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Global change biology

Ocean acidification bends the mermaid's wineglass

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Ocean acidification lowers the saturation state of calcium carbonate, decreasing net calcification and compromising the skeletons of organisms such as corals, molluscs and algae. These calcified structures can protect organisms from predation and improve access to light, nutrients and dispersive currents. While some species (such as urchins, corals and mussels) survive with decreased calcification, they can suffer from inferior mechanical performance. Here, we used cantilever beam theory to test the hypothesis that decreased calcification would impair the mechanical performance of the green alga *Acetabularia acetabulum* along a CO_2 gradient created by volcanic seeps off Vulcano, Italy. Calcification and mechanical properties declined as calcium carbonate saturation fell; algae at 2283 µatm CO_2 were 32% less calcified, 40% less stiff and 40% droopier. Moreover, calcification was not a linear proxy for mechanical performance; stem stiffness decreased exponentially with reduced calcification. Although calcifying organisms can tolerate high CO_2 conditions, even subtle changes in calcification can cause dramatic changes in skeletal performance, which may in turn affect key biotic and abiotic interactions.

1. Introduction

Ocean acidification is lowering the saturation state of calcium carbonate in seawater, making shells and skeletons more vulnerable to dissolution and increasing the energetic costs of calcification [1]. Falling calcium carbonate saturation levels have the potential to disrupt key organisms globally; coccolithophores and foraminiferans are responsible for 32–80% of the carbon transported to the ocean depths and in coastal waters, seaweeds can contribute even more carbonate than corals [2–4]. Calcification affects many aspects of algal performance and survival, including structural integrity [5], increased UV protection [6] and protection against herbivory [7] (but see [8]). Many organisms are less calcified under increased CO_2, but the degree to which loss of calcification affects their mechanical performance is largely unexplored (but see [9]).

Here, we used volcanic CO_2 seeps to assess the effects of chronic exposure to low calcium carbonate saturation on the calcified green alga *Acetabularia acetabulum* that persists across CO_2 gradients in the Mediterranean, albeit with changes in its biomineral composition [10]. Its common name, the mermaid's wineglass, aptly describes its morphology of a cup atop a long slender stem (figure 1a). The cup is an ephemeral reproductive structure that produces and releases spores, appearing from February to July. Calcification enables the thin stem to support the apical cup and extend up from the substrate, where it has improved access to light, nutrients and dispersive
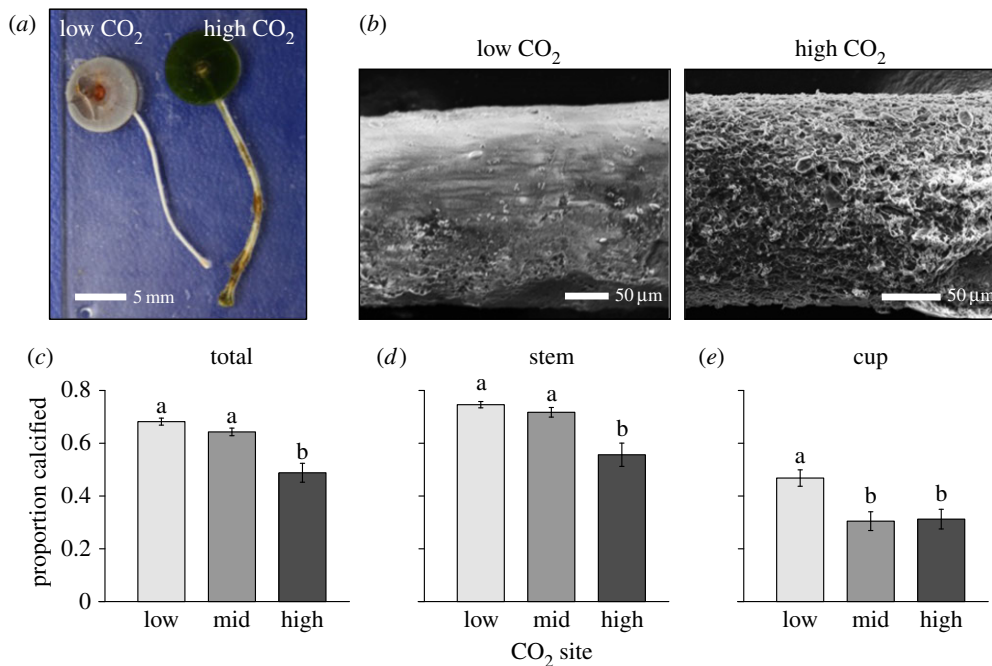
Figure 1. Characteristic appearance of specimens from the low (white and heavily calcified) and high CO_2 sites (green and less calcified) (*a*). Representative SEM images of stems showing calcium carbonate corrosion at the high CO_2 site (*b*). Algae collected in the high CO_2 site had a lower proportion of calcified tissue in the whole alga (*c*), and in the isolated stem (*d*) and cup (*e*). Letters on top of bars represent significantly different treatments, bars are means $\pm$ s.e., $n = 7 - 12$ per site. (Online version in colour.)

currents. The alga calcifies by passive precipitation of aragonite and amorphous calcium carbonate, a process in which it exhibits little biological control, creating a skeletal layer on both the inside and outside of its cell wall [11]. Specifically, we investigate whether chronic exposure to elevated CO_2 reduces calcification and stem bending stiffness (structural and material) of *A. acetabulum*.

2. Methods

The rocky north shore of Levante Bay on Vulcano Island (NE Sicily) has volcanic CO_2 seeps that create an aragonite saturation gradient running parallel to the coast (electronic supplementary material, figure S1) [12]. We sampled three sites (low, mid and high) along this 600 m gradient ranging from present day conditions (418 μatm CO_2; Ω_{arag} 3.56) to 2283 μatm CO_2 (Ω_{arag} 0.96) as described in [13] (electronic supplementary material, figure S1 and table S1). Snorkel surveys assessed the presence and appearance of *A. acetabulum*, and samples were collected for materials testing as described in the electronic supplementary material.

We applied static cantilever beam theory to each freshly collected stem to quantify the flexural stiffness (*EI*, N $\times$ m^2), an index of the droopiness of the stem *structure*, and stem stiffness (*E*, MPa), an index of the ability of each stem *material* to resist load [14] (see the electronic supplementary material, Methods for details). Briefly, the base was clamped between two horizontal glass slides, suspending the hydrated stem and cup in air. A weight was hung on the stem to exert a force (*F*, in N) to deflect (*y*, in m) the algal beam 10–15% of its length (*L*, m). Flexural stiffness (*EI*, N $\times$ m^2), a structural property, was calculated as

$$EI = \frac{FL^3}{3y},$$

where *I* is the second moment of area (m^4, see the electronic supplementary material, Methods for calculation) measured from analyses of stem cross sections imaged under a scanning electron microscope (SEM) to the nearest 10^{-6} m. Stiffness (*E*),

a material property, was calculated by dividing flexural stiffness (*EI*) by the second moment of area (*I*).

Our metric of calcification is the proportion calcified (*C*) of each algal stem and cup, measured by decalcification in 1 N HCl following methods in [15] and weighed to the nearest 10^{-5} g. A separate set of samples were stored in 70% ethanol in seawater prior to analysis with a JEOL 5000 SEM. Percent cover, proportion calcified, flexural stiffness and stiffness were compared among sites using statistical methods described in electronic supplementary material, Methods. Regression analysis compared linear with nonlinear (exponential and polynomial) curves to describe the relationship between algal calcification and stiffness, as described in the electronic supplementary material, Methods.

3. Results

Surveys in May 2014 revealed that *A. acetabulum* cover did not differ among sampling stations (high CO_2: 0.56% $\pm$ 0.41; mid CO_2: 0.41% $\pm$ 0.25; low CO_2: 0.81% $\pm$ 0.20; table 1). All surveys revealed that these algae ranged in appearance, from those with bright white cups at the low CO_2 site to green cups at the high CO_2 site (figure 1*a*). No calcified algae were present in the region nearest the seeps, where aragonite saturation levels fall below 1 [12].

SEM images revealed that all the specimens at the low CO_2 site had an intact sheath of aragonite supporting the stem, whereas those from the high CO_2 site had surface erosion and deep pits into the skeleton (figure 1*b*). Specimens from the high CO_2 site were 32% less calcified than those from the mid and low CO_2 sites (figure 1*c* and table 1). The same pattern was observed for the proportion calcified of the isolated algal stems and cups; those at the high CO_2 site were 25% and 34% less calcified, respectively (figure 1*d*,*e* and table 1). The stem was more calcified than the cup at all sites (table 1). Because there was no significant difference in

Table 1. Statistical summary of percent cover, proportion calcified and mechanical properties of *Acetabularia acetabulum* collected from three sites representing high, mid and low (ambient) levels of CO_2 off Vulcano, Italy in May 2013.

	statistical test	F	d.f.	p
percent cover	ANOVA	0.65	2	0.5
proportion calcified				
total plant	Kruskal–Wallis	17.9	2	<0.0001
	Dunn test			
	low CO_2–mid CO_2			0.05
	low CO_2–high CO_2			<0.001
	mid CO_2–high CO_2			<0.05
stem	ANOVA	14.0	2	<0.0001
	Tukey's HSD			
	low CO_2–mid CO_2			0.07
	low CO_2–high CO_2			<0.001
	mid CO_2–high CO_2			<0.01
cup	ANOVA	3.2	2	<0.05
	Tukey's HSD			
	low CO_2–mid CO_2			<0.01
	low CO_2–high CO_2			<0.01
	mid CO_2–high CO_2			0.9
site $\times$ algal region	two-way ANOVA			
	site	10.3	2	<0.001
	region	88.6	1	<0.0001
	site $\times$ region	1	2	0.4
mechanical properties				
EI (N $\times$ m^2)	Wilcoxon's signed-rank test			<0.05
I (m^4)	ANOVA			0.7
E (MPa)	Wilcoxon's signed-rank test			<0.05
calcification versus E	regression analysis			<0.001
calcification versus EI	regression analysis			<0.05

stem calcification between the low and mid CO_2 sites, these samples were pooled as low CO_2 for subsequent mechanical property analysis.

Algae from the high CO_2 site had 40% the flexural stiffness and material stiffness of those from the low CO_2 site (figure 2*a,b* and table 1). There was no difference in the second moment of area (*I*) among sites (table 1, data not shown). Stiffness of the algal stem decreased exponentially with decreasing calcification (figure 2*c*, $r^2 = 0.51$; $p < 0.001$, AIC of 2, 391, 461 for the exponential, linear and polynomial model, respectively; table 1 and electronic supplementary material, tables S2 and S3). A similar pattern was observed for flexural stiffness ($r^2 = 0.15$, $p < 0.05$, table 1, data not shown).

4. Discussion

Acetabularia acetabulum is similar to the brown algae *Padina* spp. in that it can persist in areas with unusually high CO_2 levels despite depressed net calcification owing to low aragonite saturation levels [10,16]. Specimens growing at greater than 2000 μatm CO_2 had one-third less calcification than those from sites with less than 650 μatm CO_2. Moreover, the relationship between calcification and material stiffness was exponential, not linear; even relatively small reductions in calcification led to a disproportionate drop in the ability of the material to resist a load. Because we observed no difference in *I*, this lower material stiffness translates directly to lower flexural stiffness; the stem becomes droopier in high CO_2.

A previous study has shown *A. acetabulum* growing under high CO_2 lose their orderly aragonite crystalline structure and shift to amorphous carbonate [10]. We observed pitted imperfections on stems at elevated CO_2 levels, which could create microcracks that concentrate stress and lower a material's strength and stiffness [17]. Altered material composition and the pattern of erosion could therefore explain why algae from the high CO_2 site had 40% the material stiffness and flexural stiffness compared with those growing at ambient levels of CO_2.

Loss of material stiffness could have a number of potential costs for the alga. A less rigid stem droops towards the seafloor likely reducing the distance spores can travel away from the cup [18]. The cup is also photosynthetic [19]; bending may reorient it away from incident light and increase shading
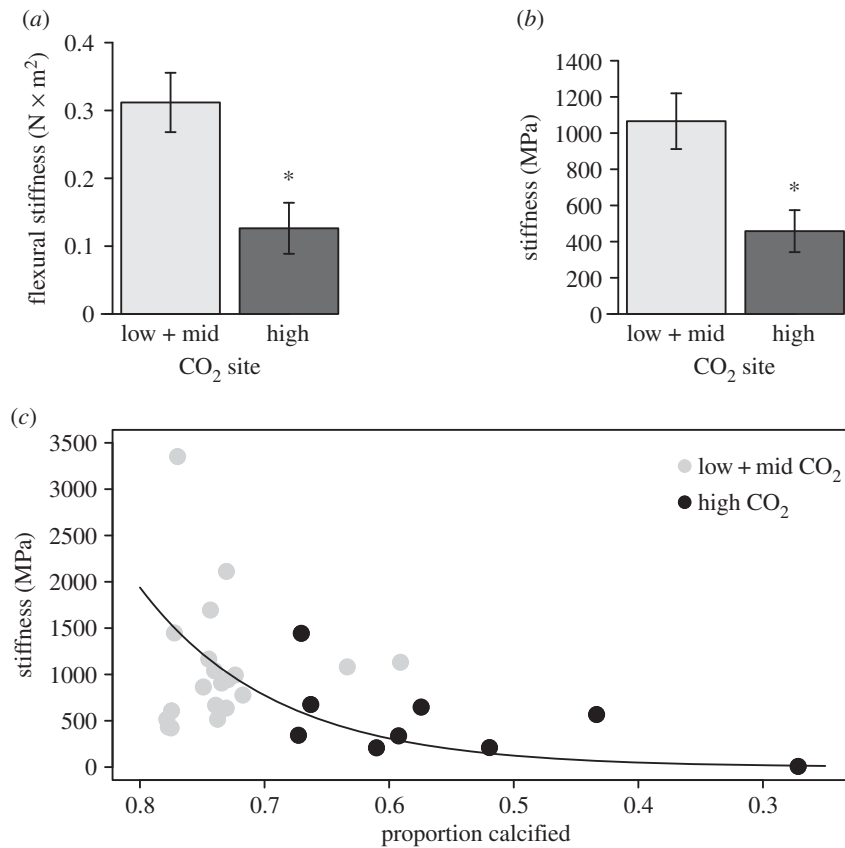
Figure 2. *Acetabularia acetabulum* from the high CO_2 site had lower mean flexural stiffness (*a*) and stiffness (*b*) than the low and mid CO_2 sites. Asterisks on top of bars represent significantly different treatments, bars are means $\pm$ s.e., $n = 7-12$ per site. Stem stiffness decreased with calcification loss (*c*), following an exponential relationship (stiffness $= 1.26\ e^{9.18*C}$, $r^2 = 0.51$).

by neighbours, thereby reducing the scope for growth [20]. While calcification can deter grazers, high CO_2 may result in the loss of grazers [13], so maintaining this defence may lose its importance and become an energetic burden. There are also potential benefits to being less stiff. A more flexible stem allows the alga to reorient in flow, reducing drag and the likelihood of dislodgement [21], and may aid in gas exchange as the stem moves back and forth like a pendulum [22]. Trade-offs between these and other costs and benefits could explain why we observed no difference in areal abundance at our three sites. Ultimately, further knowledge of the environmental context and interactions with other organisms is needed to determine the fate of organisms with reduced skeletal calcification owing to high CO_2.

This study underscores the fact that some organisms may survive ongoing ocean acidification despite reduced calcification; this facultative calcification may explain why certain calcified organisms reappear in the fossil record after mass extinctions associated with periods of high atmospheric

CO_2 [23]. Many ocean acidification studies show reduced calcification at high CO_2, but do not examine the consequences for organismal performance [24]. Our ecomaterial approach establishes these linkages between calcification and performance (and ultimately fitness) which are vital for long-term predictions of how organisms will fare in a high CO_2 world.

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