This is the pre-peer reviewed version of the following article:

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27th August, 2012

http://hdl.handle.net/2440/72604
Temperate and tropical brown macroalgae thrive, despite
decalcification, along natural CO₂ gradients.

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Keywords: ocean acidification, calcification, photosynthesis, temperate and tropical coastal
ecosystems.
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. A range of studies show that calcified coralline algae (Rhodophyta) may be especially vulnerable to ocean acidification, but there is a lack of information concerning calcified brown algae (Phaeophyta). Here we compare ecological shifts in sub-tidal rocky shore systems along CO2 gradients created by volcanic seeps in the Mediterranean and off Papua New Guinea. In both the temperate and tropical systems the abundance of grazing sea urchins fell 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. Replicated observations are required across regions to increase confidence in predictions of the ecological impacts of ocean acidification on a global scale.

Introduction

Rising anthropogenic emissions of CO2 are rapidly altering ocean chemistry since increasing pCO2 in seawater has already lowered the mean ocean surface pH by 0.1 units from pre-
industrial values, with a predicted further decrease of 0.3-0.4 units by 2100 (IPCC, 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 $p$CO$_2$ levels (Russell et al., 2009; Hepburn et al., 2011; Johnson et al., 2011; Porzio et al., 2011), 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 since the effects of elevated CO$_2$ 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 increases in CO$_2$ (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 CO$_2$ levels since 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; Doropoulos et al., 2012). Increasing concentrations of CO$_2$ can, on the other hand, enhance productivity and growth in both non-calcified (Gao et al., 1993a; Kübler et al., 1999; Connell & Russell, 2010) and calcified macroalgae (Reiskind et al., 1988; Gao et al., 1993b; Semesi et al., 2009).

Understanding the effects of ocean acidification on calcified algae is 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 $p\text{CO}_2$ 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). Consequently, there is a great need for studies targeting interactions between multiple species in order to assess the effects on strength of competition, predation and/or herbivory (Wernberg et al., in press). Here we assess the abundance of herbivores (sea urchins) and the response of brown macroalgae (Padina spp.) to increasing levels of CO$_2$ in natural settings, as interactions between these groups of organisms can drive ecological changes in benthic habitats on temperate (Sala, 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 thalli, forming concentric bands of white precipitate (Okazaki et al., 1986). Carbonate production rates of Padina sp. in one sub-tropical 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 minimising grazing damage to tissues (Littler & Littler, 1980; Padilla, 1993), increase the ability of bicarbonate and nutrient assimilation through the generation of protons (McConnaughey & Whelan, 1997), improve photosynthetic performance (McConnaughey, 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).

Ocean acidification also has the potential to reduce top-down biological control of benthic biodiversity (Widdicombe and 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 colonisation 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.

Natural CO$_2$ gradients are beginning to reveal the ecological shifts that can be expected to occur with globally increasing atmospheric CO$_2$ in both temperate (Hall-Spencer et al., 2008) and tropical ecosystems (Fabricius et al., 2011). Work has begun to understand the underlying mechanisms that cause ecological shifts along these CO$_2$ gradients, such as the influence of recruitment success (Cigliano et al., 2010) and the combined physiological effects of temperature and CO$_2$ (Rodolfo-Metalpa et al., 2011). The aim of this study was to survey populations of Padina spp. (Dictyotaceae) and sea urchins (Echinoidea) 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 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 pheaophytes.

**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., 2011). 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, characterised 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 (V-R1 pH 8.17, CI = 0.42%; V-R2 pH 8.18, CI = 0.32%; V-R3 pH 8.19, CI = 0.28%, n = 22-24). Four additional sampling stations were selected along the gradient, one located between S2 and S3 (at mean pH 7.97, CI = 1.45%, n = 16) and three at 20 m intervals between S1 and the end of the gradient (at mean pH 8.08, CI = 0.82%; pH 8.16, CI = 0.33%; pH 8.20, CI = 0.23%, n = 6-22) to allow P. pavonica and sea urchin abundance surveys to occur along the full length of the CO2 gradient. Temperature, total alkalinity, salinity and light levels were relatively constant in the shallow sub-tidal region along this gradient (Johnson et al., 2011).

Padina australis was sampled along the shallow (0.1-0.3 m, below lowest astronomic tide) shore of two sites in Milne Bay Province, Papua New Guinea (9°45’ S, 150°50’ E): Upa-Upasina and Esa’Ala along the north-western and north-eastern coast off Normanby Island (see maps in Fabricius et al., 2011) in April 2011. Tidal range in the region is <1 m. Volcanic CO2 seeps acidify the seawater, with seeping being most intense near the shore at <0.5 m depth. In these shallow shore zones, reductions in pH were greater than recorded for coral reef habitats by Fabricius et al., (2011). Two sampling stations of intermediate to low mean pH were selected at both Upa-Upasina (U-S1 pH 7.78, CI = 0.26%; U-S2 pH 7.49, CI =
0.62%, \( n = 7 \)) and Esa’Ala (E-S1 pH 7.86, CI = 1.30%; E-S2 pH 6.68, CI = 4.53%, \( n = 7-9 \)). Reference stations with 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 several hundred meters away from the seeps at comparable geophysical settings.

At all sites (Vulcano in the Mediterranean, and Upa-Upasina and Esa’Ala in Papua New Guinea), 20 quadrats (50 cm x 50 cm) were placed haphazardly within 15 x 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.

**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 hours at S3 at Vulcano), so the uncertainty inherent in using the NBS scale for seawater measurements (approximately 0.05 pH, Dickson et al., 2010) was considered acceptable for this study. Mean pH (back-transformed hydrogen ion concentrations) were calculated for each station at Vulcano (pH sampled on several occasions; 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). 95% 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 (Hoope et al., 2010). At Vulcano, TA was measured at each station, on three separate visits (Sept 2010, May 2011 and Sept 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, Georgia, 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, decalcified in hydrochloric acid (1N) and then re-dried 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 with scanning electron microscopy (JEOL JSM 5600 LV). Three fronds from each station were fixed in glutaraldehyde for 1-2 hours, and then stored in 1x PBS buffer (phosphate buffered
saline) until examination. As the size and number of crystals has been reported to vary with age of frond segment (Hills-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, 5 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. 5) 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 5µm x 5µm area were counted and averaged for each frond.

*Photosynthesis in P. pavonica*

Photosynthetic condition and performance of *P. pavonica* at Vulcano was investigated through measurements of photosynthetic pigment (Chl a and c1+c2) concentrations and Chl a fluorescence. 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 8am-10am to avoid the confounding effect of light intensity, in particularly mid-day photoinhibition, on chlorophyll content (Hädar *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 hours in the dark. The absorbance of each sample at 630, 664 and 750 nm (background absorbance) was measured (3 replicate readings were taken from each sample to obtain an average) using a Cecil CE2011 spectrophotometer. The concentration of chlorophyll $a$ and $c$ ($c_1 + c_2$) in the sample was calculated using the equations of Ritchie (2006). The volume of the solvent (in weight / g) and the weight of the frond were then used to provide a final calculated reading of chlorophyll (μg mg$^{-2}$ 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 ($r$ETR) of freshly collected, light-adapted fronds ($n = 6$ per station), were measured in small dishes using a Diving PAM fluorometer (Walz-Germany).

\[
Y = F'_m - F_t / F'_m \quad \text{(Genty, 1989)}
\]

\[
r\text{ETR} = Y \times \text{PAR} \times 0.5 \quad \text{(Beer et al., 1998)}
\]

Rapid light curves (RLC) were applied to assess the light saturation behaviour of fronds across each of the six sampling stations in Vulcano. RLC data can be useful for assessing photosynthetic capacity and potential over a wide range of ambient light intensities (Ralph & Gademann, 2005). The Diving-Pam was set to deliver red pulse-modulated light at 655 nm followed by steps of actinic light every 20 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 generalised 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 over-dispersed 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, 2012).

Results

Seawater chemistry
Table 1 shows the range in carbonate chemistry parameters for each sampling station. The median $pCO_2$ levels (calculated from median pH and mean TA) were lowest in the reference stations (276-388 µatm) and increased with proximity to the CO$_2$ seeps, with the highest values recorded at V-S3 (1428 µatm), U-S2 (2665 µatm) and E-S2 (23,095 µatm). The mean pH of the reference stations ranged from 8.17 to 8.31, while the mean pH at the seep stations ranged from 8.06 to 6.68, with increasing variance towards lower values (Fig. 1). The highest median values for $pCO_2$ 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$^{-1}$). Calcium carbonate was under-saturated at E-S2 and periods of under saturation occurred during the lowest range of pH at V-S3 ($\Omega$ 0.15 calcite and $\Omega$ 0.09 aragonite) and U-S2 ($\Omega$ 0.98 aragonite).

Padina spp. and sea urchin abundances

There were dramatic ecological shifts along all three volcanic seeps as CO$_2$ 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 CO$_2$ vents in Ischia, Italy (Fig 2a & b). These shifts were detected at median $pCO_2$ 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. 3). Benthic cover of Padina spp. increased with rising CO$_2$ and was two-three fold greater in the highest CO$_2$ stations (V-S3, U-S2 & E-S2) relative to the reference stations (Fig. 3). We detected a significant effect of pH on Padina spp. benthic cover and sea urchin abundance at all three gradients (GLM: Table 2). In contrast to Padina spp., sea urchin abundance was greatest at the reference stations and...
decreased with declining pH at all three gradients (Fig. 3 a-c, Table 2). Sea urchins were absent at stations with the highest levels of $pCO_2$ (V-S1-S3, U-S2, E-S2).

**Physiological responses of Padina spp. to elevated CO$_2$**

We found that pH had a statistically significant effect on the CaCO$_3$ content in *Padina* spp. fronds at Vulcano only (as smaller sample sizes were taken at Upa-Upasina and Esa’Ala; Fig.4, Table 2). At Vulcano, CaCO$_3$ content in *P. pavonica* was highest at the reference stations (57-63%) and decreased significantly in the CO$_2$ 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 CaCO$_3$ content from 55 % ± 1.7 at the reference station (U-R1) to 35% ± 3.6 at the intermediate station (U-S1). At Esa’Ala, CaCO$_3$ content was considerably greater in fronds from the reference station (E-R1: 66% ± 7.1) compared with those the highest CO$_2$ exposure station (E-S2: 40% ± 1.8).

Table 3 shows the abundance and morphometric data of the aragonite crystals on the surface of *P. pavonica* fronds. Over the thin calcified bands in the apical regions we detected a significant increase in crystal abundances with declining pH (GLM: slope of square root transformed data = -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 pH had a significant effect on the content of both chlorophyll *a* and chlorophyll *c* in *P. pavonica* (Fig. 5, 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* content 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 = 0.08 mg g$^{-1}$fw ± 0.002, U-S1 = 0.04 mg g$^{-1}$fw ± 0.006, U-S2 = 0.07 mg g$^{-1}$fw ± 0.007, E-S1 = 0.07 mg g$^{-1}$fw ± 0.007, E-S2 = 0.08 mg g$^{-1}$fw ± 0.007, E-S3 = 0.09 mg g$^{-1}$fw ± 0.007).
The differences observed in the photosynthetic responses of *P. pavonica* to increased CO₂ are presented in a rapid light curve in Fig. 6. The rETR max values significantly increased with declining pH (GLM: slope on forth-root transformed data = -0.54 ± 0.091, t = -5.97, *P* = 0.004). We also detected a significant effect of pH on the rETRs recorded at supersaturating irradiance; 3344 μmol quanta m⁻² s⁻¹ (slope on forth-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 effect of pH on the rETRs under a subsaturating irradiance (360 μmol quanta m⁻² s⁻¹) could be detected (slope on forth-root transformed data = -0.12 ± 0.049, t = -2.55, *P* = 0.063). We also failed to detect a significant effect of pH on the photochemical efficiencies (*Fv/Fm*) of *P. pavonica* (*P* = 0.35).

**Discussion**

To our knowledge, this is the first *in situ* demonstration of the effects of elevated CO₂ on grazer-algal population dynamics. It is also the first to provide a comparison of ecological changes along CO₂ gradients between temperate and tropical rocky shores. 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 \( pCO_2 \) levels of ~500 \( \mu \)atm in Sicily and therefore, according to climate change predictions (IPCC, 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 \( pCO_2 \) for the rocky shore shifts in Papua New Guinea were considerably higher (> 900 \( \mu \)atm) than those in Sicily, this may be due to the relatively limited range of \( CO_2 \) enriched sampling stations in Papua New Guinea. Investigating the benthos at more intermediate levels of \( CO_2 \) may have revealed lower threshold values for ecological shifts, similar to those in Sicily.

Our present knowledge of the effects of ocean acidification on calcified macroalgae is mostly derived from studies investigating the impacts of high \( CO_2 \) 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) and as a consequence, aragonitic algae have been relatively overlooked. Furthermore, the responses of calcified Pheophyta are virtually unknown (Porzio et al., 2011). To our knowledge, this is the first study to investigate the \textit{in situ} impacts of elevated \( CO_2 \) on calcification and photosynthesis in \textit{Padina} spp.

\textit{Unexpected responses of Padina spp. to elevated \( CO_2 \)}

Our present knowledge concerning the impacts of ocean acidification has raised concern for the future success of calcified macroalgae under conditions of high \( CO_2 \). Previous investigations at \( CO_2 \) vent seeps have observed dramatic reductions in the abundance of calcified macroalgae (Hall-Spencer \textit{et al.}, 2008; Martin \textit{et al.}, 2008; Fabricius \textit{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).

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 et al., 2006) and in the tropics
(Sammarco, 1982). Our recorded absence of sea urchins in the CO₂ enriched areas may
therefore explain the proliferation of Padina spp., as it becomes released from the top-down
control of these keystone grazers. Similar effects of sea urchin removal have been observed
in other Padina sp. populations (Sammarco et al., 1974) and across other Phaeophyte
assemblages (Leinaas & Christie, 1996; Ling et al., 2010).

Photosynthetic response of Padina pavonica to elevated CO₂

Increased productivity with elevated CO₂ may 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 and 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 findings, however, show the opposite. 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 with high CO2. 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 though laboratory experiments with *Halimeda* spp. which have demonstrated declines in photosynthetic efficiency (Sinutok et al., 2011) and response (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 rETR$_{max}$ and mean rETR$_{max}$ at supersaturating irradiance). *Padina pavonica* is not carbon-saturated in seawater and can utilise 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 across the gradient. Increased photosynthetic activity at high CO2 has also been observed in other calcified macroalgae (Reiskind et al., 1988, Gao et al., 1993b, Semesi et al., 2009) and non-calcified macroalgae (Gao et al., 1993a; Kübler et al., 1999; Connell & Russell, 2010; Russell et al., 2011b).

It has been established that photosynthesis can stimulate calcification in algae (Borowitzka, 1982; Gattuso et al., 1999). The co-existence of chloroplasts and aragonite deposition in the same thallus region of *Padina* indicates an intimate relationship between calcification and photosynthesis (Okazaki et al., 1986). Increased CaCO$_3$ dissolution in lower pH may therefore be offset by 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 CO₂. 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).

**Implications of elevated CO₂ 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 uncalcified 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 crystallisation 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 CO₂ 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 CO₂. 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. bio-calcification on thallus rigidity, dissolution rates and overall sediment budgets however, need further investigation.

Conclusions

Volcanic CO₂ vent systems are revealing the changes in ecological interactions and community shifts we can expect in subtidal rocky shore ecosystems under elevated CO₂. 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). Our study shows that certain calcified phaeophytes could, in fact, be amongst the ecological winners under ocean acidification scenarios, alongside fleshy macroalgae (Kübler et al., 1999; Porzio et al., 2011; Raven et al., 2011). This may be explained by a combination of; reduced sea urchin predation (and presumably other calcareous predators such as gastropods), increased photosynthetic capacity and performance and optimised energy reallocation following a reduction in carbon limitation. This work adds to evidence for proliferation of phaeophytes 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 (Russell et al., 2009; McManus & Polsenberg, 2004; Harries et al., 2007).

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 multi-factorial. An in situ,
ecosystem based approach, incorporating multi-species interactions and predator-prey dynamics, 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 (UoP) for PhD funding and the staff at the Marine Biological Association UK and the SEM unit at UoP for laboratory support. M Milazzo and M Graziano at the University of Palermo provided field assistance and pH data in Sicily, and A Beesley at Plymouth Marine Laboratory performed total alkalinity analyses. Special thanks to the Traditional Owners of the Illi Illi Bwa 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


Dickson AG (1990) Standard potential of the (AgCl + 1/2 H$_2$= Ag + HCl(aq)) cell and the association of bisulfate ion in synthetic sea water from 273.15 to 318.15 K. *Journal of Chemical Thermodynamics*, 22, 113-127.


Table 1. 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>
<thead>
<tr>
<th>Site &amp; Station</th>
<th>pH range (NBS scale)</th>
<th>( p\text{CO}_2 ) (µatm)</th>
<th>TA (mmol kg(^{-1}))</th>
<th>DIC (mmol kg(^{-1}))</th>
<th>( \text{CO}_3^{2-} ) (mmol kg(^{-1}))</th>
<th>HCO(_3^-) (mmol kg(^{-1}))</th>
<th>( \Omega_{\text{calcite}} )</th>
<th>( \Omega_{\text{aragonite}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>V R1</strong></td>
<td>max 8.35 241 2.682</td>
<td>2.402</td>
<td>0.18</td>
<td>2.206</td>
<td>4.27</td>
<td>2.69</td>
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<tr>
<td></td>
<td>median 8.17 388 (+0.12)</td>
<td>2.492</td>
<td>0.13</td>
<td>2.341</td>
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<td>1.89</td>
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<td></td>
<td>min 8.06 513</td>
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<td>0.10</td>
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<td><strong>V R2</strong></td>
<td>max 8.29 274 2.591</td>
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<td>2.177</td>
<td>3.67</td>
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<td></td>
<td>median 8.18 365 (+0.03)</td>
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<td>0.12</td>
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<td>1.31</td>
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<td>median 7.71 1244 (+0.07)</td>
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<td></td>
<td>median 7.66 1428 (+0.12)</td>
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<td>2.762</td>
<td>0.15</td>
<td>0.09</td>
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<td>1.556</td>
<td>7.47</td>
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<td>median 8.31 276</td>
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<td>7.38</td>
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<td>U R2</td>
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<td>8.22</td>
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<td>1.655</td>
<td>6.49</td>
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<td>0.04</td>
<td>2.201</td>
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</tr>
</tbody>
</table>

832
833
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841
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Table 2. Changes in (a) *Padina* spp. cover, (b) urchin abundances and (c) CaCO$_3$ content of *Padina* spp. fronds, along the three pH gradients at Esa’Ala, Upa-Upasina and Vulcano. Generalised linear model outputs. Data in bold indicate significant effect of pH ($P<0.05$).

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>SE</th>
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<th>P</th>
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<tr>
<td><strong>a)</strong></td>
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<tr>
<td>Region.Esa</td>
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<td>4.31</td>
<td>3.26</td>
<td>0.008</td>
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<td>Region.Upa</td>
<td>22.83</td>
<td>8.74</td>
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<td>-2.43</td>
<td><strong>0.033</strong></td>
</tr>
<tr>
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<td>0.96</td>
<td>-4.48</td>
<td><strong>0.001</strong></td>
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<tr>
<td>RegionVul : pH</td>
<td>-3.48</td>
<td>0.74</td>
<td>-4.70</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td><strong>b)</strong></td>
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<td></td>
<td></td>
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<td>Region.Esa</td>
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<td>3.40</td>
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<td><strong>c)</strong></td>
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<tr>
<td>RegionVul : pH</td>
<td>3.25</td>
<td>0.54</td>
<td>6.06</td>
<td><strong>0.004</strong></td>
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</table>
Table 3. 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. 5), therefore do not reflect total means for whole fronds.

<table>
<thead>
<tr>
<th>Station</th>
<th>Mean no. crystals (per 5 µm²)</th>
<th>Mean crystal length (µm)</th>
<th>Mean crystal width (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V-R1</td>
<td>94 ± 6.65</td>
<td>1.30 ± 0.05</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>V-R2</td>
<td>96 ± 7.47</td>
<td>1.44 ± 0.07</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>V-R3</td>
<td>96 ± 8.20</td>
<td>1.43 ± 0.06</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td>V-S1</td>
<td>106 ± 4.76</td>
<td>1.80 ± 0.06</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>V-S2</td>
<td>115 ± 8.74</td>
<td>1.54 ± 0.10</td>
<td>0.19 ± 0.01</td>
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<tr>
<td>V-S3</td>
<td>153 ± 6.31</td>
<td>1.52 ± 0.07</td>
<td>0.17 ± 0.01</td>
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</tbody>
</table>
Figure Legends

**Fig. 1** Range in pH_{NBS} (<0.5 m water depth) across CO_{2} gradients in a) Vulcano (Sicily; \( n = 22-27 \) per station) b) Upa-Upasina (Papua New Guinea; \( n = 6 \) per station) c) Esa’Ala (Papua New Guinea; \( n = 7 \) & S2, \( n = 9 \)). ‘R’ denotes reference stations, ‘S’ denotes elevated CO_{2} stations.

**Fig. 2** Images showing an urchin and coralline algae dominated rocky shore under ambient CO_{2} (a) in Ischia, Italy (photograph by David Liittschwager, National Geographic) and the proliferation of Phaeophyta at elevated CO_{2} at vent sites in Ischia (photograph by Luca Tiberti, Associazione Nemo) (b). *Padina australis* showing normal calcification at tropical (Papua New Guinea) reference station, Esa’Ala R1 (c; scale bar = 1 cm) and visibly low calcification at Esa’Ala S1 (d). Arrows indicate CO_{2} vent bubbles.

**Fig. 3** Mean percentage cover (histogram + SE) of *Padina* spp. and abundance of sea urchins (mean ± SE) along CO_{2} gradients at a) Vulcano b) Upa-Upasina e) Esa’Ala (\( n = 20 \) quadrats per station). Mean pH (\( n = 6-27 \) per station) of each station indicated.

**Fig. 4** a) Mean (+ SE) CaCO_{3} content of *Padina* spp. along CO_{2} gradients at a) Vulcano (\( n = 30 \) per station), b) Upa-Upasina (\( n = 15 \) per station) and c) Esa’Ala) (\( n = 5 \) per station).
Fig. 5 Mean (+ SE) chl a content in *P. pavonica* fronds along the Vulcano CO$_2$ gradient (*n* = 40 per station). Images illustrate changes in CaCO$_3$ deposition on *P. pavonica* frond surfaces at V-R2 and V-S2 along the Vulcano CO$_2$ gradient. All thalli at V-R1- V-R3 were heavily calcified, all thalli at S1-S3 were more lightly calcified, calcification appears to be limited to thin bands along apical regions (scale bar = 1 cm). Arrows indicate locations of SEM analyses.

Fig. 6 Rapid light curves of *P. pavonica* along the Vulcano CO$_2$ gradient, showing the mean (± SE) relative electron transport rates (rETR) per station (*n* = 5 for V-R3 + V-S3, *n* = 6 for all other stations) at increasing irradiance.
Figure 1
Figure 2.

a)  

b)  

c)  

d)
Figure 3.
Figure 4.
Figure 5.
Figure 6.