# Biological mechanisms supporting adaptation to ocean acidification in coastal ecosystems

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## Abstract

The direct influence of anthropogenic $CO_2$ might play a limited role in pH regulation in coastal ecosystems as pH regulation in these areas can be complex. They experience large variability across a broad range of spatial and temporal scales, with complex external and internal drivers. Organisms influence pH at a patch scale, where community metabolic effects and hydrodynamic processes interact to produce broad ranges in pH, (~ 0.3 to 0.5 pH units) over daily cycles and spatial scales (mm to m) particularly in shallow vegetated habitats and coral reefs where both respiration and photosynthetic activity are intense. Biological interactions at the ecosystem scale, linked to patchiness in habitat landscapes and seasonal changes in metabolic processes and temperature lead to changes of about 0.3 to 0.5 pH units throughout a year. Furthermore, on the scale of individual organisms, small-scale processes including changes at the Diffusive Boundary Layer (DBL), interactions with symbionts, and changes to the specific calcification environment, induce additional changes in excess of 0.5 pH units.

In these highly variable pH environments calcifying organisms have developed the capacity to alter the pH of their calcifying environment, or specifically within critical tissues where calcification occurs, thus achieving a homeostasis. This capacity to control the conditions for calcification at the organism scale may therefore buffer the full impacts of ocean acidification on an organism scale, although this might be at a cost to the individual. Furthermore, in some areas, calcifiers may potentially benefit from changes to ambient seawater pH, where photosynthetic organisms drawdown $CO_2$.

**Keywords**: Ocean acidification, biological interactions, calcification, homeostasis, pH variability

## 1. Introduction

Many marine organisms secrete calcified shells, tests, and external/internal skeletons as a supporting frame for their bodies and for protection against predators. Calcium carbonate ($CaCO_3$) skeletons are composed of either or a combination of calcite, aragonite, or high Mg-calcite. These compounds differ in their solubility in seawater with calcite being less soluble than aragonite and Mg-calcite being most soluble.

In seawater, $CO_3^{2-}$, not $Ca^{2+}$, is the limiting ion for calcification (Kleypas & Langdon 2000) with $CO_3^{2-}$ concentrations of about 240 µmol $kg^{-1}$ compared to about 10,000 µmol $kg^{-1}$ for $Ca^{2+}$ (e.g. Millero et al., 2008). The main effect of ocean acidification (OA), from increasing anthropogenic $CO_2$ emissions (Feely et al., 2004; Sabine et al., 2004; Orr et al., 2005), is the decrease in seawater pH and the accompanying reduction of the carbonate ion ($CO_3^{2-}$) concentration in seawater with decreasing pH. It is therefore the equilibrium between $CO_3^{2-}$ and $HCO_3^-$ and the effect of $CO_2$ on this balance that is of critical importance for calcifying organisms. Increasing OA thus leads to reductions in the saturation state ($\Omega$) of $CaCO_3$ minerals in seawater:

$$\Omega_{sw} = [Ca^{2+}]\,[CO_3^{2-}]/K_{spx} \qquad \text{Eq. 1}$$

where $[Ca^{2+}]$ and $[CO_3^{2-}]$ are the concentrations of dissolved calcium and carbonate ion in seawater, and $K_{spx}$ is the solubility constant for either high Mg-calcite, aragonite or calcite. From these equilibrium reactions, the generalised reaction for calcification based on the more abundant $HCO_3^-$ (1800 to 2000 µmol $kg^{-1}$) is commonly given as:

$$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + H_2O + CO_2 \qquad \text{Eq. 2}$$

indicating that calcification actually results in the production of $CO_2$ and hence also acts to decrease the pH of seawater.

Ocean acidification scenarios, derived from coupled biogeochemical and general circulation models (GCM), predict a decline in pH of ~ 0.3 units by the end of this century in the well mixed surface layer and a shoaling of the dissolution horizon for carbonate minerals, particularly aragonite, by the end of the century (Caldeira and Wickett 2005; Orr et al., 2005). Taxa likely to be most vulnerable to OA include open-ocean species, such as coccolithophores, pelagic mollusks, and foraminifera (among others), but there is also great concern for coastal calcifying organisms, including bivalves, calcifying algae, and corals (Gattuso et al., 1998; Riebesell et al., 2000; Zondervan et al., 2001; Hoegh-Guldberg et al., 2007; Doney et al., 2009).

Although there is agreement about the effect of increased anthropogenic $pCO_2$ on ocean chemistry, there is much less agreement on the extent and severity of the potential impacts of OA on coastal organisms (Gattuso et al., 2013). Recent meta-analyses of experimental results show a large variability in responses, particularly when considering other existing stresses like temperature (Hendriks et al., 2010; Hendriks and Duarte 2010; Kroeker et al., 2013). The experimental evidence, upon which these meta-analyses are based, consists of simple experiments where organisms are isolated from the ecosystem to test their performance under the conditions GCM's predict for the open ocean in year 2100 (Hendriks et al., 2010), with no consideration of the variability in pH in their natural habitats, or other factors possibly affecting their response (Hendriks et al., 2010; Hendriks and Duarte 2010). However, anthropogenic $CO_2$ plays a smaller role relative to other sources of variability, such as watershed and metabolic effects (Duarte et al., 2013), in coastal systems as compared to the open ocean (e.g. Salisbury et al., 2008; Hoffman et al., 2011; Aguilera et al., 2013; Duarte et al., 2013).

Most importantly, even more so than pelagic ecosystems, coastal ecosystems are biogeochemically and structurally complex. They include benthic engineering species (e.g. corals, seagrass, macroalgae, salt marshes, mangroves, sponges, oyster reefs) with the capacity to affect the chemical and physical conditions of the ecosystem (Gutierrez et al., 2011), and exert metabolic control on coastal seawater pH values and variability (Duarte et al., 2013). Depending on the region different drivers can be of importance, apart from vegetation i.e. freshwater run-off and organic matter input from rivers impacting pH regimes (Duarte et al., 2013). Coastal organisms evolved in this highly dynamic landscape, which is characterized by large spatial and temporal variability in pH and calcifying organisms living in coastal zones have evolved a wide range of mechanisms to cope with these variable pH conditions; these include pH up-regulation in extracellular fluids (McCulloch et al., 2012a) as well as potentially timing calcification events to coincide with favorable environmental conditions. For example, *Pinna nobilis*, the bivalve with the fastest know shell growth, inhabits Mediterranean seagrass meadows of *Posidonia oceanica* (Fig. 1a), an environment that is capable of raising pH by 0.3 to 0.5 units during the daytime (Invers et al., 1997; Hendriks et al., 2014). Blue mussels in Greenland often recruit and grow in dense intertidal *Fucus* and *Ascophyllum* beds (Fig. 1b). Hence, assessing the vulnerability of coastal calcifiers to OA, a slow, gradual change in ambient conditions, must consider the natural variability of pH within their habitats as well as the biological processes, ranging from community and species interactions to organismal adaptations, that together act to build some level of homeostasis for calcification.

Here, we examine the mechanisms for biological control of calcification in marine

organisms and the associated costs, and discuss if the observed variability of organism's responses to OA can be explained by these principles. To do so, we examine the impact of biological processes on pH variability in coastal ecosystems at different scales, from habitat to organismal scales, and the mechanisms that organisms have evolved to regulate calcification rates in such dynamic environments.

## 2. Biological-driven pH variability in coastal habitats

### 2.1 Metabolic effects on coastal seawater pH

Coastal oceans are characterized by large variability in carbonate chemistry (Aufdenkampe et al., 2011; Hofmann et al., 2011; Mercado and Gordillo 2011; Duarte et al., 2013), which may span a range of pH (approx. 0.3-0.5 units) that is equal to or even greater than the predicted decrease in global ocean pH of ~ 0.3 units by the end of the 21$^{st}$ century (Caldeira and Wickett 2005).

Ecosystem metabolism affects the pH of coastal waters through the removal of $CO_2$ by primary production during the day in net autotrophic ecosystems, and at night by $CO_2$ released from respiration (termed metabolic signal; Duarte et al., 2013). The amplitude of this effect is not only dependent on the productivity of autotrophs, but also the residence time of the affected water body in the vegetated area (Hendriks et al., 2014). Habitats like seagrass meadows and kelp forests alter water flow and attenuate waves and turbulence (Granata et al., 2001; Koch et al., 2006; Hendriks et al., 2007). This increases the residence time of water in these highly productive ecosystems, thereby amplifying their metabolic signal and raising the question of whether autotrophic communities, which exert a net drawdown of $CO_2$, are potential refugia for calcifiers under increasingly "acidified" conditions. For instance, high pH values caused by

seagrass photosynthesis enhanced calcification rates of calcareous red macroalgae (*Hydrolithon* sp. and *Mesophyllum* sp.) and the green macroalga *Halimeda renschii* growing within a tropical seagrass bed (Semesi et al., 2009). Additionally, seagrass productivity is expected to increase with future OA (Beer and Koch 1996; Hendriks et al., 2010; Jiang et al., 2010), suggesting a potential positive feedback to their ability to serve as refugia from OA (Palacios and Zimmerman 2007). A particular situation would be that of macrophytes in the high Arctic, where a high summer pH could be maintained theoretically across 24 hours during summer, due to the lack of a night and day phase. The opposite argument (24h-dark) could be used for a low winter pH, although lower respiration rates due to low temperatures could dampen this effect.

The drawdown of DIC due to photosynthesis of macrophytes can result in a local net increase of $\Omega_{Ar}$, while if the spatial patterns of the benthic community structure (i.e. percent cover of algal and coral communities) is appropriate, and the vegetation is located upstream from a calcifying reef, this change in carbonate chemistry can impact overall calcification rates on reef flats (Kleypas et al., 2011). Likewise in patchy landscape configurations, bivalves and some coral species may benefit from the increase in pH by the metabolism of the adjacent seagrass meadow (e.g. Kleypas et al., 2011; Unsworth et al., 2012).

### 2.2 pH variability at the µm scale

The no-slip condition of water velocity dictates that no water movement occurs at the organism´s surface, thus a velocity gradient develops across a DBL (Diffusive Boundary Layer). Water residence times are higher closer to the organism, thus allowing for an increased capacity to affect the pH within the DBL through metabolic

activity. The thickness of the DBL is directly influenced by surface roughness and morphology and by water speed and turbulence (Hansen et al., 2011, Hurd and Pilditch 2011), with thicker DBLs found on rougher surfaces with low water speeds. Within the DBL, transport of ions and gases to and from an organism's surface is limited by diffusion, which is, in turn, dependent on the concentration gradient across the DBL. The DBL thickness is important for highly productive organisms, as slow diffusion rates across the DBL relative to uptake rates can result in limitation of nutrients, $CO_2$, and $O_2$.

The limiting effect of the rate of mass transport through the DBL has been directly observed. For example, primary productivity of an epilithic alga on a dead coral became mass-transport limited with respect to $CO_2$ as a pH gradient developed at light irradiances over 500 µmol photons $m^{-2}$ $s^{-1}$ (maximum pH of 8.7-8.9; Larkum et al., 2003). During light-dark cycles the pH in the DBL of calcifying foraminifera and diatoms ranges from 8.0 to 9.1 (Köhler-Rink and Kühl 2005; Kühn and Raven 2008). This DBL surrounding the foraminiferal test allows the organism to create a microenvironment, greatly increasing pH (by 0.5 units) above ambient seawater. Gradients in the DBL also occur in organisms larger than foraminifera, although modification of pH at the organism's surface varies between taxa. For example, abalone did not affect the pH of the proximate water, whereas sea urchins reduced pH by about 0.35 units, and coralline algae increased pH in the adjacent water by 0.5 units in the light but decreased pH by 0.35 units in the dark (Hurd et al., 2011).

### 2.3 pH regulation in host-symbiont systems

The regulation of pH through interactions between calcifying organisms and photosynthetic organisms can occur at very small scales, as in the case of calcifying

hosts and their photosynthetic symbionts. Symbiotic relationships between calcifiers and photosymbionts are widespread, including scleractinian corals, forams, and bivalves, such as the giant clam, *Tridacna gigas*.

Most shallow water scleractinian corals that dominate tropical reef systems have developed an obligate relationship with photosymbionts (dinoflagellate genus *Symbiodinium*). Symbiont photosynthesis provides an important source of energy to the coral host, facilitating the high rates of calcification that enables coral reef formation in the oligotrophic waters characteristic of tropical shallow-water coastal environments of coral reef ecosystems. As photosynthesis by symbionts removes $CO_2$ and increases pH, it affects the conditions for calcification. Since photosynthetic rates of the symbionts like *Symbiodinium* are stimulated by light, this is an important factor for the supply of organic compounds necessary for growth (Muscatine 1990) and manipulation of the immediate calcifying environment. As a result, an increase in light up to threshold levels typically enhances coral calcification relative to dark calcification (Gattuso et al., 1990). Loss of symbionts or, coral bleaching, an event that might occur more frequently with increasing anthropogenic stressors and specifically global warming (e.g. Hoegh-Guldberg et al., 2007) therefore would affect the energy budget of corals (e.g. D'Olivo et al., 2013).

Like corals, some species of foraminifers host symbionts, which play important roles in the supply of energy from photosynthesis, enhancement of calcification, and physiological health (Lee and Zucker 1969; Erez 1983; Hallock, 2000). Bé et al., (1982) determined that symbiont–bearing *Globigerinoides sacculifer* produced 1.5 to 2.5 times more chambers, grew 1.5 to 2 times larger in size, and survived up to 4 times longer than their aposymbiotic counterparts. This is consistent with the observation that

photosynthesis and calcification are proportional to light intensity, and that calcification rate during light conditions can be 2-3 times faster than the dark phase (Duguay and Taylor 1978).

The third type of algae-invertebrate symbiosis occurs in bivalves and appears to be less efficient, as calcification/photosynthesis (C/P) ratios are between 0.2-0.9 whereas foraminifera and corals often have C/P ratios close to 1 (McConnaughey and Whelan 1997).

Therefore, to understand the response of corals and other symbiont-bearing invertebrates to OA, it is necessary to not only consider the direct effects of OA on the process of calcium carbonate calcification, but also the interactive effects of environmental change on the host photosymbiont, and the holobiont entity in general. This is true for organisms in pelagic as well as coastal benthic environments, however as the latter can be a highly fluctuating environment with large pH ranges and associated low values this interaction is of relevance as it might ameliorate the negative effects of low pH on an organismal level.

### 2.4. pH regulation at the organismal scale

The OA literature often presents biological calcification merely as a chemical reaction dependent on the concentration of carbonate ions in ambient seawater, which as discussed previously is generally thought to be the limiting constituent rather than $Ca^{2+}$. However, calcifying organisms have evolved a number of adaptations to regulate their calcifying environment both at the extracellular and intracellular levels, thereby reducing their dependence on the chemical conditions of ambient waters.

### 2.4.1. Extrapallial fluid pH regulation in bivalves

The deposition of calcium carbonate in bivalve shells is a complex and biologically controlled process (Waldbusser et al., 2011). Shell growth initiates with the formation of a periostracum (outer organic coating, Kennedy et al., 1969) in the mantle folds, followed by the deposition of an organic matrix, and then calcium carbonate (Wilbur and Saleuddin 1983; Addidi et al., 2006). Biological control of bivalve shell mineralogy is most probably achieved via: (1) the nature of the extrapallial fluid (Wilbur and Bernhardt 1984), (2) the periostracum, which is a thin, organic layer of quinone-tanned protein (Degens et al., 1967) inhibiting shell corrosion from low-pH waters, and/or (3) the organic matrix when initiating calcification (Kennedy et al., 1969; Wilbur and Bernhardt 1984).

The acid-base conditions in body fluids, like the extrapallial fluid (EPF), affect calcification and existing shell structures. Buffering hemolymp (HL) or EPF to avoid decreasing pH at calcification sites is an efficient mechanism used, for example, by teleost fish, decapod crustaceans, and cephalopod molluscs (Larsen et al., 1997; Pane and Barry 2007; Gutowska et al., 2010). However, *Mytilus edulis* does not actively buffer HL or EPF by $HCO_3^-$ accumulation to counter the effects of decreasing seawater pH (Heinemann et al., 2012). A decrease in pH of the internal fluids causes a shift of mussel metabolism to partial anaerobiosis, with a consequent degradation of proteins (De Zwaan et al., 1976) as demonstrated by increased excretion of ammonia (Pörtner et al., 2004, Michaelidis et al., 2005), which can be interpreted as an intracellular pH regulatory mechanism (Fernández-Reiriz, et al., 2012).

The effects that OA may have on shell formation are modulated by a combination

of dissolution of the external shell surface and physiological changes to the internal acid-base balance, affecting the rate of new shell deposition (Waldbusser et al., 2011). In decreasing pH conditions, both reduced shell growth (Thomsen and Melzner 2010) as well as increases in shell mass (Heinemann et al., 2012) have been reported, indicating considerable plasticity and biological control of the ability to precipitate a shell from fluids that are highly undersaturated with calcium carbonate.

### 2.4.2 Extracellular regulation of pH in scleractinian corals

Scleractinian corals do not precipitate their aragonite calcium carbonate skeleton directly from seawater, but from an extracellular calcifying medium, located at the interface between the coral polyp's basal cell layer and the underlying skeleton (e.g. Allemand et al., 2004). Corals actively manipulate the pH of the calcifying fluid by $Ca^{2+}$-ATPase pumping of Ca ions in exchange for protons (Cohen and McConnaughey 2003; Allemand et al., 2004). This process shifts the equilibrium composition of DIC in the calcifying fluid in the direction of $CO_3^{2-}$, thereby increasing the saturation state ($\Omega$) of the calcifying fluid (Cohen et al., 2009; Holcomb et al., 2009; McCulloch et al., 2012b) and thus has the potential to counter the effects of reduced carbonate saturation in seawater.

Physiological evidence (Al-Horani et al., 2003 and Venn et al., 2011) and boron isotope compositions of coral skeletons (Trotter et al., 2011, Holcomb et al., 2014) have confirmed that there is an absolute increase of pH relative to seawater during active calcification. This relationship between the pH of seawater ($pH_{sw}$) and the calcifying fluid ($pH_{cf}$) is highly systematic and species-dependent (Trotter et al., 2011; Fig. 2):

$$pH_{cf} = m\, pH_{sw} + C_{sp} \qquad \text{Eq. 3}$$

where $C_{sp}$ is the species dependent intercept and $m$ is the gradient.

From variations in the boron isotopic composition of scleractinian corals ($\delta^{11}B_{carb}$), the calculated $pH_{cf}$ relative to ambient seawater $pH_{sw}$ show exceptionally well-defined species-specific linear arrays (Trotter et al., 2011, Holcomb et al., 2014; Fig. 2). The gradients (m) of the linear arrays range from 0.24 to 0.49 and indicate that a maximum of only about 50% of the change in seawater pH is transmitted to the site of calcification. These findings have been confirmed by confocal microscopy pH sensitive dye experiments (Venn et al., 2013) and direct comparisons of the precipitated skeleton using boron isotopes (Holcomb et al., 2014). The intercept values ($C_{sp}$) are strongly species-dependent, with the temperate-water coral endemic to the Mediterranean (*C. caespitosa*) exhibiting higher up-regulation of $pH_{cf}$ (~0.6), than, for example, the tropical species (~0.4). This differential in scleractinian corals is a likely response to the different temperature regimes of these environments, as also suggested by even higher $pH_{cf}$ (~0.7 to ~1.0) in aragonitic cold water corals (McCulloch et al., 2012b; Fig. 2).

The empirical exponential rate dependence law derived for abiotic carbonate precipitation (Burton and Walter, 1987) can be used to quantify how calcification rates are affected by biological pH up-regulation when it is applied to the biologically mediated internal saturation state ($\Omega_{cf}$; McCulloch et al., 2012a). The rate of calcification ($R_{calcif}$) can then be described as:

$$R_{calcif} = k(\Omega_{cf} - 1)^n \qquad \text{Eq. 4}$$

where *k* is the rate law constant, and *n* is the order of the reaction – both constants being temperature dependent (Burton and Walter 1987).

This approach, based on the well-known concept of '*biologically induced*' calcification (Lowenstam and Weiner 1989), has been termed IpHRAC (McCulloch et al., 2012a) since it combines Internal pH Regulation with Abiotic Calcification, and provides a quantitative means to determine changes in calcification rates as a function of both ambient seawater pH and temperature.

The key reason for the lower sensitivity response of coral calcification to declining seawater pH is that pH up-regulation of the calcifying fluid results in significantly elevated internal saturation states, with $\Omega_{cf}$ of about 15 to 25, despite seawater pH decreasing from 8.1 to 7.6 (McCulloch et al., 2012a). This is consistent with studies that indicate a much lower pH threshold at which corals can continue to maintain calcification (Fine and Tchernov 2007). In fact, the IpHRAC model explains the apparently paradoxical ability of corals to calcify at a seawater saturation state of $\Omega_{sw} < 1$. This behaviour is simply attributable to $\Omega_{cf} >> \Omega_{sw}$ with the extent of enrichment being attributable to mainly increased $pH_{cf}$ through up-regulation, and for zooxanthellate corals, combined with an approximately twofold enrichment in *DIC* (McCulloch et al., 2012a). The even greater capacity of azooxanthellate aragonitic cold water corals to up-regulate pH may represent an adaptive mechanism enabling these deep-sea calcifiers (e.g. *Desmophyllum dianthus and Lophelia. pertusa*) to occupy a unique niche where the seawater saturation state is close to undersaturation (i.e. $\Omega_{sw}$ ~1), or in some cases undersaturated with $\Omega_{sw}$ <1 (Thresher et al., 2011).

In contrast to these highly consistent rates of up-regulation in scleractinian corals,

some species of calcitic foraminifera have a $pH_{cf}$ close to ambient seawater (Fig. 2), indicating that $pH_{cf}$ up-regulation is not a universal characteristic of marine calcifiers. In foraminifera, the biological control of calcification appears to be much more variable, which may be attributed to the ability of their protoplasm to modify intracellular pH and/or modify the ratio between *DIC*, bicarbonate, and carbonate ions independently of external pH and $pCO_2$ (Bentov et al., 2009; de Nooijer et al., 2009). Notably, species lacking symbionts such as hyaline and miliolid foraminifers, are able to elevate the pH at the site of calcification by one unit above seawater pH by forming intracellular high pH vesicles, which are used for calcification (de Nooijer et al., 2009). Clearly, given the variable responses of species from this phylum, it is unlikely that OA will have a consistent impact on foraminiferal physiology.

### 2.5 The cost of compensating pH differences

The modification of the intra- and extracellular carbonate chemistry of an organism to favor calcification has an associated energy cost. This has been demonstrated for several families of calcifiers, for example some foraminifera species (de Nooijer et al., 2009) and gastropods (Palmer 1992). Therefore, food availability might affect the sensitivity of a species for low pH environments.

When energy is dedicated to calcification, it cannot be used for other processes, prompting growth limitation and reduced reproductive output. In molluscs, the cost of formation of the shell organic matrix is around 10 - 60% of that of somatic growth and 15 - 150% of the cost for gamete formation (see Palmer 1992 and references therein). This cost includes: (1) the energetic content of the matrix, about 10% of the total shell-production cost, and (2) the metabolic cost of synthesizing the matrix. Overall, the total

cost of $CaCO_3$ precipitation is considerably less than the total cost of synthesizing protein (Palmer 1992). The low cost of calcification relative to other metabolic costs in marine species is caused by the high saturation constant of $CO_3^{2-}$ in seawater. However, as a consequence of lower energy availability or even a direct effect of OA, the response to a predator could be modified, like i.e. in a Chilean gastropod (Manríquez et al., 2014), even if shell growth is not (Manríquez et al., 2013), which has consequences for survival. In general slower growth would lead to a disadvantage for i.e. bivalves as it would take longer to reach a larger size class with less predation pressure (Hiddink et al., 2002).

In corals, heterotrophic feeding adds to energy reserves for processes of calcification, photosynthesis, and production of coral biomass (Houlbrèque and Ferrier-Pagès 2009). Indeed, some corals can maintain calcification rates at low pH when nutrient concentrations are elevated (Atkinson et al., 1995, Holcomb et al., 2010), and nutrient addition can reduce the effect of declining aragonite saturation state on calcification (Atkinson and Cuet 2008). Some coral species, like *Porites rus*, seem to be robust to OA during short-term exposures, whereas the negative effects seen during medium-term exposures can be mediated through increased food availability (Comeau 2013).

For photosymbiont bearing corals the energy requirements needed to increase $pH_{cf}$ above ambient seawater values are relatively minor because the free-energy change required to maintain the pH gradient is ~3 to 6 kJ $mol^{-1}$ of $CaCO_3$ precipitated, compared to ~400-500 kJ $mol^{-1}$ C available from photosynthesis by the symbionts (McCulloch et al., 2012a). Thus, the energy requirement is only <1% of that generated

by photosynthesis. Although it is only a relatively small fraction, it is nevertheless essential for maintaining pH up-regulation as illustrated by the dramatic decrease in calcification that can occur when the zooxanthellae coral symbiosis is disturbed, such as occurs for example during coral bleaching (e.g. D'Olivo et al., 2013).

## 3. Conclusions

The intense multi-scale dynamics of pH in coastal ecosystems explains the evolutionary development of mechanisms to maintain homeostasis in the calcification process of most coastal calcifiers. Calcifying organisms can biologically control the environment of carbonate deposition, by producing sharp pH gradients in the DBL, controlling the pH in extracellular fluid, or by controlling deposition in a regulated, intracellular environment. Some organisms benefit directly from the close interaction with autotrophic symbionts. However corals, which have lost their symbionts, might still be vulnerable at sites with low food availability. Coastal calcifying organisms can also benefit from the large diversity of pH niches in their habitats, where pH variability in space and time offers niches of improved conditions for calcification. Where calcifiers have a close association with macrophytes, they might adapt the window of calcification to match the most productive hours of the day, when $CO_2$ is drawn down by primary productivity, as might occur in some bivalve species associated to habitats with high autotrophic metabolic activity (Fig. 1). In addition to the direct facilitation of calcification by the elevated pH in macrophyte beds, increased food supply for filter feeders within macrophytes stands (e.g. Peterson et al., 1984) also implies that they can allocate more energy resources to maintain the homeostasis of calcification. These adaptations allow coastal organisms with calcified components to respond to highly variable pH in their

environment and buffer the negative effects of a decrease in ambient seawater pH in the future. However we caution against the generalization of the common assumption that coastal environments will provide refugia for the diverse range of calcifying species. In particular, scleractinian corals are generally adapted to high water flow conditions (Falter et al., 2004), which contrasts to the long-residence time within densely vegetated environments with associated high primary productivity.

The conditions imposed in OA experiments, typically involving stable pH conditions under an "*elevated*" and "*present*" scenario (Hendriks et al., 2010), may not adequately represent the environments that coastal organism's experience, either presently or in the future. Experiments should include the *in situ* community, which will add variability in pH through metabolic processes. However, this may introduce additional variables (e.g. species interactions) and limit the capacity to resolve individual pH effects. A first approach would be to design the treatments so that they include diel fluctuations in pH, comparable to those the organisms experience in their natural environment, the response to which are still poorly constrained.

In summary, this review of pH variability and biological mechanisms regulating calcification rates by coastal biota shows that, contrary to the open pelagic environment, coastal ecosystems can be characterized by strong variability in pH mediated by metabolic processes. Such high variability in the saturation state of carbonate minerals may have prompted the evolution of a broad range of mechanisms by which coastal organisms can maintain homeostasis for calcification in such variable environments. In addition, complex variability of pH in coastal ecosystems imply that these often do not follow the trajectories predicted from OA, the latter derived for open ocean waters

(Duarte et al., 2013). However, many coastal calcifiers have a pelagic larval stage, which may even be advected out to open ocean waters and during this critical stage of their life cycle be subject to the OA values present in open ocean systems. So, while coastal calcifiers may be generally more resistant to OA than hitherto believed, their pelagic larval stage may be the 'Achilles heel' under future OA conditions. The ability of some organisms to modify pH internally or within the BLs surrounding their body may be an adaptation to allow calcification and in general functioning in the highly variable pH environment of the coastal zone. This adaptation may offer some protection against low pH thereby possibly allowing species that are considered vulnerable to persist even under future scenarios of OA.

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## Figure Headings

Fig. 1. Bivalve calcifiers living in metabolically intense habitats A) The pen shell *Pinna nobilis* in a *Posidonia oceanica* meadow (Mediterranean, photo by I.E. Hendriks), b) Blue mussel *Mytilus edulis* in *Ascophyllum* sp. in (Greenland, photo by C.M. Duarte).

Fig. 2. Relationship of ambient seawater pH versus pH of calcifying fluid ($pH_{cf}$) as determined from boron isotope systematics in aragonitic corals and calcitic foraminifera. Elevation of $pH_{cf}$ (pH upregulation) relative to seawater, for example where pH = 8.0 (vertical dashed line) is greatest in cold water corals (~0.7 to 0.9), then temperate corals (~0.6), with tropical corals showing the lowest values (~0. to 0.5). Conversely, calcitic foraminifera (grey squares) lie on or near the abiotic line (i.e. seawater pH = $pH_{cf}$) that defines no pH upregulation. Dashed lines show regressions for $pH_{cf}$ versus seawater pH for temperate and tropical coral species subject to a range of experimental and natural conditions. All the linear regressions are significant ($p<0.01$). The cold water corals *Desmophyllum dianthus* (brown diamonds), *Caryophyllia smithii* (orange), *Lophelia pertusa* (purple), *Madrepora oculata* (aqua),and *Enallopsammia rostrata* (yellow) are from a wide range of deep-sea environments (Mediterranean, east Pacific and Southern oceans), and although show more elevated $pH_{cf}$ values do not give well defined linear regressions (McCulloch et al., 2012b). For data sources and sample details see Holcomb et al., (2014) for S*tylophora pistillata* (black), and for remaining datasets see Trotter et al., (2011) and McCulloch et al., (2012a,b).

Adaptation to OA impacts by biological mechanisms

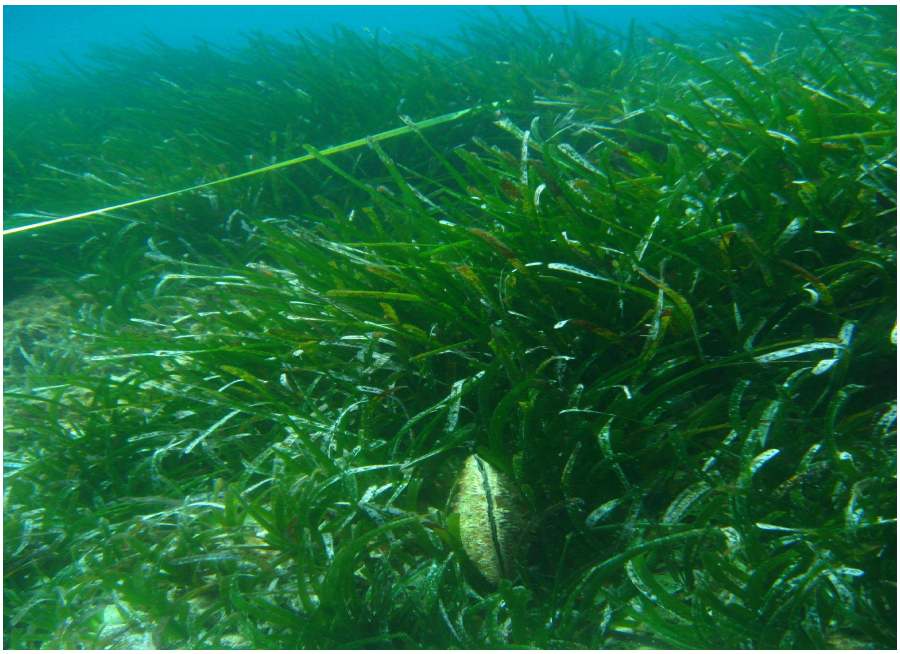
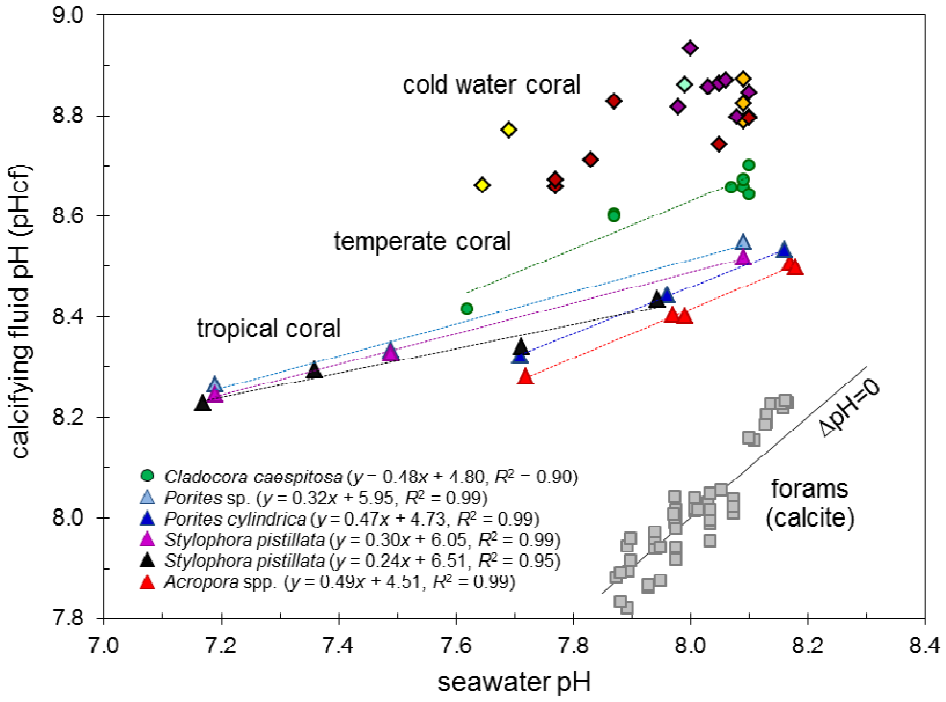


Figure 1a.

Figure 1b.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calcifying fluid pH (pHcf)
seawater pH
cold water coral
temperate coral
tropical coral
ΔpH=0
forams
(calcite)
Cladocora caespitosa (y = 0.18x + 1.80, R² = 0.90)
Porites sp. (y = 0.32x + 5.95, R² = 0.99)
Porites cylindrica (y = 0.47x + 4.73, R² = 0.99)
Stylophora pistillata (y = 0.30x + 6.05, R² = 0.99)
Stylophora pistillata (y = 0.24x + 6.51, R² = 0.95)
Acropora spp. (y = 0.49x + 4.51, R² = 0.99)

Figure 2.

**Figure(s)**
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