Sea anemones may thrive in a high CO$_2$ world

DAVID J. SUGGETT*, JASON M. HALL-SPENCER†, RICCARDO RODOLFO-METALPA†, TOBY G. BOATMAN*, ROSS PAYTON*, D. TYE PETTAY‡, VIVIENNE R. JOHNSON†, MARK E. WARNER‡ and TRACY LAWSON*

*Coral Reef Research Unit, School of Biological Sciences, University of Essex, Colchester, CO4 3SQ, UK, †Marine Biology and Ecology Research Centre, University of Plymouth, Plymouth, PL4 8AA, UK, ‡College of Earth, Ocean, and Environment, University of Delaware, 700 Pilottown Road, Lewes, Delaware 19958, USA

Abstract

Increased seawater pCO$_2$, and in turn ‘ocean acidification’ (OA), is predicted to profoundly impact marine ecosystem diversity and function this century. Much research has already focussed on calcifying reef-forming corals (Class: Anthozoa) that appear particularly susceptible to OA via reduced net calcification. However, here we show that OA-like conditions can simultaneously enhance the ecological success of non-calcifying anthozoans, which not only play key ecological and biogeochemical roles in present day benthic ecosystems but also represent a model organism should calcifying anthozoans exist as less calcified (soft-bodied) forms in future oceans. Increased growth (abundance and size) of the sea anemone (*Anemonia viridis*) population was observed along a natural CO$_2$ gradient at Vulcano, Italy. Both gross photosynthesis ($P_G$) and respiration (R) increased with pCO$_2$ indicating that the increased growth was, at least in part, fuelled by bottom up (CO$_2$ stimulation) of metabolism. The increase of $P_G$ outweighed that of R and the genetic identity of the symbiotic microalgae (*Symbiodinium* spp.) remained unchanged (type A19) suggesting proximity to the vent site relieved CO$_2$ limitation of the anemones’ symbiotic microalgal population. Our observations of enhanced productivity with pCO$_2$, which are consistent with previous reports for some calcifying corals, convey an increase in fitness that may enable non-calcifying anthozoans to thrive in future environments, i.e. higher seawater pCO$_2$. Understanding how CO$_2$-enhanced productivity of non- (and less-) calcifying anthozoans applies more widely to tropical ecosystems is a priority where such organisms can dominate benthic ecosystems, in particular following localized anthropogenic stress.

Keywords: Cnidarian, CO$_2$ vent, Ocean acidification, Productivity, Sea anemone, *Symbiodinium* spp.

Received 5 April 2012 and accepted 5 June 2012

Introduction

Rising atmospheric CO$_2$ from anthropogenic activity will significantly increase seawater CO$_2$ partial pressure (pCO$_2$) and result in lower ocean pH or ‘ocean acidification’ (OA) this century (Caldeira & Wickett, 2005). Predictions suggest that by the year 2100 surface ocean pH could be reduced by ca. 0.3–0.5 units compared to present day values (IPCC, Intergovernmental Panel on Climate Change, 2007), with profound consequences for fundamental biological processes, such as calcification and photosynthesis, and in turn the functioning of entire marine ecosystems (e.g. Hoegh-Guldberg & Bruno, 2010). The potential impact of OA upon corals reefs has particularly received much high profile attention. Coral reef ecosystems are characterized by high productivity and diversity as a result of primary productivity and calcification by coral cnidarians (Class: Anthozoa). Observations from laboratory experiments attempting to replicate future OA scenarios (e.g. Anthony et al., 2008; Edmunds et al., 2012) as well as in situ investigations at present day naturally high CO$_2$ shallow water reefs (Fabricius et al., 2011; Crook et al., 2012), generally demonstrate that calcification and growth will be impacted by elevated CO$_2$ (reduced pH) (see also Andersson et al., 2011). However, the evidence is not entirely negative:

A growing volume of studies suggest that net calcification rates may in fact not always be reduced under long-term exposure to elevated CO$_2$ (Krief et al., 2010; Rodolfo-Metalpa et al., 2010). Some calcifiers, including coral cnidarians (Rodolfo-Metalpa et al., 2011) can maintain intact their calcification rates at extremely low pH levels, most likely via tissues and other organic layers acting as a barrier to the surrounding seawater and thus reducing dissolution. Furthermore, even where calcification and growth rates are decreased, calcifying coral cover can remain high since some species are still able to survive in high CO$_2$ environments (e.g.
Porites lutea, Fabricius et al., 2011; see also Crook et al., 2012). Even more intriguing is the fact that coral primary productivity can often increase (or is unaffected) while net calcification decreases/dissolution increases for some OA scenarios (e.g. Crawley et al., 2010); consequently, anthozoans can potentially remain completely viable, but as alternative non-calcifying soft-bodied forms under extremely high CO2/low pH (Fine & Tchernov, 2007; see also Krief et al., 2010). However, how such OA-driven phenotypic responses alter the functional role of soft-bodied forms of anthozoans, or even convey longer term ecological success, is presently unknown. Such limited knowledge still reflects the fact that we do not fully understand how present day non-calcifying anthozoans, will respond to OA. Non-calcifying anthozoans such as soft corals and anemones, play important ecological and biogeochemical roles in reef environments (e.g. Fitt et al., 1982; Bak & Borsboom, 1984; Muller-Parker & Davy, 2001), but much less is known how these organisms will respond to OA (Doherty, 2009; Towanda & Thuesen, 2012) given a present bias in OA research towards calcifying organisms (Connell & Russell, 2010).

As with reef-forming scleractinian corals, many anemones (Class: Anthozoa, Subclass: Hexacorallia) harbour symbiotic algae (Symbiodinium spp.) to supplement their nutritional requirements. However, in contrast to calcifying corals, anemones are fast growing and easier to manipulate via the lack of tissue adhesion to an underlying skeleton; thus anemones are potentially model organisms for understanding the nature with which environmental change, including OA, may impact reef-forming cnidarians (e.g. Muller-Parker & Davy, 2001; Weis et al., 2008). Here we present the findings of the first in situ assessment of the effects of OA on anemones whereby elevated CO2 can enhance productivity, and in turn growth, and community dominance of anemones within a natural benthic ecosystem.

A major limitation for almost all OA studies to date has been replicating the rate (decades) and biological scale (ecosystems) at which OA operates. However, natural CO2 vents at coastal sub-tropical rocky shores (Hall-Spencer et al., 2008; Kroeker et al., 2011; Meron et al., 2012) and tropical coral reefs (Fabricius et al., 2011) provide unique experimental settings to evaluate relatively long term changes in pCO2 (pH) across many biological and spatial scales. The few studies conducted to date at CO2 vent sites have generally revealed a decrease in calcifying invertebrate and macroalgal abundance and increased contribution of non-calcifying macroalgae and/or seagrass to overall benthic cover (Hall-Spencer et al., 2008; Fabricius et al., 2011); such changes again correspond to the elevated CO2 (lower pH) induced reduction of calcification and/or enhanced shell dissolution in the dominant invertebrates (including gastropods and hermatypic corals; Hall-Spencer et al., 2008; Rodolfo-Metalpa et al., 2011).

We present novel observations across a natural coastal CO2 gradient from a shallow cold vent system (Vulcano, Italy) (Johnson et al., 2011, 2012) supporting previous observations from other vent sites that certain invertebrates increase in abundance with increasing pCO2/decreasing pH (Cigliano et al., 2010; Kroeker et al., 2011). Here, anemone (Anemonia viridis) abundance increased with pCO2 and dominated the invertebrate community at high pCO2 conditions. We tested the hypothesis that their increased dominance was driven (bottom up) via enhanced anemone productivity. Physiological analyses demonstrated an increase of gross maximum photosynthesis ($P_{\text{max}}$) and respiration rates (R) (but with $P_{\text{max}}$ > R) as well as increased dinoflagellate endosymbiont abundance (but unchanged diversity) with increasing pCO2. Our observations are the first to show that non-calcifying anthozoans can be actively selected for under OA conditions and thus it is essential that greater focus is given to better understand how this previously neglected group will contribute to ecosystem scale productivity and nutrient cycling in future oceans.

Materials and methods

Sample site environment and benthic community analyses

Data collection was conducted in the sublittoral of North Vulcano Island (38° 25′ N, 14° 57′ E) ca. 25 km North East of Sicily, 11th to 26th May 2011. Several vents naturally release CO2 in coastal waters here as a result of the close proximity to Vulcano’s active volcano (described previously, Johnson et al., 2011, 2012). As with recent investigations from this site (Johnson et al., 2012) we selected three reference sites (R1-3) away from the vents, and hence representative of ‘present day’ pCO2 conditions, and three sites (S1-3) with increasing proximity to the vents; together these six sites provided a gradient of decreasing pH (increasing pCO2) from ca. 8.2 (365 µatm) at 7.6 (1425 µatm) (see Table 1). Full details of the carbonate chemistry (and associated methodology) for these six sites are given in Johnson et al. (2012). Variability in the carbonate system also increases with proximity to the vents (Table 1), a factor that is also discussed further in Johnson et al. (2012).

All sample sites were shallow (1–2m) and investigated between 10:00 and 12:00 local time. Water temperature was measured using a HOBO® logger (Tempon, USA) throughout the sampling period and was constant across all sites at ca. 20.6–21.4 °C (data not shown). Light attenuation ($K_0$ [PAR], m$^{-1}$) within the upper 1–2m was measured via the light sensor of a Diving-PAM (Pulse Amplitude Modulated) fluorometer (Walz GmbH, Germany) as described previously by Hennige et al. (2008), and also remained constant between
Table 1  Chemical and biological characteristics measured from both reference (R) and elevated CO₂ (S) sites at Vulcano: (i) Median (min-max) pH (NBS scale) and pCO₂ (µatm) taken from Johnson et al. (2011) to demonstrate the long-term intra-site variability relevant to ecological scale processes; and (ii) Mean (± standard error) of anemone pedal disc (PD) diameter, *Symbiodinium* cell concentration per unit anemone tentacle, and parameters describing anemone productivity from across the study sites: PAM-fluorescence derived light intensity of saturated productivity (Eₖ, Eq. 1), light-limited and light-saturated electron transfer rate (x and ETRₘₐₓ, Eq 2) and ΔTCO₂ derived respiration and maximum gross photosynthesis rate (R and Pₘₐₓ; also shown is the gross photosynthesis-to-respiration ratio (Pₘₐₓ:R). In all cases n = 5 for each site except anemone PD diameter where n = 37 (R1), 32 (R2), 30 (R3), 51 (S1), 59 (S2), 177 (S3). Note that for Tukey HSD post-hoc ANOVA groupings: sites that are not significantly different from one another are grouped within square brackets; each set of square brackets indicates significantly different groups of species whereas hyphens indicate overlapping groupings.

<table>
<thead>
<tr>
<th>Site</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>ANOVA</th>
<th>Post-hoc grouping</th>
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<tbody>
<tr>
<td>pHNBS</td>
<td>8.17 (8.35–8.06)</td>
<td>8.18 (8.29–8.08)</td>
<td>8.18 (8.29–8.10)</td>
<td>8.08 (8.22–7.76)</td>
<td>7.71 (8.10–7.07)</td>
<td>7.66 (8.24–6.80)</td>
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<tr>
<td>pCO₂ (µatm)</td>
<td>388</td>
<td>365</td>
<td>364</td>
<td>510</td>
<td>1244</td>
<td>1428</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemone PD diameter (mm)</td>
<td>(1.12)</td>
<td>(1.24)</td>
<td>(1.03)</td>
<td>(1.23)</td>
<td>(1.36)</td>
<td>(0.78)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Symbiodinium</em> concentration (cells × 10⁵ cm⁻²)</td>
<td>3.76</td>
<td>2.92</td>
<td>3.92</td>
<td>8.25</td>
<td>13.05</td>
<td>15.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eₖ (µmol photons m⁻² s⁻¹)</td>
<td>466.1</td>
<td>481.6</td>
<td>472.0</td>
<td>485.4</td>
<td>469.3</td>
<td>474.7</td>
<td></td>
<td></td>
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<tr>
<td>(21.81)</td>
<td>(18.17)</td>
<td>(17.64)</td>
<td>(23.16)</td>
<td>(16.53)</td>
<td>(23.73)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>x (µmol electrons [mol photons]⁻¹)</td>
<td>0.481</td>
<td>0.441</td>
<td>0.499</td>
<td>0.601</td>
<td>0.640</td>
<td>0.706</td>
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<td>(0.008)</td>
<td>(0.029)</td>
<td>(0.011)</td>
<td>(0.014)</td>
<td>(0.006)</td>
<td>(0.025)</td>
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<td></td>
</tr>
<tr>
<td>ETRₘₐₓ (µmol electrons m⁻² s⁻¹)</td>
<td>226.9</td>
<td>212.9</td>
<td>248.0</td>
<td>272.5</td>
<td>302.1</td>
<td>319.2</td>
<td></td>
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<tr>
<td>(16.30)</td>
<td>(15.12)</td>
<td>(11.01)</td>
<td>(16.73)</td>
<td>(8.24)</td>
<td>(13.27)</td>
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<td></td>
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<tr>
<td>ΔPₘₐₓ (µmol CO₂ g⁻¹ h⁻¹)</td>
<td>0.239</td>
<td>0.236</td>
<td>0.240</td>
<td>0.269</td>
<td>0.311</td>
<td>0.423</td>
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<tr>
<td>(0.013)</td>
<td>(0.014)</td>
<td>(0.016)</td>
<td>(0.014)</td>
<td>(0.012)</td>
<td>(0.02)</td>
<td></td>
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<tr>
<td>R (µmol CO₂ g⁻¹ h⁻¹)</td>
<td>0.216</td>
<td>0.217</td>
<td>0.219</td>
<td>0.236</td>
<td>0.248</td>
<td>0.326</td>
<td></td>
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<tr>
<td>(0.011)</td>
<td>(0.008)</td>
<td>(0.015)</td>
<td>(0.010)</td>
<td>(0.013)</td>
<td>(0.019)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔPₘₐₓ:R (mol CO₂ [mol CO₂]⁻¹)</td>
<td>1.102</td>
<td>1.082</td>
<td>1.098</td>
<td>1.192</td>
<td>1.254</td>
<td>1.301</td>
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<tr>
<td>(0.027)</td>
<td>(0.029)</td>
<td>(0.034)</td>
<td>(0.023)</td>
<td>(0.020)</td>
<td>(0.014)</td>
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</table>
sites, at 0.27–0.33 m$^{-1}$. Therefore, temperature and light availability were not further considered as environmental factors significantly contributing to any ecological/physiological differences between sites.

Benthic assemblages for each of the six sites were assessed using a visual census method ca. 0.0–0.3m below low water. A quadrate measuring 0.25 m$^2$ was randomly placed for quantifying sea urchin (*Paracentrotus lividus*, *Arbacia lixula*) and anemone (*A. viridis* and *Actinia equina*) abundance; a total of 20 separate samples (per 0.25 m$^2$) were made at each site. Continuous-line intercept transects (*n* = 3 per site) of 20 m were further used for general characterization of the relative (%) cover of the major benthic groups (canopy forming macroalgae, e.g., *Sargassum vulgare*, *Cystoseira* spp., seagrass, sessile invertebrates etc.). Pedal disc size of all anemones located up to 0.5 m either side of the transect (to yield a 20 m$^2$ belt transect) were subsequently measured with vernier callipers (precision, 0.1 mm); dimensions reported here are the mean length and width.

### Anemone productivity

Anemones (*n* = 5) were randomly collected from each site and returned to the laboratory for subsequent examination of productivity via two complementary approaches; specimens from each site were examined on consecutive days: Active chlorophyll a fluorescence rapid light curves (RLCs) initially provided estimates of photosynthetic activity (as the electron transfer rate, ETR) whilst TCO$_2$-drift measurements in the light and dark subsequently provided corresponding rates of primary production and respiration. All anemones were initially blotted dry and wet-weighed (*Technico-PW01 balance, ± 0.01g*) and then maintained in aquaria with seawater filled from the corresponding sample site. Specimens were returned to the original site of collection to minimize the length of time *ex situ* post examination.

Rapid light curves, RLCs were collected using a diving-PAM programmed according to settings described previously (Hennige et al., 2008; Suggett et al., 2012) to deliver nine light steps of 20 s duration with increasing intensity from 0 to ca. 3000 μmol photons m$^{-2}$ s$^{-1}$. Optimum instrument sensitivity (gain) and induction intensity (saturation width and intensity) were verified prior to each RLC and all anemones were dark acclimated for ca. 1–2 h prior to examination. Each light step yielded a measure of the minimum and maximum fluorescence yield ($F'$ and $F'_m$, respectively, instrument units) from which the photosynthetic efficiency ([$F'_m-F']/F'_m = F'_d/F'_m$, dimensionless) could then be determined. An equation describing the light-dependency of the photosynthetic efficiency (Hennige et al., 2008) was then fit using least squares non-linear regression to the RLC measures of $F'_d/F'_m$,

$$F'_d/F'_m = \left[ \left( F'_d/F'_m^{\text{max}} \right) E_k \left( 1 - \exp(-E/E_k) \right) \right]/E \quad (1)$$

where $E$ is the light intensity for each light step (μmol photons m$^{-2}$ s$^{-1}$), $E_k$ is the saturation light intensity (μmol photons m$^{-2}$ s$^{-1}$) and $F'_d/F'_m^{\text{max}}$ is the maximum PSII photochemical efficiency (dimensionless). Mean values for $E_k$ (Table 1) and $F'_d/F'_m^{\text{max}}$ (data not shown) were not different between sites suggesting a uniform extent of photoacclimation (e.g., Suggett et al., 2009, 2012), and thus confirming that differences in light availability across the sites/sampling period were negligible.

Photosynthetic electron transport rate (ETR, μmol electrons m$^{-2}$ s$^{-1}$) was subsequently determined for each light step from the product of $E$, $F'_d/F'_m$ and a constant factor of 0.5 (Hennige et al., 2008); this factor accounts for assumptions that only 50% of all absorbed photons are utilized by PSII and that the quantum yield electron transfer of a trapped photon within a reaction centre is 1 mol electron [mol photon]$^{-1}$ (see Suggett et al., 2011, 2012). In this case ETRs do not include quantitative information as to the extent of light absorbed and are therefore relative. ETR values from each RLC were fit to a modified equation describing the light-dependency of photosynthesis (see Hennige et al., 2008) using non-linear least squares regression,

$$ETR = ETR^{\text{max}}(1 - \exp(-zE/ETR^{\text{max}})) \quad (2)$$

where $z$ (mol electrons [mol photons]$^{-1}$) and $ETR^{\text{max}}$ (μmol electrons m$^{-2}$ s$^{-1}$) describe the light-limited and light-saturated electron transfer rate respectively.

Anemones were subsequently transferred to one of 5 × 100 mL glass Duran bottles filled with seawater from the corresponding site for TCO$_2$ drift determinations. Two additional bottles were filled with seawater alone to provide a simultaneous control of any TCO$_2$ drift induced by activity other than the anemones since the seawater was unfiltered. Lids for the bottles were pre drilled to fit the pH and temperature probes (Hydrocheck CD7000; *WPA Ltd*, Cambridge, UK; pH ± 0.01 and °C ± 0.1) and set to log every 15 min over a 90 min incubation period; the pH sensor was calibrated daily using pre-made NIST buffer solutions (pH 4.0 and 7.0, Hanna Instruments, Leighton Buzzard, UK) to yield pH total scale (*pHT*) measurements. All bottles were placed in a makeshift water bath where water was changed every 15 min and mixed with additional refrigerated water (4 °C) to maintain the temperature within 1–2°C of that *in situ* at the time of sampling (~21 °C). All vessels were incubated outdoors (under natural light), and covered with neutral density filter to provide intensities similar to those *in situ* (ca. 600–1000 μmol photons m$^{-2}$ s$^{-1}$ at noon local time), to determine maximum net photosynthesis rates (e.g., Anthony et al., 2008). Incubations were performed between 12:00 and 13:30 (local time), and subsequently repeated in darkness following sunset (20:00–21:30, local time) to determine corresponding respiration rates.

Measurements of temperature, salinity, *pH$_4$* along with those for total alkalinity (TA) for each site (see Johnson et al., 2012), were used to calculate the total concentration of inorganic carbon (= TCO$_2$, μmol CO$_2$ L$^{-1}$, as the sum of free CO$_2$, HCO$_3$– and CO$_3^{2-}$) via CO2SYS software (Lewis & Wallace, 1998) using the constants of Roy et al. (1993) and Dickson (1990) for KSO$_4$. The concentration of TCO$_2$ was determined at the start and end of each incubation and we assumed that TA did not significantly vary during the drifts since the dominant biology, the anemones, are non-calculifying. Rates of maximum
net photosynthesis and respiration ($P_{max}^G$ and $R$, $\mu$mol CO$_2$ g$^{-1}$ h$^{-1}$) were subsequently calculated as,

$$P_{max}^G(R) = \frac{[(\delta TC\text{O}_2(\text{sample}) - \delta T\text{C}O_2(\text{control}))v]}{I (Tw)} \quad (3)$$

where $v$ and $w$ are the water volume (L) and anemone wet weight (g) while $\Delta T$ and $\Delta T\text{C}O_2$ are the difference of temperature (h) and TCO$_2$ ($\mu$mol CO$_2$ L$^{-1}$) at the start and end of incubation; the mean of both $\Delta T\text{C}O_2$ (control) values was subtracted from each $\Delta T\text{C}O_2$ (sample). Respiration rates were multiplied by a factor of $-1$ to yield positive values. Maximum gross photosynthesis rates ($P_{max}^G$, $\mu$mol CO$_2$ g$^{-1}$ h$^{-1}$) were finally calculated as $P_{max}^G = P_{max}^G + R$.

**Microalgal symbiont characteristics**

Cellular density and the genetic characterization by ITS-2 (internal transcribed spacer region-2) of the anemone's microalgal endosymbiont (Symbiodinium spp.) were determined to support interpretation of any potential changes in anemone productivity. Small tentacle samples were randomly removed from each anemone used to determine productivity. Tentacle surface area (SA$_T$, cm$^2$) of the sample was measured again using vernier callipers prior to storage in glutaraldehyde (1%). Each tentacle sample was later ground in hand-held, glass tissue-homogenizers in water (0.6 mL = $V_T$) and an aliquot transferred to a haemocytometer for cell counts (eight counts per sample) using light microscopy. Symbiodinium density (cells cm$^{-2}$) was thus determined as,

$$\text{Cells cm}^{-2} = \text{cells mL}^{-1} \cdot (V_T/SA_T) \quad 4$$

Nucleic acid extractions were conducted using a modified *Promega Wizard* genomic DNA extraction protocol (following Lajeunesse et al., 2003). Symbiont identity was characterized by denaturing gradient gel electrophoresis (DGGE) fingerprinting of the partial 5.8S and internal transcribed spacer (ITS) region 2 (Lajeunesse, 2002); this region was amplified using a touch-down thermal cycle profile described in the legend for Table 1) as [R1–S2, S3]. Urchin and anemone abundance data were collected from 20 quadrat counts per site. ANOVAs upon the untransformed data: $F_{5,30} = 36.88$ with sites grouped via the post hoc Tukey test (as described in the legend for Table 1) as [R1–R2]–[R3–S3]–[S1–S2]. Urchin and anemone abundance data were collected from 20 quadrat counts per site. ANOVAs upon the untransformed data were not significant for the two urchin species; however, the ANOVA for *A. viridis* was $F_{5,114} = 23.49$ with sites grouped as [R1–R2–R3]–[S1–S2].

**Results**

**Benthic community structure and anemone distribution**

Total macroalgal cover (canopy forming macroalgae, as a % of total benthic cover) during the anemone and urchin surveys was generally higher for the elevated CO$_2$ sites, ca. 40–60%, than at the reference sites, ca. 20–30% (Fig. 1). However, cover at the site with the highest pCO$_2$ (S3) was not significantly different from that at R3 (see Fig. 1 legend for ANOVA results). Increased macroalgal cover was accompanied by a change in dominance from both calcifying and non-calcifying species at the reference sites (not shown) to fleshy macroalgal cover at the high CO$_2$ sites (phaeophytes, Johnson et al., 2012).

Reciprocal changes were observed between the sea urchin (*Arbacia lixula* and *P. lividus*) and anemone (*A. viridis*) population abundances for the reference vs. high CO$_2$ sites. Both sea urchin species exhibited consistent abundances, ca. 1–2 m$^{-2}$ (*P. lividus*) and 4–6 m$^{-2}$ (*A. lixula*), across the reference sites but were completely absent from any of the higher CO$_2$ sites (see also Johnson et al., 2012). In contrast, *A. viridis* abundance remained consistent (of ca. 10 m$^{-2}$) across the reference sites but significantly increased to ca. 20 and 40 m$^{-2}$ at sites S1–S2 and S3 respectively (see Fig. 1). In addition to abundance, *A. viridis* size (pedal disc) increased from ca. 21–23 mm at the reference sites to...
ca. 27–31 mm at the higher CO₂ sites (Table 1). The largest anemones were observed at sites S2–S3.

**Anemone productivity**

Light-limited (z) and light saturated ETRs (ETR_{max}) as well as the maximum gross photosynthesis rate (P_{max}) generally increased from the reference to the high CO₂ sites (Table 1; also Fig. 2a). Values for z, ETR_{max} and P_{max} were ca. 0.4–0.5 mol electrons [mol photons]⁻¹, 210–250 μmol electrons m⁻² s⁻¹ and 0.22 μmol CO₂ g⁻¹ h⁻¹, for the ambient pCO₂ reference sites (R1-3, Table 1); as with anemone size (above), values for these parameters describing primary productivity all increased by ca.10–20% at S1 but were not statistically different from those at R3. Highest values of z, ETR_{max} and P_{max} were observed at the highest pCO₂ sites (S2–S3), ca. 0.65–0.70 mol electrons [mol photons]⁻¹, 300–320 μmol electrons m⁻² s⁻¹ and 0.31–0.42 μmol CO₂ g⁻¹ h⁻¹.

Both measures of primary productivity, ETR_{max} and P_{max}, increased in proportion with pCO₂ across the reference (R1–R3) sites, S1 and S2 (Fig. 2a); thus the yield of CO₂ assimilation from PSII photosynthetic activity was highly conserved as pCO₂ increased (pH decreased). However, the increase of P_{max} (ca. 40%) exceeded that of ETR_{max} (ca. 10%) between S2 and S3 indicating an increased yield of CO₂ assimilation from PSII photosynthetic activity for the site with the highest pCO₂.

Respiration rates also increased with pCO₂, i.e. from ca. 0.22 μmol CO₂ g⁻¹ h⁻¹ (R1-3) to ca. 0.24–0.33 μmol CO₂ g⁻¹ h⁻¹ (S1–S3) (Table 1); however, the increase of R was less than that of P_{max} such that the P_{max}:R increased from ca. 1.1 at the reference sites to ca. 1.2–1.3 mol: mol from S1 to S3 (Table 2) and hence with increasing pCO₂ (Fig. 2b). Both increases of P_{max} and P_{max}:R suggest that greater anemone size/abundance with pCO₂ may be driven, at least in part, by enhanced metabolic activity, and notably from increased primary productivity over respiration.

**Microalgal symbiont characteristics**

*Symbiodinium* populations across all sites/anemones were identified to be the same ITS2 ‘type’ of clade A. This symbiont, designated here as A19, was previously isolated from the Mediterranean (Genbank accession 449406) and belongs to a lineage within clade A previously described as ‘A’ that appears geographically restricted, yet regionally abundant, to the temperate locations of the Mediterranean and the north-eastern Atlantic (Savage et al., 2002; Visram et al., 2006). Thus, acclimation via a single *Symbiodinium* type regulates the enhanced primary productivity with lower pH/higher CO₂.

Cell density (per cm⁻² tentacle) was constant with tentacles containing ca. 3–4 x10⁵ cells cm⁻² for the reference sites R1–R3 but increased with pCO₂ to 13–15 x10⁵ cells cm⁻² by S2–S3 (Table 2); cells contained ca. 20% more dividing cells at S3 compared to sites R1–R3 (mean ± standard error mitotic index of 1.71 ± 0.12% for R1–R3 vs. 2.08 ± 0.18% for S3, t-test, P < 0.05; not shown). Overall, the changes of *Symbiodinium* cell density were linearly correlated with those of ETR_{max} (and to a lesser extent P_{max}), r² = 0.892 (r² = 0.813) (see Fig. 2a) across all sites. As such, the increased ETR_{max} and P_{max} with pCO₂/decreasing pH was predominantly driven by increased *Symbiodinium*.
cell density as opposed to increased Symbiodinium productivity per cell.

Discussion

Anemones in present day benthic systems promote biodiversity by supporting mutualisms and numerous predators (e.g. Holbrook & Schmitt, 2005), reducing macro- and filamentous-algal recruitment (Taylor & Littler, 1982) and overgrowth (Bak & Borsboom, 1984), and maintain primary productivity rates that can be as high as that of algae (Fitt et al., 1982). Thus, the enhanced metabolic activity (productivity) and, in turn growth (size and abundance), of anemones with increasing pCO₂/reduced pH suggest that OA could alter the role of anthozoans in benthic communities with fundamental implications to ecosystem function. At Vulcano, concomitant increases in anemone abundance with pCO₂ corresponded with enhanced P_C, i.e. a response comparable to that of the macroalgae at this site (Johnson et al., 2012). Although the increases in anemone abundance were perhaps not as pronounced as for the microalgae, the increase in P_C (also>P) would suggest that under OA, anemones could contribute significantly to driving the ecosystem metabolism towards greater net autotrophy and CO₂ sequestration. Such a biogeochemical response is potentially important in offsetting OA impacts to reef environments (Anthony et al., 2011), but this comes at a cost to the diversity of species and function.

Seawater acidification has been shown to reduce ecological diversity (e.g. Hall-Spencer et al., 2008; Fabricius et al., 2011), in particular where enhanced dissolution impedes growth of calcifying invertebrates (Rodolfo-Metalpa et al., 2011) and therefore limits their ability to keep the biomass of algae, or indeed other space competitors, in check. As non-calcifiers that supplement heterotrophy with autotrophy (although our higher values of P_C over R would suggest autotrophy dominates), anemones are already at an advantage over other invertebrates to lower pH conditions. Calcifying invertebrates that can compete under lower pH conditions inevitably exhibit reduced growth rates (Fabricius et al., 2011) as more energy is likely redirected to maintain calcification (e.g. Krief et al., 2010); however, in the case of the anemones their success via elevated productivity may be further exacerbated by their capacity to compete with algae for space via chemical deterrents (Bak & Borsboom, 1984). Indeed, a decrease in macroalgal cover was observed at the highest CO₂ site (S3), compared to S1 and S2, where the corresponding anemone abundance was almost a factor of 2 greater at S3 than at S1–S2. Anthozoans are well known for their aggressive control of space, perhaps giving soft-bodied anthozoa a competitive edge should they persist under OA; such enhanced fitness could indeed be crucial where elevated CO₂ already enhances algal competition (Díaz-Pulido et al., 2011; Johnson et al., 2012).

Of course understanding the driving mechanisms behind enhanced competition under OA also requires knowledge of whether or not anemone predation is also affected. Anemones are predated by a huge diversity of organisms, including crustaceans (decapods), molluscs (gastrapods), echinoderms (starfish) (e.g. Ottaway, 1977), which are all likely impacted at some stage of their life history by ocean acidification (e.g. Andersson et al., 2011). Unfortunately, little is known as to the predator-prey dynamics of Mediterranean A. viridis populations and thus we cannot presently address this issue; instead we focus on the enhanced P_G, which clearly provides evidence for some fundamental role of OA in enhancing anemone success from the bottom up.

Regulation of anemone productivity by environmental factors such as light availability (e.g. Bythell et al., 1997; Muller-Parker & Davy, 2001) is reasonably well understood; however, the role of CO₂ (pH) has been largely neglected. Some OA-focussed lab experiments have been recently performed on temperate anemones of the temperate genus Anthopleura, but with contrasting results between studies/species: An increase of P_C and R (but P_C>R) as well as more abundant (and larger) Symbiodinium cells with decreasing pH were evident for Anthopleura elegantissima (Towanda & Thuesen, 2012), i.e. results consistent with our observations at Vulcano for A. viridis. However, only small increases of Symbiodinium cell density and an increase of R exceeding that of P_C were observed at lower pH for A. aureoradiata (Doherty, 2009). Unfortunately, neither experiment was performed for long enough to determine whether either of these OA responses was conveyed into longer term differences in anemone fitness, such as growth and reproduction.

The underlying reason for these contrasting experimental results is not clear, and may simply reflect species-specific differences, but could equally reflect differences in conditions other than CO₂ availability; for example, although both species were provided with similar feeding regimes (every 4–5 days), A. elegantissima and A. aureoradiata were maintained under temperature and light conditions of 12°C and 660 µmol photons m⁻² s⁻¹ (14 : 10 L : D) and 16°C and 275 µmol photons m⁻² s⁻¹ (12 : 12 L : D) respectively. Both light and temperature moderate the extent of CO₂ uptake (and hence the likely responses observed to OA, e.g. Rodolfo-Metalpa et al., 2011) but it is not possible to determine which may be contributing to the contrasting responses from the data currently available.
Our observations from Vulcano and those of Towanda & Thuesen (2012) and Doherty (2009) would suggest that *Symbiodinium* productivity in anemones is limited by inorganic carbon (iC) availability. As with other symbiotic cnidarians, *Symbiodinium* in anemones are separated from the external inorganic carbon supply by several membranes and thus limited by the supply of iC (e.g. Benazet-Tambutte et al., 1996; Muscatine et al., 1998; Davy & Cook, 2001; see also Brownlee, 2009). To overcome iC limitation the host cnidarian employs external (likely membrane-bound) carbonic anhydrase to convert HCO$_3^-$ to CO$_2$ and enhance the iC supply from seawater to the *Symbiodinium* cells (e.g. Ganot et al. (2011) for *A. viridis*; see also Weis (1993), Weis & Reynolds, 1999). Increased iC availability promotes autotrophy (PG), which in turn would increase the metabolic exchange with the host (Brownlee, 2009) and host respiration (Harland & Davies, 1995). Such a response would be consistent with the greater OA-induced increase of PC over R; that said, changes of PC may not fully account for those of R, in particular where PC does not fully meet the metabolic demands of the host (Davy et al., 1996). Although previous observations from the anemone *A. elegantissima* (Towanda & Thuesen, 2012) suggest that the host receives more of their respiratory carbon from PC under OA, we cannot presently discount that differences in food supply (particulate organic carbon, POC) may also exist between reference and elevated sites to explain the differences of R, and thus account for the relative role of iC vs. POC availability upon elevated growth.

Importantly the increased PC with pCO$_2$ observed here corresponded with enhanced *Symbiodinium* cell density whereas ETR$^{\text{max}}$ and P$_{\text{max}}$ cell$^{-1}$ remained relatively constant. *Symbiodinium* can invest into enhanced growth (over cell-specific productivity) under OA (Brading et al., 2011) but such a response does not seem consistent with previous OA observations from calcifying anthozoans (e.g. Crawley et al., 2010; Krief et al., 2010; Meron et al., 2012) (see below). However, *Symbiodinium* cells are often nutrient-limited within (anemone) hosts, and the host environment likely setting the upper limit on the rate of *Symbiodinium* cell-cycle progression (Smith & Muscatine, 1999; see also Muller-Parker & Davy, 2001). Thus, increases in cell density can occur where densities are simply not optimum (at ‘steady state’ levels) and environmental conditions become more favourable for growth (Jones & Yellowless, 1997; Muscatine et al., 1998); as such, *Symbiodinium* cell densities at the lowest CO$_2$ sites could be lower than required for optimum growth. It is important to also note that our approach for normalizing *Symbiodinium* cell density to tentacle SA may influence how changes of pCO$_2$ drive those of cell density. Specifically, tissue protein content per unit area appears to increase with pCO$_2$ for both anemones (Towanda & Thuesen, 2012) and scleractinian corals (Meron et al., 2012). Therefore, our observations of increased cells cm$^{-2}$ with pCO$_2$ may reflect the capacity of increased tissue content (per cm$^{-2}$ tentacle) to simply harbour more cells. Even so *Symbiodinium* cell densities ultimately are increased with pCO$_2$.

An increase of PC with CO$_2$, i.e. ‘CO$_2$ enrichment’, is somewhat analogous to that expected from addition of other inorganic nutrients essential for *Symbiodinium* growth (Weis 1933); for example, NH$_4^+$ additions have been repeatedly shown to increase *Symbiodinium* cell number and productivity by anemones (e.g. Cook et al., 1988; Muscatine et al., 1998, Smith & Muscatine, 1999) suggesting that mutualistic associations occur between anemones and other organisms that excrete NH$_4^+$, e.g. anemone fish in tropical systems, provide nutritional benefits that can promote anemone growth (e.g. Holbrook & Schmitt, 2005). In fact under extreme nutrient-addition events, e.g. eutrophication of tropical reef systems, phase shifts dominated by enhanced anemone abundance may occur (Tkachenko et al., 2007). Such responses raise a critical point here:

Stimulation of productivity by inorganic carbon (OA) would require that nutrients such as nitrate and phosphate are available to build additional organic skeletons and thus ‘fuel’ the benefits of CO$_2$ availability into growth; indeed, reports do exist of high NH$_4^+$ excretion rates by temperate anemones (Jensen & Muller-Parker, 1994), suggesting higher intrinsic nutrient availability/storage inherent to temperate cnidaria (see also Davy et al., 2006) could support elevated PC under OA, but such reports are rare. Stimulated growth of anemones can occur under increased nutrient availability without reductions of seawater pH (Tkachenko et al., 2007). In this case the actual benefit to the host, in terms of carbon translocated (and hence R), may not increase unless more CO$_2$ is available (Davy & Cook, 2001). Our data does provide some evidence that nutrient availability may be enhanced at the lowest pH site since ETR$^{\text{max}}$ becomes more closely coupled with P$_{\text{max}}$ (see Fig. 2); such a response is typical of stress relief, where electrons would otherwise be funnelled into alternative acceptors and/or cyclic flow (e.g. Suggett et al., 2011). So are the OA responses we observe at Vulcano potentially influenced by nutrient availability?

At present we cannot determine whether changes in particulate organic nutrient supply occur along the CO$_2$ gradient; however, some data were collected for dissolved inorganic nutrients (nitrate plus nitrite, DIN, as well as phosphate, DIP) but not NH$_4^+$ (L. Al-Moosawi, Personal communication) from surface samples during May 2011: DIP concentrations remained undetectable
(<10 nM) across the pH gradient. DIN concentrations also remained low (ca. 0.2 μM) for waters with a pH of 8.2–8.0, but did increase from ca. 0.2–0.8 μM as pH decreased from 7.6 to 7.2. Thus, it is possible that DIN concentrations are elevated periodically for S3 and, to a lesser extent, S2 (as per the pH range for these sites, Table 1). Even so, it is important to point out that (i) these elevated DIN concentrations at the lower pH are still somewhat low to sustain the substantially enhanced productivity here, (ii) a CO₂ response is still somewhat low to sustain the substantially increased growth with P_C (Rodolfo-Metalpa et al., 2010; Krief et al., 2010; Towanda & Thuesen, 2012). However, these increases may often be relatively small, ca. 10–20% increases with a twofold increase in pCO₂ (Rodolfo-Metalpa et al., 2010) and/or, restricted to only moderate changes in pCO₂ (Crawley et al., 2010); indeed some experiments have shown that P_C and/or P_N is either unchanged or can even decrease with increasing pCO₂ (e.g. Schneider & Erez, 2006). Such diversity of responses is perhaps not surprising since other factors likely regulating productivity, e.g. light availability (see Langdon & Atkinson, 2005) and the role of calcification in modifying DIC availability (Schneider & Erez, 2006; Krief et al., 2010), are again not well accounted for between experiments. Furthermore, reconciling these contrasting responses is confounded by a lack of knowledge of Symbiodinium genetic identity, which is almost never reported from coral OA studies but can fundamentally determine whether or not P_C is likely to respond to OA (Brading et al., 2011). Indeed, differences between Symbiodinium spp. may also explain why the OA response for anemones, i.e. enhanced P_C via increased cell density over productivity cell⁻¹, appears to differ from that previously observed for corals.

At Vulcano, enhanced P_C observed for the anemones was driven by increased growth (but not P_C cell⁻¹) of a specific ITS2 type (A19) with CO₂. Such a response of increased growth with pCO₂ has been observed for another clade A-type, isolated from a tropical anemone (A13, Condylactis gigantea) (Brading et al., 2011); this same study also demonstrated enhanced P_C cell⁻¹ for an A-type originally isolated from the scleractinian coral Montastrea faveolata, further suggesting that some clade A Symbiodinium may be particularly affected by OA. Thus, OA enhancement of productivity for anemones (and other non-calcifying cnidarians) may be expected where Symbiodinium within clade A dominates, such as in Mediterranean and/or European waters (Savage et al., 2002; Visram et al., 2006). Conservation of a single Symbiodinium type across natural pCO₂ gradients is consistent with recent reports from scleractinian corals (Balanophyllia europaea and Cladocora caespitosa) at another Mediterranean vent site (Ischia, Meron et al., 2012); although whether or not this single type also exhibited enhanced P_C with pCO₂ at Ischia is presently not known. The experiments by Fine & Tchernov (2007) showing viable cnidarian growth under extremely low pH have only been demonstrated on Mediterranean corals to date.

Whether or not strong Symbiodinium driven responses of anthozoan productivity go beyond harbouring A-types in the Mediterranean is currently unknown; however, OA-induced stimulation of anemone productivity occurs with Symbiodinium from clade B as well (A. elegantissima, Towanda & Thuesen, 2012). Even so, the strong OA response observed with Symbiodinium A19 at Vulcano could imply that OA enhanced productivity would perhaps not be as prominent in tropical reefs where anemones and other cnidarians, such as hard and soft corals, harbour genetically different symbionts within clade A in addition to those from other clades (e.g. Finney et al., 2010; LaJeunesse et al., 2010). Strong geographical and temporal delineations of Symbiodinium diversity do exist for anemone populations (Venn et al., 2009; Sanders & Palumbi, 2011). Thus, further understanding how OA responses in cnidarians are moderated by symbiont identity will be key, in particular where specific phylotypes may further determine how cnidarians can respond to additional stressors, such as anomalous temperatures.

Overall, our data show that productivity and in turn growth of anthozoans can be substantially up-regulated by OA-like conditions; these factors appear to contribute, at least in part, to a bottom-up enhancement of anemone abundance in Vulcano’s benthic community. Similar OA-induced enhancement of (non-calcifying) cnidarian abundance from other present day CO₂ vent sites has not been previously observed (Hall-Spencer et al., 2012).
et al., 2008; Fabricius et al., 2011) but may represent a complex mix of factors regulating the responses; notably (i) ecological interactions modifying whether enhanced $P_C$ results in increased net growth/abundance (e.g. enhanced predation), (ii) interactive influences of other environmental conditions and (iii) Symbiodinium identity. How the responses we currently observe at Vulcano translate to broader (ecosystem)-level responses by non-calculifying cnidarians under OA cannot currently be determined but is an obvious priority: Non-calculifying cnidarians, such as soft corals and anemones, can play key ecological and biogeochemical roles and often (re)populate reefs that have been impacted by local stressors (Tkachenko et al., 2007). Consequently, understanding how this group of organisms responds to longer term climatic change, and the combination of factor(s) that drives ‘success’ alongside elevated $pCO_2$ will be an essential component of confidently predicting the future form and function of benthic ecosystems.

Acknowledgements
We wish to thank Marco Milazzo (University of Palermo) for essential academic and logistical support throughout. We are indebted to Simon Davy for critically reviewing and improving the final version of the manuscript. This work contributes to the EU FP7 project on ‘Mediterranean Sea Acidification under a changing climate’ (MedSeA grant agreement no. 265103) and to the UK Ocean Acidification Research Programme (NERC); also we gratefully acknowledge additional funding support by NERC (grant NE/G020116/1 to DJS and TL), Save Our Seas Foundation (JHS), The National Science Foundation (grant 1040940 to MEW) and The Earls Colne and Halstead Educational Charity (TGB).

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