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Coral calcifying fluid pH dictates response to ocean acidification

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Ocean acidification driven by rising levels of CO_2 impairs calcification, threatening coral reef growth. Predicting how corals respond to CO_2 requires a better understanding of how calcification is controlled. Here we show how spatial variations in the pH of the internal calcifying fluid (pH_{cf}) in coral (*Stylophora pistillata)* colonies correlates with differential sensitivity of calcification to acidification. Coral apexes had the highest pH_{cf} and experienced the smallest changes in pH_{cf} in response to acidification. Lateral growth was associated with lower pH_{cf} and greater changes with acidification. Calcification showed a pattern similar to pH_{cf}, with lateral growth being more strongly affected by acidification than apical. Regulation of pH_{cf} is therefore spatially variable within a coral and critical to determining the sensitivity of calcification to ocean acidification.

The seawater environment that marine organisms inhabit is changing due to rising atmospheric CO_2, which is causing a decline in seawater pH or 'ocean acidification'[1]. Declining seawater pH and the resultant decrease in the calcium carbonate saturation state are expected to lead to reduced calcification rates for a wide range of calcifying organisms[2,3]. This is because bio-calcification, which generally occurs in an internal semi-isolated environment created and controlled by the organism, is nevertheless influenced by the external environment[4–8].

Among the organisms expected to be affected by ocean acidification are scleractinian corals. The effects of acidification on coral calcification have been extensively studied[9–17], with a wide range of responses being observed[18,19]. Such variability likely reflects the degree to which corals are able to control the chemistry at the site of calcification. One of the mechanisms by which corals are thought to facilitate calcification is through up-regulation of the pH of the calcifying fluid (pH_{cf})[19–21]. An increase in pH is generally linked to an increase in the aragonite saturation state due to HCO_3^- converting to CO_3^{2-} at elevated pH, thus favoring the precipitation of skeletal aragonite ($CaCO_3$). The aragonite saturation state, represented by $\Omega_{Aragonite}$, is defined as:

$$\Omega_{Aragonite} = \frac{[CO_3^{2-}][Ca^{2+}]}{K_{SP}} \quad (1)$$

where K_{SP} is the solubility of aragonite.

The decline in calcification rate often observed with ocean acidification has been suggested to be linked to a decline in pH in the calcifying fluid induced by lower pH in the external seawater environment[19,22,23]. Understanding how the pH at the site of calcification changes in response to changes in the external seawater environment, both in the modern ocean and over evolutionary timescales, represents a critical step in predicting how calcifying organisms will fare under future conditions of ocean acidification.

In addition to the importance of pH_{cf} for the calcification process itself, understanding how pH_{cf} relates to seawater pH is also of critical importance for the development of seawater pH proxies. Boron is of particular interest in this regard as it exists in seawater in one of two forms – borate or boric acid. The relative proportion of borate versus boric acid is determined by pH. Further there is a large (~27‰) difference in the isotopic composition of borate relative to boric acid, so the isotopic composition of borate is a function of pH[24]. Boron in the coral skeleton is thought to be derived almost entirely from borate present in the calcifying fluid[25] and boron in the calcifying fluid is thought to come directly from seawater, which has a known boron isotopic composition[26]. Thus measurements of the isotopic composition of boron in corals can be used to calculate pH_{cf}[6,27]. Since pH_{cf} is related to seawater pH[28] and the boron isotopic composition of the coral skeleton[6,29], boron isotopes can be used to calculate seawater pH, thus allowing the estimation of seawater pH for periods prior to instrumental records.

Here we investigate the sensitivity of corals to ocean acidification at intra-colony spatial scales by measuring pH_{cf} and calcification rates in different regions within coral colonies exposed to reduced seawater pH. Branch tips (apexes) from colonies of the tropical coral *Stylophora pistillata* were attached to glass cover-slips and allowed to grow out laterally across the cover-slip (basal-lateral growth), and to extend the original branch tip (apical growth) (Fig. 1a). Measurements of pH_{cf} were made in different regions of coral colonies using analytical methods suited to each region. Confocal microscopy with the pH indicator SNARF-1 was used to measure pH_{cf} during the early stages of basal-lateral growth at the growing edge of the colonies where skeletal material is limited, thus facilitating observations with confocal microscopy. Boron isotope measurements were made on skeletal calcium carbonate samples from two different regions: 1) the lateral region (Fig. 1a), which included the material from the growing edge where confocal microscopy was undertaken, as well as the more mature skeletal regions overlying the material initially formed at the growing edge; and 2) the upwardly growing tips of the apexes, or apical region (Fig. 1a). The apical region is the region in which most growth normally occurs.

Results

Corals were maintained in seawater with varying degrees of acidification (Supplementary Table S1) ranging from near ambient, pH_T = 7.94 (pCO_2 = 550 µatm), to pH_T = 7.17 (pCO_2 = 4140 µatm, used to achieve undersaturation with respect to aragonite). Corals produced new skeleton under the full range of pH treatments. However lateral growth rates (measured as changes in skeletal area) declined progressively with decreasing seawater pH (Fig. 1b), and growth was significantly reduced at pH_T = 7.16 relative to ambient conditions ($F_{3,32}$=5.81, $p<0.01$)[28]. In contrast to lateral growth, the apical extension of branches showed no significant change ($F_{3,52}$=1.1, $p=0.36$) in response to acidification (Fig. 1b).

Measurements of $\delta^{11}B$ showed a consistent decline in $\delta^{11}B$ values associated with a decline in seawater pH_T (Fig. 2a). For all regions, although pH_{cf} was invariably elevated relative to seawater pH_T (Fig. 2b), pH_{cf} decreased with a decline in seawater pH_T (Fig. 2b). The decline in pH_{cf} was smaller than the corresponding pH_T change in the seawater, thus the difference between seawater pH_T and pH_{cf} (ΔpH^e) increased as seawater pH_T declined (Fig. 2b and Supplementary Fig. S1). Although measurements of both apical and lateral growth showed the same general pattern, lateral growth generally had lower pH_{cf} values than apical growth, and the difference between lateral and apical regions increased as pH_T declined (Fig. 2b). Apexes thus had smaller changes in pH_{cf} in response to acidification than adjacent lateral skeletal growth. For the growing edge, pH_{cf} estimates were even lower and showed the largest declines in pH_{cf} in response to acidification.

Discussion

Our estimates of the effects of ocean acidification on the extension of branch tips (apical growth) are similar to those of other studies on *S. pistillata* colonies[9,30,31], being fairly stable over a wide range of pH_T conditions (Fig. 1b). In contrast, extension of the lateral part of the colonies (lateral growth) was more strongly affected by ocean acidification, similar to other studies that have examined lateral growth in *S. pistillata*[30]. Boron isotope-based estimates of pH_{cf} exhibited patterns similar to calcification data. Lateral growth, which showed the greatest decline in calcification in response to acidification also showed a greater decline in pH_{cf} in response to acidification than adjacent apical growth. Thus spatial differences in the regulation of pH_{cf} may account for changes in calcification, consistent with the IpHRAC[19] model in which internal pH_{cf} regulation controls abiotic calcification rates.

Variation in the ability of the overlying tissue layer to control the pH at the site of calcification, as suggested by pH_{cf} estimates (Fig. 2), may be linked to a number of differences existing between the apically and basal laterally growing regions[32,33]. The growth of these two regions is fundamentally different: apical growth occurs in a largely unrestricted environment whereas basal lateral growth occurs at an interface between the coral and a substrate. Thus the growing edge (initial basal lateral growth) faces a number of potential challenges not generally encountered by the rest of the coral tissue, including competing with other organisms for substrate, and isolating new substrates from the surrounding environment to allow crystal growth to occur. The devotion of resources to compete for substrate could limit the energy available for calcification and in-turn reduce the ability of the tissue to up-regulate pH when faced with a more acidified environment. Or the isolation of new substrates from the surrounding environment may not be as complete as the isolation of existing skeletal regions, thus allowing higher rates of seawater ingress resulting in a more pronounced effect of acidification. Regardless of the underlying mechanism(s), pH_{cf} and thus calcification, was more strongly affected by acidification in the basal, laterally

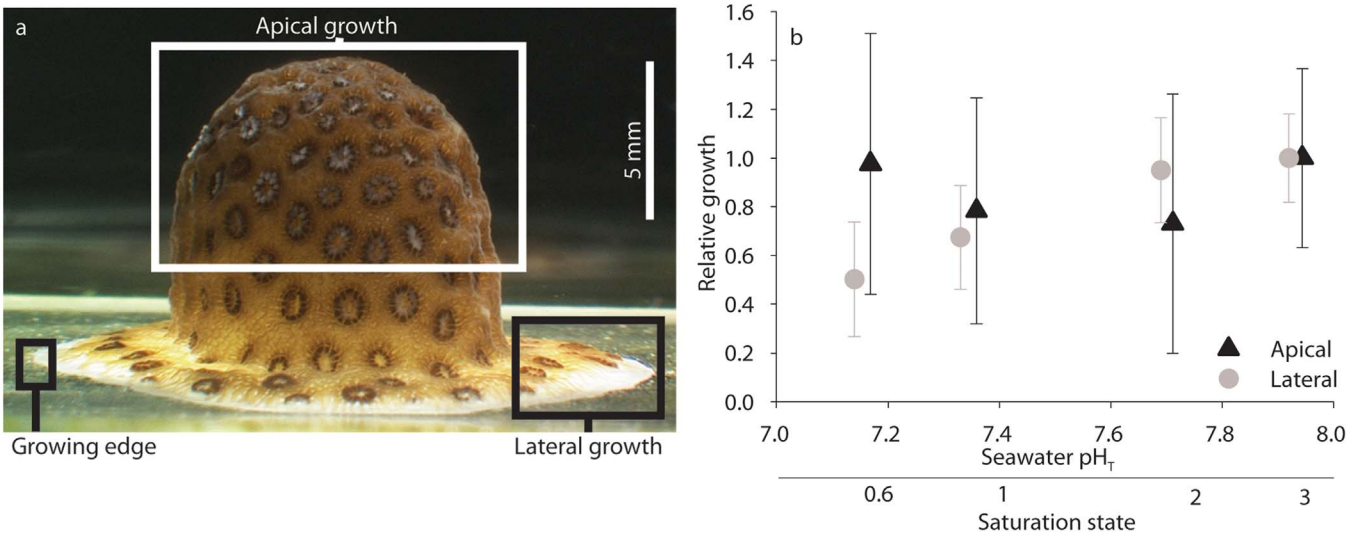

Figure 1 | Regions sampled for pH_{cf} measurements and growth rates of different regions. (a) Sampling locations for boron isotope samples and SNARF based measurements. (b) Relative growth rates for lateral expansion[28] and apical extension, values are expressed relative to the mean rate at ambient seawater pH for the given measurement period. Symbols are means, error bars are standard deviation.

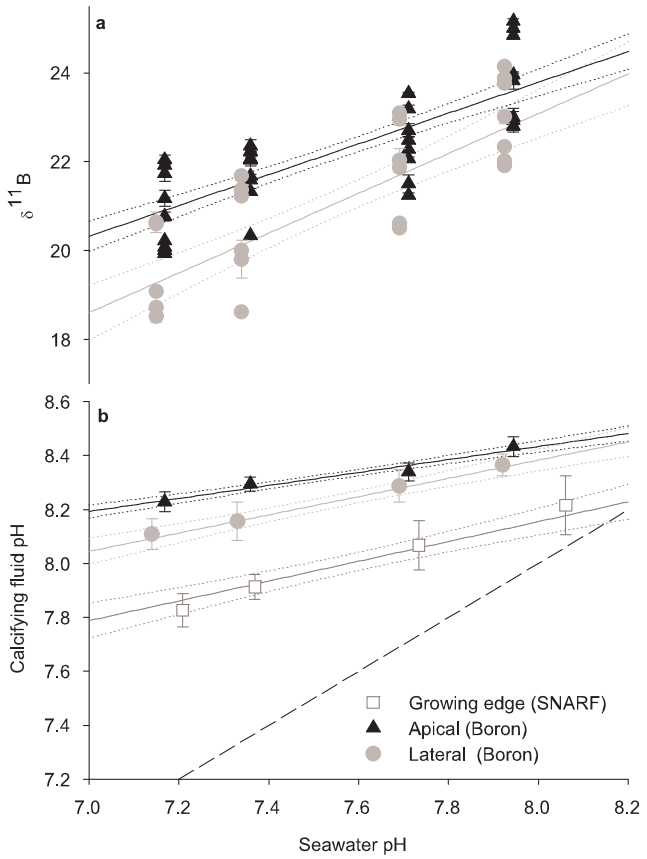

Figure 2 | Measured $\delta^{11}B$ and pH_{cf} estimates for different regions of the coral plotted against seawater pH_T. (a) $\delta^{11}B$ values measured in apical (black) and lateral (grey) skeletal regions plotted against average seawater pH. Symbols are average measurements of a given sample, error bars are standard error. (b) Calcifying fluid pH estimates plotted against average seawater pH (aquarium pH_T for boron isotope based estimates, perfusion chamber pH_T for SNARF based estimates). Symbols are weighted means, error bars are standard deviation, with n=3 time-points. Symbols for apical and lateral measurements are offset on the x-axis for clarity. Solid lines are weighted regression fits to the data, dotted lines are 95% confidence intervals for the regressions. For reference, the 1:1 line is shown as a dashed line, this is where pH_{cf} = seawater pH_T. All pH values are on the total scale (pH_T).

growing regions than the apical growth. Thus stages of coral growth that involve extension over new substrates are likely to be more strongly affected by ocean acidification. This may be particularly relevant to the larval-stage of coral growth when all calcification is occurring on a new substrate, to coral fragments that must cement themselves to a new substrate, as well as to damaged corals attempting to re-grow over exposed skeleton; all represent growth stages in which lateral growth over a substrate plays an important role. These stages are therefore least likely to be able to maintain pH_{cf} under acidified conditions and thus are likely to be more adversely affected by ocean acidification.

Since calcification within a coral differs spatially in its sensitivity to ocean acidification, and that variations in pH_{cf} appear to correspond to these differences in calcification, pH_{cf} may help to predict how calcification will respond to ocean acidification. Measurements of pH_{cf} thus represent an important tool for identifying stages of coral growth (e.g. colonization of new substrates) and particular species that will most likely be adversely affected by ocean acidification. Measurements of pH_{cf} using boron isotopes can allow pH_{cf} to be estimated over long time scales and allow variations in biologically controlled pH up-regulation to be linked to events (e.g. bleaching, storms, etc) in the natural environment which may further impact the ability of corals to regulate pH_{cf}. Collectively such data can help to better predict how corals will respond to the range of conditions they face.

Methods

See the supplemental material.

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Author contributions

M.H., A.A.V., E.T., S.T., D.A., J.T. and M.M. designed the experiments. M.H., A.A.V. and E.T. performed research. M.H., A.A.V., E.T., S.T., D.A., J.T. and M.M. were involved in the preparation of the manuscript.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

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