

ORIGINAL ARTICLE

Effects of ocean acidification on sponge communitiesClaire Goodwin¹, Riccardo Rodolfo-Metalpa², Bernard Picton¹ & Jason M. Hall-Spencer²¹ National Museums Northern Ireland, Holywood, County Down, UK² Marine Biology and Ecology Research Centre, Plymouth University, Plymouth, UK**Keywords**CO₂ vents; Mediterranean; ocean acidification; Porifera; sponge; volcanic vents.**Correspondence**

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Abstract

The effects of ocean acidification on lower invertebrates such as sponges may be pronounced because of their low capacity for acid–base regulation. However, so far, most studies have focused on calcifiers. We present the first study of the effects of ocean acidification on the Porifera. Sponge species composition and cover along pH gradients at CO₂ vents off Ischia (Tyrrhenian Sea, Italy) was measured at sites with normal pH (8.1–8.2), lowered pH (mean 7.8–7.9, min 7.4–7.5) and extremely low pH (6.6). There was a strong correlation between pH and both sponge cover and species composition. *Crambe crambe* was the only species present in any abundance in the areas with mean pH 6.6, seven species were present at mean pH 7.8–7.9 and four species (*Phorbas tenacior*, *Petrosia fici-formis*, *Chondrilla nucula* and *Hemimycale columella*) were restricted to sites with normal pH. Sponge percentage cover decreased significantly from normal to acidified sites. No significant effect of increasing CO₂ levels and decreasing pH was found on spicule form in *Crambe crambe*. This study indicates that increasing CO₂ concentrations will likely affect sponge community composition as some demosponge species appear to be more vulnerable than others. Further research into the mechanisms by which acidification affects sponges would be useful in predicting likely effects on sessile marine communities.

Introduction

Atmospheric CO₂ concentration is currently approximately 383 parts per million by volume (ppmv), the highest level for at least 650,000 years and, due to anthropogenic influences, is projected to increase by 0.5% per year throughout the 21st century (Guinotte & Fabry 2008). The oceans are a major sink for CO₂ and have absorbed approximately half of all anthropogenic emissions (Sabine *et al.* 2004). Since CO₂ dissolves in seawater, it shifts the carbonate system towards higher concentrations of CO₂, bicarbonate (HCO₃⁻) and protons H⁺, therefore decreasing the seawater pH and the carbonate ions used by calcifiers to build their shells and skeletons. Since the early 1900s, a 30% increase in H⁺ concentration of surface waters has resulted in a pH decrease of 0.1 units, and a further drop in surface seawater pH of up to 0.5 units has been projected by 2100 (Caldeira & Wickett 2005).

The adverse effect of seawater acidified with CO₂ has been most apparent on marine calcifiers, such as coral-line algae (Kuffner *et al.* 2008; Martin *et al.* 2008), foraminiferans (Moy *et al.* 2009; Dias *et al.* 2010), corals (e.g. Silverman *et al.* 2009), echinoderms (e.g. Michaelidis *et al.* 2005) and molluscs (Gazeau *et al.* 2007). Among these organisms, rates of calcification have been predicted to fall by up to 60% within this century, depending on the physiology of the species and their mineralogy (Kleypas *et al.* 2006). However, although it is in calcifiers that the effects are most obvious, studies using high CO₂ levels indicate that unifying principles define sensitivity to CO₂ in both calcifying and non-calcifying animals (Pörtner 2008). The effects of ocean acidification on lower invertebrates such as sponges are likely to be pronounced because of their low capacity for acid–base regulation (Pörtner 2008). As yet no studies have examined the effect of ocean acidification on the Porifera.

Recently, CO₂ vents have been used as natural laboratories to advance our understanding of ocean acidification at the ecosystem level (Hall-Spencer *et al.* 2008; Cigliano *et al.* 2010; Porzio *et al.* 2011). While most vents emit hot gases, including toxic sulphur compounds (Dando *et al.* 1999), vents off Ischia island near the volcano of Vesuvius in Italy eject >90% CO₂ at ambient temperature without toxic sulphur (Hall-Spencer *et al.* 2008), acidifying coastal seawater on the north and south sides of Castello Aragonese for centuries, maybe millennia (Tedesco 1996). Hall-Spencer *et al.* (2008) studied species community composition of the area along gradients of normal pH (8.1–8.2), lowered pH (mean 7.8–7.9, min 7.4–7.5) to extremely low pH 6.6. They recorded 64 species of plant, alga and animal, many of which were resilient to the acidified conditions. Within sponges, eight genera were found with an apparent decrease in their distribution from normal to extremely low pH values (Hall-Spencer *et al.* 2008, supporting information). However, no sampling or full quantitative analysis of sponge species composition was undertaken. Although other studies have examined the biodiversity of sponges associated with hydrothermal vent sites (Morri *et al.* 1999; Pansini *et al.* 2000), none has looked at the effect of small-scale variations in pH on sponge community composition.

The sponge fauna of Ischia is relatively well studied, with some 86 species reported from the area (Sara 1959). Both demosponges, which possess skeletons of siliceous spicules, and calcarea, which have calcium carbonate spicules, are present. Here we present the results of a complete survey of the sponge fauna at CO₂ vents, taking account of both qualitative (species composition) and quantitative (percentage cover of the substratum) aspects of the sponge community composition along pH gradients.

Methods

Study site and seawater carbonate measurements

Sponge community composition was measured during May 2008 on the north and south sides of the Castello Aragonese (40°043.849' N, 13°57.089' E) off Ischia in Italy (Fig. 1). Six sites were identified along gradients of increasing pCO₂ to the north and south of the Castello: two normal pH stations (N1 and S1; Fig. 1); two stations that had reductions in mean pH of 0.2–0.4 units (N2 and S2; Fig. 1) and two stations with reductions in mean pH of 0.6–1.5 units (N3 and S3; Fig. 1) which are more representative of the localised effects to be expected from deliberate CO₂ sequestration rather than from global ocean acidification. Sites were chosen on the basis of the carbonate chemistry of the vents, mapped out by Hall-Spencer *et al.* (2008), Rodolfo-Metalpa *et al.* (2010) and

Cigliano *et al.* (2010), who repeatedly measured pH_T (in total scale), total alkalinity (TA), temperature and salinity. Water samples were collected at 1 m depth using glass bottles, and the pH_T was measured immediately using a metre accurate to 0.01 pH units (Metrohm 826 pH mobile, Metrohm, Herisau, Switzerland) calibrated using TRIS/HCl and 2-aminopyridine/HCl buffer solutions (DOE 1994). Seawater samples were then passed through 0.45-µm pore size filters (GF/F Whatman, Maidstone, Kent, UK) and poisoned with 0.05 ml of 50% HgCl₂ (AnalaR: Merck, Darmstadt, Germany) to avoid biological alteration, and stored in the dark at 4 °C. Three replicate 20-ml sub-samples were analysed at 25 °C using a titration system composed of a pH meter with an ORION pH electrode (see Hall-Spencer *et al.* 2008 for further details). Parameters of the carbonate system [pCO₂, CO₃²⁻, HCO₃⁻, and saturation state of calcite (Ω_{calcite})] were calculated from pH_T, TA, temperature and salinity (38‰) using the free-access CO₂ Systat package (Systat Software Inc., Chicago, Illinois, USA) (Table 1).

Sponge community composition

Quantitative and qualitative measurements of sponge community composition were assessed at each of the six study sites (N1–N3, S1–S3). At each site, 10 quadrats of 30 cm² were sampled, deployed haphazardly on very steep or vertical bedrock faces in a depth range of 1–3 m. Each quadrat was photographed with a Nikon D70 camera (Nikon Inc., Tokyo, Japan.) in an Ikelite housing using an 18–70 mm zoom lens for quantitative sponges cover. The number of species in each quadrat was counted and a small sample of each species was scraped off from the rock using a knife and placed in a ziplock bag. Samples were preserved in 70% denatured ethanol and examined microscopically to confirm identification (see Picton & Goodwin 2007 for detailed methodology).

Change in species composition along a pH gradient (7.09–8.14 pH) was examined on a 240-m transect. Three 30-cm² quadrats were haphazardly deployed at 25 stations spread at 10-m intervals. Quadrats were deployed on very steep or vertical bedrock faces between 1 and 3 m in depth and the composition of sponge communities was measured as described above. Only sponges on the north side of the Castello Aragonese were quantified in this way. Percentage sponge cover per square meter was calculated using IMAGEJ (Rasband 1997–2008). Percentage data were arcsine-square root-transformed. Differences in percentage cover were tested with the Kruskal–Wallis test.

Ten choanosomal style spicules from five samples of *Crambe crambe* were measured for each of the southern sites (S1–S3). The aim of this was to examine any effects of increasing CO₂ and decreasing pH on spicule form.

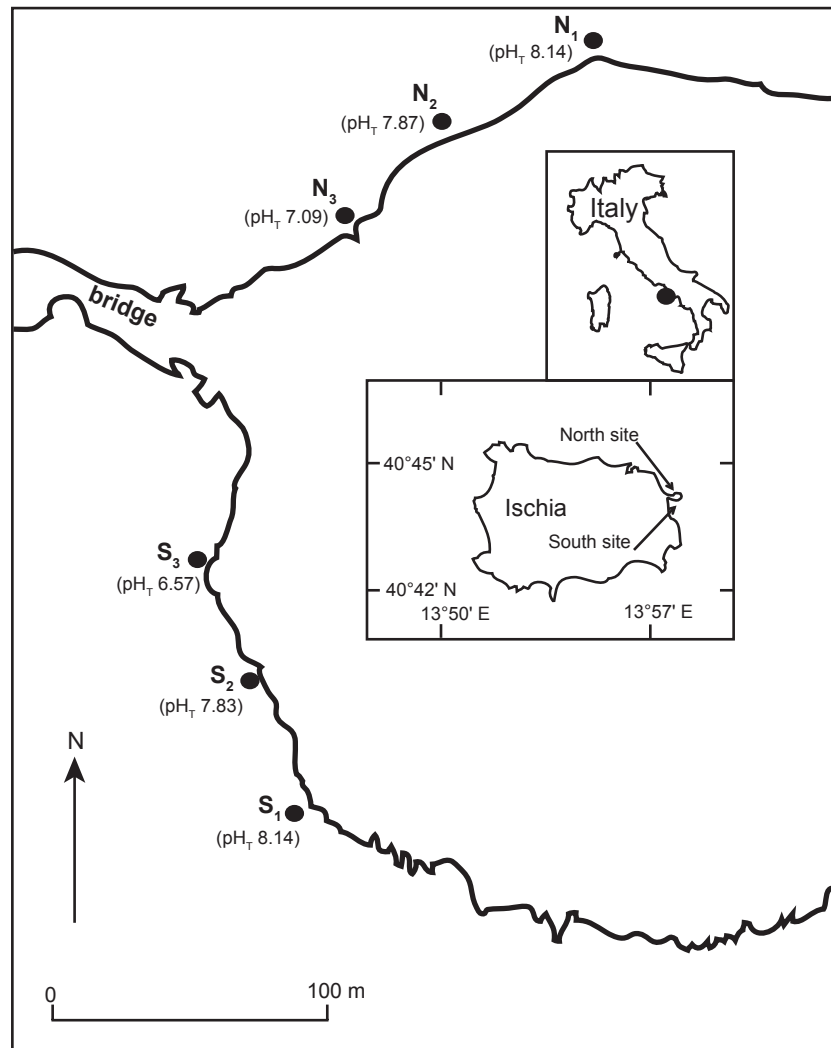


Fig. 1. Map of CO₂ vent sites north and south of Castello Aragonese, off Ischia Island, Italy. Mean surface pH is shown at 35-m-wide stations.

Only *C. crambe* was examined as it was the only species present at all three sites. The species has siliceous spicules.

Results

There was a significant difference in total percentage sponge cover on both south sites (Kruskal–Wallis $H = 16.250$, $P < 0.0003$) and north sites (Kruskal–Wallis $H = 16.979$, $P < 0.0002$). The difference was most marked between the highly acidified sites (N3, S3) and the control sites (N1, S1) (Figs 2 and 3).

In total 14 sponge species were recorded from the study area (Table 2). Species composition varied between sites. The only species present in any abundance in the extremely acidified sites (S3, N3) was *Crambe crambe*, although small patches of *Microciona* cf. *toxitenus* and *Spirastrella cunctatrix* were also present. A greater variety

of sponges was present at sites of intermediate pH (mean 7.8–7.9). Four species (*Phorbas tenacior*, *Petrosia ficiformis*, *Chondrilla nucula* and *Hemimycale columella*) were restricted to the normal pH sites (N1, S1).

Species composition on the north side of Castello Aragonese varied along the transect with very little sponge cover in the most acidified areas (Fig. 4). *Crambe crambe* was the most abundant species and was the only species present in the most acidified areas (Table 2).

There was no significant difference in mean spicule morphology or length (Kruskal–Wallis $H = 3.86$, $P < 0.145$) between sites for *Crambe crambe*.

Discussion

Whereas vertebrates and higher invertebrates have some capacity for acid–base regulation, sponges lack intercellu-

Table 1. Summary of pH_T (in total scale), and carbonate chemistry data from 2007 to 2008 at CO₂ vents off Ischia (from Hall-Spencer *et al.* 2008; Cigliano *et al.* 2010; Rodolfo-Metalpa *et al.* 2010). Water samples were taken from rocky shore zones N₁–N₃ and S₁–S₃ NB: min pH had the max pCO₂ levels and vice versa.

Site	pH _T	pCO ₂ (μatm)	CO ₃ ²⁻ (μmol·kg ⁻¹)	HCO ₃ ⁻ (μmol·kg ⁻¹)	Ω _{calc}	Ω _{arag}
N ₁						
Min	7.96	286	190	1810	4.48	2.92
Mean (n = 18)	8.13	353	263	1949	5.93	3.86
Max	8.19	552	370	2830	6.58	4.28
N ₂						
Min	7.49	309	70	1850	1.55	1.00
Mean (n = 24)	7.85	854	159	2127	3.71	2.41
Max	8.17	1908	270	2450	6.20	4.03
N ₃						
Min	6.31	654	5	2090	0.11	0.07
Mean (n = 23)	7.13	7553	48	2392	1.12	0.73
Max	7.90	29,885	170	2480	3.89	2.53
S ₁						
Min	8.09	304	240	1830	5.61	3.62
Mean (n = 12)	8.13	347	258	1920	6.02	3.91
Max	8.17	395	280	2000	6.50	4.24
S ₂						
Min	7.27	314	50	1840	1.11	0.73
Mean (n = 23)	7.79	1119	148	2149	3.49	2.27
Max	8.16	3278	270	2470	6.35	4.14
S ₃						
Min	6.07	886	3	2220	0.07	0.04
Mean (n = 35)	6.68	18,313	21	2462	0.46	0.30
Max	7.79	51,997	140	2490	3.27	2.13

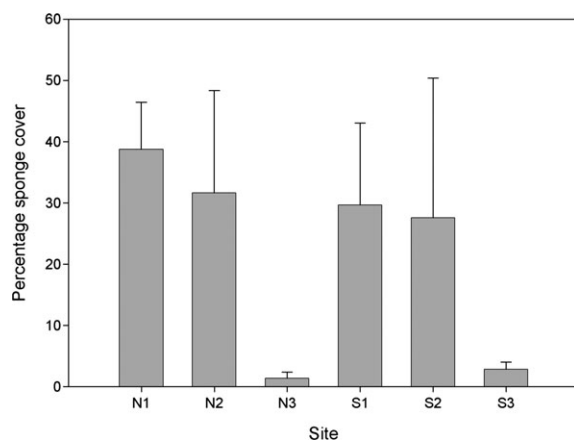


Fig. 2. Sponge percentage cover per square meter (mean + SE, n = 10) at sampling stations along the rocky cliff of Castello Aragonese. North stations: N1 (mean pH 8.14), N2 (mean pH 7.87) N3 (mean pH 7.09). South stations: S1 (mean pH 8.14), S2 (mean pH 7.83), S3 (mean pH 6.57).

lar junctions and other ultrastructural connections which allow the epithelial layers of other animals to act as barriers (Pörtner 2008). Their interstitial fluid is very similar to that of their environment (Ruppert & Barnes 1994). Consequently they are unable to regulate for changes in

acidity and may be particularly vulnerable to alterations in environmental conditions.

Sponge community species composition and abundance has been shown to be affected by substratum inclination (Preciado & Maldonado 2005), exposure to light (Jokiel 1980), degree of siltation (Brien *et al.* 1973; Bell & Barnes 2000b), exposure to predators (Dunlap & Pawlik 1996) and algal abundance (Bell & Barnes 2000a, 2002). Differences in these environmental factors were controlled for as far as possible by sampling from similar habitats at all sites (vertical rock faces between 1 and 3 m in depth). However, orientation of rock face and therefore exposure to light could not be controlled for. Species abundance and sponge cover were slightly lower on the south sampling sites, possibly due to the greater light exposure. Despite this, a decrease in sponge abundance with increased pH was observed on both south and north sites, suggesting that this is the dominant factor affecting sponge community composition in the study area. There has been little research into the effects of pH on sponge metabolism but there is some evidence that it may affect reproductive success and regeneration. Changes in environmental pH have been shown to affect the calcium-induced cell aggregation of sponge cells, possibly by altering the level of intracellular Ca²⁺ or by affecting cell

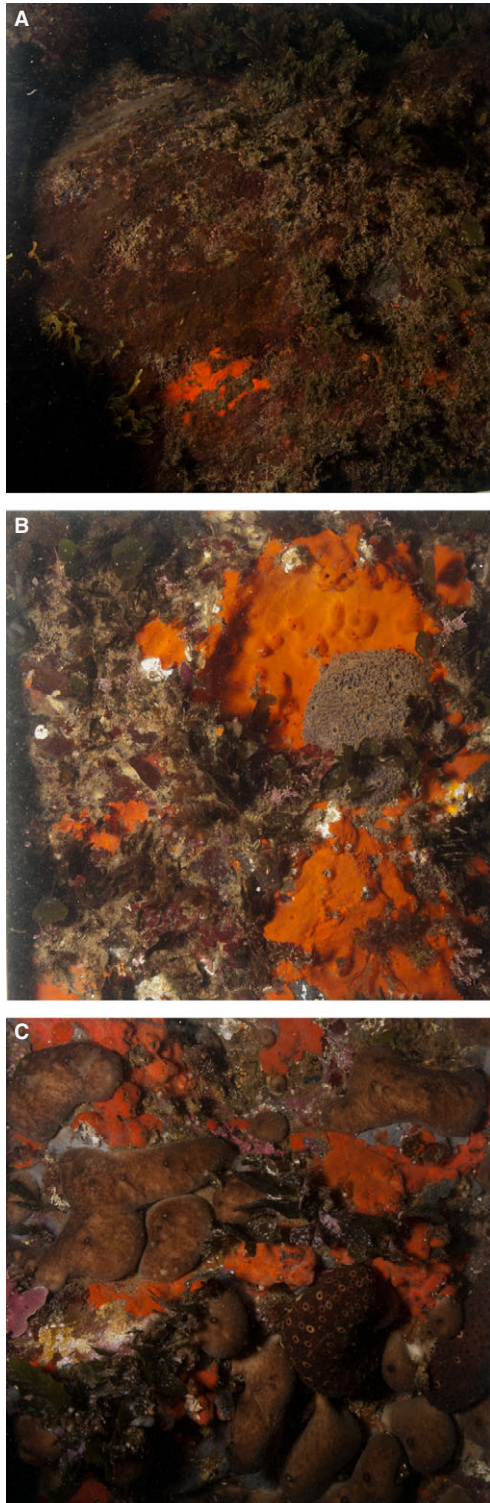


Fig. 3. Representative 25-cm² sponge cover quadrats from north side transect. (A) Quadrat 1, N3 (*Microciona* cf. *toxitenus*). (B) Quadrat 9 N2 (*Crambe crambe*, *Ircinia variabilis*, *Chondrosia reiniformis*, *Phorbas tenacior*). (C) Quadrat 9, N1 (*Crambe crambe*, *Phorbas tenacior*, *Chondrosia reiniformis*).

permeability (Phillip 1997). Re-aggregation is thought to be one process involved in sponge regeneration (Bergquist 1978). It is possible that the difference in species composition between sites reflects differential effects on re-aggregation mechanisms and consequently potential to recover from disturbance.

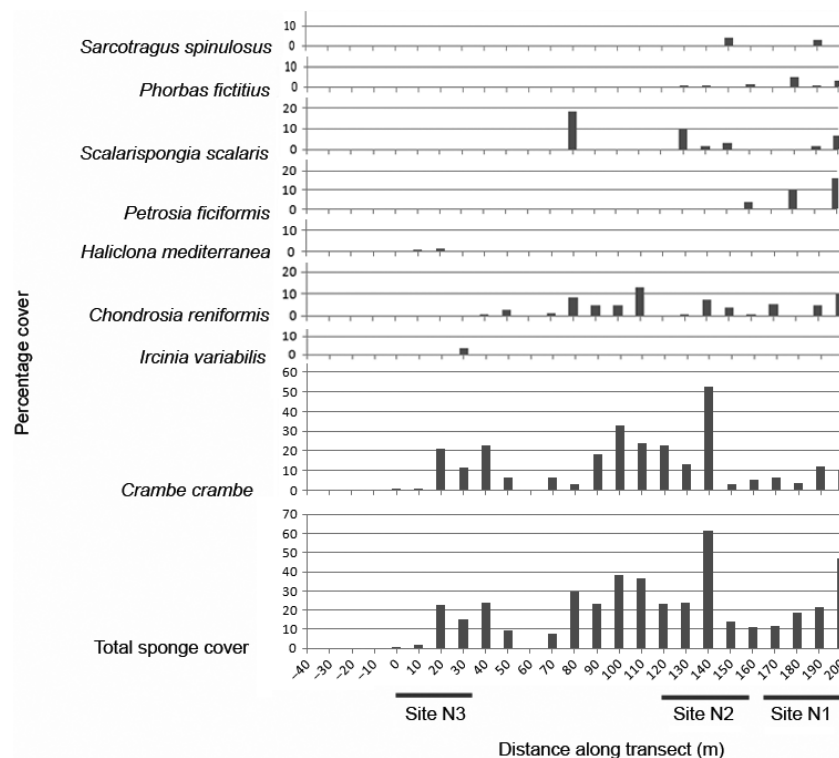
Exposure to low pH (5.8–6.5) has been found to affect hatching rate and hatchability of gemmules in the freshwater sponge *Ephydatia mülleri* (Benfey & Reiswig 1982). Larval phases of marine organisms may be particularly vulnerable to the effects of even small alterations in pH, with increases resulting in larval mortality and developmental abnormalities (Dupont *et al.* 2008) and a significant effect of pH on settlement has been shown for other groups at the study site (Cigliano *et al.* 2010). A variety of developmental forms was represented by the species present. *Crambe crambe*, the dominant species at the acidified sites, reproduces sexually with internal fertilisation, lecithotrophic larvae of the parenchymella type are released, spending 24–72 h in the plankton before settlement (Uriz *et al.* 1998). A range of reproductive modes was present in those species only found at the normal pH sites, including both direct development with no larval phase in *Petrosia ficiformis* (Gil 2007) and development via a non-tufted parenchymella larvae *Phorbas ficiticius* (Mariani *et al.* 2006). Consequently, it does not seem that mode of development can account for the difference in distribution between species; other factors may be affecting larval settlement efficiency.

Crambe crambe is one of the most widespread littoral sponges in the Mediterranean (Uriz *et al.* 1992). It is an opportunistic species with high reproductive output, and larval swimming behaviour which results in wide dispersal (Uriz *et al.* 1998). A trade-off between defence and growth rate has been found to occur, with allocation to chemical and physical defences being negatively correlated with reproduction and growth (Becerro *et al.* 1997) and spatial competition being a key factor. The absence of competitors in the acidified sites may favour *C. crambe* by enabling it to devote more resources to growth; it was the only species to decrease in abundance at the normal pH sites. There was no noticeable effect of acidity on spicule formation in *C. crambe*. Spicule formation has been found to be affected by other environmental factors such as water temperature and silica concentration (Elvin 1971; Bavastrello *et al.* 1993). During the late Permian mass extinction, possibly linked to elevated CO₂ levels, in South China there was a 88–92% decrease in spicule diversity: the surviving spicule forms were smooth and simple (oxeas, strongyles, oxy-orthopentactines and oxy-orthohexactines; Liu *et al.* 2008), mirroring the trend for simplified skeletons in other groups (Knoll *et al.* 1996). It is possible that CO₂ affects silica formation, preventing

Table 2. Sponge species recorded on the north (N1–N3) and south side of Castello Aragonese along gradients of pH. Mean percentage cover/m² (n = 10).

Species/Area	N1 (pH 8.14)	N2 (pH 7.87)	N3 (pH 7.09)	S1 (pH 8.14)	S2 (pH 7.83)	S3 (pH 6.57)
<i>Crambe crambe</i>	●●	●●	●	●●	●●	●
<i>Chondrosia reiniformis</i>	●●	●		●		
<i>Phorbas tenacior</i>	●	●				
<i>Scalarispongia scalaris</i>	●	●			●	
<i>Petrosia ficiformis</i>	●			●		
<i>Ircinia variabilis</i>	●	●			●	
<i>Sarcotragus</i> sp.		●				
<i>Microciona</i> cf. <i>toxitenus</i>			●			
<i>Hemimycale columella</i>				●		
<i>Spirastrella cunctatrix</i>						●
<i>Phorbas fictitius</i>						
<i>Chondrilla nucula</i>				●		
<i>Haliclona mediterranea</i>		●				
<i>Eurypon</i> cf. <i>cinctum</i>	●					
Total number of species	6	7	2	5	3	2

● = <1%; ● = 1–5%; ● = 5–10%; ● = 10–20%; ● = 20–30%.

**Fig. 4.** Mean cover of sponge species (n = 3) from quadrats taken at 10-m intervals along a transect along the north wall of Castello Aragonese. The transect included sampling stations N1 (mean pH 8.14), N2 (mean pH 7.87) and N3 (mean pH 7.09).

the formation of complex spicules and consequently preventing species with more complex skeletons from colonising the vent sites. Experimental studies would be useful in determining whether this is the case.

Other studies on the vent sites have noted a shift from typical rocky shore communities with abundant calcareous organisms at the control sites to communities lacking scleractinian corals with significant reductions in sea

urchin and coralline algal abundance (Hall-Spencer *et al.* 2008). Although total algal cover remained relatively consistent throughout the sites, there was a shift from calcareous algal dominance to dominance by non-calcareous algae at the low pH sites (for example *Caulerpa*, *Cladophora*, *Asparagopsis*, *Dictyota* and *Sargassum*; Hall-Spencer *et al.* 2008; Porzio *et al.* 2011). However, many of the sites at which no sponge cover was recorded lacked macroorganisms, consequently greater competition from non-calcareous than calcareous algae is unlikely to be a controlling factor.

Studies of other hydrothermal vent communities in the Mediterranean have indicated that although sponge species biodiversity can be higher at vent than non-vent sites (Morri *et al.* 1999; Pansini *et al.* 2000), proximity to vents had no influence on sponge percentage cover (Pansini *et al.* 2000). In these studies no direct measurements of pH or attempts to correlate sponge biodiversity with pH were made. The hydrothermal vent fluids were recorded as having pH varying from pH 5.2 to 7.1 (Dando *et al.* 1999) but when diluted by the surrounding seawater the pH is likely to be higher. The study sites were large, encompassing a depth range of several metres and consequently even those in closest proximity to the vents will have encompassed a range of pH conditions.

Elevated CO₂ levels have been proposed as a causative factor for the late Permian mass extinction (Knoll *et al.* 1996; Pörtner *et al.* 2004). The extinction was particularly pronounced among reef-building metazoans such as calcareous sponges and rugose corals (Pruss & Bottjer 2005) and other organisms with carbonate skeletons (Knoll 2003; Dias *et al.* 2010). Disassociation of skeletal elements makes the study of siliceous species more difficult, but in South China extinction rates of 88–92% of spicule forms have been shown at the Permian–Triassic boundary, indicating extinction of the majority of siliceous sponge species, including all hexactinellids and species with tetraxons (Liu *et al.* 2008). Although the presence of a calcified skeleton is recognised as a factor in sensitivity to elevated CO₂ (Knoll *et al.* 1996; Pörtner *et al.* 2004), other aspects, also found in siliceous sponges, such as low-metabolic rate, absence of a circulatory system and elaborated gas-permeable surfaces, may also result in increased vulnerability (Knoll *et al.* 1996). Sponges are important functional components of marine ecosystems; playing roles in substrate modification and benthic-pelagic coupling, as well as having a large number of associations with species from other phyla (Bell 2008). Consequently, a future CO₂ rise may have significant ecological effects on sponges. Our study indicates that some demosponge species are likely to be more vulnerable than others and further research into the underlying mechanisms would be useful in predicting likely outcomes.

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