Using B isotopes and B/Ca in corals from low saturation springs to constrain calcification mechanisms

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Ocean acidification is expected to negatively impact calcifying organisms, yet we lack understanding of their acclimation potential in the natural environment. Here we measured geochemical proxies (δ¹¹B and B/Ca) in *Porites astreoides* corals that have been growing for their entire life under low aragonite saturation (Ωsw: 0.77–1.85). This allowed us to assess the ability of these corals to manipulate the chemical conditions at the site of calcification (Ωcf), and hence their potential to acclimate to changing Ωsw. We show that lifelong exposure to low Ωsw did not enable the corals to acclimate and reach similar Ωcf as corals grown under ambient conditions. The lower Ωcf at the site of calcification can explain a large proportion of the decreasing *P. astreoides* calcification rates at low Ωsw. The naturally elevated seawater dissolved inorganic carbon concentration at this study site shed light on how different carbonate chemistry parameters affect calcification conditions in corals.
Ocean acidification is projected to lead to negative effects on calcifying organisms, particularly tropical corals. Our understanding of the potential fate of corals in the face of changing pCO2 in the ocean is based primarily on controlled laboratory studies (e.g. refs. 4,5), mesocosm studies mimicking coral community composition6-8, alkalisation versus carbon dioxide-enrichment studies in natural coral reef sites9,10, and a number of field studies with naturally reduced calcium carbonate saturation state (Ωaragonite)1,11-14. These efforts have provided strong evidence that the calcification rates of a large number of coral species investigated to date will decline in response to projected pCO215. However, some studies also report that certain coral species were able to maintain high calcification rates or even benefit from elevated pCO21,16-18, suggesting a high resilience potential of some coral species to changing carbonate chemistry19. Specifically, the ability of an organism to control the biomineralization process clearly determines its ecological and physiological success under reduced pH conditions14. The process of calcification in corals is linked to their ability to control the pH at the site of calcification (pHcf) by removing protons out of the calcicoblastic space between the tissue and skeleton, where calcification takes place2. This enables corals to sustain pHcf well above seawater pH (pHsw)5,19,20. The physiological capacity of corals to control pHcf may alleviate the decline in coral growth and increase coral resilience to future climate change19. Knowledge about internal calcifying fluid pHcf in corals has been derived from a few direct measurements under the calcifying cell layer either using microsensors21,22 or pH-sensitive dyes23. These studies confirmed an elevated pHcf of between 0.4 and 2 pH units above ambient seawater in the calcicoblastic space. Indirectly, boron isotopes (δ11B) of coral skeletons, which represent the pHcf of the calcifying solution, also suggest an elevated pHcf (e.g. refs. 19,24-26). Boron isotopes are more readily accessible compared to direct measurements and have the additional benefit that they integrate pHcf history over longer time periods19,20,24,27. Studies suggest that pHcf is an important driver affecting pHsw25,28. However, it was recently demonstrated that changes in seawater dissolved inorganic carbon (DIC) or total alkalinity (TA) can also affect pHcf regulation23. Using B/Ca as a proxy for internal carbonate ion concentration (CO32−-cf), provided geochemical evidence that corals can also modulate and adjust the internal DIC (DICcf) concentration. Together—the potential to upregulate DICcf and pHcf—allows for higher carbonate ion concentrations at the site of calcification and hence a higher Ωcf that facilitates calcification29,30.

Over the last decade, a growing body of literature has provided evidence that corals subjected to daily and seasonally fluctuating environmental conditions are able to exert a stronger control over their internal physiological attributes, potentially allowing them to better cope with future changes (reviewed in ref. 31). For instance, in situ flume experiments mimicking natural (daily, seasonal) fluctuating conditions coupled with future pCO2 conditions showed that corals from acidified treatments could maintain a constant calcification pHcf irrespective of changes in seawater pHsw27. The authors argued that the fluctuating conditions the corals were exposed to likely favour this strong control on internal conditions. That year long experiment, however, cannot tell whether corals can maintain such strong control when exposed to reduced mean seawater aragonite saturation state (Ωsw) for their entire life span. Corals living for their entire life under continuously low Ωsw and variable environmental conditions can be used to test whether corals can maintain pHcf homeostasis over long time spans in their natural ecosystem with its complex biological interactions. Because many natural ocean acidification sites also show strongly fluctuating conditions11,32,33 these settings may be ideal for testing the relationship between environmental variability and acclimation potential of corals to low Ωsw34.

Here we measured geochemical proxies in the uppermost recently formed skeletal parts of Porites astreoides corals that were collected along a natural aragonite saturation gradient at sub-marine springs (locally known as ojos) in Puerto Morelos, Mexico1. These geochemical proxies (δ11B-derived estimates of pHcf and B/Ca derived estimates of CO32−-cf) allowed us to infer carbonate chemistry conditions at the site of calcification, which provide valuable new insights into the internal calcification regulation mechanisms in corals exposed to persistent low Ωsw as well as fluctuating carbonate chemistry conditions35,36. Our results, combined with bio-inorganic calcification models19,30, identified critical regulation mechanisms and the inability of corals to fully acclimate to these conditions and sufficiently elevate their Ωcf to sustain growth rates similar to the same species of corals growing at ambient Ωsw.

**Results**

**Natural conditions at the ojos.** We used 12 cores from the coral P. astreoides: 5 cores collected from the centre of the low Ωsw ojos and 7 from control present-day Ωsw sites adjacent (within a few meters) to the ojos41. Porites astreoides, the species used in this study, represents one of only three calcifying coral species found growing within the discharge impacted area, while nine coral species are found nearby under ambient present-day Ωsw. Previous studies indicate that although the abundance of P. astreoides was not significantly reduced at the low Ωsw ojos, its growth rate (measured as net calcification) decreased significantly by 37% compared to the same species collected at control sites11. The control sites have a relatively consistent Ωsw (on average: 3.92 ± 0.03 sd) year round compared to the ojos where Ωsw is always <2 and ranges from 0.77 to 1.85 (on average: 1.49 ± 0.14 sd, Supplementary Table 1 11).

**Skeletal δ11B and thus pHcf as a function of Ωsw.** The δ11B in the 12 corals analysed ranged from 23.1%o to 27.6%o, with slight but significantly lower values for corals affected by the ojo discharge where Ωsw was low (Fig. 1a, Supplementary Table 2; t-test: p = 0.022; r2 = 0.29, p = 0.040). These δ11B values translate into pHcf that are slightly lower (but not statistically significant) in the corals from sites with Ωsw < 2 with an average internal pHcf of 8.46 (±0.03 sem) compared to 8.54 (±0.01 sem) at the control sites (t-test: p = 0.085, Fig. 1b, Supplementary Table 2). The pHcf difference between the corals is relatively small (0.08 pH units) compared to the difference in environmental seawater pHsw of ~0.54 pH units between the sites. Hence, compared to Ωsw in their surrounding environment, corals at the ojo centres maintained a higher pH gradient between seawater and the calcifying fluid (ΔpH) in comparison to corals at control sites (Fig. 1c, Supplementary Table 2; t-test: p = 0.002, r2 = 0.89, p < 0.001).

**Skeletal B/Ca, thus DICcf and CO32−-cf as a function of Ωsw.** Changes in coral skeletal B/Ca were determined along with δ11B. This ratio varied between 442 and 721 µmol mol−1 and did not significantly correspond to Ωsw (Fig. 2a, p = 0.86). Using the δ11B and B/Ca skeletal proxies together to constrain the carbonate system at the site of calcification suggests an elevation of CO32−-cf not only due to shifts in internal pHcf but also due to an increase in DICcf (Supplementary Table 2, Fig. 2b). The ratios of DICcf/DICsw—a measure of the upregulation of DICcf compared to seawater—were significantly higher at the control sites than at the low Ωsw sites (Fig. 2c, Supplementary Table 2; p = 0.036, r2 = 0.33, p = 0.029). This is mainly due to the higher than ambient DICsw (Supplementary Table 1) at the ojos because the DICcf did
Discussion

Coral calcification is one of the most fundamental processes in reef ecosystems and is essential for reef accretion and ecosystem diversity; however, calcification may be impacted by changes in seawater carbonate chemistry. Although corals are sensitive to changes in ocean carbonate chemistry, the underlying physiological mechanisms that determine vulnerability are far from understood. Natural sites with low aragonite saturation that select for genotypes that can calcify under such conditions and permit decade-long developmental acclimation to changes in $\Omega_{sw}$ are invaluable model systems for understanding the resilience of corals and coral calcification processes. Here we reveal that corals grown for their entire lifetime at low aragonite saturation conditions in their natural environment, at ojos in the Caribbean, exert strong control on both pH$_{cf}$ and DIC$_{cf}$, thereby modulating CO$_3^{2-}$, $\Omega_{sw}$, and calcification rate. At the calcification site, both parameters that control $\Omega_{sw}$ (pH$_{cf}$ and DIC$_{cf}$) decreased only slightly along the ambient $\Omega_{sw}$ gradient in which the analysed corals live, highlighting the strong control of Porites astreoides corals over the biomineralization process. Yet the combined change in pH$_{cf}$ and DIC$_{cf}$ corroborate the observed decline in calcification rate along the environmental gradient (Fig. 4b).

Interestingly, at this field site ojos with low $\Omega_{sw}$ had elevated DIC$_{sw}$ but this did not result in higher DIC$_{cf}$ concentrations in the calcifying fluids, indicating a decoupling of internal and external DIC concentrations. This indicates that corals have significant control over the carbonate chemistry of the calcifying fluid, likely mediated by bicarbonate transporters (NBC, SLC4 family of ion transporters) that are localised in the calcicoblastic epithelium, as well as other, not yet identified acid–base relevant transporters$^{35}$. Carbonate chemistry at the calcification site clearly differs between coral growing at the control and ojo locations. The difference explains 41% of the observed difference in calcification rate; however, it still leaves 59% of the variation in calcification rate unexplained (Fig. 4b).

In our study, we took advantage of the inherent conditions of this submarine springs system, including the strong environmental fluctuations and the fact that carbonate chemistry is controlled by saline groundwater discharge, allowing us to provide new facets on drivers of coral calcification in natural settings affected by ocean acidification. In the subsequent discussion we will outline the novel insights we derive from the observed internal carbonate chemistry conditions at this natural low $\Omega_{sw}$ site, discuss potential mechanisms that control calcification rates, add to the ongoing discussion on how seawater carbonate chemistry affects regulations of internal conditions at the site of calcification (e.g. ref. 23), and emphasise the importance of deciphering internal calcium regulation$^{36–38}$.

The ability of organisms to modify pH$_{cf}$ reflects the strong effect of intracellular biological processes on coral calcification and is manifested in skeletal isotopic composition. The control of pH$_{cf}$ represents one mechanism to counter external seawater conditions$^{39}$. The boron isotopic-derived pH$_{cf}$ values we report are similar to those reported in other studies for Porites$^{25–27}$. The
Fig. 2 Porites astreoides internal CO$_3^{2-}$ and DIC$_{cf}$ based on skeletal proxies. Coral skeletal B/Ca ratio a from naturally different seawater aragonite saturation state ($\Omega_{sw}$) sites were translated into internal calcifying carbonate ion concentration (CO$_3^{2-}$) values b and dissolved inorganic carbon (DIC$_{cf}$) concentration, as well as upregulation compared to seawater (DIC$_{cf}$/DIC$_{sw}$) (c, d respectively). Circles represent values for each individual coral colony (mean ± confidence interval). Filled and non-filled symbols denotes the different locations: filled are the centres of the ojos with lower $\Omega_{sw}$ and non-filled the control high $\Omega_{sw}$ site. Triangles in c represent seawater DIC$_{sw}$ concentrations. Dashed line represents regression line for site-specific significant different values for DIC$_{cf}$/DIC$_{sw}$ with $\Omega_{sw}$ ($r_{adj}^2 = 0.33$, $p = 0.029$) and grey area denotes the 95% confidence band. Individual values are mean ± 95%-confidence interval

Fig. 3 Growth response of Porites astreoides corals. The modelled growth response displays relative changes in calcification rate (relative calcification rate = mean control/individual colony). Calcification rates were calculated following the IpHRAC model$^{19}$ (internal pH regulation and abiotic calcification: Calcification = $k^\prime (\Omega_{cf} - 1)^n$) with calcifying fluid aragonite saturation state ($\Omega_{cf}$) was calculated from the average internal calcifying fluid pH (pH$_{cf}$) of individual colonies and the dissolved inorganic carbon (DIC$_{cf}$); in a dependent variable $\Omega_{cf}$ is based on DIC$_{cf}$ and pH$_{cf}$ and b depicts the respective calculated calcification rates. Circles represent values for each individual coral colony (mean ± confidence interval). Filled and non-filled symbols denotes the different locations: filled are the centres of the ojos with lower seawater aragonite saturation state ($\Omega_{sw}$) and non-filled the control high $\Omega_{sw}$ site. We compared calculated values with measured data$^{11}$ (for better comparison also calculated as relative rate, open triangles). Individual values are mean ± 95%-confidence interval
sensitivity of pH_{cf} to changes in the environmental pH_{sw}, however, differed between the different studies, as the environments the corals originated from were distinct^{27,40}. All studies observed that pH_{cf} stays within a narrower range (8.2–8.6) compared to large changes in seawater pH_{sw}. They all highlight the generally strong control corals exert on pH_{cf}. Despite this capacity for regulation, however, the observed pH_{cf} was lower at lower Ω_{sw} (e.g. refs. 20,25,28,41). Irrespective of whether corals maintained high pH_{cf}, the corals exposed to low Ω_{sw} maintained a higher proton gradient at lower pH_{sw} (Fig. 2b). A potential driving force that fosters acclimation to various changes a coral may experience is the environmental history corals have been exposed to during their lifetime^{31}. For example, pH homeostasis—the maintenance of internal pH_{cf} irrespective of the external seawater pH_{sw}—was observed in corals that live in a highly dynamic naturally variable environment^{5,42}. The underlying assumption is that these corals are better able to buffer external changes by exerting a stronger control over the calcifying fluids or by better exploiting times of favourable conditions^{27,40}. Although the ojos represent a highly dynamic system^{33,43,44}, coral performance measured in terms of net calcification was lower at these sites relative to the same species collected at control sites at the same location. Here, life-long exposure to variable and persistently low Ω_{sw} (e.g. <2) did not lead to full acclimation^{11}. It is likely that there is a critical Ω_{sw} threshold beyond which corals are no longer able to fully compensate for external acid–base changes. Such a critical threshold has been observed for corals grown at a Papua New Guinea CO_{2} seep site, where pH_{cf} homeostasis was only found for Ω_{sw} of >7.8 and Ω_{sw} of >2.3. Beyond that, pH_{cf} could not reach the same values as those under control conditions, and likely the coral’s physiological limit to compensate for changes was reached^{40}. This lack of ability to fully compensate for the lower pH_{sw} may be responsible for the slight differences in pH_{cf} observed in this study.

The use of our dual geochemical proxy data to model coral growth (e.g. IpHRAC^{19,30}) allowed us to further pinpoint potential mechanisms of how external seawater conditions affect internal calcifying conditions and ultimately skeletal growth. Calcification was once thought to be a passive diffusion process of seawater that brings external DIC to the site of calcification (potentially gaining DIC from metabolic CO_{2} by passing through the paracellular pathways)^{45} and by active ion transporters^{46} that result in an elevation of pH_{cf}, thereby facilitating precipitation. At our study sites, DIC_{sw} is significantly higher at the low Ω_{sw} sites (in average 2790 μmol kg^{-1} compared to control average DIC_{sw} of 2050 μmol kg^{-1}) allowing us to decipher the role of external DIC_{sw} in modulating calcification regulation processes. If corals modify internal DIC_{cf} by simply up-regulating DIC_{cf} from the external concentrations baseline, we would expect higher DIC_{cf} values for the ojo corals where DIC_{sw} is higher. Under such assumption the elevated DIC_{cf} compensates for the slightly lower pH_{cf} effect on Ω_{cf} and calcification rates would essentially be similar between sites (Supplementary Fig. 1). However, our data clearly demonstrate that DIC_{cf} is not directly linked to external concentrations and can differ significantly from that of seawater^{22,30,47} (reported DIC_{cf}-upregulation values range from 1.6 to 3.2^{30,38}, with the ojo corals at our sites at the lower end), and this impacts Ω_{cf} (more precisely CO_{3}^{2−}) and calcification. A recent study under laboratory conditions with Stylophora pistillata^{23} observed that changes in DIC_{sw} concentration modulates internal pH_{cf} regulation, with higher external DIC_{cf} facilitating higher internal pH_{cf}, resulting in a clear correlation between seawater DIC_{sw}/H^{+}_{sw} and pH_{cf}. Since DIC_{sw} at the ojos is significantly higher than at the control sites, one could expect this to compensate reduced pH_{cf} up-regulation induced only due to changes in seawater pH_{sw}. Yet we do not see a strong correlation between pH_{cf} and seawater DIC_{sw}/H^{+}_{sw}, suggesting different drivers for Ω_{cf} regulation in P. astroides compared to those observed in S. pistillata^{23}. Nevertheless, the change in pH_{cf} and the limited ability to upregulate DIC_{cf} at the ojos corroborates the observed calcification rate decrease of corals at the ojos. However, these parameters may not be the only drivers for the decline in growth. Recent studies identified internal calcium (Ca^{2+}) regulation as an additional player in coral calcification responses and emphasised that regulation of Ca^{2+} can contribute to a corals’ resistance to future ocean changes^{36–38,48}. In this sense, the good agreement of our model with the observed calcification response may imply that internal average steady-state calcium concentrations (Ca^{2+}) at the ojos are lower by some proportion that is related to the pH_{cf} changes, since our model based on pH_{cf} and
CO$_3^{2-}$, can explain only 41% of the observed calcification decline. This suggests a strong link between Ca$^{2+}$ and pH$_{cf}$ and supports the idea of a plasma-membrane Ca-ATPase (49, but see ref. 50) responsible for pH$_{cf}$ regulation. However, it is possible that pH$_{cf}$ and Ca$^{2+}$ were both regulated by additional and/or different ion transport mechanisms (e.g. potentially ion exchangers, Ca$^{2+}$-channels)56,57.

The present study also indicates that the calcification process in different corals encompass some degree of flexibility in terms of the relative role of pH$_{cf}$ and DIC$_{cf}$ regulation in increasing the $\Omega_{sw}$, with some individuals compensating by adjusting their internal pH$_{cf}$ and others primarily by DIC$_{cf}$ modulation. This may also be true of the role of Ca$^{2+}$ upregulation. The relative amount, source, and transportation pathways of DIC, H$_2$CO$_3$ and Ca$^{2+}$ to the site of calcification are still not fully understood52 and transport processes may differ between different coral species and even individual corals of the same species. Another potential driver for the observed differences among studies could be the number and type of symbionts the corals are hosting. Corals at the ojos harbour a higher density of symbionts35 that may potentially account for the higher energy demands for H$_2$CO$_3$ upregulation resulting in the relatively small difference in the internal conditions (pH$_{cf}$, DIC$_{cf}$) we see. Recent work provided the first evidence that coral symbionts (e.g. by modulating the chemical microenvironment within the diffusive boundary layer surrounding the coral that may buffer external changes54) and host genotypes can jointly affect coral calcification rates55. Similarly, possible interactions with the microbiome (e.g. restructuring of the corals microbiome56) or changes in energy acquisition and allocation processes to overcome environmental gradients57,58 can affect coral growth. Environmental factors may also affect pH$_{cf}$ and DIC$_{cf}$ explaining some of the observed differences between the ojos and ambient corals at our study site. Studies have shown that a decrease in pH$_{cf}$ and DIC$_{cf}$ is associated with increasing temperature38, yet at our sites the temperatures at the ojos is actually lower, on average, than at control sites. Salinity might also influence regulation processes, yet the measured average values (32.2 psu) as well as the salinity range measured (26–36 psu)44 at the springs can be tolerated by corals and the long-term exposure to such conditions may have allowed them to develop mechanisms to better cope and adapt to this variable environment59. Overall, these environmental and biological parameters may be responsible for the observed internal conditions in the calcifying fluid but likely also affect rates of processes that ultimately affect calcification, and thus contribute to the unexplained component in our relationship between calcification and the geochemically derived DIC$_{cf}$ and pH$_{cf}$. Our geochemical model approach assumes steady-state equilibrium conditions; however, the rates of the various transport processes involved in regulating the chemistry of the calcifying fluid will ultimately dictate the calcification response60. These rates may differ between individual coral genotypes, further contributing to the offsets between the model output and observations.

In this study, we utilised a dual geochemical proxy approach ($\delta^{18}$B and B/Ca) to constrain calcifying fluid carbonate chemistry in P. astreoides corals that spent their entire life (decades) under acidified low $\Omega_{sw}$ conditions. We found that at the pH$_{cf}$ for corals at the low $\Omega_{sw}$ was slightly lower than at the ambient conditions indicating inability to achieve optimal calcification conditions. We also determined that pH$_{cf}$ and DIC$_{cf}$ are independently regulated and corroborated the calcification response in P. astreoides at this site. The study provides new insights into calcification responses of P. astreoides under changing environmental conditions and sheds light on the potential of corals to acclimate36,41,47,61,62. Using the geochemical proxies in combination with the bio-inorganic model brought forward by McCulloch et al. 30, we could explain 41% of the variability in coral growth rates along a $\Omega_{sw}$ gradient. The variability which is not explained indicates that additional physiological and environmental processes contribute to the control of calcification rates in natural environments. This provides promising new avenues towards studying acclimation and adaptation potential of long-lived marine invertebrates such as corals.

Methods
Site description and coral core collection. Corals from colonies of P. astreoides were collected at the ojos—natural springs of low-pH water—in the National Maritime Park at Puerto Morelos, Mexico (see refs. 19, 39, for more details). Five corals were drilled in close proximity to the low pH discharge and seven corals were drilled from control sites outside the ojos discharge influence (~2–5 m away). After collection, cores were dried at 50 °C before further analysis. Water chemistry was measured at the different sites (summarised in Supplementary Table 1 and for more details see refs. 13,33,35,41,63,64) and used to calculate carbonate chemistry (see Supplementary Table 1). In general, corals were collected from sites that have similar light conditions, differ marginally in temperature (<1 °C lower at the ojos averaged over all seasons with temperatures cooler than ambient in summer and slightly warmer in winter), have consistently lower salinity (2–4 units lower than ambient), and are considerably different in $\Omega_{sw}$ (Supplementary Table 1)13,33,35. We note that these submarine springs are not perfect analogues for future ocean acidification. Specifically, the conditions creating low-pH seawater at the ojos differ from those of the ocean acidification scenario as the high CO$_2$ in the discharging water at the ojos is derived from brackish water that has interacted with soil and rock, and supplied by a spring water that is characterised by lower TA but similar calcium (Ca$^{2+}$) concentration compared to the ambient conditions away from the spring influence. The corals at these ojos are constantly exposed to these discharging water (Supplementary Table 1), as discussed in detail in refs. 13,33,35,64, and they represent settings with persistent low $\Omega_{sw}$. In particular, these environmental conditions have persisted at the ojo discharge sites at least since the last deglaciation (~18,000 years ago65) the corals at these sites were exposed to low $\Omega_{sw}$ for their whole life span, potentially allowing enough time for acclimation. Moreover, it is quite likely that strong selection processes have resulted in successful colonisation of the ojos by a fraction of the coral population that is better adapted to low pH$_{sw}$ and high CO$_2$65.

Water samples were also taken for seawater boron concentrations (measured on a ICP-MS Finnigan Element XRF following Krupinski and colleagues66) ± 8 µM, with no difference between ojos and control) and a boron isotopic composition ($\delta^{11}$B$_{bw}$) of 59.15 (1δ = 0.12, n = 3) for the control site and 38.85 (1δ = 0.17; n = 5) for the low pH ojos. Boron in the spring water isotopic composition (38.85) was not explained indicating that additional physiological and environmental processes contribute to the control of calcification rates in natural environments. This provides promising new avenues towards studying acclimation and adaptation potential of long-lived marine invertebrates such as corals.

Sample preparation and geochemical analysis. Collected coral cores were cut in half. One was bleached for 24 h, thoroughly washed with milli-Q and dried overnight at 50 °C. Subsequently, the slab surfaces were carefully ground (Stuers, 60º, 400 µm SiC grade paper, Dur 9 µm polishing suspension) in preparation for boron analysis using a Struers TegraPol-21 with TegraForce-5 head (Grinder and Polisher). The $\delta^{18}$B and B/Ca composition was measured simultaneously by laser ablation multi-collector inductively coupled plasma mass spectrometer (Thermo Fisher MC-ICP-MS AXIOM, coupled to a New Wave Research, 213 fs laser ablation system equipped with an excimer 193 nm laser). The measurement procedure followed Fietzke et al.68 and Wall et al.69 with slight modifications. Specifically, we used Multiplier and Faraday cup simultaneously to collect data for B$_{bw}$ and B$_{bw}$ (both on multiplier) as well as C$_{bw}$ (Faraday cup). This allows us to derive B/C and B/Ca from the same measurement. Calculations were performed for a regular baseline (every 2–4 days). The tubes going from the ablation cell to the plasma torch were checked for material deposition and cleaned by high flow rates overnight and/or mobilisation of the debris by increased flow rates transporting it out of the tubes. Prior to each measurement session the standard and samples were pre-ablated to remove surface contaminations (spot size was one user bigger than during analysis). A standard-sample-bracketing method was used. The data of one measurement session contained 5–6 brackets. Both C$_{bw}$ and the variation of the standard (NIST SRM610) for each session were used to check for instrument stability and contaminations. Sessions were repeated when the standard drift was too high. The $\Omega_{sw}$ of the standard was calculated by conversion of the standard to the stable form of Ca$^{2+}$ ($\Omega_{sw}$). 20 individual laser tracks (25 × 500 µm) were placed as close as possible to the edge of the skeletal section (expecting to mainly ablate the soft organic part of the skeleton). Standards were then used to check for instrument stability and contaminations. The internal reproducibility (RSD) of the standards was 0.17% for the control site and 0.5% for the low pH ojos. Boron and 4 units lower than at the ambient conditions indicating inability to achieve optimal calcification conditions. We also determined that pH$_{cf}$ and DIC$_{cf}$ are independently regulated and corroborated the calcification response in P. astreoides at this site. The study provides new insights into calcification responses of P. astreoides under changing environmental conditions and sheds light on the potential of corals to acclimate36,41,47,61,62. Using the geochemical proxies in combination with the bio-inorganic model brought forward by McCulloch et al. 30, we could explain 41% of the variability in coral growth rates along a $\Omega_{sw}$ gradient. The variability which is not explained indicates that additional physiological and environmental processes contribute to the control of calcification rates in natural environments. This provides promising new avenues towards studying acclimation and adaptation potential of long-lived marine invertebrates such as corals.
afterwards) or increased organics and excluded this parts from analysis, and (b) aimed for 20 tracks of ~25 x 500 x 20 µm on all individuals to have a representative δ11B dataset per individual. By this approach we expect to cover a representative sample set and minimise the natural intra-skeletal variability and cover similar proportions in each of the different corals (assuming that COC to fibre ratio in coral grown under various environmental conditions stays constant). The accuracy of our δ11B measurements has been checked by repeated analyses of Portites coral standard Jcp-1 and NIST SRM610, measured against a pellet of primary boron standard NSB951 (boric acid) (see Supplementary Fig. 2).

**δ11B determination.** The data reduction followed Fietzke et al.68. This yields one δ11B value per sample and session with an average precision of <1‰ (1 SD) for ~1.7 µg of carbonate sample. A minimum of 15 and up to 20 values of δ11B spread over the core surface in the upper few mm of each coral colony (below the tissue, representing ~1 year of growth) were measured to obtain a representative data set per sample. The data set reflects the high variability in δ11B for a single colony, and replicates were averaged afterwards to yield values that reflect the mean δ11B value, hence the mean internal calcification conditions (see below).

**B/Ca determination.** B/C elemental ratios have been determined simultaneously with the boron isotope ratios via LA-ICP-MS. Boron isotope data (δ10B and 11B) have been collected using a pair of ion counters, while carbon (12C) had been determined using a Faraday cup. B/C data are based on the integrated boron intensities (10B + 11B) divided by the 12C intensity. The calibration (conversion from intensity ratios to concentration ratios) has been done using a natural L. pertusa coral sample covering a B concentration range of about 450–950 µmol/mol, which had been determined before using LA-ICP-MS relative to standard NIST—SRM610 using 4Ca as internal standard. This calibration procedure resulted in: B/C (µmol/µmol) = 78.800 B/C (cps/cps); (cps—counts per second, ion beam intensity). We used stoichiometric ratio of C/Ca = 1 as approximation for natural carbonates and translated B/C ratios in B/Ca (µmol/µmol) ratios.

**δ18O as internal pHc proxy.** All δ18O values were translated into internal pHc following Eq. (1) with a seawater δ18Osw of 38.85 for ooe centries and 39.15 for control sites, a fractionation factor (αo) of 1.027269 and pKr averaged for the two sites (see Supplementary Table 1).

\[
\text{pHc} = \text{pKr} - \log \left( \left( \frac{\delta^{18}O_{sw}}{18} \right) \left( \text{αo} + 1 \right) \right)
\]

Following the method in Trotter and colleagues54 the superimposed physiological pH control was calculated with the equation:

\[
\Delta \text{pH} = \text{pHc} - \text{pH}_{\text{sw}}
\]

and related to the seawater aragonite saturation state (Ωsw) to quantify the extent of the physiological control on the internal pHc.

We note here, that the local variability in carbonate chemistry at the ojos and hence, associated changes in pKr and seawater δ11B can add some uncertainty to the derived pHc and overestimate or underestimate its actual value. To test the sensitivity to changes in pKr we used our dataset and recalculated pHc values. We applied a range of seawater δ18Osw that encompasses the average measured δ18Osw per site but also seawater isotopic composition beyond this level ranging from 38.55% to 39.45% and recalculated pHc (Supplementary Fig. 3a). This allowed us to decipher the combined role of site specific pKr and seawater δ18Osw for a range of skeletal δ11B (Supplementary Fig. 3b). In general, the δ11B-derived pHc decreases slightly with increasing seawater δ18Osw. Changes in seawater δ18Osw in the corals surrounding will either over or underestimate pHc, and calculated changes in pHc range from 0.019 to 0.023 pH units per 0.3 change in δ18Osw (Supplementary Fig. 3c). The average difference between our sites; or change from 0.056–0.065 for the entire seawater δ18Osw range tested). Compared to the pHc range (8.2–8.8) derived from individually measured skeletal δ11B values such changes are minor (Supplementary Fig. 3a; in contrast to the individual coral’s pHc standard deviation of 0.04–0.13, Supplementary Table 2).

**B/Ca as CO32⁻ and DICcf proxy.** All individual B/Ca data were used to estimate CO32⁻ based on the δ11B-derived δ11B data and further used to calculate the DICcf following the approach of McCulloch et al.90. This allows to use the following simplified relationship to determine the CO32⁻ concentration from B/Ca:90

\[
[\text{CO}_3^{2-}]_{\text{cf}} = \frac{\text{[B(OH)]}\text{cf} + K_D^{\text{Ca}}}{\text{B/Ca}}
\]

and the distribution coefficient is determined for synthetic aragonite and follows the equation:

\[
K_D^{\text{Ca}} = 0.00297 \exp(-0.0202[H^+]_{\text{cf}})
\]

based on the internal pH.54,90 Both pHc and [CO32⁻] are then used to calculate DICcf.
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Author contribution

M.W., J.F., and A.P. designed the experimental analyses. E.D.C. collected the samples. M.W. prepared the samples. M.W. and J.F. analysed the samples. E.D.C. and A.P. provided background data. M.W. analysed data. M.W., J.F., A.P., and E.D.C. were involved in the preparation of the manuscript.

Additional information

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