Temperate and tropical brown macroalgae thrive, despite decalcification, along natural CO₂ gradients

VIVIENNE R. JOHNSON*, BAYDEN D. RUSSELL†, KATHARINA E. FABRICIUS§, COLIN BROWNLEE† and JASON M. HALL-SPENCER*

*Marine Biology and Ecology Research Centre, University of Plymouth, Plymouth, PL4 8AA, UK, †The Marine Biological Association of the United Kingdom (MBA), The Laboratory, Citadel Hill, Plymouth, PL1 2PB, UK, § Southern Seas Ecology Laboratories, Earth and Environmental Sciences, The University of Adelaide, Adelaide, SA 5005, Australia, § Australian Institute of Marine Science, PMB 3, Townsville, QLD 4810, Australia

Abstract

Predicting the impacts of ocean acidification on coastal ecosystems requires an understanding of the effects on macroalgae and their grazers, as these underpin the ecology of rocky shores. Whilst calcified coralline algae (Rhodophyta) appear to be especially vulnerable to ocean acidification, there is a lack of information concerning calcified brown algae (Phaeophyta), which are not obligate calcifiers but are still important producers of calcium carbonate and organic matter in shallow coastal waters. Here, we compare ecological shifts in subtidal rocky shore systems along CO₂ gradients created by volcanic seeps in the Mediterranean and Papua New Guinea, focussing on abundant macroalgae and grazing sea urchins. In both the temperate and tropical systems the abundances of grazing sea urchins declined dramatically along CO₂ gradients. Temperate and tropical species of the calcifying macroalgal genus Padina (Dictyoaceae, Phaeophyta) showed reductions in CaCO₃ content with CO₂ enrichment. In contrast to other studies of calcified macroalgae, however, we observed an increase in the abundance of Padina spp. in acidified conditions. Reduced sea urchin grazing pressure and significant increases in photosynthetic rates may explain the unexpected success of decalcified Padina spp. at elevated levels of CO₂. This is the first study to provide a comparison of ecological changes along CO₂ gradients between temperate and tropical rocky shores. The similarities we found in the responses of Padina spp. and sea urchin abundance at several vent systems increases confidence in predictions of the ecological impacts of ocean acidification over a large geographical range.

Keywords: calcification, ocean acidification, photosynthesis, temperate and tropical coastal ecosystems

Received 29 March 2012; revised version received 29 March 2012 and accepted 31 March 2012

Introduction

Rising anthropogenic emissions of CO₂ are rapidly altering ocean chemistry as increasing pCO₂ in seawater has already lowered the mean ocean surface pH by 0.1 units from preindustrial values, with a predicted further decrease of 0.3–0.4 units by 2100 (IPCC (Intergovernmental Panel on Climate Change), 2007). The resulting decrease in calcium carbonate saturation levels compromises the ability of many marine organisms to form shells and skeletons (Orr et al., 2005; Doney et al., 2009). This, in combination with the diverse responses of photosynthetic organisms to increased pCO₂ levels (Russell et al., 2009; Hepburn et al., 2011; Forzio et al., 2011; Johnson et al., 2012), is expected to alter the structure of biological communities along coastlines worldwide (Barry et al., 2011). However, the potential effects of altered community structure on ecosystem functioning are unclear as the effects of elevated CO₂ levels on organism interactions have only recently begun to be addressed (Diaz-Pulido et al., 2011; Doropoulous et al., 2012).

Seagrasses and many macroalgal species are notably tolerant of, or even benefit from increases in CO₂ (Connell & Russell, 2010; Fabricius et al., 2011; Porzio et al., 2011; Roleda et al., 2011). However, studies from polar, temperate and tropical latitudes have revealed that settlement, calcification, growth and abundance of calcified macroalgae can be negatively affected by increasing CO₂ levels as this lowers carbonate saturation states which can corrode the algal skeletons (Kuffner et al., 2008; Martin et al., 2008; Martin & Gattuso, 2009; Robbins et al., 2009; Russell et al., 2009; Büdenbender et al., 2011; Price et al., 2011; Sinutok et al., 2011; Doropoulous et al., 2012). Increasing concentrations of CO₂ can, on the other hand, enhance productivity and growth in both noncalcified (Kübler et al., 1999; Connell
& Russell, 2010) and calcified macroalgae (Reiskind et al., 1988; Semesi et al., 2009).

Understanding the effects of ocean acidification on calcified algae is of a high priority as they play a crucial role in the ecology of coastal ecosystems (Nelson, 2009). Most studies to date have been single species laboratory experiments that last a year at most (Martin & Gattuso, 2009). Such experiments provide important information on species’ responses to increased pCO2 but fail to account for the effects of long-term exposure. They are also unrepresentative of natural ecosystems since, for example, they remove the effects of species interactions (Barry et al., 2011). In contrast to short laboratory experiments, CO2 gradients in natural settings, where whole ecosystems have been exposed to elevated levels of pCO2, allow us to investigate changes in the interactions, competition, predation and/or herbivory that involve long-lived metazoan species in benthic marine ecosystems.

Volcanic CO2 gradients are beginning to reveal the ecological shifts that can be expected to occur with globally increasing atmospheric CO2 in both temperate (Hall-Spencer et al., 2008) and tropical ecosystems (Fabricius et al., 2011). Work has begun to show the underlying mechanisms that cause ecological shifts along these CO2 gradients, such as the influence of recruitment success (Cigliano et al., 2010) and the combined physiological effects of temperature and CO2 (Rodolfo et al., 2011). Drawbacks associated with using this approach include the fact that these are open systems surrounded by waters that are unaffected by the vents, a situation that is unrealistic as the global oceans acidify (Hall-Spencer 2011). Despite such limitations, this mensurative approach provides insights that are complimentary in scope and scale to the prevalent ex situ approaches (Wernberg et al., 2012). Here we assess the abundance of herbivores (sea urchins) and the response of brown macroalgae (Padina spp.) to increasing levels of CO2 in natural settings, as interactions between these groups of organisms can drive ecological changes in benthic habitats on temperate (Sala et al., 1998; Hernández, et al., 2008) and tropical shores (McClanahan, 1994; Mumby et al., 2006).

Padina is one of only two genera of Phaeophyta that calcify and is an important producer of calcium carbonate and organic matter in both temperate and tropical shallow waters (Bathurst, 1971; Milliman, 1974). Calcium carbonate is deposited as aragonite needles on the surface of fan-shaped thallii, forming concentric bands of white precipitate (Okazaki et al., 1986). Carbonate production rates of Padina sp. in one subtropical system have been calculated to be around 240 gm⁻² yr⁻¹, considerably higher than for other erect calcified algal genera such as Halimeda (50 gm⁻² yr⁻¹) and Penicillus (30 gm⁻² yr⁻¹) (Wefer, 1980). Several roles have been suggested for calcification in macroalgae. It is thought to offer structural defence, providing mechanical resistance to herbivores and minimizing grazing damage to tissues (Little & Littler, 1980; Padilla, 1993), increase the ability for bicarbonate and nutrient assimilation through the generation of protons (McConnaughey & Whelan, 1997), improve photosynthetic performance (McConnaghey, 1998) and provide protection from excess irradiance (Bürger & Schagerl, 2010). Therefore changes in macroalgal calcification as a result of ocean acidification have the potential to alter physiological and ecological fitness, by altering photosynthetic efficiency, thallus rigidity, growth rates and mortality (Nelson, 2009).

Our present knowledge of the effects of ocean acidification on calcified macroalgae is mostly derived from studies investigating the impacts of elevated CO2 on calcifiers with high magnesium calcite skeletons, such as the family Corallinaceae (Anthony et al., 2008; Kuffner et al., 2008; Martin et al., 2008; Martin & Gattuso, 2009; Semesi et al., 2009; Gao & Zheng, 2010; Büdenbender et al., 2011). The surface seawater saturation state of aragonite (Ω 3–4) is greater than that of high magnesium calcite (Ω 2–3), so algae that precipitate the latter are expected to have more difficulty producing their CaCO3 skeletons under increasing CO2 than aragonite species (Kleypas et al., 1999) and, as a consequence, aragonitic species have been relatively overlooked. Furthermore, the responses of calcified Phaeophyta are virtually unknown (Porzio et al., 2011). Padina spp. are not obligate calcifiers and deposit CaCO3 extracellularly (on the thallus surface), so their response may differ to that of Corallinaceae which are obligate calcifiers with intercellular deposition (within cell walls).

Ocean acidification also has the potential to reduce top-down biological control of benthic biodiversity (Widdicombe & Spicer, 2008). Sea urchins are dominant grazers in many marine habitats and play an important role in controlling the structure and composition of macroalgal communities. They often act as keystone species (Sala et al., 1998) and, as a consequence, reduction in their abundance or removal from an ecosystem can result in rapid colonization of benthic habitats by macroalgae (Villouta et al., 2001; Behrens & Lafferty, 2004). Sea urchins are particularly susceptible to reductions in pH (Miles et al., 2007) and a mean pH of 7.8 appears to be the critical level below which Mediterranean sea urchins do not survive (Hall-Spencer et al., 2008). Adverse impacts of ocean acidification on echinoderms would be likely to have significant consequences at the ecosystem level (Barry et al., 2010; Dupont et al., 2010). It has the potential to release algae from the control of grazing by sea urchins, resulting in...
cascade effects throughout benthic food webs, with potentially profound implications for the structure and function of marine communities.

The aim of this study was to survey populations of sea urchins (Echinoidea) and Padina spp. (Dictyotaceae) along pH gradients in both temperate and tropical ecosystems, and to measure in situ effects of elevated CO2 on calcification and photosynthesis in this common phaeophyte. We present data on the long-term effects of natural exposure to low pH and high CO2 on Padina pavonica (Linnaeus) Thivy at shallow, volcanic CO2 seeps on the island of Vulcano, NE Sicily and on Padina australis Hauck at comparable seeps in the D’Entrecasteaux Island group, Papua New Guinea. To our knowledge, this is the first study to compare ecological responses to CO2 gradients in temperate and tropical systems. We observed strikingly similar ecological shifts along both tropical and temperate rocky shores as CO2 levels increased to those previously recorded at CO2 vents off Ischia, Italy (Hall-Spencer et al., 2008), with the loss of sea urchins and coralline algae together with an increased abundance of phaeophytes.

Material and methods

Temperate and tropical rocky shore surveys

Padina pavonica was sampled along a stretch of rocky coast off the island of Vulcano (38°25’ N, 14°57’ E, part of the Aeolian Island chain, NE Sicily) in September 2010 and May 2011 (see maps in Johnson et al., 2012). This is a microtidal region where volcanic CO2 vent activity acidifies the seawater producing a pH gradient ranging from ~8.2 to ~6.8, running parallel to the coast. Within the vent area, three shallow (<0.5 m depth) sampling stations were selected as they lay along a CO2 gradient, characterized by intermediate to low mean pH (V-S1 pH 8.06, CI = 0.59%; V-S2 pH 7.54, CI = 1.59%; V-S3 pH 7.46, CI = 2.03%, n = 24–27). Three reference stations located outside the vent area were selected on the basis of their normal, relatively stable pH (U-R1 pH 8.31, CI = 0.12%; U-R2 pH 8.22, CI = 0.10%; E-R1 pH 8.19, CI = 0.77%, n = 6–9) were chosen within several hundred meters away from the seeps at comparable geophysical settings. There was variation in pH at stations exposed to the CO2 seepage, particularly at Vulcano (stations S2 and S3). This is the result of variable mixing of ambient seawater during calm vs. windy periods and is inherent at CO2 vent systems (Fabricius et al., 2011; Hoffmann et al., 2011; Kerrison et al., 2011).

At all sites (Vulcano in the Mediterranean, and Upa-Upasina and Esa’Ala in Papua New Guinea), 20 quadrats (50 cm × 50 cm) were placed haphazardly (‘blind throws’) within 3 m survey zones (<0.5 m depth) at each station along the CO2 gradients. Within each quadrat, the percentage cover of Padina spp. was estimated and the total number of sea urchins (Paracentrotus lividus & Arbacia lixula in the Mediterranean, Diadema spp. & Echinometra sp. in Papua New Guinea) recorded. Visual estimates were conducted by VRJ and JH-S who compared their techniques to minimize inter-observer variability.

Carbonate chemistry measurements

A calibrated pH meter was used to measure pH (NBS scale) at each sampling station at Vulcano (YSI 556 MPS, three-point calibration) and Papua New Guinea (Hach or Oakton, two-point calibration, with readings cross-checked against a Tris buffer seawater standard). Temperature and salinity were also measured alongside each pH reading. We recorded rapid pH fluctuations along this coastal gradient (over 1 unit in under ~4 h at S3 at Vulcano), so the uncertainty inherent in using the NBS scale for seawater measurements (approximately 0.05 pH, Dickson, 2010) was considered acceptable for this study. Mean pH (back-transformed hydrogen ion concentrations) was calculated for each station at Vulcano (pH sampled on several occasions, at various times of the day; September–October 2009, April 2010, July 2010, September–October 2010, May 2011, September–October 2011, n = 22–27) and Papua New Guinea (25th and 29th April 2011, n = 6–9). Ninety-five percentage confidence intervals were calculated and presented as a percentage of the mean pH.

Total alkalinity (TA) was measured alongside pH to calculate the other parameters constraining the carbonate chemistry of the seawater (Hoppe et al., 2010). At Vulcano, TA was measured at each station, on three separate visits (September 2010, May 2011 and September 2011), from a water sample after 0.2 μm filtration and storage in the dark at 4 °C, using an AS-Alk 2 Total Alkalinity Titrator (Apollo SciTech Inc, Bogart, GA, USA). Total alkalinity data for Papua New Guinea were taken from Fabricius et al. (2011). The remaining parameters
of the carbonate system were calculated using the CO2 SYS software (Lewis & Wallace, 1998).

**Padina spp. calcium carbonate analysis**

Large (>2 cm) *Padina* spp. fronds were collected from each sampling station at Vulcano in the Mediterranean (n = 30 per station) and from a reference and high CO2 station at both Upa-Upasina (U-R1 & U-S1, n = 15 per station) and Esa’Ala (E-R1 & E-S2 n = 5 per station) in Papua New Guinea. Samples were stored in 70% ethanol until analysis. Calcium carbonate (CaCO3) content of each frond was determined through a weight loss after acidification protocol (Martone, 2010). Fronds were dried, weighed and decalcified in hydrochloric acid (1N) overnight. This resulted in the complete dissolution of a thin layer of CaCO3; the fronds were then redried and reweighed. The CaCO3 content, expressed as a percentage of dry weight, was calculated from the difference between dried mass and decalcified dry mass.

Images of *P. pavonica* aragonite crystals were examined for size and abundance using scanning electron microscopy (JEOL JSM 5600 LV: JEOL Ltd., Tokyo, Japan). Three fronds from each station were fixed in glutaraldehyde for 1–2 h, and then stored in 1× PBS buffer (phosphate buffered saline) until examination. As the size and number of crystals has been reported to vary with age of frond segment (Hillis-Colinvaux, 1980), we only compared the apical segments of *P. pavonica* fronds between stations. Prior to viewing under the SEM, samples were air dried, mounted on aluminium stubs with carbon adhesive tape and coated in gold. For each of the 18 samples, five images were taken at random locations (using image coordinates and random number generator) over calcified regions of the apical surface only (see images in Fig. 4) and the average length and width of 10 randomly selected crystals per image was measured digitally using Image J software (v 1.43; National Institutes of Health, Bethesda, MD, USA). In addition, for each image, the number of crystals within a randomly selected 5 µm × 5 µm area were counted and averaged for each frond.

**Photosynthesis in Padina pavonica**

Photosynthetic capacity and performance of *P. pavonica* at Vulcano was investigated through measurements of photosynthetic pigment (Chl a and c1+c2) concentrations and Chl a fluorescence respectively. These physiological measurements were performed in summer months (May and September) when algal productivity is high. For pigment analysis, fronds were collected from each sampling site at Vulcano in September 2010 and September 2011 (n = 40 per station), rinsed in distilled water and frozen for transportation back to the laboratory. Fronds were collected between 8:00 and 10:00 hours to avoid the confounding effect of light intensity, in particularly mid-day photoinhibition, on chlorophyll content (Häder et al., 1996). To prevent chlorophyll degradation during storage, samples were kept at −20 °C in the dark during the sampling period on Vulcano and at −80 °C when longer periods occurred before analysis. Chlorophyll was extracted from all samples within <2 weeks of sampling.

Prior to extraction, fronds (~0.70 g samples) were homogenized in 90% acetone by pestle and mortar. Chlorophyll was extracted in 90% acetone at 4 °C for 24 h in the dark. The absorbance of each sample at 630, 664 and 750 nm (background absorbance) was measured (three replicate readings were taken from each sample to obtain an average) using a Cecil CE2011 spectrophotometer (Cecil Instruments Ltd, Cambridge, UK). The concentration of chlorophyll a and c (c1+c2) in the sample was calculated using the equations of Ritchie (2006). The volume of the solvent (in weight g−1) and the weight of the frond were then used to provide a final calculated reading of chlorophyll (µg mg−1 fresh weight). Values for both September sampling periods were pooled to calculate a mean for each station.

In May 2011, the effective quantum yield (Y) and relative electron transport rates (rETR) of freshly collected, light-adapted fronds (n = 6 per station, stored in seawater from site of collection), were measured in small dishes using a Diving-PAM fluorometer (Heinz Walz GmbH, Effeltrich, Germany) in a dimly lit room.

\[
Y = \frac{F_m - F_i}{F_m} = \frac{Y}{C2} \\
\text{rETR} = Y \times PAR \times 0.5
\]

where; \(F_m\) = maximum fluorescence yield of light adapted fronds, \(F_i\) = steady-state level of fluorescence under illumination at time \(t\) (Genty et al., 1989); PAR = photosynthetic active radiation and 0.5 is a constant assuming both PSI and PSII absorb equal amounts of the incoming photons (Beer et al., 1998).

Rapid light curves (RLC) were applied to assess the light saturation behaviour of individual, whole fronds across each of the six sampling stations in Vulcano. Rapid light curves data can be useful for assessing photosynthetic capacity and potential over a wide range of ambient light intensities (Ralph & Gademan, 2005). The Diving-PAM was set to deliver red pulse-modulated light at 655 nm followed by steps of actinic light from 1 to 3344 µmol photons m−2s−1 delivered every 20 s over a period of 160 s (other settings: gain = 4, actinic light factor = 0.5, light curve intensity y = 5, saturation width = 0.8, saturation intensity = 3, signal damping = 2).

**Statistical analyses**

To test for significant effects of mean pH on variations in *Padina* spp. we used generalized linear models (GLM), with pH as the explanatory variable and Site (Vulcano, Upa Upasina and Esa’Ala) as a covariate. Data were averaged across stations and transformed where necessary to approximate normality and equal variance. For count data with many zeroes (e.g., sea urchin abundances) or overdispersed data, a quasi-poisson link function was used, whereas for proportional, ETR and yield data, a quasi-binomial link function, and for the remaining data the Gaussian link function were used. All statistical
analyses were performed using R (R Development Core Team, 2011).

Results

**Seawater chemistry**

The mean pH of the reference stations in all three systems ranged from 8.17 to 8.31, whereas the mean pH at the seep stations ranged from 8.06 to 6.68, with increasing variance towards lower values (Fig. 1a). Carbonate chemistry parameters for each sampling station

are presented in the supplementary material (Table S1). The range in pCO₂ and aragonite saturation along the gradients is displayed in Fig.1b. The median pCO₂ levels (calculated from median pH and mean TA) were lowest at the reference stations (276–388 μatm) and increased with proximity to the seeps, with the highest values recorded at V-S3 (1428 μatm), U-S2 (2665 μatm) and E-S2 (23 095 μatm). The highest median values for pCO₂ and DIC were found at V-S3 (1428 μatm and 3.79 mmol kg⁻¹ respectively), U-S2 (2665 μatm and 2.03 mmol kg⁻¹) and E-S2 (23 095 μatm and 2.85 mmol kg⁻¹). Aragonite saturation decreased with increasing levels of CO₂ (Fig.1b) and periods of under-saturation occurred at stations V-S2, V-S3, U-S2 and E-S2.

**Padina spp. and sea urchin abundances**

There were dramatic ecological shifts along all three volcanic seeps as CO₂ levels increased. We observed a loss of sea urchins and coralline algae together with an increased abundance of phaeophytes that was strikingly similar to that recorded at CO₂ vents in Ischia, Italy (Fig 2a and b). These shifts were detected at median pCO₂ levels of 510 μatm (median pH 8.08), 1218 μatm (median pH 7.78) and 914 μatm (median pH 7.89) along the gradients at Vulcano, Upa Upasina and Esa’Ala respectively (Fig. 3a). Benthic cover of Padina spp. increased with rising CO₂ and was twofold–three-
fold greater in the highest CO2 stations (V-S3, U-S2 & E-S2) relative to the reference stations (Fig. 3a). We detected a significant relationship between pH and both Padina spp. benthic cover and sea urchin abundance at all three gradients (GLM: Table 1). In contrast to Padina spp., sea urchin abundance was greatest at the reference stations and decreased with declining pH at all three gradients (Fig. 3a; Table 1). Sea urchins were absent at stations with the highest levels of pCO2 (V-S1-S3, U-S2, E-S2).

Changes in Padina spp.; CaCO3 content, crystal structure and photophysiology along the CO2 gradients

We found that the CaCO3 content in Padina spp. fronds was significantly related to pH at Vulcano only (as smaller sample sizes were taken at Upa-Upasina and Esa’Ala; Table 1). At Vulcano, CaCO3 content in P. pavonica was highest at the reference stations (57–63%) and decreased significantly in the CO2 enriched stations; S1 (35% ±1.4), S2 (15% ±1.3) and S3 (14% ±0.9). Analysis of P. australis from Upa-Upasina in Papua New Guinea also revealed a large reduction in CaCO3 content from 55% ±1.7 at the reference station (U-R1) to 35% ±3.6 at the intermediate station (U-S1). At Esa’Ala, CaCO3 content was considerably greater in fronds from the reference station (E-R1: 66% ±7.1) compared with those from the highest CO2 exposure station (E-S2: 40% ±1.8).

The abundance and morphometric data of the aragonite crystals on the surface of P. pavonica fronds are presented in the supplementary material (Table S2). Over the thin calcified bands in the apical regions we detected a significant increase in crystal abundances with declining pH (GLM: slope = 0.23 ± 0.077, t = 2.99, P = 0.037) and a reduction in the width of crystals (slope = 0.23 ± 0.067, t = 3.42, P = 0.026), but no effect on crystal length (P = 0.85).

The content of both chlorophyll a and chlorophyll c in P. pavonica was significantly related to pH (Fig. 4, GLM: slope = -0.24 ± 0.065, t = -3.78, P = 0.019; slope = -0.028 ± 0.0055, t = -5.21, P = 0.006, for chlorophyll a and c respectively). Both the chlorophyll a and c con-
tent increased with declining pH (Chl c: V-S1 = 0.05 mg g⁻¹ fw ± 0.002, V-S2 = 0.06 mg g⁻¹ fw ± 0.002, V-S3 = 0.07 mg g⁻¹ fw ± 0.003 compared with those in the reference stations: V-R1 = 0.04 mg g⁻¹ fw ± 0.002, V-R2 = 0.04 mg g⁻¹ fw ± 0.004, V-R3 = 0.04 mg g⁻¹ fw ± 0.003).

The differences observed in the photosynthetic responses of *P. pavonica* to increased CO₂ are presented in a rapid light curve in Fig. 5. The rETR max values significantly increased with declining pH (GLM: slope on fourth-root transformed data = –0.54 ± 0.091, t = –5.97, P = 0.004). We also found that the rETRs recorded at supersaturating irradiance; 3344 μmol quanta m⁻² s⁻¹ were significantly related to pH (slope on fourth-root transformed data = –0.49 ± 0.098, t = –4.95, P = 0.008) where the greatest values were recorded at S2 and S3 (137.43 μmol electrons m⁻² s⁻¹ ± 10.12, 134.45 ± 7.97 respectively), however no significant relationship between pH and the rETRs under a subsaturating irradiance (360 μmol quanta m⁻² s⁻¹) could be detected (slope on fourth-root transformed data = –0.12 ± 0.049, t = –2.55, P = 0.063). We also failed to detect a significant relationship between pH and the photochemical efficiencies (Fv/Fm) of *P. pavonica* (P = 0.35).

**Discussion**

To our knowledge, this is the first *in situ* observation of the changes of both grazers and macroalgae along gradients of increasing CO₂. It is also the first to provide a comparison of ecological changes along CO₂ gradients between temperate and tropical rocky shores. This study reveals dramatic shifts in benthic community structure that were strikingly similar to those documented at another CO₂ vent site in Italy (Hall-Spencer et al., 2008). Along both temperate and tropical rocky shores there was a reduction in sea urchin abundances alongside a proliferation of *Padina* spp., as CO₂ levels increased. We propose that the elevated CO₂ levels may influence algal-grazer dynamics as species assemblages change, causing profound structural and functional changes in rocky shore habitats. The changes in benthic community composition were detected at threshold pCO₂ levels of ~500 μatm in Sicily and therefore, according to climate change predictions (IPCC (Intergovernmental Panel on Climate Change), 2007), indicate that we may begin to witness these ecological shifts occurring in temperate rocky shores from around the midpoint of this century. Threshold values of pCO₂ for the rocky shore shifts in Papua New Guinea were considerably higher (>900 μatm) than those in Sicily, this may be because of the relatively limited number of midrange CO₂ enriched stations sampled in Papua New Guinea. Investigating the benthos at more intermediate levels of CO₂ may have revealed lower threshold values for ecological shifts, similar to those in Sicily.
Unexpected responses of Padina spp. to elevated CO2

Our present knowledge concerning the impacts of ocean acidification has raised concern for the future success of calcified macroalgae under conditions of high CO2. Previous investigations at CO2 vent seeps have observed dramatic reductions in the abundance of calcified macroalgae (Hall-Spencer et al., 2008; Martin et al., 2008; Fabricius et al., 2011). The results from this investigation, however, indicate that some calcified algae may thrive as the oceans acidify despite expected reductions in calcification. We discovered that tropical and temperate Padina spp. can proliferate with CO2 enrichment, as similarly recorded for some genera of fleshy macroalgae (Hall-Spencer et al., 2008; Fabricius et al., 2011; Porzio et al., 2011). That such algae are abundant at CO2 vents may not, however, necessarily imply that they will be winners in a high CO2 world as they can recruit from outside the vent areas; new work on seagrasses at volcanic vents reveal that chronic exposure to increased CO2 levels adversely affects their ability to defend themselves with phenolic compounds (Arnold et al., in press).

In both P. pavonica and P. australis, the content of CaCO3 in thalli decreased with reductions in pH. This is consistent with other calcification studies on aragonitic macroalgae (Price et al., 2011; Sinutok et al., 2011) and high magnesium calcitic macroalgae (Martin & Gattuso, 2009; Semesi et al., 2009). Reductions in CaCO3 content implies that Padina spp. herbivore defence may be compromised under low pH, potentially leading to an increase in grazing mortality and reduction in benthic cover. This was not, however, reflected in situ. Sea urchins are major grazers on Padina spp. and their presence can cause significant reductions in the abundance of these algae in the Mediterranean (Hereu, 2006) and in the tropics (Sammarco, 1982). Our recorded absence of sea urchins in the CO2 enriched areas may be one explanation for the proliferation of Padina spp., as it becomes released from the top-down control by these keystone grazers. This effect of sea urchin removal has been observed in other Padina sp. populations (Sammarco et al., 1974) and across other Phaeophyte assemblages (Leinaas & Christie, 1996; Ling et al., 2010). In situ manipulations, such as those carried out on grazing gastropods by Rodolfo et al. (2011), are required to test the cause of our observed correlations.

Photosynthetic response of Padina pavonica to elevated CO2

Increased productivity with elevated CO2 may also contribute to the success of Padina at low pH. Laboratory studies of other calcified macroalgae have revealed declines in photosynthetic pigments in high CO2/low pH treatments (Gao & Zheng, 2010; Sinutok et al., 2011) which are indicative of chlorophyll degradation, a reduction in photosynthetic unit size and/or a reduction in PSII reaction centres (Sinutok et al., 2011). Our data, however, show the opposite of the findings from these laboratory studies. We found that Chl a and Chl c content in P. pavonica was greater in the CO2 enriched stations indicating an increase in photosynthetic capacity under conditions of higher CO2. A possible cause for the lower Chl a content (μg mg−2) in fronds from ambient pH may be because of the higher CaCO3 contents relative to those in low pH which have undergone decalcification. In this case however, CO2 levels appear to be a more likely cause for the variations as fronds from S2 and S3 shared similar CaCO3 contents, yet Chl a content was higher in S3 relative to S2.

It has been speculated that pH stress may negatively impact photosynthetic performance through the disruption of the CO2 accumulating pathway at the site of Rubisco, or interference with electron transport (Anthony et al., 2008). This has been supported through laboratory experiments with Halimeda spp. which have demonstrated declines in photosynthetic efficiency (maximum quantum yield; Fv/Fm) (Sinutok et al., 2011) and response (rETRmax) (Price et al., 2011) under elevated CO2. In contrast, we did not observe significant effect of pH on photosynthetic efficiency (Fv/Fm), along gradients of CO2. Indeed, we found a significant effect on the in situ photosynthetic responses of P. pavonica with CO2 enrichment (increases in rETRmax and mean rETRmax at supersaturating irradiance). Whilst some species of Padina are thought to possess carbon concentrating mechanisms (Raven et al., 2002; Enríquez & Rodríguez, 2006) P. pavonica is not believed to be carbon-saturated in ambient seawater and, at times, has been shown to utilize more inorganic carbon if it is provided as CO2 (Einav et al., 1995). The positive photosynthetic response of P. pavonica to CO2 enrichment therefore indicates a direct enhancement of carbon fixation along the gradient. Increased photosynthetic activity at high CO2 has also been observed in other calcified macroalgae (Reiskind et al., 1988; Semesi et al., 2009) and noncalcified macroalgae (Kübler et al., 1999; Connell & Russell, 2010; Russell et al., 2011b). As our photosynthetic measurements are from one season we cannot assess whether the photosynthetic responses of P.pavonica vary seasonally; these data provide a snapshot of responses along gradients of increasing CO2.

It has been established that photosynthesis can stimulate calcification in algae (Borowitzka, 1982; Gattuso et al., 1999). Okazaki et al. (1986) showed that aragonite deposition in Padina begins in the intracellular space.
CO$_2$ vent systems as proxies for ocean acidification

Volcanic vent sites can have highly variable CO$_2$ levels, with steep gradients in pH and carbonate saturation, so caution is required in using information derived from vent studies in projecting future high-CO$_2$ scenarios (Riebesell, 2008; Gazeau et al., 2011). Variability in CO$_2$ levels was seldom considered in the early stages of ocean acidification research, as perturbation experiments mainly investigated the responses of organisms to constant low pH, yet the pH of coastal systems is highly variable with macroalgal communities that can experience diurnal fluctuations of pH 7.5–9.0 (Middelboe & Hansen, 2007; Hoffmann et al., 2011). Volcanic vent systems are useful as they can reveal ecological responses to long-term moderate increases in CO$_2$ levels that retain natural pH variability (Fabricius et al., 2011; Kerrison et al., 2011). They are also useful for examining response boundaries and determining which organisms are the most resistant to chronic exposures to elevated CO$_2$ levels (Barry et al., 2010). Communities of organisms exposed to decades of high CO$_2$ levels provide insights into what to expect in areas that are expected to receive higher than average levels of CO$_2$, such as areas that may be exposed to CO$_2$ leaks following subseabed sequestration (Blackford et al., 2009), those with enhanced acidification due to eutrophication events or hypoxic conditions (Brewer & Peltzer, 2009; Cai et al., 2011) or those areas where CO$_2$-rich waters well-up from the deep into coastal systems (Feely et al., 2008).

Although CO$_2$ vent systems are much larger and longer lasting than the mesocosm and aquarium experiments that have taken place to date, they still only affect relatively small areas of the seabed. Being open systems, their ecology is affected by surrounding areas that have lower CO$_2$ levels, allowing recruitment and migration of organisms from unaffected habitats (Cigliano et al., 2010; Hall-Spencer, 2011). Thus CO$_2$ vent systems cannot mimic the effects of global acidification, they are too small and ephemeral, but they augment predictions based on laboratory and modelling experiments since they show long-term responses of coastal systems to increases in CO$_2$ levels at a variety of locations worldwide (Wernberg et al., 2012).

Implications of findings

Our study shows that certain calcified phaeophytes could be amongst the ecological winners under ocean acidification scenarios, alongside fleshy macroalgae (Kübler et al., 1999; Porzio et al., 2011; Raven, 2011). This work adds to evidence for proliferation of phaeo-

Implications of elevated CO$_2$ on Padina spp. calcification

There is a lack of laboratory evidence of the effects of low pH on Padina spp. calcification to confirm whether decreased calcification is a direct response to reduced pH as opposed to, for example, the reduced grazing pressure in this in situ experiment. An investigation of Caribbean Padina sp. (Lewis et al., 1987) however, revealed that in heavily grazed areas the algae existed in the form of an uncalkified turf whereas in areas of low grazing activity it grew as calcified, foliose blades. The fact that these algae still calcify when grazing intensity is low suggests that the reduced calcification recorded in this study may indeed be a direct response to lowered pH and not the changes in grazing pressure. It has been suggested that calcium carbonate crystal morphology and abundance may be associated with seawater chemistry: thinner, more abundant crystals have been shown to indicate reduced pH conditions as crystallization events are thought to be initiated and terminated more frequently (Robbins et al., 2009; Sinutok et al., 2011). Over the thin calcified band in the apical region of P. pavonica fronds in the CO$_2$ enriched stations, we recorded more abundant aragonite crystals than in the reference stations and we also observed a decreasing trend of crystal width with increasing levels of CO$_2$. These results therefore support the theory of pH dependent changes in calcium carbonate crystal morphology and deposition in calcified macroalgae. The implications of changes in Padina spp. biocalcification on thallus rigidity, dissolution rates and overall sediment budgets however, need further investigation.

formed by the infolded apical margin of the thallus and, since chloroplasts also occur in this region, the authors suggest that this may indicate a relationship between the initiation of calcification and photosynthesis. Photosynthesis-induced calcification has also been demonstrated in the interutericular spaces of the aragonitic genus Halimeda (Borowitzka, 1989). Increased CaCO$_3$ dissolution in lower pH may therefore be offset by the increased photosynthesis in those regions with chloroplasts. This may help to explain why we found that even in the lowest pH conditions, P. pavonica and P. australis were still able to calcify, seemingly from the enhancement of photosynthesis under high levels of CO$_2$. Alternatively, the high pH variability in the vent zone, caused by transient exposure to ambient pH conditions (i.e., periods of high winds increasing the mixing of vent waters with surrounding high pH seawater), has the potential to buffer the effects of acidification by relieving physiological stress (Hoffmann et al., 2011).
phytes in a high-CO₂ world (Hall-Spencer et al., 2008; Connell & Russell, 2010; Diaz-Pulido et al., 2011; Russell et al., 2011b) and has potentially profound consequences for the structure, function and resilience of a variety of benthic ecosystems globally (McManus & Polsenberg, 2004; Harries et al., 2007; Russell et al., 2009). Indeed, the structure and function of ecosystems under future conditions is likely to represent changes to the balance between productivity and consumption (Connell et al., 2011).

Large differences in the impacts of CO₂ enrichment between Padina spp. and other calcified species have been made apparent by this study. This highlights the importance of studying a wide range of genera to better inform global predictions of the impacts of ocean acidification on marine ecosystems (Russell et al., 2011a). This study has demonstrated that the response of Padina spp. to CO₂ enrichment is complex and potentially multifactorial. An in situ, ecosystem based approach, incorporating multispecies interactions, provides more accurate insights into the responses of marine organisms, highlighting the importance of natural CO₂ gradients as a valuable tool in the study of ocean acidification. The similarities we found in the responses of Padina spp. and sea urchin abundance at several vent systems increases the robustness of our predictions over a large geographical range. Similar comparisons should be adopted for other marine biota in future ocean acidification studies.

Acknowledgements

VJ thanks the Marine Institute, University of Plymouth (UiP) for PhD funding and the staff members at the Marine Biological Association UK and the SEM unit at UiP for laboratory support. M Milazzo and M Graziano at the University of Palermo provided field assistance and pH data in Sicily, D Suggett at the University of Essex for the use of Diving-PAM and A Beesley at Plymouth Marine Laboratory performed total alkalinity analyses. Special thanks to the Traditional Owners of the Illi Illi Bwa and Esa’Ala reefs for allowing us to survey their reefs. This work contributes to the EU FP7 project ‘Mediterranean Sea Acidification under a changing climate’ (grant agreement no. 265103), with additional funding for JHS from Save Our Seas Foundation. Funding for the PNG study was provided by the Australian Institute of Marine Science and an International Science Linkages Grant of the Australian Commonwealth Department of Innovation, Industry, Science and Research. An Australian Research Council grant funded BDR.

References

integrate the effects of climate change across entire systems. *Biology Letters*, 8, 164–166.


### Supporting Information

Additional Supporting Information may be found in the online version of this article.

**Table S1.** Seawater carbonate chemistry measurements for each study station off the island of Vulcano (V) and in Papua New Guinea; Upa-Upasina (U) and Esa’Ala (E), R = reference station, S = elevated CO₂ station. In Vulcano, temperature (range 18.6–27.7 °C), pH and salinity (=38) were measured in Sept-Oct 2009, April 2010, July 2010, Sept-Oct 2010, May 2011, Sept-Oct 2011. In Papua New Guinea, temperature (range 28.2–31.4 °C), pH and salinity (=34) were measured in April 2011. The pH and total alkalinity (Vulcano: mean TA, n = 3; PNG: median TA values taken from Fabricius *et al.*, 2011) were used to calculate the remaining parameters using CO₂ SYS programme (using the constants of Roy *et al.*, 1993 and Dickson, 1990 for KSO₄).

**Table S2.** Mean (±SE) abundance, length and width of aragonite crystals deposited by *Padina pavonica* along the Vulcano CO₂ gradient. Data derived from SEM analysis of fronds (n = 3 fronds per station), over calcified apical regions only (see frond images in Fig. 4), therefore do not reflect total means for whole fronds.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.