LETTERS
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Coral and mollusc resistance to ocean acidification adversely affected by warming

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Increasing atmospheric carbon dioxide (CO_2) concentrations are expected to decrease surface ocean pH by 0.3–0.5 units by 2100 (refs 1,2), lowering the carbonate ion concentration of surface waters. This rapid acidification is predicted to dramatically decrease calcification in many marine organisms[3,4]. Reduced skeletal growth under increased CO_2 levels has already been shown for corals, molluscs and many other marine organisms[4–9]. The impact of acidification on the ability of individual species to calcify has remained elusive, however, as measuring net calcification fails to disentangle the relative contributions of gross calcification and dissolution rates on growth. Here, we show that corals and molluscs transplanted along gradients of carbonate saturation state at Mediterranean CO_2 vents are able to calcify and grow at even faster than normal rates when exposed to the high CO_2 levels projected for the next 300 years. Calcifiers remain at risk, however, owing to the dissolution of exposed shells and skeletons that occurs as pH levels fall. Our results show that tissues and external organic layers play a major role in protecting shells and skeletons from corrosive sea water, limiting dissolution and allowing organisms to calcify[10,11]. Our combined field and laboratory results demonstrate that the adverse effects of global warming are exacerbated when high temperatures coincide with acidification.

It is projected that anthropogenic CO_2 emissions will decrease surface ocean pH by 0.3–0.5 units, lowering the carbonate ion concentration ($[CO_3^{2-}]$) and the saturation states of calcite (Ω_c) and aragonite (Ω_a) by 2100 (refs 1,2). These alterations in carbonate chemistry have been used to predict calcification responses to ocean acidification, based on experiments that quantify decreased skeletal growth in corals, molluscs and many other marine organisms as CO_2 levels increase[4–9]. Previous work has not determined the impact of acidification on the ability of individual species to calcify, because net calcification (that is, gross calcification minus dissolution) was measured, thus failing to disentangle the relative contributions of gross calcification and dissolution rates. A decrease in net calcification could result from a decrease in gross calcification, an increase in dissolution rates, or both. Recent studies indicate inconsistencies in the use of carbonate saturation state to predict marine calcification, because some species are able to maintain, or even increase, their net calcification under low pH conditions[9,10,12–15]. It is thought that this species-specific sensitivity is due to the presence and composition of external organic layers[10] and an ability to elevate pH and $[CO_3^{2-}]$ at sites of calcification[10,16]. For example, measurements of the boron isotopic composition in *Cladocora caespitosa* held in aquaria[15] at pH_T (on the total scale) 7.8 and from CO_2 vents showed that this Mediterranean coral was able to maintain a higher internal pH and hence ΔpH relative to ambient seawater pH (ref. 17), therefore allowing calcification in undersaturated sea water. Calcification is known to occur between the tissue and the shell or skeleton, where extrapallial fluid (in molluscs) and extracellular calcifying fluid (in corals) pH is 0.5 to >1 units higher than in ambient sea water[16–20].

We used a series of transplantation experiments (Supplementary Fig. S1 and Table S1) along natural pCO_2 gradients at volcanic vents[21] off Ischia (Tyrrhenian Sea, Italy) to investigate whether the projected reduction in calcification in key marine habitats by 2100 and beyond is a result of the suppression of gross calcification, carbonate dissolution or both. We compared (1) a mollusc that has a periostracum covering the outside of the shell (*Mytilus galloprovincialis*) with one that lacks a periostracum (*Patella caerulea*) and (2) the zooxanthellate coral *Balanophyllia europaea*, which has a skeleton completely covered in tissue, with *C. caespitosa*, which has skeletal parts that are exposed to the surrounding sea water (Fig. 1). These organisms characterized shallow rocky habitats outside the vent systems, but only the limpets were found below mean pH_T 7.8. Shell and coral dissolution rates and coral net calcification rates were measured by buoyant weighing samples, whereas gross calcification rates of all four species were measured in aquaria with the radiotracer ^{45}Ca after incubation *in situ* (see Supplementary Tables S2, S3 for pH and carbonate chemistry measurements at transplantation sites).

Effects of acidification on molluscs. Dead mussel and limpet shells held for 21 days in aquaria dissolved faster at lower pH ($F_{2,31} = 478$; $P < 0.001$; see Supplementary statistical analyses for details). At pH_T 7.8, dissolution only occurred in *Mytilus* shells ($0.003 \pm 0.007\,mg\,cm^{-2}\,d^{-1}$), whereas at pH_T 6.8, dead limpet shells dissolved nine times faster than the mussel shells (9 ± 1 versus $1.1 \pm 0.3\,mg\,cm^{-2}\,d^{-1}$). The outer shell layer is calcitic in both species, yet the mussels probably dissolved more slowly than limpets because their periostracum protected the shell. Protective organic layers are produced by many marine calcifiers and seem

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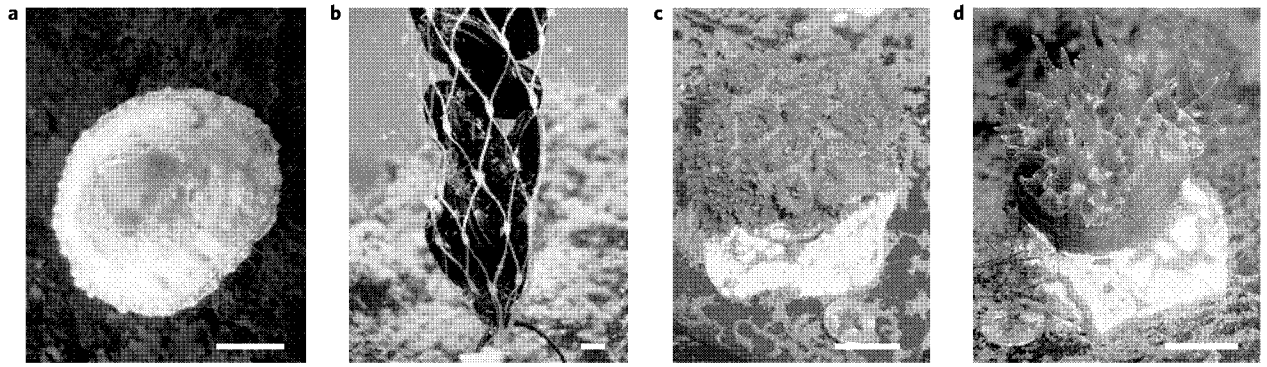

Figure 1 | Species transplanted along a pH gradient near volcanic CO_2 vents off Ischia, Italy. a, *P. caerulea* naturally occurring at mean pH_T 6.8 (note severe shell dissolution). **b**, Transplanted *M. galloprovincialis* after five months at mean pH_T 7.2. **c**,**d**, Transplanted corals *C. caespitosa* and *B. europaea* after three months at mean pH_T 7.3. Scale bars = 1 cm.

to be key in determining their relative susceptibility to dissolution owing to ocean acidification[10,22]. Dead mussel shells transplanted for three months to mean pH_T 7.2 exhibited marked dissolution (Supplementary Fig. S2), whereas live mussels maintained their periostracum and were still growing after five months at pH_T 7.2. Dissolution only occurred where the periostracum at the umbo, the oldest part of the shell, had been abraded owing to adjacent mussels rubbing together. These mussels had larger areas of damaged periostracum than those transplanted to mean pH_T 8.1 (6.4±3.1% and 0.4±0.9%, respectively; $F_{1,42} = 127$; $P < 0.001$), suggesting a decreased ability to repair periostracum at low pH.

Both of the molluscs were able to precipitate calcium carbonate in sea water undersaturated with calcite, as previously found for *Mytilus edulis* in the Baltic[23]. Gross calcification in mussels increased linearly (overall slope $F_{1,182} = 376$; $P < 0.001$) at the same rate over two weeks at both pH_T 7.4 and 8.0 (Fig. 2, May), but was 70% at mean pH_T 7.4 compared with mean pH_T 8.1 in June (planned contrast: $P < 0.001$). Adult limpets were found at the CO_2 vents at mean pH_T 6.5, although in lower abundances than outside the vents[21]. Their shells were highly corroded, as they were permanently exposed to undersaturated conditions ($\Omega_c = 0.39$) and lacked a protective periostracum. Shell dissolution was pronounced on the oldest parts of large individuals (Fig. 3a). We found that the gross calcification rates were consistently higher in individuals living within the vent area (pH: $F_{1,54} = 4$; $P < 0.042$) than in individuals living outside the vent area, both at pH_T 8.1 and 6.8 (Fig. 3b). Transplanted limpets grew a distinctive new shell rim after only one week, at both mean pH_T 8.0 and 6.5, as they grew to conform to the rock surface. The newly secreted shell was easily detected, as it was the normal brown colour; it started to lose colour and dissolve after the first week at mean pH_T 6.5. Limpets living in the low-pH area had up-regulated their calcification rates, which helped counteract higher shell dissolution rates. It is likely that the limpets may sustain the higher energetic cost required to maintain elevated calcification rates[12] thanks to the abundant algal turf, on which they feed, in the high CO_2 areas[21,24].

Effects of acidification on corals. Dead samples of both coral species did not dissolve after three weeks in aquaria at constant pH_T 7.8, a value often projected for the year 2100. Dissolution was measured at pH_T 7.4 (0.3–0.6 mg g^{-1} d^{-1}) and 6.8 (3.7–4.2 mg g^{-1} d^{-1}), and at the vents at mean pH_T 7.3 (4.0–4.5 mg g^{-1} d^{-1}). Dissolution of live samples transplanted at the vents was species-specific. *C. caespitosa*, which has large parts of its skeleton exposed, showed evident marks of dissolution (Supplementary Fig. S3), whereas *B. europaea*, which has a skeleton that is completely covered in tissue, was apparently unaffected (Fig. 4 and Supplementary Fig. S4). The morphology of the exposed skeletons

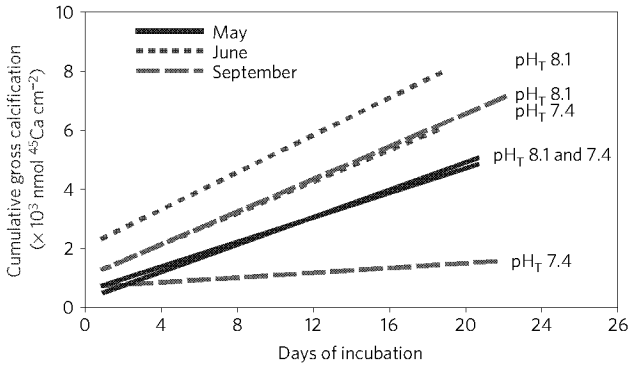

Figure 2 | Gross calcification of *M. galloprovincialis* measured in aquaria over a three-week period at pH_T 7.4 and 8.1. Measurements were repeated in May and after two-and five-month transplant periods at mean pH_T 7.2 and 8.0 (Supplementary Tables S2b and S3). Points omitted for clarity. Linear regressions of cumulative gross calcification over time were significant ($n = 30$–36, all $P < 0.001$, see Supplementary statistical analyses).

of live corals, as well dead samples of both corals maintained at mean pH_T 7.3 for three months, showed disordered aragonite crystals. In contrast, skeleton covered in tissue had organized bundles of fine aragonite crystals, as in samples maintained at normal pH. Just as periostracum protected mussel shells, coral tissues protected the skeletons from corrosive ($\Omega_a < 1$) sea water. This protective role may explain why some corals increase tissue thickness when seawater pH is lowered[14].

As expected from other coral studies[3,14], net calcification after three months decreased significantly with decreasing pH in our coral transplants (*C. caespitosa*: $F_{3,44} = 50$; $P < 0.001$; *B. europaea*: $F_{3,52} = 17$; $P < 0.001$; Fig. 5a). *C. caespitosa* had significantly slower linear growth rates as pH decreased ($F_{2,65} = 62$; $P < 0.001$; mean±s.d.: 0.19±0.03, 0.32±0.05 and 0.34±0.07 mm $month^{-1}$, at mean pH_T 7.5, 7.8 and 8.1 respectively). At mean pH_T 7.3, all the transplanted colonies had dissolved after approximately five months, so no measurements were possible. In *C. caespitosa*, net calcification rates became negative at mean $pH_T < 7.5$ (mean $\Omega_a = 1.69$, minimum $\Omega_a = 0.40$), but remained positive for *B. europaea* even at mean pH_T 7.3 (mean $\Omega_a = 1.13$, minimum $\Omega_a = 0.33$). Their tissue coverage partially explains the different susceptibility of the two corals to undersaturated sea water. However, as net calcification does not distinguish gross calcification from skeletal dissolution, it is unclear from these data whether corals exposed to lower pH decreased their calcification rates and/or underwent greater dissolution.

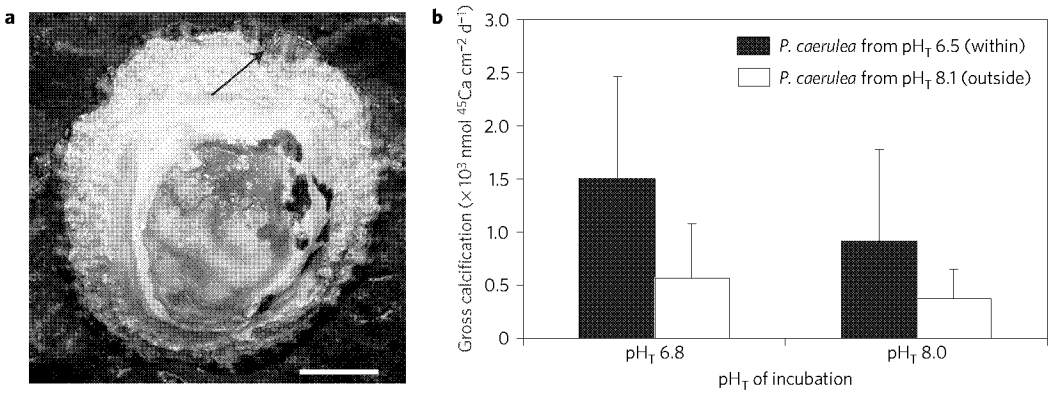

Figure 3 | Calcification of *P. caerulea* measured in aquaria at pH_T 6.8 and 8.0. a, Limpets collected at mean pH_T 6.6 and transplanted at the same pH. The arrow shows new brown shell secreted one week after transplantation. Scale bar = 1 cm. **b**, Gross calcification of *P. caerulea* collected within and outside volcanic CO_2 vents and incubated in aquaria with ^{45}Ca-labelled sea water both at pH_T 6.8 and 8.0 (Supplementary Tables S2c and S3). Data are means ± s.d. ($n = 14$).

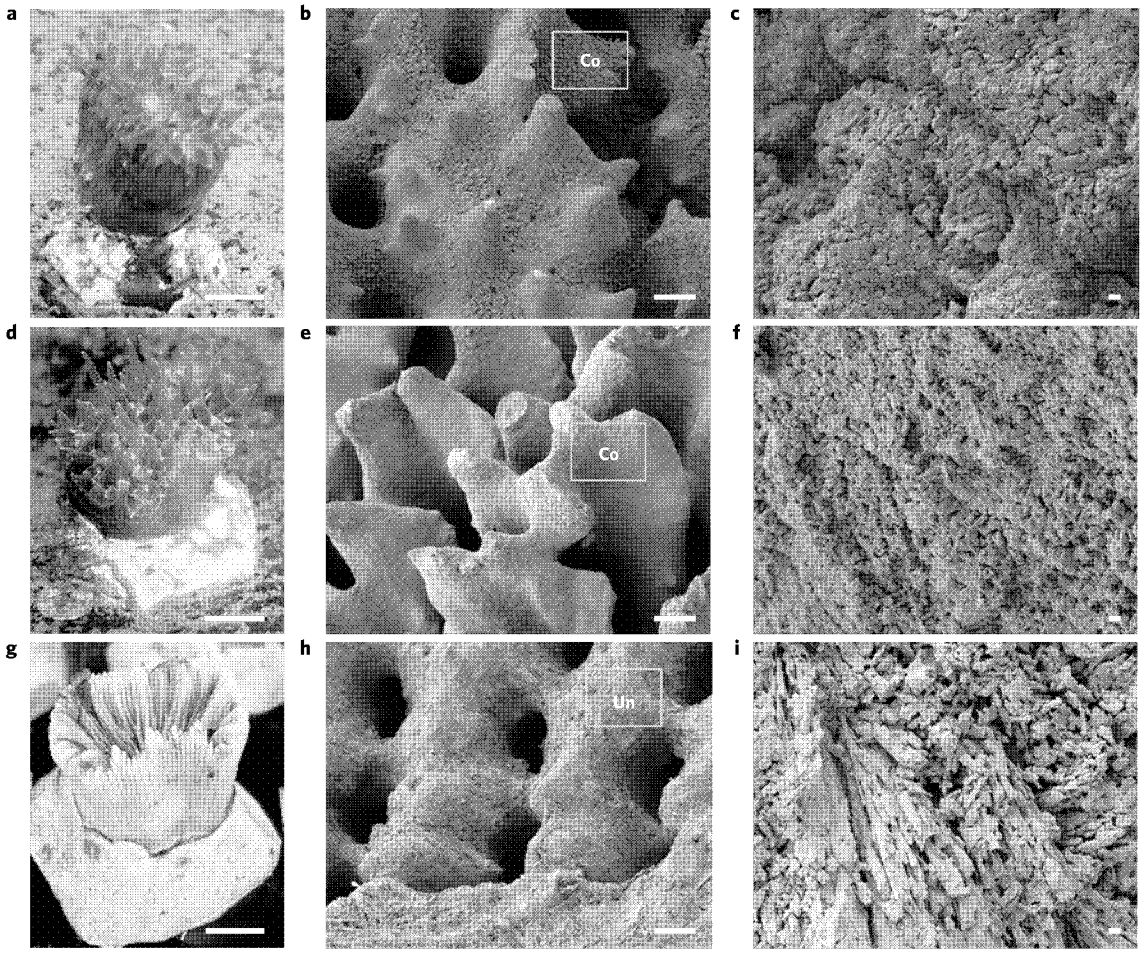

Figure 4 | Underwater and scanning electron microscopy images of *B. europaea* transplanted along a CO_2 gradient off Ischia. a–f, Live coral after seven months at mean pH_T 8.1 (**a–c**) and 7.3 (**d–f**). **g–i**, Dead coral after three months at mean pH_T 7.3 (Supplementary Table S2a). Details of the outer corallite wall showing normal skeleton when covered (Co) in tissue (**b**,**e**) and dissolved skeleton when uncovered (Un) in tissue (**h**) . Enlargements (yellow boxes on **b**, **e**, **h**) show organized (**c**,**f**) and dissolved (**i**) bundles of aragonite crystals. Scale bars = 1 cm (**a**,**d**,**g**), 100 μm (**b**,**e**,**h**) and 1 μm (**c**,**f**,**i**).

Labelling with ^{45}Ca showed that, remarkably, both coral species were able to maintain gross calcification at pH levels projected for 2100 and beyond (Fig. 5b). Gross calcification in *C. caespitosa* was similar at pH_T 8.1 and 7.7 and decreased by 30% at pH_T 7.4 ($F_{2,45} = 10$; $P < 0.001$), whereas gross calcification in *B. europaea* significantly increased with decreasing pH in June and July ($F_{2,54} = 26$; $P < 0.001$). This raises the possibility, as in other corals[13], that *Balanophyllia* may rely on HCO_3^- for calcification.

Combined stress of warming and acidification. The ability of corals to calcify in undersaturated conditions was disrupted when acidification was combined with elevated temperatures.

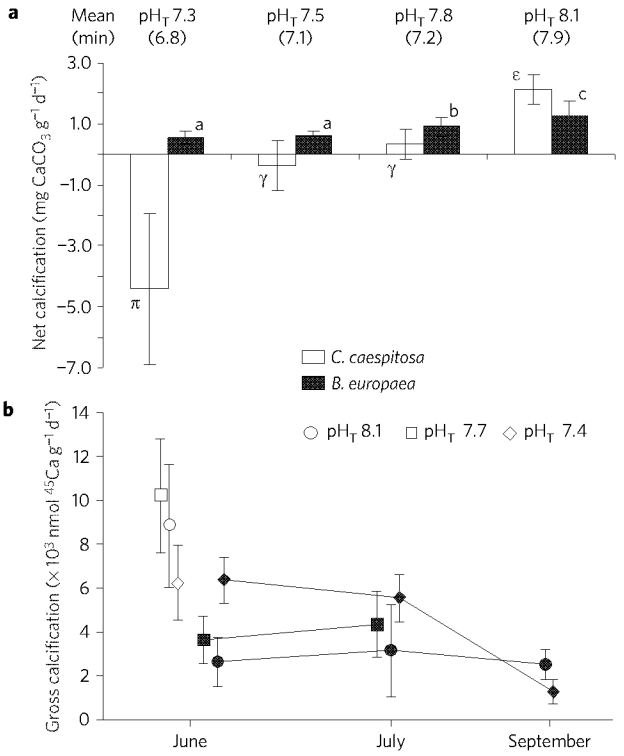

Figure 5 | Calcification rates of corals transplanted along a CO_2 gradient off Ischia. a, Net calcification measured after three months in 2008 (Supplementary Table S2a). Different symbols (for *C. caespitosa*) and letters (for *B. europaea*) are significantly different ($P < 0.05$). Data are means ± s.d. ($n = 12$ for *C. caespitosa*; $n = 14$ for *B. europaea*). **b**, Gross calcification measured in aquaria with ^{45}Ca-labelled sea water at pH$_T$ 7.4, 7.7 and 8.1 (Supplementary Table S3) on samples transplanted up to seven months in 2009 (Supplementary Table S2b). *C. caespitosa* gross calcification was measured only in June 2009 after three months. Data are means ± s.d. ($n = 16$ for *C. caespitosa*; $n = 10$ for *B. europaea*).

Transplanted organisms experienced a prolonged period of unusually high (28.5 °C) seawater temperature from June to September 2009 (Supplementary Fig. S5). We also observed the widespread mortality of gorgonians, corals and molluscs in several locations around Ischia Island during this period. Such mass mortality events are being reported with increasing frequency in the Mediterranean as heatwaves become more intense[25], suggesting that in summer some organisms are living near their upper thermal limits. Mortality rates of transplanted *B. europaea* were higher at mean pH$_T$ 7.1 and 7.2 (80 and 50%, respectively) than at mean pH$_T$ 8.0 (30%). Gross calcification rates of surviving *B. europaea* and *M. galloprovincialis* measured in September 2009 were close to zero at mean pH$_T$ 7.4 (Figs 2 and 5b); at mean pH$_T$ 8.0 they calcified at similar rates to those measured in May and June. It is therefore likely that the decrease in gross calcification was due to the synergistic effects of low pH and elevated temperatures (interaction pH x months $F_{2,185} = 24$, $P < 0.001$, and $F_{3,66} = 12$, $P < 0.001$ for *Mytilus* and *Balanophyllia* respectively).

Previous investigations of Ischia CO_2 vents[21] showed that an ecological tipping point occurred whereby subtidal calcifiers were absent below mean pH$_T$ 7.8. Our transplantation experiments show that the underlying cause for this major ecological shift is not necessarily impaired calcification, as each of the species we examined was able to calcify (that is gross calcification) and some calcified faster at pH values well below those projected for global surface waters by 2100. Limpets underwent the most striking dissolution when exposed to sea water undersaturated with calcite, because of their lack of periostracum, yet those that occurred naturally in areas with very high CO_2 levels had adapted physiologically to the effects of ocean acidification. They had faster rates of calcification than limpets sampled at background CO_2 levels, but their tolerance to acidification was limited by high shell-dissolution rates. The other three species we examined also had higher or similar gross calcification rates at low pH levels, but had protective organic layers that cover parts or all of their shells or skeletons. Their tolerance to acidification depended on their ability to maintain this protection at increased levels of CO_2. The fact that corals and mussels were not found at mean pH$_T$ below 7.8 probably reflects an inability to meet the increased metabolic costs of coping with high CO_2 levels in the oligotrophic, food-limited conditions of the Tyrrhenian Sea, combined with a decreased ability to compete with uncalcified seaweeds[8,26], which grew well in the high CO_2 levels at the vents[21,24]. We have shown that although some organisms can up-regulate calcification at lowered carbonate saturation states, they rely on protective organic layers to avoid dissolution, and projected levels of ocean acidification are likely to increase erosion of unprotected biogenic carbonate structures[27]. Worryingly, the coastal calcifiers that we transplanted along natural CO_2 gradients were more vulnerable to the effects of ocean acidification when the water was warmest, indicating that ocean acidification will probably exacerbate the mass benthic mortality events that have been recorded with increasing frequency in the warming Mediterranean Sea.

Methods

Seawater carbonate chemistry. Seawater temperature was measured using Hobo Onset loggers. Total alkalinity (A_T), determined by Gran titration[28], and pH$_T$ (in total scale) were measured frequently *in situ* and during aquarium experiments (Supplementary Tables S2, S3). Carbonate system parameters (pCO_2, CO_3^{2-}, HCO_3^-, dissolved inorganic carbon (C_T) and the saturation state of calcite (Ω_c) and aragonite (Ω_a)) were calculated from pH$_T$, mean A_T, temperature and mean salinity (38) using CO_2SYS (ref. 29).

Field transplantation and experimental design. Supplementary Table S1 summarizes the experiments and measurements performed *in situ* and in aquaria. Briefly, samples of *B. europaea* and *C. caespitosa* were collected around Castello Aragonese in March 2008 and 2009, glued (HoldFast, Ohio, USA) onto individual PVC plates and transplanted near CO_2 vents, where pH$_T$ was reported to vary from 7.2 to 7.9 units[21], and 100–150 m away from the vents in normal pH (Supplementary Fig. S1). In 2008, all individuals were collected after three months to measure their net calcification (live samples) and dissolution rates (dead samples), except for three live *C. caespitosa* colonies marked with Alizarin red that were collected after seven months to measure their linear extension rates. In 2009, coral samples were collected after three, four and seven months (only *B. europaea*) to measure their gross calcification rates in aquaria.

In 2009, mussels were collected from the port of Ischia; some were used to measure gross calcification rates at pH$_T$ 7.4 and 8.0 (May 2009), the rest were transplanted to mean pH$_T$ 7.2 and 8.0 to measure dissolution rates (after two months on dead samples) and gross calcification rates after two and five months. Pictures were analysed using UTHSCSA Image Tool (http://ddsdx.uthscsa.edu/dig/download.html) to estimate the surface area of shell lacking periostracum. One valve of each mussel was photographed using scanning electron microscopy.

In 2009, limpets with high degrees of shell dissolution were collected within CO_2 vents (mean pH$_T$ 6.6–6.8) and outside the vents (mean pH$_T$ 8.1–8.2). Some of them were incubated in aquaria using an orthogonal experimental design to measure gross calcification rates, whereas other limpets were re-transplanted at the site of collection. Some limpets collected from mean pH$_T$ 8.1 were prepared to measure shell dissolution rates at the vents at mean pH$_T$ 6.5.

Biotic responses. Coral net calcification and dissolution rates were measured using the buoyant weight technique[15,22] on live and dead samples, respectively. Dissolution of dead skeletons and shells was measured *in situ* as well as in aquaria under controlled pH and temperature conditions. Coral skeletons were cleaned by dissolving their tissues in H_2O_2 and dead shells were obtained by removing the animal with a scalpel. The inner shell surface of limpets and mussels were coated in transparent water-resistant paint to simulate the presence of the animal restricting dissolution to the outer shell surface.

Coral, limpet and mussel gross calcification rates were measured using the ^{45}Ca technique[30], which measures the rate of calcium deposited by the animal and is largely unaffected by dissolution in our experimental set-up. Animals were held for

a week in aquaria spiked with $^{45}CaCl_2$ (final activity of 50 Bq ml^{-1}) at temperature and pH conditions similar to their collection sites. pH_{NBS} was controlled in each aquarium to within ±0.05 pH units using a continuous pH-stat system (IKS, Karlsbad), which bubbled pure CO_2 into aquaria that were continuously aerated with CO_2-free air. Shells and skeletons were then prepared (see Supplementary Methods) and dissolved in HCl, and the amount of ^{45}Ca deposited was measured in 10 ml of scintillation medium using a liquid scintillation counter (2100 TR Packard; Tricarb). Non-biological incorporation of ^{45}Ca (adsorption) on exposed skeletons and shells was estimated using an identical protocol on dead skeletons and incubated shells. Adsorbed [^{45}Ca] (less than 5%) was subtracted from the total amount measured on live samples.

Statistical analyses. Details of statistical analyses performed and all the results are in the Supplementary Methods; tests were performed in SPSS 17.0 (SPSS, Chicago). Data were analysed by Type III Sums of Squares GLM.

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Author contributions

R.R-M. and F.B. designed the study. Most experiments were performed by R.R-M., who wrote the paper in collaboration with F.H., J.M.H-S., M.F. and J-P.G. Coral radiotracer incorporation was performed by F.H. Scanning electron microscopy was performed by E.T. on corals and C.B. on mussels. A.F. performed the statistical analysis. All authors read and commented on the manuscript.

Additional information

The authors declare no competing financial interests. Supplementary information accompanies this paper on www.nature.com/natureclimatechange. Reprints and permissions information is available online at http://www.nature.com/reprints. Correspondence and requests for materials should be addressed to R.R-M.