Physiological advantages of dwarfing for life in high-CO₂ oceans

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Excessive carbon dioxide (CO₂) in the present-day ocean-atmosphere system is causing ocean acidification, and is likely to cause a severe biodiversity decline in the future¹, mirroring effects in many past mass extinctions²–⁴. Fossil records demonstrate that organisms surviving such events were often smaller than those before⁵,⁶, a phenomenon called the 'Lilliput effect'. Here, we show that two gastropod species adapted to acidified seawater at shallow-water CO₂ seeps were smaller than those found in normal pH conditions and had higher mass specific energy consumption but significantly lower whole animal metabolic energy demand. These physiological changes allowed the animals to maintain calcification and to partially repair shell dissolution. These observations of long-term chronic effects of increased CO₂ levels warn of changes we can expect in marine ecosystems as CO₂ emissions continue to rise unchecked, and support the hypothesis that ocean acidification contributed to past extinction events. The ability to adapt through dwarfing can confer physiological advantages as the rate of CO₂ emissions continues to increase.

The present rate of ocean acidification is a global concern because many of the mass extinction events that affected evolution on Earth are associated with evidence for elevated CO₂ and global warming, triggered by large-scale continental volcanism. These include the largest known extinction event, which occurred in the late Permian Alone, where atmospheric CO₂ levels are estimated to have increased by a factor of four to six²,³, and the Late Triassic event that saw a doubling in CO₂ levels⁴ and was the most severe extinction to have affected extant groups such as scleractinian corals⁵. Evidence that ocean acidification due to volcanism played a significant role in past marine extinctions comes from analyses of physiological selectivity²,³, and changes in shell mineralogy and lithology⁵. In the immediate aftermath of the mass extinction events, many of the survivors were smaller than before² (for example, brachiopods⁶, gastropods⁷, bivalves and shelled cephalopods); a phenomenon termed the 'Lilliput effect'. After the most severe Late Permian extinction, gastropod species remained relatively small for millions of years⁸. One hypothesis is that this dwarfing was an adaptation to ocean acidification to mitigate against the increased energetic cost of carbonate secretion⁹. Calcifiers use the ion transporter Ca²⁺ATPase to build shells/skeletons which pumps protons out of the extracellular calcifying medium, increasing the internal pH and favouring calcification. This is an energetically expensive process¹⁰, the cost of which increases for animals exposed to high Pₐ conditions. For instance, scleractinian corals have an extra metabolic cost of about 10% per 0.1 unit decrease in seawater pH (ref. 11). It is possible that faced with an increase in calcification costs, some species may adapt by decreasing in size¹².

Areas with naturally high levels of CO₂ provide opportunities to study the adaptation of organisms exposed to chronic hypercapnia¹³,¹⁴. At such sites, increased CO₂ levels cause biodiversity loss on sufficiently large spatial and temporal scales to reveal ocean acidification effects at the ecosystem level¹⁵,¹⁶. Off Vulcano Island, Sicily¹⁷, seep gas composition is 97–98% CO₂, which acidifies the surrounding waters down to pH 5.64 near the main seeps, rising to ambient levels of pH 8.2 over a distance of around 400 m (ref. 23). Traces of other hydrothermal gases (H₂, CH₄ and H₂S) are also present near the seeps but become undetectable around 5 m away¹⁸. Seawater temperature and oxygen levels reach ambient values a few tens of metres from the main seep area. At about 100 m from the main seeps the nassariid gastropods Nassarius corculus and Cyclope neritea are abundant on coarse sand and gravel (Fig. 1). These species are widespread in coastal lagoons and salt marshes in the Mediterranean as well as at shallow-water hydrothermal seeps (for example, off Milos²⁰ and Pantelleria²¹). We know that populations of N. neritea and N. corculus had developed at the CO₂ seeps because their shells had paucispiral protoconches indicating these snails lack a planktotrophic larval stage (see Supplementary Information for more details). Seawater off Vulcano has been acidified since the late Pleistocene epoch (ref. 13) and a dwarf population of N. corculus has been present least 30 years²², providing an opportunity to study chronic effects of ocean acidification on gastropods submitted to high CO₂ levels over multiple generations.

Here, we compared gastropods living in naturally acidified shallow-water conditions near CO₂ seeps off Vulcano with those at sites with ambient seawater pH (Supplementary Fig. 1) to test
for their ability to cope with acidification and, potentially, adapt.  
Shell morphology, dissolution and repair were examined using scanning electron microscopy. Animals collected in September and November 2011 were incubated in aquaria at CO$_2$ levels similar to those measured at reference and CO$_2$ seep sites (Supplementary Table 1) and their gross calcification rates$^{16}$ were measured using $^{44}$Ca at both seawater pH levels. Rates of metabolic oxygen uptake of individuals from CO$_2$ seeps and a reference site were determined by stop-flow respirometry at pH$_{NBS}$ 6.5 or pH$_{NBS}$ 8.1, respectively, within 24 h of collection.

Gastropods were collected from the seep site at mean pH$_T$ 7.41 ± 0.13 (corresponding to $\Omega_{arv}$ of 1.10 ± 0.28; Supplementary Table 1) where seawater pH varied during a 24-h cycle from about 6.6 to 7.7 pH$_T$ units (Supplementary Fig. 2) and from three sites at pH$_T$ 8.1. Considerably fewer specimens of both species were found at reference sites (on average 1 specimen m$^{-2}$ versus 7 m$^{-2}$). Overall shell shapes were significantly different between sites (GLM $p < 0.001$, Supplementary Tables 2–13; PERMANOVA $p < 0.001$, Supplementary Tables 14–15) and individuals within species were significantly smaller at CO$_2$ seeps than at control sites, showing 1.26 and 1.37 mean (log) volume ratios between ambient and acidified conditions (Table 1 for raw data; Tukey's LSD tests for Vulcano (acidified) versus ambient controls $p < 0.001$, Supplementary Tables 6 and 13). These shell volume shifts, which approximate to biomass shifts, provide a means of directly comparing experimental and fossil data$^{35}$. In his global study of clade-level size change in gastropods Payne$^{33}$ recorded a shell volume shift of 1.45 mean (log) volume between the Late Permian and Early Triassic periods, which is of the same order as that recorded by our experimental data (Supplementary Fig. 3).

In N. corniculus, shell integrity was clearly affected by the corrosive seawater at CO$_2$ seeps; pockmarks were present on the teleoconch in almost all samples (Fig. 2a), corresponding to zones where the periostracum was affected by swelling and breaking (Fig. 2b). Deterioration of the periostracum is the first step before mineralized shell layers undergo dissolution (Fig. 2c), as previously shown in mussels at shallow$^{20}$ and deep CO$_2$ seeps$^{26}$. For C. neritea the early shell whorls were also corroded at the CO$_2$ seeps, with loss of the protoconch and pitting on the first teleoconch whorl (Fig. 1a).

At reference sites, fully grown N. corniculus had 7–7.5 whorls, whereas all the N. corniculus collected at CO$_2$ seeps had only 1–2 whorls (Fig. 1b and Supplementary Fig. 4). Acidic environment gradually corroded gastropod shells, especially in the sutural area (Fig. 2d), suggesting that different truncations might affect a single individual during its life history. Although similar shell truncation is recorded in fossil gastropods from other seep environments$^{27}$, it is difficult to assess whether shell damage due to corrosive seawater was a common feature in gastropods surviving past extinctions$^{28}$, because shell dissolution may also occur post mortem, through fossilization processes as well as during collection and separation of the fossils from the rock.

All the N. corniculus specimens from CO$_2$ seeps had the most severe surface injuries and the truncated apices neatly plugged by...
were removed from elevated CO$_2$ calcification rates than those at the reference site even when they
for both months separately, overall GLM
showing adaptation to acidified environments rather than plasticity
Supplementary Table 16b), whereas they were consistently greater
Nassarius corniculus
were generally lower than samples from the reference site (pH
to ocean acidification are a matter of debate$^{18}$, many benthic
organisms calcify in waters undersaturated with carbonate, although they may incur elevated energetic costs that may
result in reduced fitness, growth, reproduction and predation
response. Limpets that live in high-CO$_2$ areas$^{27}$ are not dwarfed, but counteract shell dissolution by upregulating their calcification
rates$^{19}$, and therefore have to meet the higher energetic cost required
to calcify$^{11}$. Other organisms in which calcification rates are affected
at high CO$_2$ levels (including economically important shellfish
such as clams, oysters and mussels$^{29}$) may adapt ocean acidification
through reductions in size$^{9}$, therefore reducing the total whole
animal energy demand but at the same time increasing the mass
specific energy demand necessary to maintain calcification and
compensate for calcium carbonate dissolution.

We found that metabolic oxygen consumption corrected for body
mass (mass-adjusted MO$_x$), which is the best descriptor of the animal’s metabolic/maintenance costs, was significantly higher at
CO$_2$ seeps (pH$_{seep}$ 6.5) compared with the samples from a reference
site (pH$_{ref}$ 8.1) for both $C$. neritea and $N$. corniculus (Fig. 3d; GLM $p < 0.001$, Supplementary Table 18b); however, because of
dwarfing, whole animal metabolic demands were significantly
lower at the CO$_2$ seeps in both species (Fig. 3e; GLM $p < 0.001$, Supplementary Table 18b). Size reduction may compensate for loss
of metabolic efficiency at high CO$_2$ in addition to the mass specific
increased costs of maintenance. Our results support the conclusions
of ref. 19, which demonstrated both physiological acclimatization
and metabolic adaptation to high $P_\text{CO}_2$ and reductions in size in
seep-associated populations of bentho-polychaetes. We therefore
corroborate the observation that $P_\text{CO}_2$-tolerant species seem more
likely to compensate for environmentally stressful conditions by
elevating metabolic rates at the expense of growth.

Instead of being merely a result of changes in energy partitioning
leading to changes in the rates of growth/calcification, small body
size may be of selective advantage in maintaining mass specific rates

Table 1 | Mean and standard deviation (s.d.) of shell morphometric parameters, shell volume (V) and average size decrease (%) of $Cyclope$ $neritea$ and $Nassarius$ $corniculus$ from CO$_2$ seeps and from reference sites C1-C3 (Stagnone di Marsala; Lampedusa Island; San Giovanni Li Cuti, respectively).

<table>
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<tr>
<th>Data</th>
<th>Ht</th>
<th>Hlw</th>
<th>W</th>
<th>Ws</th>
<th>Ha</th>
<th>Olt</th>
<th>V</th>
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<td>(± s.d.)</td>
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<td>(0.19)</td>
<td>(0.53)</td>
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<td>(0.37)</td>
<td>(0.03)</td>
<td>(8.89)</td>
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<tr>
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<td>10.72</td>
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<td>(± s.d.)</td>
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<td>(0.36)</td>
<td>(0.91)</td>
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<td>(0.50)</td>
<td>(0.21)</td>
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<td>41.3</td>
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<td>(0.72)</td>
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<td>(0.60)</td>
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<td>(0.52)</td>
<td>(0.25)</td>
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<tr>
<td>Size decrease (%)</td>
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<tr>
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<td>37.7</td>
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Shell morphometric parameters (mm) are: (Ht) total height; (Hlw) last whorl height; (W) last whorl width; (Ws) width at the suture between last and penultimate whorl; (Ha) aperture height; (Olt) outer lip thickness. For $N$. corniculus, Ht was not measured because all the Vulcano adult shells were more or less markedly truncated in their apical part. Volume (V) is in mm$^3$. The last whorl width (Ws) was measured only for $N$. corniculus, allowing calculation of volume by approximating shells as a truncated cone (see Supplementary Information for more details). For $C$. neritea $n = 25$ for both CO$_2$ seeps and reference site C1, whereas for $N$. corniculus $n = 40$ (seeps and reference site C1); $n = 20$ and 10 for reference sites C2 and C3 respectively.
Figure 3 | Gross calcification (GC) and metabolic oxygen consumption (MO$_2$) in *Nassarius corniculus* and *Cyclope neritea* across normal and acidified sites. a, GC in September and November at pH measured at collection sites. b,c, GC of *N. corniculus* in September (a) and November (b) using a two-way orthogonal experimental design (acclimation state/Origin x pH) to measure the response of samples collected within the seeps and at reference sites and incubated at crossed pH treatments. Arrows show the GC change on samples incubated at pH of collection (CO$_2$ seeps or reference site C1) and the pH of the incubation (pH$_T$ 8.0 and 7.2). For September, numbers of *N. corniculus* and *C. neritea* were $n=14$–39 and $n=16$–20 (see Supplementary Information). Data are means ± s.d. d,e, Oxygen uptake as an index of metabolic rates (MO$_2$) expressed as body-mass-adjusted MO$_2$ (d) and whole animal MO$_2$ (e). Sample replicates are $n=9$; data are means ± s.d.

...of energy consumption within an energy-limited environment. This would allow individuals to consume less energy, trading off growth and calcification against lifetime reproductive output. Furthermore, this would have conferred additional advantages during past global warming crises where changes in productivity may have impacted the availability of food resources$^{1,3,9}$. Geologic evidence for mass extinctions associated with elevated CO$_2$ levels provides a stark warning for today, as the current rate of ocean acidification and warming is more rapid than during historical events and is acting synergistically with other global anthropogenic stressors. Understanding the ramifications of the potential current mass extinction is limited, as we do not know which organisms will be able to evolve and how they might adapt to survive these changes. Organisms that have been exposed over multiple generations to elevated CO$_2$ levels provide valuable insights both into changes we can expect in marine ecosystems...
as CO₂ emissions continue to rise unchecked, and into past mass extinctions; not only do they demonstrate a similar magnitude and direction of body size change as fossil organisms, but they also reveal the physiological advantages of dwarfing. It is critical that we understand the mechanisms by which certain species survive chronic exposure to elevated CO₂, as emissions of this gas are already having adverse effects on marine food webs and putting food security at risk.

Methods

Methods and any associated references are available in the online version of the paper.

Received 20 November 2014; accepted 23 March 2015; published online XX Month XXXX

References


Acknowledgements

We thank F. Houbrique, J. F. Comanducci and F. Oberland for their help during radiotracer experiments. We also thank H. Graham, D. Small and C. Bertolini for assisting in the field. The International Atomic Energy Agency is grateful to the Government of the Principality of Monaco for the support provided to its Environment Laboratories. This work contributes to the EU ‘Mediterranean Sea acidification under a changing climate’ project (MedSeA; grant agreement 265103) and the NERC UK Ocean Acidification Research Programme (Grant no. NE/H02543X/1). S.P.S.R. was funded by a NERC UK Ocean Acidification Research Programme AVA fellowship. This is UMR ENTROPIE scientific contribution n. 028.

Author contributions

V.G. and R.R-M. designed the study and wrote the paper in collaboration with M.M., A.F., R.J.T., S.P.S.R. and J.M.H.S.; V.G. and D.P. performed scanning electronic microscopy analyses; R.R-M. performed gastropod radiotracer incorporation; S.P.S.R. and M.M. performed metabolic rate experiments; A.F. performed statistical analysis; M.M., D.S. and L.B. helped during sampling; all authors read and commented on the manuscript.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to V.G. or R.R-M.

Competing financial interests

The authors declare no competing financial interests.
Methods

Sampling sites. For shell morphometric parameters, scanning electronic microscopy analyses, gross calcification and metabolic rate measurements, *Nassarius corniculus* and *Cyclope neritea* were collected in September and November 2011 at CO₂ seeps off Vulcano Island (northern Sicily, Aeolian Archipelago, Tyrrhenian Sea), and at reference site C1 in the Stagnone of Marsala (western Sicily; Supplementary Fig. 1). Specimens of *N. corniculus* were also collected at two other reference sites—Lampedusa Island (C2; southern Sicily) and San Giovanni Li Cuti (C3; eastern Sicily)—and their shell morphometric parameters were measured.

Seawater carbonate chemistry. Seawater carbonate chemistry variations at CO₂ seeps were frequently measured during our previous studies (for example, ref. 22). Seawater carbonate chemistry parameters were measured.

Shell morphometric and scanning electron microscopy analyses. Samples were preserved in 70% ethanol after collection. Morphometric parameters, such as total shell height (Ht), shell maximum width (W), shell width at the suture between last and penultimate whorl (W₀), last whorl height (H₀), aperture height (Ha) and thickness of the outer lip (Ol), were measured on adult *C. neritea* and *N. corniculus* shells collected at CO₂ seeps and the three reference sites C1–C3 using a stereomicroscope. *Nassarius corniculus* shell macro- and microstructures were investigated using a LEO 420 scanning electron microscope on samples from Vulcano and the reference site C1.

Aquarium experiments and gross calcification (GC). After collection, both in September and November 2011, samples were transported to the IAEA-MESL laboratory in Monaco. They were maintained in flow-through aquaria containing sediments collected from sampling sites. *C. neritea* were divided per site of collection at field pH (sites C1, pH 8.0 and CO₂ seep, pH 7.2–7.3) whereas *N. corniculus* were incubated using a two-way orthogonal experimental design (acclimation state/Origin × pH) to measure the GC rates of samples collected within and outside the vents at crossed pH treatments. Half of the *N. corniculus* from both sites were incubated at temperature and pH conditions similar to in situ values (CO₂ seep; pH 7.2–7.3; site C1: pH 8.0; Supplementary Table 1), whereas the remaining specimens were incubated at pH 7.2–7.3 and pH 8.0 for specimens from reference and CO₂ seep sites, respectively. After two weeks, samples were transferred in separated 6 litre aquaria (two to three replicate tanks for each site/pH treatment) and their GC were measured with the radiotracer ⁴⁰Ca (Supplementary Information).

Metabolic rates. Metabolic oxygen consumption (MO₂) was determined for *C. neritea* and *N. corniculus* from both CO₂ seeps and reference site C1 within 24 h of collection. During individual incubations, gastropods were allowed to acclimatize to the respirometry chambers, receiving fully oxygenated water from their site of collection at pH 39.86, 6.5 or pH 46.81 for individuals collected at CO₂ seeps and reference site C1, respectively. After 1 h acclimatization the chambers were closed, measurement run for another hour and the decrease in PO₂ within each respirometer stop-flow chamber measured using a polarographic O₂ electrode (E-5046 electrode, Radiometer) connected to an oxygen meter (Strathkelvin Oxygen Meter 781). Then, specimens were killed to measure their shell-free wet-body mass for use as a covariate in GLM analysis of MO₂.

Statistical analysis. Univariate data on shell morphometric parameters (Supplementary Information), GC and metabolic rates were analysed using GLM in SPSS 21.0; data are presented as mean ± s.d. Pair-wise comparisons between treatments were performed using a priori contrasts based on estimated marginal means and Tukey's LSD tests. Conformity to assumptions of GLM was confirmed by homogeneity of variances testing and inspection of analytical residuals; transformations were used when necessary. Multivariate analyses of overall organism shape were conducted using PERMANOVA in PRIMER version 6.1 (see Supplementary Information for all details and results).
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