

# Effects of naturally acidified seawater on seagrass calcareous epibionts

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**Surface ocean pH is likely to decrease by up to 0.4 units by 2100 due to the uptake of anthropogenic CO<sub>2</sub> from the atmosphere. Short-term experiments have revealed that this degree of seawater acidification can alter calcification rates in certain planktonic and benthic organisms, although the effects recorded may be shock responses and the long-term ecological effects are unknown. Here, we show the response of calcareous seagrass epibionts to elevated CO<sub>2</sub> partial pressure in aquaria and at a volcanic vent area where seagrass habitat has been exposed to high CO<sub>2</sub> levels for decades. Coralline algae were the dominant contributors to calcium carbonate mass on seagrass blades at normal pH but were absent from the system at mean pH 7.7 and were dissolved in aquaria enriched with CO<sub>2</sub>. In the field, bryozoans were the only calcifiers present on seagrass blades at mean pH 7.7 where the total mass of epiphytic calcium carbonate was 90 per cent lower than that at pH 8.2. These findings suggest that ocean acidification may have dramatic effects on the diversity of seagrass habitats and lead to a shift in the biogeochemical cycling of both carbon and carbonate in coastal ecosystems dominated by seagrass beds.**

**Keywords:** acidification; CO<sub>2</sub>; carbonate production; calcareous epibionts; coralline algae

## 1. INTRODUCTION

One-third of the CO<sub>2</sub> released into the atmosphere through human activities is taken up by the ocean (Sabine *et al.* 2004). As a result, the increasing CO<sub>2</sub> partial pressure ( $p\text{CO}_2$ ) in surface water lowers the pH. This decreases the carbonate (CO<sub>3</sub><sup>2-</sup>) ion concentration and lowers the saturation state ( $\Omega$ ) of calcium carbonate (CaCO<sub>3</sub>). Global ocean models forced with atmospheric CO<sub>2</sub> projections predict surface reductions of 0.2–0.4 pH units over the course of this

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century (Caldeira & Wickett 2005). This could have a major impact on calcifying organisms such as corals, coralline algae, foraminifera and coccolithophorids (see a review by Kleypas *et al.* 2006). Most studies have investigated the response of marine calcifiers to elevated  $p\text{CO}_2$  (and lower pH) through *in vitro* and short-term experiments on isolated organisms and mesocosms, an approach that raises questions about the impacts of containment and whether acute responses are representative of future, long-term effects. There is a lack of information about the impacts of lower pH on marine organisms in their natural environment and on their capacity to acclimatize and adapt to elevated  $p\text{CO}_2$  over long periods of time. Areas with naturally elevated  $p\text{CO}_2$  have the potential to provide additional insights into those obtained by modelling and mesocosm experiments, such as indicating which groups of organisms can acclimatize and adapt to long-term acidification. Here, we investigate the response of calcareous epibionts of *Posidonia oceanica* to reductions in pH in aquaria and at a volcanic CO<sub>2</sub> vent area recently described by Hall-Spencer *et al.* (2008). These calcareous epibionts are of paramount importance in the carbon cycle of seagrass ecosystems, being major contributors to CO<sub>2</sub> fluxes through their high CaCO<sub>3</sub> production and dissolution (Barrón *et al.* 2006).

## 2. MATERIAL AND METHODS

Fieldwork was conducted from 23 to 27 April 2007 at Castello Aragonese (Island of Ischia, Italy; 40°43.84' N, 13°57.08' E; figure 1), where volcanic vents occurred in shallow waters emitting  $1.4 \times 10^6 \text{ l d}^{-1}$  of gas comprising 90–95% CO<sub>2</sub>, 3–6% N<sub>2</sub>, 0.6–0.8% O<sub>2</sub>, 0.2–0.8% CH<sub>4</sub> and 0.08–0.1% Ar, without toxic sulphur (Hall-Spencer *et al.* 2008). The work was carried out within a *P. oceanica* meadow at five stations situated at approximately 3 m depth in order to have similar incident irradiance (approx.  $7500 \text{ lx d}^{-1}$ ). One reference station (St 1) was situated 400 m from the gas vent area, three stations (St 2–4) were adjacent to the vents and one station (St 5) was in the vent area. *Posidonia oceanica* shoot density and productivity was homogeneous between stations except at station 5 where shoot density was 30 per cent higher (Hall-Spencer *et al.* 2008). Bottom water samples were collected for determination of pH and total alkalinity (TA) on eight cardinal points distributed on a 6 m diameter circle (figure 1) at each station at around midday. pH (in total scale; DOE 1994) was measured immediately after collection on 2–4 different days, including calm and rough conditions, in order to determine variability due to sea state. Salinity and temperature were also assessed using a YSI/25 FT probe. Samples for determination of TA were prepared and stored according to DOE (1994) and were measured by potentiometric Gran titration. Parameters of the carbonate system were calculated from pH, TA, temperature and salinity using the program CO2SYS (Lewis & Wallace 1998). At each station, *P. oceanica* blades were collected manually in eight 1 m<sup>2</sup> quadrats situated on the eight cardinal points previously described. Ten 8–9 mm width blades were selected haphazardly from each quadrat and the distal 15 cm were sampled. Epibionts were identified and their per cent surface cover was estimated. The mass of CaCO<sub>3</sub> per blade was assessed by weighing the blades before and after dissolving CaCO<sub>3</sub> by an acid treatment (see Perry & Beavington-Penney 2005). To test the short-term effect of increased CO<sub>2</sub>, the distal 12 cm of *P. oceanica* blades heavily encrusted with coralline algae (50–70% cover) were collected at station 1 on 16 September 2004. Two sets of nine blades were maintained in two 5 l aquaria filled with water from station 1, one bubbled with CO<sub>2</sub> to maintain a pH of 7.0 and the other bubbled with air (pH 8.1, control). The coralline algal cover was estimated after two weeks. Correlation between environmental parameters and epibiont-related parameters was examined using Pearson's correlation test.

## 3. RESULTS

Coralline algae (*Hydrolithon boreale*, *Hydrolithon cruciatum*, *Hydrolithon farinosum*, *Pneophyllum confervicola*,

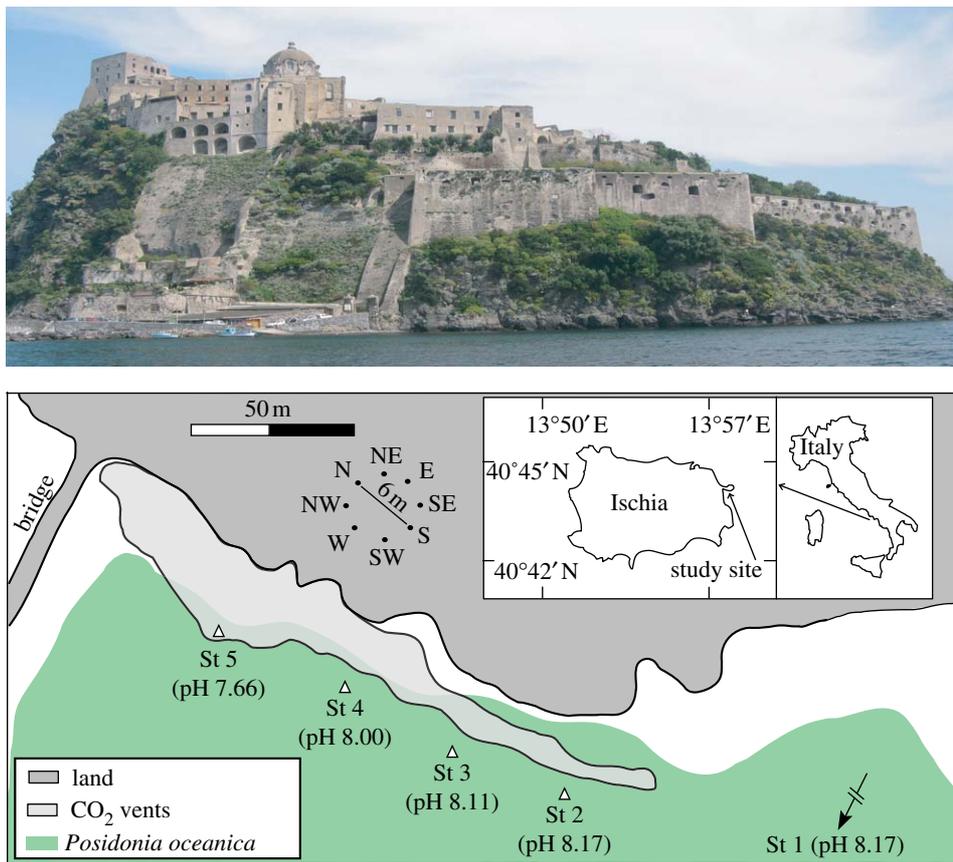


Figure 1. Locations of the sampling stations (St 1–5) at the Castello Aragonese site. Details of the eight sampling points (S, south; SE, southeast; E, east; NE, northeast; N, north; NW, northwest; W, west; SW, southwest) are given.

*Pneophyllum fragile* and *Pneophyllum zonale*) dominated the epiphytic community at stations 1–4 (18–69% cover), where the pH averaged 8.0–8.2, but were absent at station 5 where the mean pH was 7.7 (table 1; figure 2). Bryozoans (*Callopora lineata*, *Electra posidoniae*, *Microporella ciliata* and *Tubulipora* spp.) covered 1–6% of the blade surface, while non-calcified organisms such as hydrozoans had less than 1 per cent cover. Coralline cover and epiphytic  $\text{CaCO}_3$  were highly correlated with pH and the other parameters of the carbonate chemistry (Pearson's correlation test,  $p < 0.0001$ ), except TA ( $p > 0.05$ ) that was homogeneous between stations, while bryozoans ( $r = 0.28$ ,  $p = 0.08$ ) and hydrozoans ( $r = -0.29$ ,  $p = 0.07$ ) were not. Correlations between pH and epiphytic cover and  $\text{CaCO}_3$  were independent of seagrass meadow parameters being highly significant ( $p < 0.001$ ) at stations 1–4 where the meadow was homogeneous. In aquaria, epiphytic coralline algae were completely dissolved after two weeks at a pH of 7.0, whereas control samples showed no discernable change in coralline cover. No significant correlations were observed with the other measured environmental parameters such as temperature ( $p > 0.05$ ). Epiphytic  $\text{CaCO}_3$  and surface cover did not show any significant relationships with environmental parameters within a station ( $p > 0.05$ ). In particular, the pH was spatially homogeneous within a station in spite of temporal variability from day to day, with the lowest values on calm days and increasingly from station 1 to 5 (see the electronic supplementary material).

#### 4. DISCUSSION

The present study shows a significant reduction in epiphytic coralline algal cover with increasing acidification of seawater due to natural  $\text{CO}_2$  vents. Although a range of factors may be responsible for this observed shift in seagrass epiphytism, lowered pH and reduced calcite saturation levels are the most likely factors affecting coralline algal cover. Coralline algae were absent where the pH periodically fell below 7 and their calcimass was greatly affected where the pH ranged from 7.7 to 8.2. Our preliminary short-term shock experiment conducted in an aquarium at pH 7 verified that elevated  $p\text{CO}_2$  levels could cause the dissolution of calcareous epiphytes of the Mediterranean seagrass *P. oceanica*. Previous studies have shown that tropical crustose coralline algae are highly sensitive to lowered pH in mesocosm experiments. Jokiel *et al.* (2008) reported skeleton dissolution rather than growth for the species *Lithophyllum*, *Hydrolithon* and *Porolithon* sp. at a pH of approximately 7.9, relative to a normal pH of 8.2, while Kuffner *et al.* (2008) reported a drop in recruitment rate and per cent cover of 78 and 92 per cent, respectively, at a pH of 7.9. Accordingly, we found more than a 50 per cent decrease in epiphytic crustose coralline cover and  $\text{CaCO}_3$  mass at station 4, where the mean pH was 8.0. This result for coralline algae exposed to low pH for a long time confirms predictions based on short-term experiments on isolated organisms and mesocosms. Located in the vicinity of the vent area, stations 2–5 may be expected to reach lower pH than those reported. In addition, the

Table 1. Mean ( $\pm$ s.d.,  $n=16-32$ ) environmental (temperature, salinity and parameters of the carbonate system) and biological ( $\text{CaCO}_3$  mass and surface cover of epibionts) parameters at each station. (Minima and maxima are given in parentheses.)

	St 1	St 2	St 3	St 4	St 5
<i>environmental parameters</i>					
temperature ( $^{\circ}\text{C}$ )	19.4 $\pm$ 0.2 (19.0–19.5)	18.5 $\pm$ 0.0 (18.5–18.5)	19.0 $\pm$ 0.0 (19.0–19.0)	19.2 $\pm$ 0.2 (19.0–19.5)	19.3 $\pm$ 0.3 (19.0–19.5)
salinity	38.0	38.0	38.0	38.0	38.0
pH (total scale)	8.17 $\pm$ 0.01 (8.15–8.18)	8.17 $\pm$ 0.03 (8.13–8.20)	8.11 $\pm$ 0.03 (8.05–8.15)	8.00 $\pm$ 0.16 (7.67–8.16)	7.66 $\pm$ 0.32 (6.98–8.14)
TA ( $\mu\text{Eq kg}^{-1}$ )	2514 $\pm$ 6 (2504–2524)	2515 $\pm$ 5 (2506–2520)	2512 $\pm$ 5 (2505–2520)	2531 $\pm$ 3 (2528–2536)	2508 $\pm$ 7 (2497–2515)
pCO <sub>2</sub> (ppm)	311 $\pm$ 7 (298–324)	306 $\pm$ 24 (280–349)	365 $\pm$ 27 (325–435)	549 $\pm$ 258 (319–1187)	1564 $\pm$ 1169 (335–6273)
CO <sub>2</sub> ( $\mu\text{mol kg}^{-1}$ )	10 $\pm$ 0 (10–11)	10 $\pm$ 1 (9–12)	12 $\pm$ 1 (11–14)	18 $\pm$ 8 (10–38)	51 $\pm$ 38 (11–202)
CO <sub>3</sub> <sup>2-</sup> ( $\mu\text{mol kg}^{-1}$ )	274 $\pm$ 5 (265–282)	270 $\pm$ 12 (249–284)	246 $\pm$ 11 (220–264)	209 $\pm$ 54 (108–270)	124 $\pm$ 80 (24–262)
HCO <sub>3</sub> <sup>-</sup> ( $\mu\text{mol kg}^{-1}$ )	1849 $\pm$ 12 (1830–1871)	1858 $\pm$ 29 (1824–1909)	1914 $\pm$ 27 (1871–1978)	2025 $\pm$ 132 (1877–2269)	2207 $\pm$ 195 (1873–2450)
DIC ( $\mu\text{mol kg}^{-1}$ )	2133 $\pm$ 7 (2121–2146)	2138 $\pm$ 18 (2117–2170)	2172 $\pm$ 17 (2146–2212)	2252 $\pm$ 86 (2157–2416)	2382 $\pm$ 147 (2145–2676)
$\Omega_{\text{calcite}}$	6.39 $\pm$ 0.11 (6.18–6.58)	6.31 $\pm$ 0.28 (5.82–6.63)	5.75 $\pm$ 0.25 (5.14–6.16)	4.88 $\pm$ 1.26 (2.53–6.30)	2.90 $\pm$ 1.87 (0.56–6.11)
$\Omega_{\text{aragonite}}$	4.16 $\pm$ 0.07 (4.02–4.28)	4.10 $\pm$ 0.18 (3.78–4.31)	3.74 $\pm$ 0.17 (3.34–4.00)	3.17 $\pm$ 0.82 (1.65–4.09)	1.89 $\pm$ 1.22 (0.36–3.98)
<i>biological parameters</i>					
CaCO <sub>3</sub> (mg blade <sup>-1</sup> )	41 $\pm$ 6 (4–64)	24 $\pm$ 10 (0–80)	14 $\pm$ 10 (0–51)	11 $\pm$ 8 (0–51)	3 $\pm$ 1 (0–23)
corallines (% cover)	69 $\pm$ 15 (10–95)	61 $\pm$ 25 (3–95)	18 $\pm$ 22 (0–80)	29 $\pm$ 29 (0–95)	0 $\pm$ 0 (0–0)
bryozoans (% cover)	2 $\pm$ 5 (0–30)	4 $\pm$ 7 (0–30)	6 $\pm$ 9 (0–35)	1 $\pm$ 3 (0–15)	2 $\pm$ 3 (0–21)
hydrozoans (% cover)	0.2 $\pm$ 0.5 (0–3)	0.2 $\pm$ 0.5 (0–3)	0.2 $\pm$ 0.6 (0–3)	0.1 $\pm$ 0.5 (0–3)	0.3 $\pm$ 0.5 (0–8)

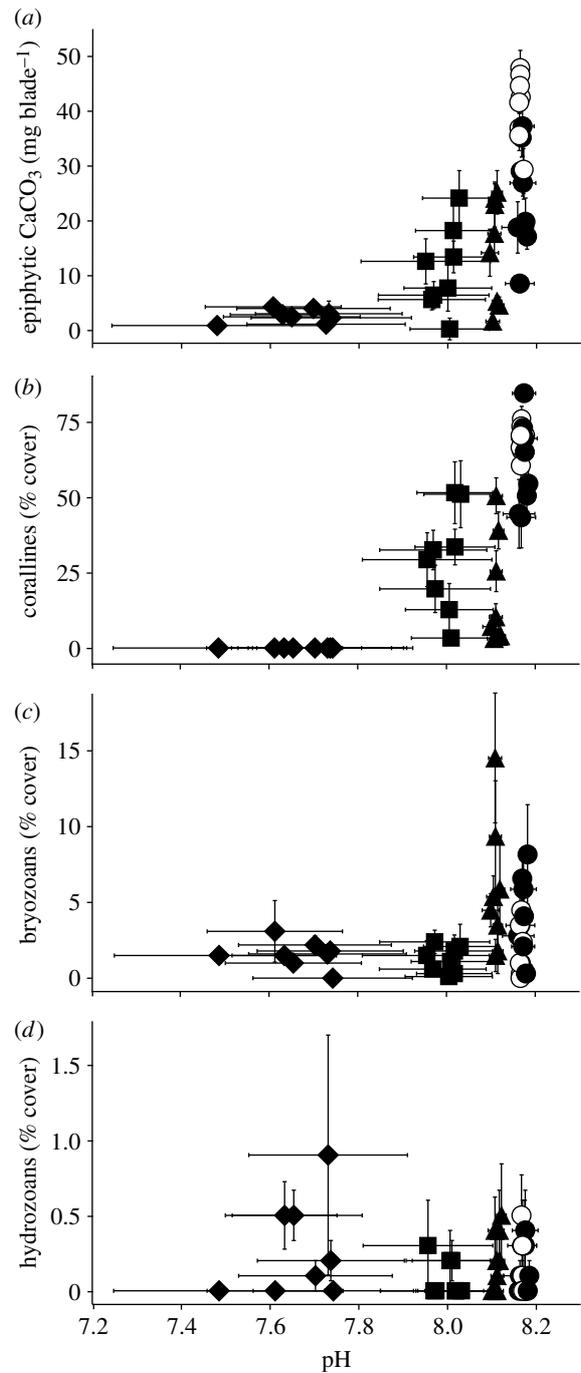


Figure 2. Mean ( $\pm$ s.e.,  $n=10$ ) weight of (a) epiphytic  $\text{CaCO}_3$  and per cent surface cover of (b) coralline algae, (c) bryozoans and (d) hydrozoans on *P. oceanica* blades measured at each sampling point of the five stations (open circles, St 1; filled circles, St 2; filled triangles, St 3; filled squares, St 4; filled diamonds, St 5). Mean pH are shown  $\pm$ s.e. ( $n=2-4$ ).

rapid pH perturbation, which can occur in these stations, could have increased physiological consequences. Accordingly, for a similar mean pH value, the coralline per cent cover and epibiont  $\text{CaCO}_3$  at station 2 represented only 90 and 60 per cent, respectively, of those measured at station 1 where pH values exhibited smaller day-to-day changes. Settlement of epiphytic coralline algae appeared to be precluded at station 5 at pH periodically lower than 7, while non-calcified organisms such as hydrozoans were not affected by such a pH decrease. Some calcified organisms, such as

bryozoans, were able to survive pH lower than 7 and tolerated high temporal pH fluctuations of more than 1 unit. The mineralogy of the bryozoans may explain the differences in their ability to resist to low pH. The skeleton of coralline algae is high magnesian calcite with Mg concentration varying from 3.5 to 6 per cent (Milliman 1974). The mineralogy of encrusting species of bryozoans tends to be calcitic but have a lower Mg content than coralline algae (0.1–3% Mg; Milliman 1974), which makes them more resistant to chemical dissolution at low pH.

The decreases in pH measured in the present study (0–0.5 units) are close to the range predicted for surface pH reductions by the end of the century (0.2–0.4 units; Caldeira & Wickett 2005). The sensitivity of coralline algae to projected seawater acidification may thus lead to drastic changes in the well-known high diversity of seagrass meadows (Hemminga & Duarte 2000) and their rate of carbonate production. Coralline algae pioneer the colonization of seagrass, making the blade more hospitable for other species such as diatoms, sponges, foraminifera, worms or turf algae (Corlett & Jones 2007). The decrease in the per cent cover of epiphytic coralline algae could therefore affect a large range of epiphytic organisms and cause subsequent changes in the associated food webs. Conversely, the seagrass *P. oceanica* itself was remarkably tolerant of low pH (Hall-Spencer *et al.* 2008). Seagrass photosynthetic rates have been shown to be higher at low pH (6–7) than at normal pH (8.2; Invers *et al.* 2001). Seagrasses could also benefit from the exclusion of encrusting epibionts since the presence of epibionts on their blades can reduce their photosynthetic rate both by acting as a barrier to carbon uptake and by reducing light intensity (Sand-Jensen 1977). Coralline algae are of significant importance, especially in the north-western Mediterranean Sea where they are the major contributors to biogenic carbonate production, and their contribution to sediment accumulation in *P. oceanica* meadows is suggested to be significant (Canals & Ballesteros 1997). They also contribute to a high CaCO<sub>3</sub> dynamic that significantly influences CO<sub>2</sub> fluxes in seagrass ecosystems (Barrón *et al.* 2006). Our results suggest that CaCO<sub>3</sub> production by epiphytic coralline algae may decrease by more than 50 per cent by the year 2100. Such a decrease may lead to drastic changes to local sediment budgets and biogeochemical cycles of carbon and carbonate in shallow-water coastal systems.

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