that facilitating DNA demethylation in incompletely reprogrammed cells — using a chemical inhibitor of the DNA-methylating enzyme DNMT1 — leads to a considerable response. After treatment with the inhibitor, a sizeable fraction of the stable intermediate cells exhibit three iPS-cell-like characteristics: significant demethylation of the pluripotency genes; reactivation of genes normally expressed in ES cells; and an ability to form teratomas (benign tumours) composed of all three embryonic cell layers when injected under the skin of adult mice.

There was also a time-dependent aspect to the inhibitor's effect. Incorporating this demethylating agent into the early stages of the reprogramming protocol interfered with reprogramming, whereas its addition at later stages increased the number of ES-cell-like colonies fourfold. But the demethylating agent was much more effective at enhancing reprogramming than a theoretically more specific inhibition of DNMT1 using the technique of RNA interference (RNAi). The demethylation results therefore remain open to interpretation, because the drug might have indirect or nonspecific effects, and/or the specific RNAi approach might be much less efficient at decreasing methylation.

In one of the stable intermediate cell lines, inhibitor-induced DNA demethylation alone was not sufficient to increase reprogramming efficiency. This suggests that the other potential impediment — incomplete repression of genes specifying a particular cell type — may block full reprogramming in these cells. But inhibiting the expression of several such genes with RNAi did not help. Only when RNAi-mediated inhibition of transcription factors was combined with chemically induced DNA demethylation did these intermediate cells become more amenable to reprogramming. The authors conclude that incomplete suppression of such genes and failure to demethylate DNA both interfere with reprogramming of adult cells, and that removing these impediments will enhance the efficiency of direct reprogramming. It remains to be seen whether the iPS cells generated from this more efficient protocol can contribute to the germ line when injected into a blastocyst (70–100-cell embryos), a rigorous test of their functional similarity to ES cells.

Research into direct reprogramming is advancing rapidly. Reprogramming protocols that exclude the cancer-associated gene c-*myc* have been developed[5,13]. Differentiated human cells have now been reprogrammed with the same four-gene cocktail, including cells from young and older individuals[5–7]. And the therapeutic potential of iPS cells has been demonstrated in a 'humanized' mouse, in which globin genes were replaced with human globin genes so as to model sickle-cell anaemia[14]. It will be interesting to know whether the DNA demethylating agent or inhibition of cell-type-specific factors that Mikkelsen and colleagues[2] describe will improve the efficiency of the reprogramming protocols used for human cells, and of protocols lacking c-*myc* (refs 5, 13).

iPS cells and the relatively simple methods used to generate them are of fundamental importance to biology. Reprogramming shatters the long-standing concept that the identity of differentiated adult cells is indelible. That DNA demethylation is essential for direct reprogramming is particularly interesting as this process is also strictly necessary for reprogramming by nuclear transplantation, and is a common mechanism in human cancers. Although we know very little about how DNA demethylation happens naturally, or how to manipulate it in a gene-specific way, this process clearly guides several essential cellular transition events[15]. Another puzzle is whether DNA demethylation associated with direct reprogramming involves just a few crucial genes, or occurs genome-wide. Given the current international investment in comprehensively mapping DNA methylation and other epigenetic modifications genome-wide, the 'red-hot' iPS cells will undoubtedly garner even more attention. On research into iPS cells, E. E. Cummings might have commented "into the strenuous briefness, again". ■

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CLIMATE CHANGE

Acid test for marine biodiversity

Ulf Riebesell

Rising levels of atmospheric carbon dioxide lead to acidification of the oceans. A site in the Mediterranean, naturally carbonated by under-sea volcanoes, provides clues to the possible effects on marine ecosystems.

Much of the carbon dioxide released into Earth's atmosphere by human activities is absorbed by the oceans[1]. When dissolved in water, CO_2 forms carbonic acid. Anthropogenic carbon emissions are therefore leading to global acidification of the surface ocean[2], with uncertain consequences for marine life.

On page 96 of this issue, Hall-Spencer *et al.*[3] describe conditions off the island of Ischia near Naples, Italy (Fig. 1). Here, the release of CO_2 from under-sea volcanoes causes local acidification of sea water by as much as 1.5 pH units below the average ocean pH of 8.1–8.2. Although surrounded by a diverse rocky shore community with abundant calcareous organisms, the CO_2 venting site is impoverished in sea urchins and coralline algae, and is bare of stony corals. The shells of snails found in this area are weakened, and snail juveniles are completely absent. Are these changes a foretaste of the fate of the oceans in general?

Adverse effects of ocean acidification, particularly on organisms that build shells and skeletons from calcium carbonate, have been reported from experiments on individual species and enclosed communities[4,5]. Such experiments rely almost exclusively on abrupt and short-term changes in CO_2 concentrations, raising questions about the relevance of the observed responses to marine ecosystems exposed to high CO_2 and low pH over periods of years or decades. This includes uncertainties about the ability of marine organisms to adapt to the projected ocean acidification, and whether species sensitive to high CO_2 and low pH might be replaced by more robust forms of life without jeopardizing the overall functioning of the ecosystem.

Hall-Spencer *et al.*[3] take research in this field an important step forwards by investigating the long-term biological effects of permanent exposure to high CO_2 concentrations on a natural ecosystem. In addition to confirming laboratory-based results on individual species, they see a substantial shift in the benthic community composition, with no indication of adaptation or replacement of sensitive species by others capable of filling the same ecological niche. As predicted from previous work[6], however, there are winners as well as losers in ocean acidification and carbonation. Although calcareous groups generally decline in abundance or vanish completely, photosynthetic groups such as sea grasses and brown algae benefit from the higher CO_2 availability by increasing their biomass.

R. RODOLFO-METALPA

Figure 1 | Sparkling sea water. **Venting of volcanic CO_2 at a Mediterranean site off the island of Ischia provides the opportunity to observe changes in the community structure of a rocky shore ecosystem along gradients of decreasing pH close to the vents. Groups such as sea urchins, coralline algae and stony corals decline in abundance or vanish completely with decreasing pH. Sea grasses and brown algae benefit from elevated CO_2 availability close to the vent by increasing their biomass. Similar high CO_2/low pH conditions are on the verge of progressively developing ocean-wide through the uptake of fossil-fuel CO_2 by the surface ocean.**

This study[3] is a compelling demonstration of the usefulness of natural CO_2 venting sites in assessing the long-term effects of ocean acidification on sea-floor ecosystems, an approach that undoubtedly needs to be further explored. But there are considerable differences between such systems and the situation arising from global-scale ocean acidification caused by rising atmospheric CO_2. For example, temporal and spatial variability in CO_2 and pH perturbations, induced in part by changes in the direction and intensity of water currents, complicate the determination of a reliable dose–response relationship. Large but short-term variation in pH may itself be stressful to some organisms owing to the extra physiological burden of acclimating to ever-shifting conditions. In addition, mobile species and planktonic stages continually move or are carried into the venting area, providing a supply of organisms previously unexposed to high CO_2 and low pH. This further complicates the extrapolation of CO_2 effects from volcanic vents to global-scale ocean acidification. Invasion of non-adapted organisms may also cause short-term stress to those organisms, possibly amplifying the range of high-CO_2 responses.

In the case of unabated CO_2 emissions, ocean acidification may develop to pose an unprecedented threat to marine life. Our understanding of the processes that underlie its observed effects on ecosystems and biogeochemistry is still rudimentary, as is our ability to forecast its impacts. There is an urgent need to develop tools to assess and quantify such impacts across the entire range of biological responses, from subcellular regulation to ecosystem reorganization, and from short-term physiological acclimation to evolutionary adaptation.

Hall-Spencer *et al.*[3] provide independent support for conclusions, reached by experimental studies, that ocean acidification can cause a loss of biodiversity and trigger shifts in ecosystem structure and function[4–6]. They also demonstrate that, although natural CO_2 venting sites are not precise analogues of global-scale ocean acidification, they can provide essential information about high-CO_2 effects on spatial and temporal scales, which are otherwise difficult to address. Tackling this emerging threat to marine biota calls for a coordinated research effort and requires "a coherent global vision ... to better determine the impacts of climate change on marine systems"[7]. ■

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See also page 16.

50 & 100 YEARS AGO

50 YEARS AGO

At a meeting of the Linnean Society on July 1, attended by members of the Darwin and Wallace families, representatives of other societies and institutions and members of the Linnean Society, the president, Dr. C. F. A. Pantin, unveiled a plaque in the meeting room commemorating the centenary of the reading before the Society on July 1, 1858, of the joint communication by Charles Darwin and Alfred Wallace on their theory of evolution by natural selection. At the meeting a hundred years ago neither Darwin nor Wallace was present: Darwin because of family bereavement and illness, and Wallace was still in Ternate. The papers were communicated by Sir Charles Lyell and Dr. (later Sir) J. D. Hooker ... Hooker, writing to Francis Darwin at a later date giving an account of the meeting, said " ... The interest excited was intense, but the subject too novel and too ominous for the old School to enter the lists before armouring. It was talked over after the meeting, 'with bated breath' ..."
From *Nature* 5 July 1958.

100 YEARS AGO

The list of honours issued on the occasion of His Majesty's birthday includes the name of a few men distinguished for their work in pure or applied science ... Some reference has been made in the daily papers to the ratio of honours awarded to naval and military men, the suggestion being that the Army receives an undue share of these distinctions. With the demands of the two services for recognition we are not concerned, but the question induces us to ask what ratio exists between the award of honours to men who devote their lives to work which promotes the scientific progress of the country and those who do not? ... Probably the reason is that ministers and officials who are chiefly concerned with the affairs of State and Court live in a world in which science and the results of science are almost unknown.
From *Nature* 2 July 1908.

LETTERS

Volcanic carbon dioxide vents show ecosystem effects of ocean acidification

Jason M. Hall-Spencer[1], Riccardo Rodolfo-Metalpa[1], Sophie Martin[2], Emma Ransome[1], Maoz Fine[3,4], Suzanne M. Turner[5], Sonia J. Rowley[1], Dario Tedesco[6,7] & Maria-Cristina Buia[8]

The atmospheric partial pressure of carbon dioxide (p_{CO_2}) will almost certainly be double that of pre-industrial levels by 2100 and will be considerably higher than at any time during the past few million years[1]. The oceans are a principal sink for anthropogenic CO_2 where it is estimated to have caused a 30% increase in the concentration of H^+ in ocean surface waters since the early 1900s and may lead to a drop in seawater pH of up to 0.5 units by 2100 (refs 2, 3). Our understanding of how increased ocean acidity may affect marine ecosystems is at present very limited as almost all studies have been *in vitro*, short-term, rapid perturbation experiments on isolated elements of the ecosystem[4,5]. Here we show the effects of acidification on benthic ecosystems at shallow coastal sites where volcanic CO_2 vents lower the pH of the water column. Along gradients of normal pH (8.1–8.2) to lowered pH (mean 7.8–7.9, minimum 7.4–7.5), typical rocky shore communities with abundant calcareous organisms shifted to communities lacking scleractinian corals with significant reductions in sea urchin and coralline algal abundance. To our knowledge, this is the first ecosystem-scale validation of predictions that these important groups of organisms are susceptible to elevated amounts of p_{CO_2}. Sea-grass production was highest in an area at mean pH 7.6 (1,827 µatm p_{CO_2}) where coralline algal biomass was significantly reduced and gastropod shells were dissolving due to periods of carbonate sub-saturation. The species populating the vent sites comprise a suite of organisms that are resilient to naturally high concentrations of p_{CO_2} and indicate that ocean acidification may benefit highly invasive non-native algal species. Our results provide the first *in situ* insights into how shallow water marine communities might change when susceptible organisms are removed owing to ocean acidification.

Short-term laboratory experiments show that many calcareous organisms may be unable to build their skeletons as oceans acidify over the next 100 years[6,7]. This may combine with other stresses, such as global warming, to drive tropical coral reefs towards functional collapse[8]. However, attempts to determine whether expectations on the basis of laboratory experiments and modelled predictions translate to field conditions have been hindered by the difficulty of imitating ocean acidification conditions *in situ* for sufficient periods to affect communities of macroorganisms.

Natural CO_2 flux from volcanic vents and high heat flow areas amounts to less than 0.5% of anthropogenic emissions to the global carbon budget, but can alter local ocean chemistry[9,10]. Marine CO_2 vents are abundant in the Mediterranean, especially around Italy and Greece where they typically eject volcanic fluids containing up to 1–2% hydrogen sulphide[10,11]. Some marine CO_2 vents are at ambient seawater temperature and lack toxic sulphur compounds; such vents can prevail for years to millenia[12] and may be used as natural experiments to advance our understanding of ocean acidification at the ecosystem level.

We studied cold vent areas off Ischia in Italy (Fig. 1) where sea water was being acidified by gas comprising 90.1–95.3% CO_2, 3.2–6.6% N_2, 0.6–0.8% O_2, 0.08–0.1% Ar and 0.2–0.8% CH_4 (no sulphur). Salinity (38%) and total alkalinity (2.5 mequiv. kg^{-1}) were homogeneous between survey stations and temperature-matched ambient seasonal fluctuations (13–25 °C). Vents occurred on the north and south sides of Castello d'Aragonese (40° 043.84′ N; 13° 57.08′ E) adjacent to a steeply sloping rocky shore. At the south vent site gas was emitted at 1.4×10^6 litre day^{-1} in an area of about 3,000 m^2 (mainly >5 vents m^{-2}); at the north site gas was emitted at 0.7×10^6 litre day^{-1} in an area of about 2,000 m^2 (mainly <5 vents m^{-2}). No seasonal, tidal or diurnal variation in gas flow rates was detected in 2006–07. The pH and saturation states (Ω) of calcite and aragonite varied with sea state, being lowest on calm days, and showed large decreases as p_{CO_2} amounts increased from approximately 300 to more than 2,000 µatm through the venting gas fields (Fig. 2 and Supplementary Table 2). Here we examine ecological tipping points along gradients of increasing p_{CO_2}, comparing normal pH stations (N_1, S_1 and P_1–P_2) with three stations that had reductions in mean pH of 0.2–0.4 units (N_2, S_2 and P_3; Fig. 1) and three stations (P_4, N_3 and S_3) with reductions in mean pH of 0.6–1.5 units which are more representative of the localized effects to be expected from deliberate CO_2 sequestration[13] rather than from global ocean acidification.

Rocky-shore stations with a mean pH of 7.8–7.9 (mean p_{CO_2} 804–957 µatm) showed a 30% reduction in species numbers (notably calcifiers) compared with the normal pH stations (Supplementary Tables 3 and 4). Temporal variability in p_{CO_2} will have contributed to the pronounced biodiversity shifts observed, as these stations experienced short periods of pH as low as 7.4–7.5. Organisms with aragonite skeletons were common outside the vents (for example, *Halimeda* algae and the corals *Caryophyllia*, *Cladocora* and *Balanophyllia*) but were absent at mean $\Omega_{arag} \leq 2.5$ (minimum Ω_{arag} 0.8–1.2), providing *in situ* support for predictions of global coral reef dissolution at these concentrations[8]. Although scleractinians can survive skeletal dissolution as polyps in the laboratory[14], reduced calcification due to low Ω_{arag} may result in increased risk to predation or competition in open ecosystems. The only Cnidaria in waters undersaturated with aragonite were anemones such as *Anemonia viridis*, which may benefit from increased p_{CO_2} for photosynthesis of its endosymbiotic dinoflagellates. Although atmospheric diffusion of CO_2 is not predicted to result in aragonite undersaturation in shallow waters of the Mediterranean,

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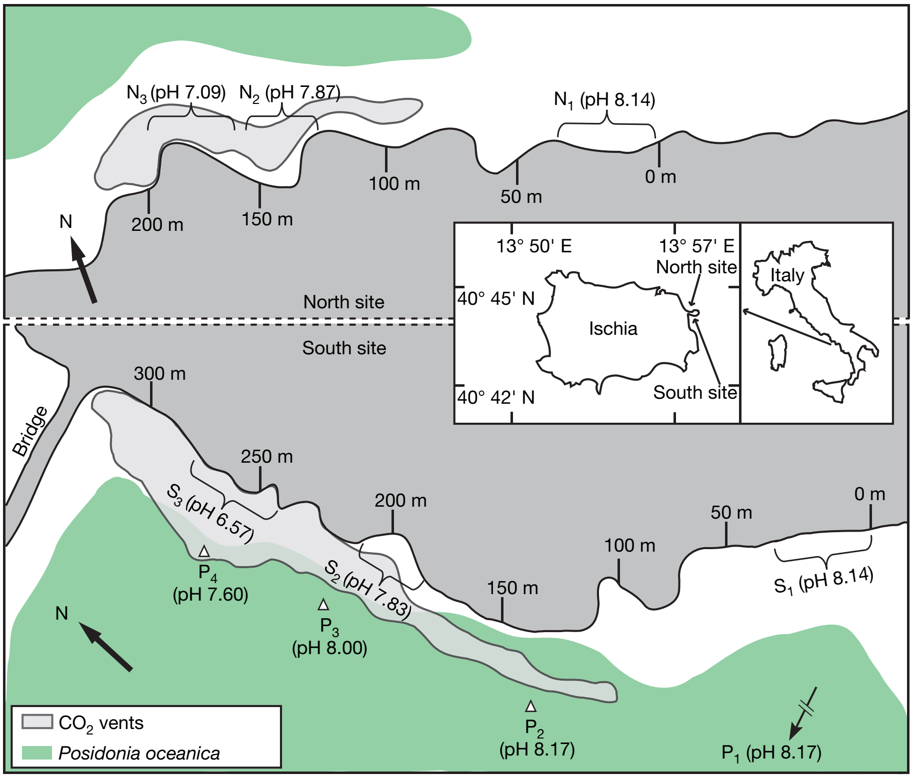

Figure 1 | Map of CO_2 vent sites north and south of Castello d'Aragonese, off Ischia Island, Italy. Mean surface pH is shown at 35-m-wide rocky-shore stations N_1–N_3 and S_1–S_3. Mean subtidal pH is shown at stations P_1–P_4, together with the distributions of CO_2 vents and *P. oceanica* sea-grass meadows. Reference station P_1 was at a 3-m depth, 400 m from the arrow shown.

observations of such areas are relevant to the localized effects caused by deliberate CO_2 sequestration and to the widespread effects predicted for areas that at present have low Ω_{arag}, given that high-latitude pteropods and coral reefs may be unable to make their skeletons by the year 2100 (refs 7, 13).

Mesocosm experiments have led to predictions that Corallinaceae, which help to protect against coral reef erosion in the tropics, are vulnerable to ocean acidification due to the solubility of their high magnesium calcite skeletons[15,16]. We found that Corallinaceae cover was significantly reduced at lowered pH (Table 1 and Supplementary Tables 2–4). As coralline algal cover fell from >60% outside the vent area to zero within it, non-calcareous algal cover increased significantly from near zero to >60% (Fig. 2 and Table 1). A suite of algal genera proved to be resilient to naturally high amounts of p_{CO_2} (for example, *Caulerpa*, *Cladophora*, *Asparagopsis*, *Dictyota* and *Sargassum*), some of which include invasive alien species that have begun to alter shallow marine ecosystems worldwide[17]. This adds to previously scant experimental information about the sorts of marine phototrophs that have enhanced growth and undiminished rates of photosynthesis at elevated concentrations of CO_2 (refs 4, 5, 18, 19).

The analysed *Posidonia oceanica* shoots were >10 yr old at the subtidal study sites and will have integrated the effects of lowered pH over this time. Sea-grass leaves at P_1 (pH 8.2) had 75% cover of calcified epiphytes but only 2% cover at P_4 (mean pH 7.6) with a significant reduction in epiphytic calcium carbonate per leaf (Table 1 and Figs 3 and 4). When heavily epiphytised leaves were transplanted from station P_1 to P_4 they showed complete dissolution of Corallinaceae in 2 weeks, whereas transplants moved within P_1 were unaffected. Mesocosm experiments have shown that sea-grass production can be enhanced at high p_{CO_2} (ref. 19). We found no difference (Table 1) in the photosynthetic performances of individual *P. oceanica* leaves between the four stations (mean ± s.e.m., photosynthetic efficiency (F_v/F_m) 0.74 ± 0.01 and electron transport rates $(ETR)_{max}$ 8.4 ± 1.9, $n = 40$) but sea-grass production was

Table 1 | Analysis of ecological tipping-points along marine acidity gradients

Category, site	*F* (d.f.)	*P* value	Tukey's test, site comparison
Corallinaceae cover, north	$F_{2,21} = 43.8$	0.000	$N_1 > N_2 > N_3$
Corallinaceae cover, south	$F_{2,21} = 48.0$	0.000	$S_1 > S_2 = S_3$
Non-calcareous crustose algal cover, north	$F_{2,21} = 0.31$	0.74	NS
Non-calcareous crustose algal cover, south	$F_{2,21} = 62.5$	0.000	$S_1 = S_2 < S_3$
Sea-grass epiphyte weight, south	$F_{3,315} = 176.2$	0.000	$P_1 > P_2 > P_3 > P_4$
Sea-grass F_v/F_m, south	$F_{3,36} = 0.13$	0.93	NS
Sea-grass ETR_{max}, south	$F_{3,36} = 0.06$	0.98	NS
Sea-grass shoot density, south	$F_{3,16} = 67.6$	0.000	$P_1 = P_2 = P_3 < P_4$
Sea urchin abundance, north	$F_{2,9} = 14.7$	0.001	$N_1 > N_2 = N_3$
Sea urchin abundance, south	$F_{2,9} = 65.3$	0.000	$S_1 > S_2 = S_3$
C. stellatus abundance, north	$F_{2,21} = 0.72$	0.50	NS
C. stellatus abundance, south	$F_{2,21} = 29.4$	0.000	$S_1 = S_2 > S_3$
O. turbinata abundance, north	$F_{2,21} = 3.50$	0.049	$N_1 = N_2 > N_3$
O. turbinata abundance, south	$F_{2,21} = 6.39$	0.007	$S_1 = S_3 < S_2$
P. caerulea abundance, north	$F_{2,21} = 22.8$	0.000	$N_1 > N_2 > N_3$
P. caerulea abundance, south	$F_{2,21} = 9.24$	0.001	$S_1 = S_2 > S_3$

Significant differences were assessed using one-way analysis of variance (ANOVA, *F*) and Tukey's HSD (honestly significant difference) post-hoc tests. Data are from stations north and south of Castello d'Aragonese, Ischia, Italy in spring 2007. d.f., degrees of freedom, NS, not significant.

highest at mean pH 7.6 (biomass increased by 2.8 g m^{-2} day^{-1} at mean p_{CO_2} 1,827 μatm) where shoot density was significantly higher (Table 1 and Fig. 3) and approximately 30% higher than that known anywhere else around Ischia[12].

Sea urchins (*Paracentrotus lividus*, *Arbacia lixula*), which have high magnesium calcite skeletons, were the most common large invertebrates on sublittoral rock outside the vents but their abundance was significantly reduced where pH reached minima of 7.4–7.5 (Table 1 and Fig. 2). This supports physiological studies showing that sea urchins are vulnerable to a rise in CO_2, and is a concern as sea urchin loss can drive deteriorations in ecosystem complexity and stability[20,21]. Although sea urchins cannot close off their supply of ambient sea water, some organisms can do this to avoid pH minima. Other calcitic organisms, such as the barnacle *Chthamalus stellatus*, for example, may survive pH minima by closing their rostral plates as their abundance was not significantly reduced until extremely low mean pH 6.6 (Table 1 and Fig. 2). Juveniles of *Osilinus turbinata* and *Patella caerulea* gastropods were absent in areas with pH minima ≤7.4, where all adult gastropod shells (including *Hexaplex trunculus* and *Cerithium vulgatum*) were weakened by the acidified sea water (Figs 2 and 4, Table 1 and Supplementary Video), an effect which probably increases their risk of predation[22].

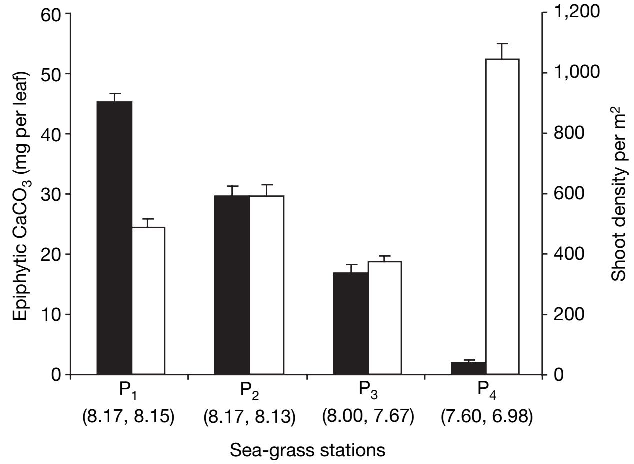

Figure 3 | Sea-grass shoot density and amount of epiphytic $CaCO_3$ on leaves growing at differing pH levels south of Castello d'Aragonese. Shoot density (open column, $n = 4$, mean and s.d.) and epiphytic $CaCO_3$ (filled column, $n = 80$, mean and s.d.) for data from 18 April to 9 May 2007 at various pH levels (mean and minimum values are shown; P_1 $n = 30$, P_2 $n = 16$, P_3 $n = 23$ and P_4 $n = 37$).

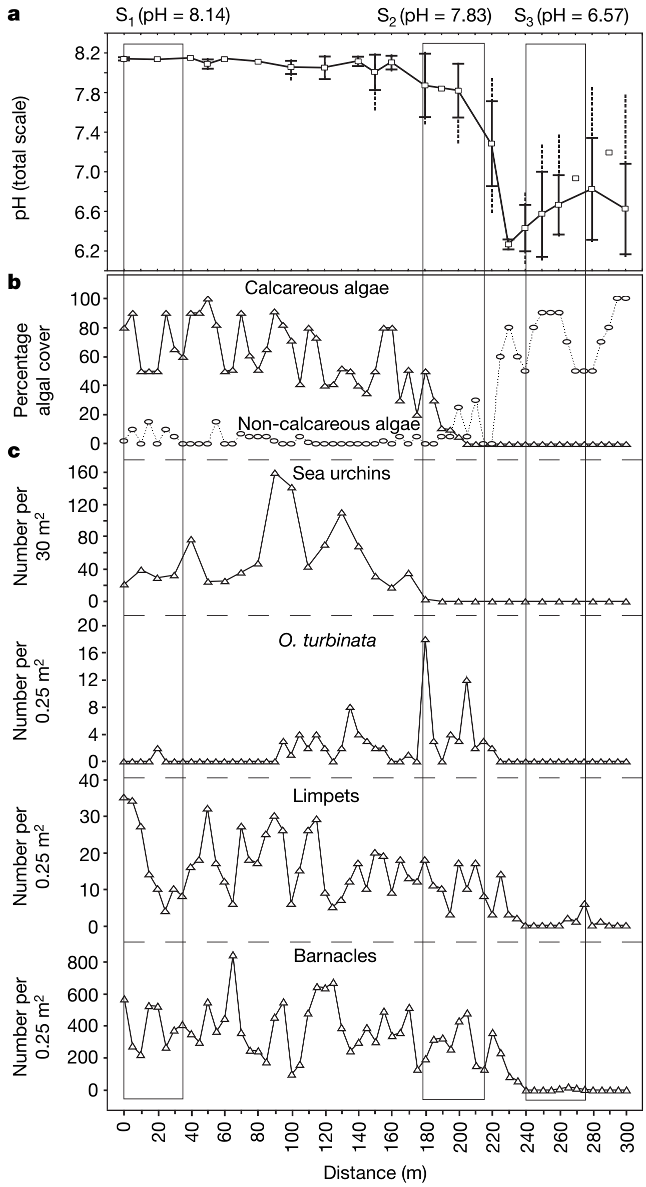

Figure 2 | Variation in pH, cover of algae and abundance of species at CO_2 vents south of Castello d'Aragonese. Data are from stations S_1–S_3 (see Fig. 1) from 18 April to 9 May 2007. **a**, The mean pH ± s.d. (cross bars) is shown. Ranges are denoted by the dotted line; $n = 6$ at 0 m, $n = 11$ at 50 m, 100 m, 250 m and 300 m, $n = 9$ at 220 m, 260 m, 280 m and $n = 12$ at 150 m and 200 m. **b**, The percentage cover of calcareous (triangles) and non-calcareous algae (circles) is shown. **c**, The abundances of sea urchins, *O. turbinata*, limpets and barnacles.

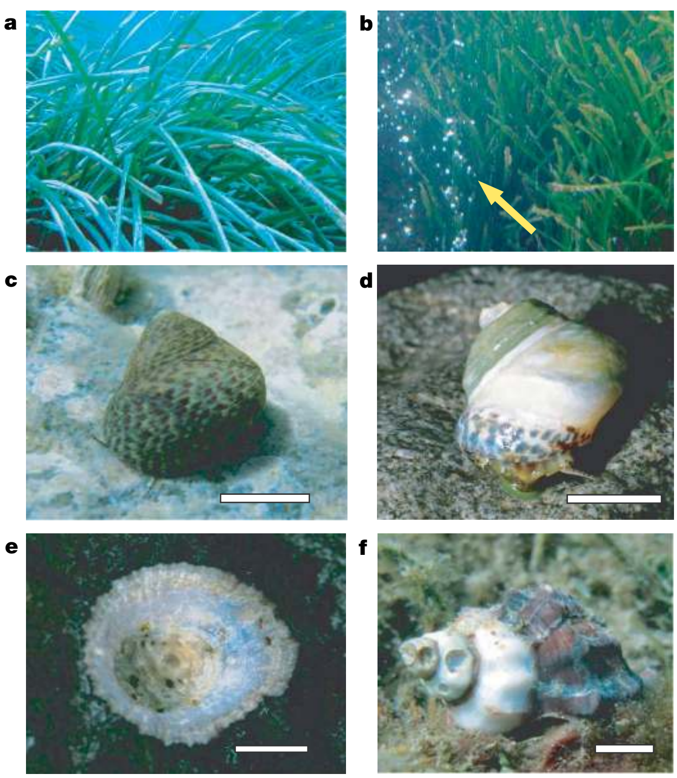

Figure 4 | Dissolution of calcified organisms due to naturally acidified sea water. **a**, **b**, *Posidonia oceanica* with heavy overgrowth of Corallinaceae at pH 8.2 (**a**) and lacking Corallinaceae at mean pH 7.6 (**b**); arrow indicates bubbles from the CO_2 vent field. **c**, **d**, Typical examples of *O. turbinata* with the periostracum intact at pH 8.2 (**c**) and with old parts of the periostracum removed at mean pH 7.3 (**d**). **e**, **f**, Live *P. caerulea* (**e**) and *H. trunculus* (**f**) showing severely eroded, pitted shells in areas of minimum pH 7.4. Scale bars represent 1 cm.

Vent systems are not perfect predictors of future ocean ecology owing to temporal variability in pH, spatial proximity of populations unaffected by acidification and the unknown effects of other global changes in parameters such as temperature, currents and sea level. However, such vents acidify sea water on sufficiently large spatial and temporal scales to integrate ecosystem processes such as production, competition and predation. Lush stands of sea-grass and brown algae can thrive along natural pH gradients where aragonitic and then calcitic calcareous organisms are lost owing to skeletal dissolution. This confirms experimental and modelling predictions that differential responses of benthic species to decreased pH can lead to substantial changes in community structure[4–8,13–16]. Many of the organisms that were adversely affected by reductions in pH at our study sites belong to groups that existed before and after periods of similar reductions in the past (for example, calcified algae, corals and sea urchins)[14]. It is unknown whether there will be sufficient refugia or enough time for these groups to adapt to survive the rapid rate of ocean acidification predicted due to anthropogenic CO_2. This opportunity to observe the tipping points at which principal groups of marine organisms are affected by lowered pH proves that, even without global warming, the projected rise in atmospheric CO_2 concentration is hazardous, as ocean acidification will probably bring about reductions in biodiversity and radically alter ecosystems.

METHODS SUMMARY

Vent gases were collected in pre-evacuated glass flasks partly filled with 0.1 M $Cd(OH)_2$ and 4 N NaOH solution (see Supplementary Video). Uncondensable gases were collected in the headspace, inorganic residual gas compounds were analysed using thermal conductivity chromatographs, methane was analysed with a flame ionization detector and ion chromatography was used to analyse condensable gases such as CO_2 dissolved during collection. Between 18 April and 9 May 2007, surface and bottom water samples were regularly taken for measurements of the spatial and temporal variability in pH (in total scale), total alkalinity and salinity in various weather conditions. In winter 2006, and spring and autumn 2007, intertidal and subtidal SCUBA surveys were made of the main macroorganisms present within and adjacent to the vents to 3 m depth. Epibiont calcium carbonate on *P. oceanica* leaves was quantified along a gradient of pH; leaves that were heavily encrusted with Corallinaceae were transplanted from a reference site into an area with mean pH 7.6 then reassessed after 2 weeks. *Posidonia oceanica* production, growth dynamics and shoot density was estimated at stations P_1–P_4 where their photosynthetic efficiency (F_v/F_m) and electron transport rates (ETR) were measured *in situ* using a diving pulse amplitude modulation (PAM), and in the laboratory using an imaging PAM.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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