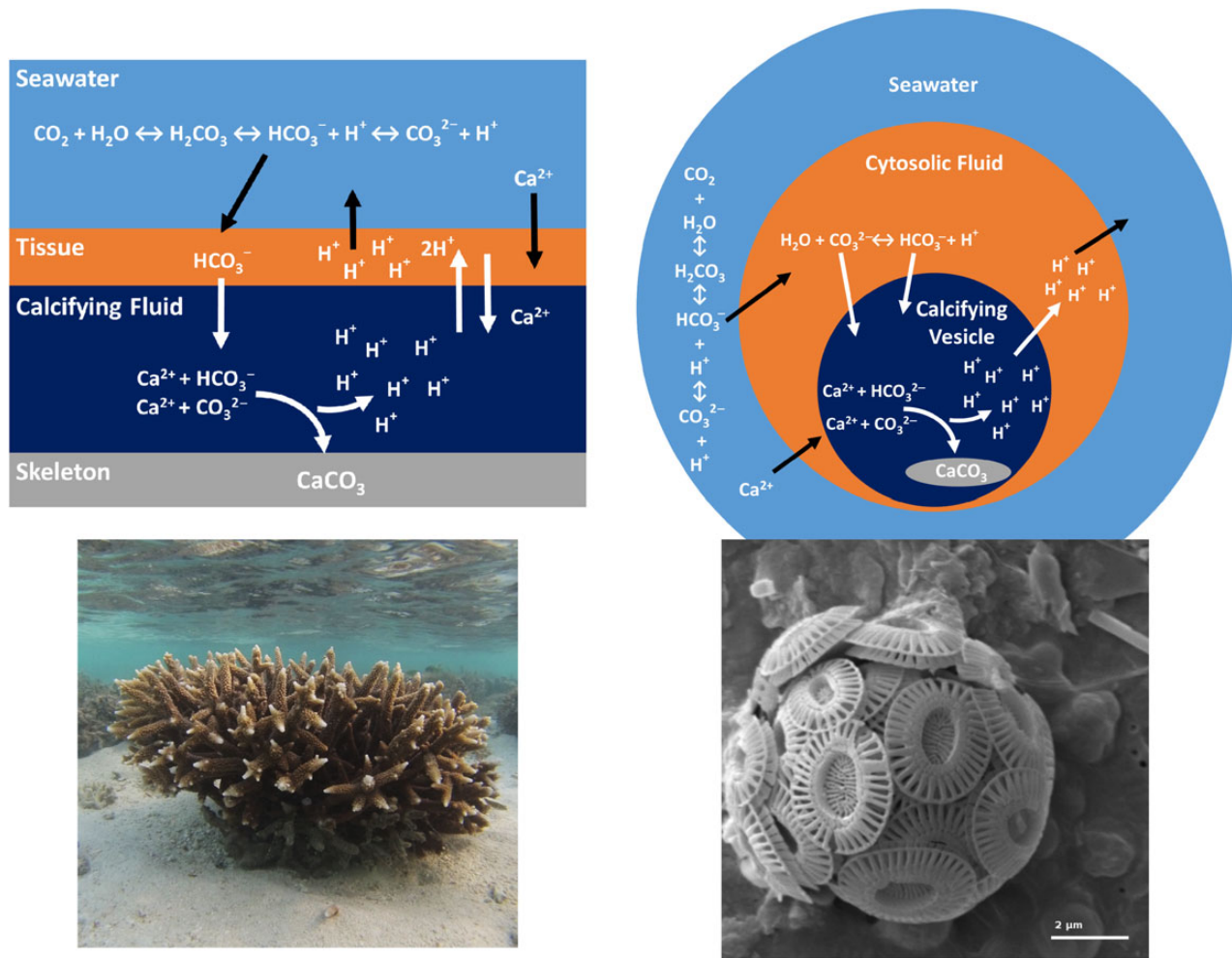


Figure 1. A simplified schematic demonstrating the internal build-up of protons during the calcification process in corals and coccolithophores. Corals (left panel) must dissipate excess protons produced by calcification through a boundary layer and into the water column as proposed by Jokiel (2011b). Internally, corals most likely actively pump HCO_3^- ions into the calcifying fluid where protons build-up as CaCO_3 is precipitated. To maintain favourable conditions for precipitation in the calcifying fluid, corals likely actively pump 2H^+ out and Ca^{2+} in using a Ca^{2+} -ATPase (Allemand et al., 2011). To maintain the pH inside their tissue corals must remove protons, which becomes more energetically demanding when the gradient between the tissue and seawater $[\text{H}^+]$ is less pronounced due to ocean acidification. Suffrian et al. (2011) demonstrated that internal cellular pH (pH_i) in coccolithophores like *Emiliania huxleyi* (right panel) is directly affected by the surrounding seawater pH. This is most likely because *E. huxleyi* uses passive gated H^+ channels to control cytosolic pH, which are forced to work against a less pronounced H^+ gradient in an acidifying ocean. Black arrows represent fluxes between the organism and external seawater while white arrows represent fluxes occurring within the organism.

plankton enhances calcification rates (Ferrier-Pages et al., 2003; Houlbrèque et al., 2004; Towle et al., 2015). Another hypothesis explaining light enhanced coral calcification is in direct agreement with the proton flux limitation hypothesis. Moya et al. (2006) suggested that increased pH in the gut of coral polyps during the day (Furla et al., 2000; Al-Horani et al., 2003) decreases the gradient against which H^+ ions are pumped out of the calcifying fluid. It has also been suggested that the carbon concentrating mechanism (CCM) used by coral endosymbionts during daytime photosynthesis, which produces OH^- ions, can absorb excess H^+ (Furla et al., 2000). In coccolithophores, the cytosolic pH is regulated by H^+ efflux via passive voltage-gated H^+ channels (Suffrian et al. 2011). However, under ocean acidification scenarios, the electrochemical gradient becomes less and less favourable, most likely affecting pH and Omega inside the calcifying vesicle.

Results from field and mesocosm studies of coral reef ecosystems are also consistent with the proton flux limitation model. Venti et al. (2014) recently demonstrated that light and temperature had much greater control than seawater Ω on changes in seasonal calcification rates of corals in the field. Also, a diel hysteretic pattern between external seawater Ω and calcification rates, with the highest rates of calcification occurring before the daily peak in bulk seawater Ω , has been observed in all levels of coral ecosystems from carbonate sediments (Cyronak et al., 2013b), to corals and macroalgae (Jokiel et al., 2014), and entire ecosystems (McMahon et al., 2013; Shaw et al., 2015). The hysteretic pattern observed in these studies is most likely due to the influence that benthic organisms have on seawater carbonate chemistry in coral reef ecosystems, with benthic production and calcification driving diel changes in seawater Ω , not the other way around. Importantly, these studies demonstrate the

complexity in extrapolating the effects of OA based on short term natural changes in Ω . There is also a strong correlation between primary production and calcification in a range of studies (e.g. Gattuso *et al.*, 1999), indicating that photosynthesis is a dominant control on coral calcification.

Field and laboratory evidence across organisms and ecosystems indicates that calcification in an acidifying ocean is not controlled by bulk seawater Ω , but most likely inhibited by an increase in the gradient of protons between the calcifying fluid and external seawater. Therefore, a much better indicator of the influence of seawater chemistry on calcification may be the ratio of the substrate (dissolved inorganic carbon; DIC or $[\text{HCO}_3^-]$) to the waste product ($[\text{H}^+]$) (Jokiel, 2011a, 2013). In fact, recent experimental evidence in mussels demonstrated that the seawater $[\text{HCO}_3^-]$ to $[\text{H}^+]$ ratio is important in controlling calcification rates (Thomsen *et al.*, 2015), indicating that pH homeostasis may be important in controlling biologically mediated calcification in a diverse range of organisms. In contrast, a recent study suggested that bivalve larvae were particularly sensitive to Ω (Waldbusser *et al.*, 2015). However, Waldbusser *et al.* (2015) did not distinguish between the effects of Ω on gross calcification and dissolution, and their results may be due to the dissolution of CaCO_3 shells exposed to under saturated seawater. Furthermore, an additional explanation for the lack of correlative power between changes in calcification rates and pH (instead of Ω) in some studies with un-coupled carbonate chemistry manipulations, such as in Langdon *et al.* (2000) or Waldbusser *et al.* (2015), is that increasing $[\text{HCO}_3^-]$ could partially compensate for unfavourable pH levels.

It is also important to remember that calcification is influenced by many other factors such as light availability and temperature, which may be more important in driving future changes in calcification rates (McNeil *et al.*, 2004). The coupling of seawater pH and Ω under ocean acidification scenarios (i.e. inputs of CO_2) can lead to positive correlations between calcification and both pH and Ω . However, pH and Ω can become decoupled or negatively correlated on geological time scales due to land-based weathering processes (Hönisch *et al.*, 2012), and on more modern timescales due to opposite effects of temperature on pH and Ω (Figure 2) (Gattuso *et al.*, 1999). While this decoupling is not likely on the timescale of modern OA in the open ocean, it could occur in some coastal areas with pronounced diel temperature variability.

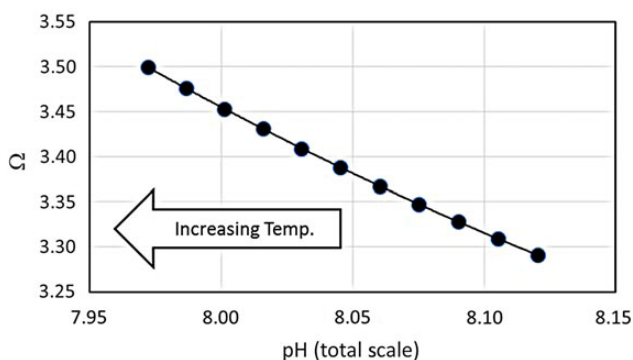


Figure 2. Correlation of pH and aragonite saturation state (Ω) when temperature is increased from 20 to 30°C. pH and Ω were calculated at constant TA and DIC concentrations of 2300 and 2000 $\mu\text{mol kg}^{-1}$, respectively, while temperature was varied by 1°C from 20 to 30°C. It is important to note that global warming will not drive the uncoupling of pH and Ω due to ongoing CO_2 equilibration between the atmosphere and ocean.

Current predictions of the fate of coral reefs in an acidifying ocean which are reliant on the relationship between net ecosystem calcification and Ω (e.g. Figure 6 in Shamberger *et al.*, 2011) could be based on a basic misconception about the factors driving changes in coral physiology. With this in mind, it becomes vitally important to grasp the correct mechanistic understanding of how increasing CO_2 inhibits the calcification of marine organisms. We are not the first to stress this point. A recent perspective article noted a disconnect between the more recent OA literature and older studies on calcification physiology (Roleda *et al.*, 2012). Other recent work is beginning to highlight the sensitivity of CaCO_3 dissolution to OA, which may pose a more serious threat to coral reef ecosystems than changes in calcification (Andersson *et al.*, 2009; Cyronak *et al.*, 2013a; Eyre *et al.*, 2014). Multiple theories exist for the influence of OA on marine calcifiers (Allemand *et al.*, 2011). However, the prevailing notion in the OA literature that calcification is inhibited through a reduction in seawater $[\text{CO}_3^{2-}]$, and thus Ω , is most likely incorrect. Rather, as outlined above, it is most likely the decrease in seawater pH and associated problems of pH homeostasis within organisms that governs changes in calcification rates under OA conditions.

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