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Effects of ocean acidification on invertebrate settlement at volcanic CO₂ vents

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Abstract We present the first study of the effects of ocean acidification on settlement of benthic invertebrates and microfauna. Artificial collectors were placed for 1 month along pH gradients at CO₂ vents off Ischia (Tyrrhenian Sea, Italy). Seventy-nine taxa were identified from six main taxonomic groups (foraminiferans, nematodes, polychaetes, molluscs, crustaceans and chaetognaths). Calcareous foraminiferans, serpulid polychaetes, gastropods and bivalves showed highly significant reductions in recruitment to the collectors as *p*CO₂ rose from normal (336–341 ppm, pH 8.09–8.15) to high levels (886–5,148 ppm) causing acidified conditions near the vents (pH 7.08–7.79). Only the syllid polychaete *Syllis prolifera* had higher abundances at the most acidified station, although a wide range of polychaetes and small crustaceans was able to settle and survive under these conditions. A few taxa (*Amphiglena mediterranea*, *Leptochelia dubia*, *Caprella acanthifera*) were particularly abundant at stations acidified by intermediate amounts of CO₂ (pH 7.41–7.99). These results show that increased

levels of CO₂ can profoundly affect the settlement of a wide range of benthic organisms.

Introduction

Increasing atmospheric CO₂ concentrations are causing a rise in *p*CO₂ concentrations at the ocean surface (Houghton et al. 1992; Keeling and Whorf 1994) due to atmospheric CO₂ ocean exchange on time scales of several months (Zeebe and Wolf-Gladrow 2001). By 2100, the concentration of CO₂ in the ocean is expected to rise to 750 ppm, which is about twice the present 385–390 ppm (Feely et al. 2004; Raven et al. 2005). As CO₂ dissolves in the surface ocean, it reacts with water to form carbonic acid (H₂CO₃), which dissociates to bicarbonate (HCO₃⁻), carbonate ions (CO₃²⁻) and protons (H⁺). With increasing atmospheric *p*CO₂, the equilibrium of the carbonate system will shift to higher CO₂ and HCO₃⁻ levels, while CO₃²⁻ concentration and pH will decrease.

These changes in carbonate chemistry, often referred to as ‘ocean acidification’, are already occurring and are expected to intensify in the future. Models predict that the pH of surface seawater will drop by 0.4 units by the year 2100 (Caldeira and Wickett 2003). Consequently, the rise of CO₂ in ocean waters leads to more corrosive conditions for calcifying organisms, making it more difficult for them to build and maintain their carbonate skeletons (Raven et al. 2005). Calcification rates of several species, including coralline algae, coccolithophores, corals, bivalves and echinoderms, decreases with increasing *p*CO₂ (e.g. Kleypas et al. 2006; Fabry et al. 2008), although the response is species specific with the up-regulation of calcification in some species (Wood et al. 2008; Ries et al. 2009; Jury et al. 2009; Rodolfo-Metalpa et al. 2010a). The recruitment rate

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and the growth of crustose coralline algae is severely inhibited under elevated $p\text{CO}_2$, suggesting that changes in benthic community structure may occur owing to the impact of ocean acidification on recruitment and competition for space (Kuffner et al. 2007; Hall-Spencer et al. 2008; Jokiel et al. 2008). Initial results by Porzio et al. (2008) demonstrate significant loss of algal diversity and changes in macroalgal community structure in a naturally acidified environment.

However, effects of ocean acidification on larval pelagic stages of invertebrates are still poorly understood (Vézina and Hoegh-Guldberg 2008). Many laboratory studies have shown that the early life history stages of several organisms are negatively impacted by acidified seawater, including work on echinoderms, crustaceans and molluscs (Kurihara and Shirayama 2004; Kurihara et al. 2004, 2007; Dupont et al. 2008; Kurihara and Ishimatsu 2008; Ellis et al. 2009; Findlay et al. 2009). However, potential shifts in benthic recruitment that may result from these effects on early life history changes are unknown due to the difficulties of maintaining mixed populations of delicate larval stages in laboratory conditions.

Hall-Spencer et al. (2008) have shown that natural CO_2 venting sites may be useful for assessing the long-term effects of ocean acidification on benthic biota and sea-floor ecosystems. They indicate that, although natural CO_2 venting sites are not precise analogues of global-scale ocean acidification, they can provide essential information about high- CO_2 effects on spatial and temporal scales which are otherwise difficult to address. Here, we provide first data on the effects of acidification on invertebrates and microfauna settled on artificial collectors placed at various distances from CO_2 vents, creating a gradient of different pH conditions.

Study site

Castello Aragonese, located at the north-eastern side of Ischia island (Fig. 1, $40^\circ 43.84' \text{ N}$, $13^\circ 57.08' \text{ E}$) is part of a 132,000 years old volcano (Rittmann and Gottini 1981) where gas vents occur in shallow water (Tedesco 1996). The gas comprises 90–95% CO_2 , 3–6% N_2 , 0.6–0.8% O_2 , 0.2–0.8% CH_4 and 0.08–0.1% air and bubbles at about $1.4 \times 10^6 \text{ l d}^{-1}$ at ambient temperature. The site is microtidal (0.30–0.50 m range) and the CO_2 vents lack sulphur (Tedesco 1996); they acidify normal salinity and alkalinity of seawater along a pH gradient from 8.17 down to 6.57 for 300 m running parallel to the rocky shore on the north and south sides of the Castello (Hall-Spencer et al. 2008). The south side is more sheltered from wave action and has a shallow (0.5 m depth) *Posidonia oceanica* meadow forming a reef-like structure (*sensu* Augier and Boudouresque

1970), where leaves float on the water surface at low tide with the highest mean shoot density recorded around Ischia (up to 900 shoots/ m^2 , Buia et al. 2003). Previous studies at the Castello report rich algal (Bourdouresque and Cinelli 1971, 1976) and sponge communities (Sarà 1959; Pulitzer Finali 1970; Pulitzer Finali and Pronzato 1976), as well as diverse invertebrate and fish faunas associated with the *Posidonia oceanica* meadows (Russo et al. 1984a; Guidetti and Bussotti 1998; Scipione 1999; Gambi and Cafiero 2001) in the areas nearby and partially influenced by the vents. Hall-Spencer et al. (2008) reported a total of 64 megabenthic taxa along the gradient at the vents areas, where reductions in the diversity of adult populations are caused partly by the dissolution of calcified species due to lowered pH (Martin et al. 2008; Rodolfo-Metalpa et al. 2010b). Loss of macroalgal diversity was also shown by Porzio et al. (2008). Although CO_2 vents are localised and highly variable in pH, they provide information about the ecological effects of long-term exposures to high CO_2 levels encompassing the life cycles of interacting macrobenthic organisms as well as the feedbacks and indirect effects that occur within natural marine systems (Hall-Spencer et al. 2008; Riebesell 2008). The aim of this study was to use artificial collectors to determine whether invertebrates and microfauna varied along gradients in pH where acidification of water by CO_2 affects natural marine communities. We focus on early-settled stages of invertebrates and foraminiferans, a fauna component which was not considered at the vents initial survey by Hall-Spencer et al. (2008). Our null hypothesis was that there would be no significant differences in species composition and community structure in the collectors placed along the pH gradients studied.

Materials and methods

Artificial collectors (scouring pads) were placed in situ along 300 m transects on the north and south sides of Castello Aragonese at six stations (N1, N2, N3, S1, S2, S3) where Hall-Spencer et al. (2008) had recorded significant differences in pH due to CO_2 vents (Fig. 1). N1 and S1 are monitoring stations located under normal pH conditions, N2 and S2 are intermediate stations, characterized by high pH fluctuations and mean intermediate values, while N3 and S3 are characterized by low pH, acidified conditions. Water samples ($n = 3\text{--}5$) were taken at buoyed stations by SCUBA divers using glass bottles (250 cc volume) during the month of collector deployment, and the pH_T (total scale) was measured immediately using a meter accurate to 0.01 pH units (Metrohm 826 pH mobile) calibrated using TRIS/HCl and 2-aminopyridine/HCl buffer solutions (DOE 1994). Seawater samples were then passed through 0.45- μm

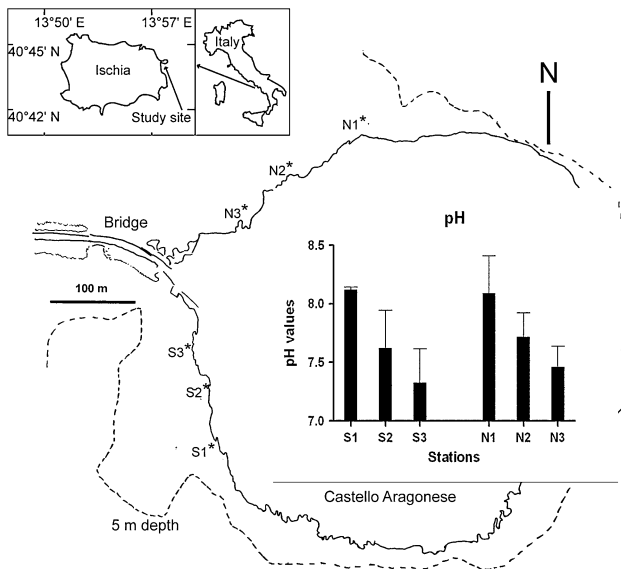


Fig. 1 Map of the study area at the Castello Aragonese (Ischia island, Italy) with location of the sampling stations on the north and south sides along a pH gradient from normal (N1, S1) to acidified (N3, S3) conditions. The graph inside the map represents the mean and SD of the pH values measured during the sampling period in April–May 2008 (see also Table 1)

pore size filters (GF/F Whatman) and poisoned with 0.05 ml of 50% HgCl_2 (Merck, Analar) to avoid biological alteration and stored in the dark at 4°C. Total alkalinity (TA) was measured using a titration system composed of a pH meter with an ORION pH electrode and a 1 ml automatic burette (METHROM). TA was calculated from the Gran function applied to pH variations from 4.2 to 3.0 as mEq l^{-1} from the slope of the curve HCl volume vs. pH. Parameters of the carbonate system [pCO_2 , CO_3^{2-} , HCO_3^- and saturation state of calcite (Ω_{calcite})] were calculated from pH_T , TA, temperature and salinity (38) using the CO_2 Systat package.

Artificial collectors were 8 cm diameter rounded scouring pads formed by an enrolled coarse nylon net. These collectors were chosen as they have been widely used to collect both larval and adult stages of benthic invertebrates (e.g. Menge 1992; Kendall et al. 1996; Porri et al. 2006; Gobin and Warwick 2006). At each station (N1, N2, N3, S1, S2, S3), three scouring pad collectors (labelled as a, b, c) were fixed to buoyed moorings 1 m from the bottom adjacent to the rocky shore at 1.5–2.0 m depth from the surface for a total of 18 samples. The artificial collectors were placed in situ on the 18th April 2008 and removed after 1 month (19th May). After removal, scouring pads were immediately fixed in 4% formalin in sea water. They were later unrolled and the nylon net was rinsed with sea water on a 100- μm sieve and the retained material transferred to 70% alcohol for sorting under a $\times 40$ stereomicroscope. All organisms were sorted by high taxonomic groups (Phylum,

Class and Order) and counted, the foraminiferans, polychaetes, molluscs, isopods and amphipods were classified to genus and, where possible, to species.

Data analyses

Number of species, abundance, Shannon diversity (H') and Pielou's evenness (J) were calculated for each collector and plotted as means \pm SD ($n = 3$ at each station). Differences among stations along the pH gradient and between sides (North vs. South) were tested using 2-way ANOVA for pH and carbonate parameter values, for the faunal structural parameters, as well as for most common taxonomic groups and the four most abundant species. Homogeneity of variances was verified using Cochran test ($P < 0.05$) and for those variables that were not homogeneous, an appropriate data transformation was applied. ANOVA pair-wise comparisons among stations were also performed. All statistical analyses were performed using the STATISTICA 8 free package.

Structural analysis at community level was performed using the cluster analysis (Bray-Curtis similarity, group average on square-root-transformed abundance data to smooth and down-weight the importance of highly abundant taxa) and the non-parametric multi-dimensional scaling (nMDS) (Bray-Curtis similarity on square-root transformed abundance data; PRIMER v6). In the nMDS graph, sample points were circled according to clusters obtained with the Bray-Curtis cluster analysis and that were significant using the SIMPROF test ($P < 0.05$). For the multivariate analysis, each sample (scouring pad) was separately considered (labelled as a, b, c in the nMDS graph). The SIMPER analysis was performed on the species matrix to highlight the species that most contribute to distribution pattern of the stations. ANOSIM was applied to test the significance level of the station factor (pH gradient), of the side factor (North vs. South) and of the side/station interaction.

Results

Monitoring stations N1 and S1 (Fig. 1) had normal pH (range 8.06–8.15); N2 and S2 had low pH (range 7.27–7.99 and 7.49–7.89, respectively); N3 and S3 had very low pH (range 7.26–7.60 and 7.08–7.79, respectively), but among stations, there were no differences in temperature (range 15.9–22.4°C), total alkalinity (range 2.5–2.6) or salinity (always 38) throughout the experimental period (Table 1). Table 1 shows measured and calculated differences in carbonate chemistry of the stations with pCO_2 peaks of $>3,000$ ppm and periods of aragonite under-saturation recorded at stations N3, S2 and S3 and periods of calcite

Table 1 Seawater carbonate chemistry measured (pH_T , temperature and total alkalinity TA) and calculated using CO2 Systat software (salinity = 38) during the deployment of settlement collectors on the

north (N1, N2, N3) and south (S1, S2, S3) side of the Castello Àragonese (Ischia, Italy) in April–May 2008

Station	Temperature (°C)	TA (mmol kg ⁻¹)	pCO_2 (μatm)	pH_T (total scale)	CO_2 (mmol kg ⁻¹)	HCO_3^- (mmol kg ⁻¹)	CO_3^{2-} (mmol kg ⁻¹)	DIC (mmol kg ⁻¹)	$\Omega_{\text{aragonite}}$	Ω_{calcite}
N1										
18-April	16	2.568	336	8.15	0.012	1.97	0.25	2.229	3.70	5.73
08-May	20.3	2.576	431	8.06	0.014	1.99	0.24	2.253	3.67	5.62
10-May	19.9	2.571	430	8.06	0.014	1.99	0.37	2.250	3.61	5.53
N2										
18-April	16.0	2.609	1,908	7.49	0.680	2.45	0.07	2.583	1.00	1.55
08-May	19.9	2.596	955	7.77	0.030	2.27	0.14	2.445	2.11	3.23
10-May	20.2	2.614	697	7.89	0.022	2.19	0.18	2.387	2.70	4.14
N3										
18-April	16.0	2.581	2,838	7.32	0.062	2.41	0.07	2.544	1.06	1.64
08-May	20.0	2.596	1,461	7.60	0.014	1.99	0.24	2.253	3.67	5.62
10-May	19.9	2.563	3,316	7.26	0.106	2.45	0.05	2.604	0.70	1.08
S1										
17-April	15.9	2.568	345	8.14	0.012	1.98	0.24	2.237	3.62	5.61
06-May	19.9	2.580	333	8.15	0.011	2.00	0.28	2.186	4.24	6.50
08-May	20.0	2.571	395	8.09	0.012	1.97	0.25	2.229	3.82	5.85
10-May	20.6	2.581	384	8.10	0.012	1.95	0.26	2.225	3.98	6.1
18-May	18.7	2.614	370	8.12	0.012	1.99	0.26	2.261	3.92	6.04
S2										
17-April	15.9	2.570	524	7.99	0.019	2.13	0.18	2.329	2.75	4.26
06-May	20.0	2.580	618	7.93	0.019	2.13	0.19	2.336	2.86	4.38
08-May	20.0	2.585	3,278	7.27	0.104	2.47	0.05	2.622	0.73	1.11
10-May	20.3	2.561	2,316	7.41	0.073	2.41	0.07	2.543	0.99	1.51
12-May	22.4	2.561	1,775	7.52	0.073	2.40	0.06	2.542	0.99	1.55
S3										
17-April	16.0	2.567	2,226	7.42	0.079	2.43	0.06	2.566	0.85	1.31
06-May	20.0	2.585	3,786	7.21	0.120	2.48	0.04	2.647	0.64	0.98
08-May	20.8	2.570	5,148	7.08	0.160	2.49	0.03	2.685	0.49	0.75
10-May	20.7	2.556	4,543	7.13	0.140	2.47	0.04	2.648	0.54	0.83
18-May	19.7	2.552	886	7.79	0.028	2.22	0.14	2.38	2.13	3.27

under-saturation only recorded at station S3. Although pH and carbonate chemistry values were quite variable at the vents stations (especially in the intermediate, N2 and S2, and very low pH stations N3 and S3) compared to the control stations (N1, S1), 2-way ANOVA showed that pH values ($F = 9.551$, $P < 0.001$), pCO_2 ($F = 5.303$, $P = 0.004$), $\Omega_{\text{aragonite}}$ ($F = 6.550$; $P < 0.001$) and Ω_{calcite} ($F = 8.540$; $P < 0.001$) were significantly different between stations. Such differences were mainly due to stations S3 and N3 which showed the lowest values. In contrast, the DIC values did not change significantly among stations ($F = 0.336$; n.s.).

A total of 4,463 individuals of benthic invertebrates and microfauna were sampled in the collectors and separated into 79 taxa (Table 2). Half the individuals collected were

Crustacea (Copepoda 1136, Amphipoda 398, Tanaidacea 408, Isopoda 148, Ostracoda 148, Cumacea 11 and Decapoda 2), they were followed in abundance by Mollusca (770 Bivalvia and 216 Gastropoda), by Polychaeta (876 individuals), while Foraminifera (239 individuals), Nematoda (82 individuals) and Chaetognatha (29 individuals) made up <10% of the individuals sampled. Foraminifera, Polychaeta, Amphipoda, Tanaidacea, Isopoda and Gastropoda were identified to species level giving a total of 64 species. Although similar numbers of organisms settled in the collectors across the stations with no clear trend in Evenness (J), there were significant differences ($P < 0.01$) between the numbers of taxa which were higher from collectors placed in normal pH stations than in intermediate and low pH stations on both transects, such that the normal

Table 2 List of species and taxa collected at the studied stations and their abundances (number of individuals)

Taxa/Samples	S1a	S1b	S1c	S2a	S2b	S2c	S3a	S3b	S3c	N1a	N1b	N1c	N2a	N2b	N2c	N3a	N3b	N3c	
Foraminifera																			
<i>Elphidium complanatum</i> (d'Orbigny, 1839)		3	4											7					
<i>Elphidium aculeatum</i> (d'Orbigny, 1846)	3	14	5				1	2		14	12	12	28	66	23				
<i>Elphidium depressulum</i> Cushman, 1933			2				2			1		1	6	1	2				
<i>Massilina secans</i> (d'Orbigny, 1826)	1		1							5	2	10							
<i>Milionella subrotundata</i> (Montagu, 1803)												3							
<i>Quinqueloculina parvula</i> Schlumberger, 1894			1								2								
<i>Quinqueloculina</i> aff. <i>parvula</i> Schlumberger, 1895												1							
<i>Quinqueloculina berthelotiana</i> d'Orbigny, 1839												1							
<i>Triloculina plicata</i> Terquem, 1876												1							
<i>Triloculina schreiberiana</i> d'Orbigny, 1839	1		1																
<i>Vertebralina striata</i> d'Orbigny, 1826				1							12		3	9	2	8	12	9	
Nematoda																			
Polychaeta																			
<i>Amphiglena mediterranea</i> (Leydig, 1851)	3	6	6	4	22	21	1	1	10		5	4	1	5	4	2	4	4	
<i>Ceratonereis costae</i> (Grube, 1840)					1														
<i>Exogone (Paraxogone) meridionalis</i> Cognetti, 1955	2		4	2	14		1	3		1		5	1		11	3		24	
<i>Exogone (Exogone) naidina</i> (Orsted, 1845)	8	22	16	4	27	8		10		6	18	11	5	10	20	8	25	15	
<i>Gyptis propinqua</i> Marion & Bobretzky, 1875	3	3	1							2	7	5		1					
<i>Neodextospira pseudocorrugata</i> (Bush, 1905)	11	4	13							7	12	6							
<i>Platynereis dumerilii</i> (Audouin & Milne-Edwards, 1833)	1	1	1	7	8	30	5	17	19		3	7	3	1	2	6	5		
<i>Polyophthalmus pictus</i> Dujardin, 1839	1	4	3		4					5	3	5	1	2	5				
<i>Spio decoratus</i> Bobretzky, 1870	1	2	1		1	1	1	1				4	1						
<i>Spirorbis marioni</i> Caullery & Mesnil, 1897		3	5								12	4							
<i>Syllis prolifera</i> Krohn, 1869	6	14	16	5	7	16	7	46	29		30	4		11	8	2	15	9	
<i>Trichobranchus glacialis</i> Malmgren, 1866									1										
Mollusca																			
Gastropoda																			
<i>Alvania subcrenulata</i> (Bucquoy, Dautzenberg & Dollfus, 1884)	1																		
<i>Clanculus jussieui</i> (Payreaudeau, 1826)																			1
<i>Dikoleps nitens</i> (Philippi, 1884)								6											
<i>Fossarus ambiguus</i> (Linné 1758)			1																
<i>Gibbula fanulum</i> (Gmelin, 1791)	1																		
<i>Gibbula richardi</i> (Payreaudeau, 1826)																			1

Table 2 continued

Taxa/Samples	S1a	S1b	S1c	S2a	S2b	S2c	S3a	S3b	S3c	N1a	N1b	N1c	N2a	N2b	N2c	N3a	N3b	N3c
<i>Gibbula varia</i> (Linnè, 1758)									11									
<i>Hydrobia</i> sp.											1							
<i>Jujubinus gravinae</i> (Dautzenberg, 1881)										6				1				
<i>Melanella polita</i> (Linnè, 1758)	1										3							
<i>Osilinus turbinatus</i> (Von Born, 1778)	12	4	15							1	12		1	3				
<i>Pusillina margininia</i> (Nordsteck, 1972)															1			
<i>Pusillina</i> sp.	4										2	1				1		
<i>Rissoa variabilis</i> (Von Muehlfeldt, 1824)		5	7	7		11		13	10	16	7	8	7	12	4		4	4
<i>Rissoa ventricosa</i> (Desmarest, 1814)																1		
<i>Rissoa violacea</i> (Desmarest, 1814)	1										2	1						
<i>Tricolia pullus pullus</i> (Linnè, 1758)	1	1								1								
Bivalvia	70	80	112	45	20	18	2	28	0	23	46	25	26	96	68	18	80	13
Crustacea																		
Ostracoda	2	0	1	12	3	8	2	20	21	4	7	0	0	4	5	13	36	10
Copepoda	20	29	67	102	97	106	68	117	33	23	50	48	2	47	48	54	102	123
Amphipoda																		
<i>Ampithoe ramondi</i> Audouin, 1826				8	3	10		7	12						1	3		1
<i>Leptocheirus pilosus</i> Zaddach, 1844										1		1						1
<i>Microdeutopus</i> spp.	2	3	3	3	8	5	1	7	16		1	1	1	2		2	1	1
<i>Dexamine spiniventris</i> (A.Costa, 1853)															2	1	1	1
<i>Apherusa</i> cf. <i>chierighinii</i> Giordani Soika, 1950							2	1	1									
<i>Hyale camptonyx</i> (Heller, 1866)	7	3	1	3	2	2	1	1	1	2			5					1
<i>Protohyale schmidti</i> (Heller, 1866)								6										
<i>Iphimedia minuta</i> G.O.Sars, 1882	1																	
<i>Gammaropsis palmata</i> Stebbing & Robertson, 1891								1					1					
<i>Erichthonius punctatus</i> (Bate, 1857)							1							2				
<i>Ischyrocerus inexpectatus</i> Ruffo, 1959	1									1	1	1	1	1	1			2
<i>Jassa marmorata</i> Holmes, 1903				2	1	1					1		1	3				
<i>Lysianassa costae</i> Milne Edwards, 1830									1									
<i>Elasmopus rapax</i> A.Costa, 1853	1							2			1							
<i>Gammarella fucicola</i> (Leach, 1814)																		
<i>Quadrimaera inaequipes</i> (A.Costa, 1857)	2																	
<i>Stenothoe cavimana</i> Chevreux, 1908				1														
<i>Stenothoe monoculoides</i> (Montagu, 1813)																		
<i>Caprella acanthifera</i> Leach, 1814	1	8	23	9	45		11	17	6	8	14	3	16	21	1	24	12	

Table 2 continued

Taxa/Samples	S1a	S1b	S1c	S2a	S2b	S2c	S3a	S3b	S3c	N1a	N1b	N1c	N2a	N2b	N2c	N3a	N3b	N3c
<i>Caprella</i> sp.										1								
Tanaidacea																		
<i>Parapseudes latifrons</i> Grube, 1864						1	3	3	2									
<i>Tanais dulongii</i> (Audouin, 1826)								1										
<i>Leptochelia dubia</i> (Kroyer, 1842)	30	28	29	64	66	77	5	34	20	4	5	1	3	5	3	4	8	6
Tanaidacea indet.	1					1	3										1	
Isopoda																		
<i>Cleanthis prismatica</i> (Risso, 1826)	1																	
<i>Paranthura nigropunctata</i> (Lucas, 1846)												1						
<i>Paranthura</i> indet.		2	1								1							
<i>Cymodoce truncata</i> (Leach, 1814)	12	13	4							1	1	1						
<i>Cymodoce hanseni</i> (Dumay, 1972)																		
<i>Dynamene cf. bifida</i> Torelli, 1930				5	13	3		62	25									1
Cumacea	3	1	0	0	0	0	0	0	0	0	2	4	0	0	0	0	0	1
Decapoda	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chaetognata	4	11	3	0	0	0	0	0	0	0	4	0	3	3	0	1	0	0
Total number of individuals	218	260	336	303	299	371	114	404	221	132	269	205	100	308	234	122	323	244
Total number of species	28	22	25	14	12	15	13	22	14	20	22	30	16	18	17	11	12	15
Total number of taxa	33	28	31	18	15	20	18	27	18	24	29	31	20	24	21	16	17	21
Diversity (H' index)	3.69	3.59	3.37	2.91	3	3.17	2.19	3.42	3.51	3.75	3.86	4.1	3.18	3.06	3.2	2.6	2.76	2.68
Evenness (J')	0.73	0.75	0.68	0.71	0.77	0.75	0.55	0.74	0.88	0.83	0.81	0.81	0.75	0.69	0.74	0.66	0.71	0.62

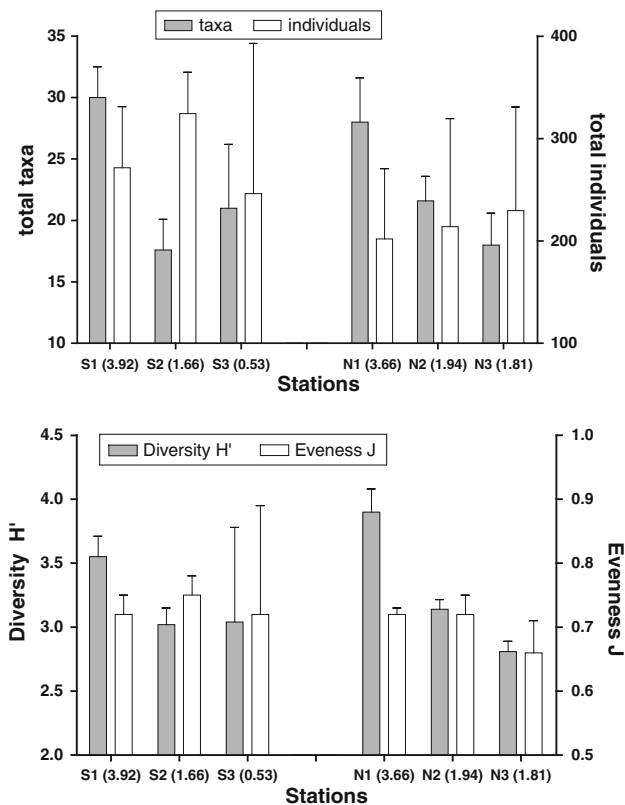


Fig. 2 Trend of the number of taxa and of individuals (*above graphs*) and Diversity (Shannon H') and Evenness (Pielou J) (*below graphs*) in the studied stations. In parentheses, next to station name are mean values of $\Omega_{\text{aragonite}}$

pH collectors were significantly ($P < 0.001$) more diverse, as also shown by the Shannon H' (Fig. 2, Table 3). A significant interaction between station and side was also observed (Table 3).

Foraminifera (11 taxa) were represented exclusively by benthic forms and were dominated by *Elphidium aculeatum*; they showed significantly fewer individuals ($P < 0.01$) and number of taxa ($P < 0.001$) in the low pH conditions (Fig. 3, Table 3). **Nematoda** and **Chaetognatha** occurred in low numbers throughout the pH range and were not identified further, precluding any investigations into species-specific responses. **Polychaeta** (12 taxa) were significantly more diverse ($P < 0.01$) at normal pH conditions with the calcareous tube-dwelling spirorbids *Spirorbis marioni* and *Neodexiospira pseudocorrugata* only occurring at normal pH stations. Polychaetes showed mixed responses to the pH gradient, with *Syllis prolifera* most abundant at the lowest pH sites (Fig. 4) and *Amphiglena mediterranea* significantly more abundant at intermediate pH station S2 (Table 3, Fig. 4). **Mollusca** (18 taxa) were mostly juvenile stages and had significantly fewer individuals ($P < 0.01$) and species ($P < 0.01$) at acidified sites. The bivalves were difficult to identify, as they were small and with damaged shells, but the most common genera were tentatively identi-

fied as *Tellina* and *Macoma*. Gastropod species were significantly more diverse at normal pH conditions ($P < 0.01$) although adults of the most abundant species, *Rissoa variabilis*, were also found at the lowest pH stations. **Crustacea** (34 taxa) also had significantly fewer individuals ($P < 0.01$) at the most acidified stations, but the total number of taxa did not differ significantly as there were a variety of ostracods, copepods, amphipods, tanaids and isopods that were tolerant of high CO_2 conditions (Fig. 3). When the responses of crustacean taxa are examined individually, they exhibit mixed responses with some responding positively and others negatively along the pH gradient, and with some showing peak abundance at intermediate pH. For example, copepods (the numerically dominant crustacean group represented by both pelagic and benthic forms) had significantly fewer individuals at low pH (Table 3), whereas *Leptochelia dubia* (the most abundant of the three species of tanaids present) and *Caprella acanthifera* (the most abundant of the 20 species of amphipods present) were significantly more abundant at intermediate pH station S2 (Table 3, Fig. 4), whereas *Dynamene cf. bifida* (the most abundant isopod) was common at the most acidified site.

The ANOVA pair-wise analysis (Table 4) showed that for most taxa and species considered, the differences were mainly between station S1 and S2–S3, and with a lower frequency between N1 and N2–N3. In general, stations S2 and S3, and N2 and N3 showed less pronounced differences.

Cluster analysis of the taxa/station matrix, based on Bray-Curtis similarity (not shown), separated three principal groups of samples at 57% similarity level and which are significant at the SIMPROF test ($P < 0.05$): the group including normal pH samples, N1b–c, S1a–c, the group with only samples N1a and N2a which are however closer to the first group, and the group including all the other intermediate and low pH samples, except the outliers S3a and N3a.

The nMDS ordination (based on Bray-Curtis similarity) grouped samples in a way very consistent with the cluster analysis (circled samples correspond to significant clusters at the SIMPROF test) with samples from normal pH conditions well separated from the others (Fig. 5). According to station (pH gradient), this ordination was significant (ANOSIM for station factor: Global $R = 0.424$, $P < 0.1\%$). The side factor North vs. South was not significant, while the interaction between side and station was significant (ANOSIM for side/station factor: Global $R = 0.649$, $P < 0.1\%$). SIMPER analysis showed that the species/taxa that most contribute to the dissimilarity between normal pH stations (S1, N1) and the low pH stations (S3, N3) were Bivalvia, Copepoda, *Neodexiospira pseudocorrugata* and *Elphidium aculeatum* (all calcifiers, except Copepoda). Those taxa which differentiate the normal pH from the intermediate stations (S2, N2) were *Leptochelia dubia*,

Table 3 ANOVA analyses of the main taxa and species, according to side (North vs. South) and station (along the pH gradient)

Taxa	Variable	Factors (<i>F</i> values)		Interaction
		Station	Side	
Foraminifera	Individuals	7.23**	n.s.	3.60*
	Species	7.76**	n.s.	8.98**
Polychaeta	Individuals	n.s.	n.s.	n.s.
	Species	7.43*	n.s.	9.67***
Gasteropoda	Individuals	5.91**	n.s.	7.76**
	Species	8.64**	n.s.	7.78*
Bivalvia	Individuals	3.72*	n.s.	5.01**
Total Mollusca	Individuals	3.77*	n.s.	5.06*
Copepoda	Individuals	3.55*	n.s.	3.94*
Amphipoda	Individuals	n.s.	n.s.	n.s.
	Species	n.s.	n.s.	n.s.
Tanaidacea	Individuals	44.23***	22.13**	n.s.
	Species	n.s.	5.60*	n.s.
Total Crustacea	Individuals	5.17**	6.15*	n.s.
	Species	n.s.	n.s.	n.s.
Abundant species				
<i>Syllis prolifera</i>	Individuals	n.s.	n.s.	n.s.
<i>Amphiglena mediterranea</i>	Individuals	n.s.	n.s.	n.s.
<i>Leptochelia dubia</i>	Individuals	42.72***	18.75**	n.s.
<i>Caprella acanthifera</i>	Individuals	n.s.	n.s.	n.s.
	Total n. taxa	8.03**	n.s.	10.72***
	Total n. individuals	n.s.	n.s.	n.s.
	Diversity (H')	7.95***	n.s.	8.51***
	Evenness (J')	n.s.	n.s.	n.s.

* $P < 0.05$; ** $P < 0.01$;*** $P < 0.001$;

n.s., Not significant

Copepoda, *Elphidium aculeatum*, *Neodexiospira pseudo-corrugata* and Bivalvia (again most are calcifiers). While those differentiating the intermediate from the low pH stations were Bivalvia, *E. aculeatum*, *Leptochelia dubia*, Copepoda, *Caprella acanthifera* and *Exogone naidina*. The species/taxa which most contribute in differentiating south from north side samples were *L. dubia*, Bivalvia and Copepoda.

Discussion

Ocean acidification causes changes in seawater chemistry that may have a large impact on marine life and biogeochemical processes; however, these impacts are still poorly understood at the ecosystem level (Vézina and Hoegh-Guldberg 2008). Here, we provide a first assessment of which benthic faunal groups successfully settle out along a pH gradient in natural CO₂ vents. Such data are required to advance our understanding on how marine coastal benthic communities may adjust to ocean acidification. There are few experimental studies that explore the effects of elevated CO₂ levels on benthic ecosystems, although mesocosm

work has shown effects on primary production and the export of organic material (Riebesell et al. 2007) as well as nutrient flux in sediments (Widdicombe and Needham 2007), bioturbation (Dashfield et al. 2008) and community changes in tropical corals (Jokiel et al. 2008).

Although pH was variable during the study period, significant differences among stations were observed on averaged values, especially in the most acidified stations (S3 and N3). Such variability in pH values is due to variable local conditions at small and medium scale and in time, and it is a problem to face when working in situ. This variability is consistent with what has been observed in other short-medium-term measurements performed in the area, both in the acidified as well as in the intermediate stations (S2 and N2) (Hall-Spencer et al. 2008; Rodolfo-Metalpa et al. 2010b; Lombardi et al. unpublished data). The intermediate stations had higher fluctuations in pH values as they were located between areas with high venting activity and normal pH ones and show which organisms can tolerate highly variable pH and carbonate chemistry conditions.

After 1 month, our artificial collectors (scouring pads) hosted a relatively abundant and rich group of species, particularly at stations with normal pH, as indicated by the

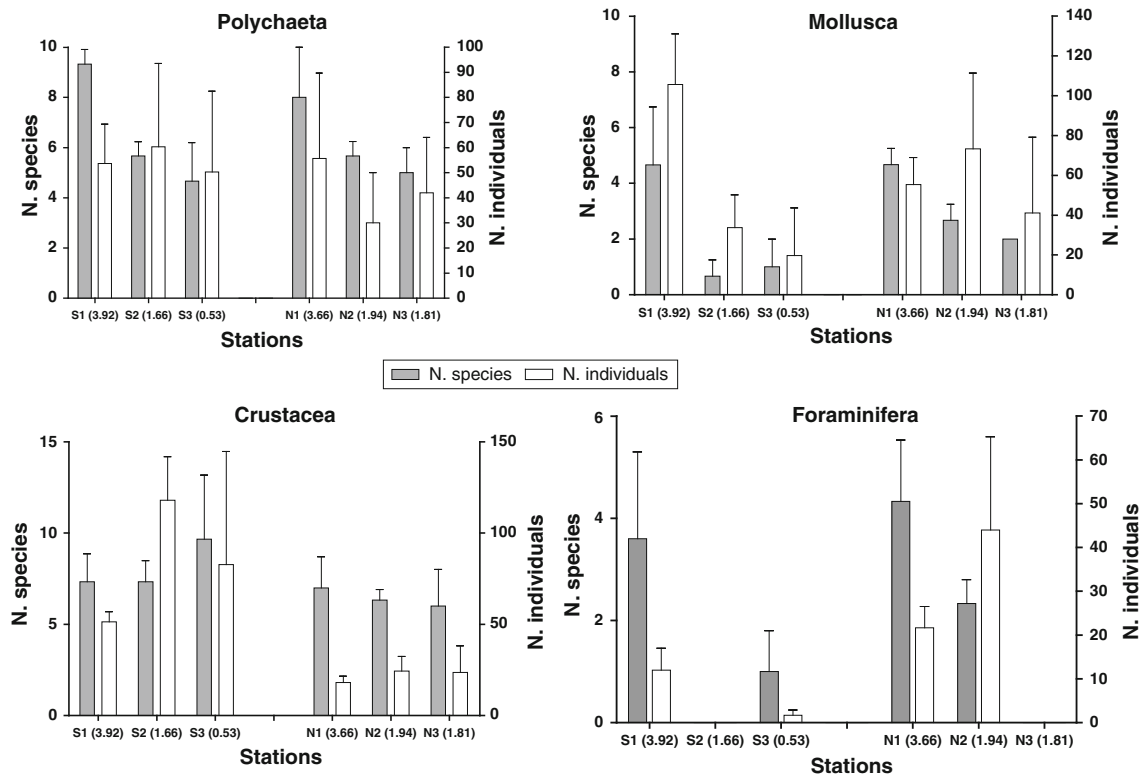


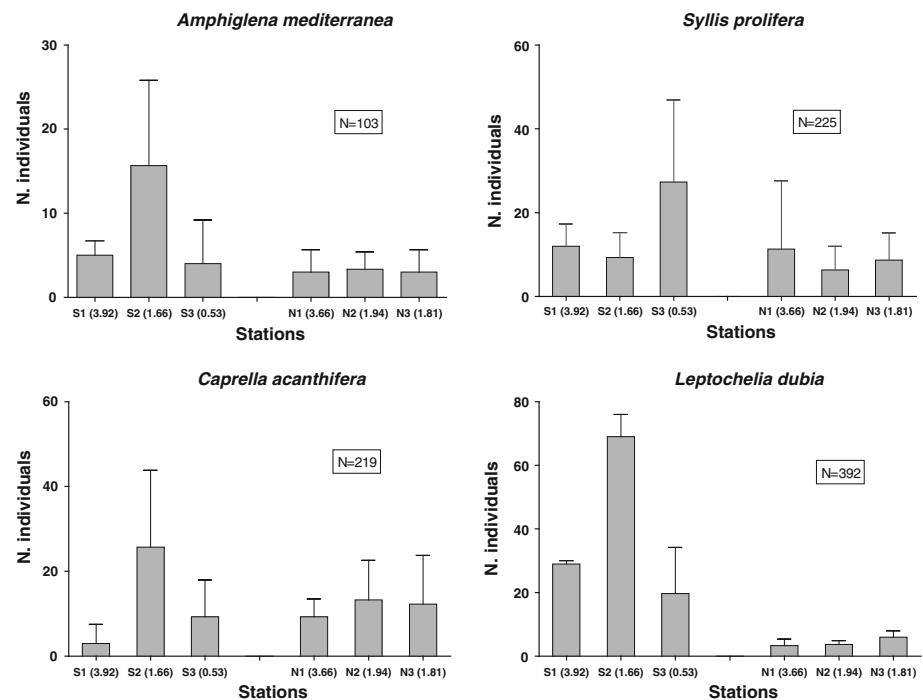
Fig. 3 Trend of the abundance (number of individuals) and number of species of the main higher taxa collected in the studied stations. In parentheses are mean values of $\Omega_{\text{aragonite}}$

values of diversity (H'), as well as by the total number of taxa/species recorded (79 and 64, respectively). Among the few abundant taxa, calcifiers were mainly restricted to the normal pH stations, such as most of the Foraminifera, *Neodexiospira pseudocorrugata* and *Spirobis marioni* (Spirorbidae), the gastropod *Osilinus turbinatus*, most of Bivalvia and the isopod *Cymodoce truncata*. Other species occurred all along the pH gradient, both on the north and south sides, such as the polychaetes *Exogone naidina*, *Amphiglena mediterranea* and *Platynereis dumerilii*, the gastropod *Rissoa variabilis* and the amphipod *Caprella acanthifera*. Finally, only the polychaete *Syllis prolifera* and the tanaid *Leptochelia dubia* showed higher abundances in some intermediate and low pH stations. On the whole, a clear decrease in species diversity is evident along the pH gradient on both sides of Castello Aragonese. This is mainly due to disappearance or strong reduction in the low pH stations of most of the calcifying organisms, such as the majority of Foraminifera, Bivalvia, many Gastropoda and all Spirorbidae polychaetes. This pattern seems clearly related to the negative effect that low pH exert on the calcareous structure of such organisms, as observed in the same area in the study of Hall-Spencer et al. (2008) with similar and other calcified groups. These organisms, which rely on the production of calcified tests or shells for survival, e.g. corals (Seibel and Fabry 2003), molluscs

(Lindinger et al. 1984; Michaelidis et al. 2005), crustaceans (deFur and McMahon, 1984) and calcified algal species (Riebesell et al. 2000), are therefore particularly vulnerable to lowering of the pH or its fluctuations. Recent studies have shown that the calcification rate within calcifying organisms decreases with increasing $p\text{CO}_2$, even in seawater supersaturated with respect to CaCO_3 (Gattuso et al. 1998; Riebesell et al. 1993, 2000; Thornton and Shirayama 2001; Bijma et al. 2002; Green et al. 2004; Kleypas et al. 2006; Gazeau et al. 2007; Rodolfo-Metalpa et al. 2010a). Other studies have shown that calcareous macroalgae (Ries et al. 2009), coccolithophores (Iglesias-Rodriguez et al. 2008), foraminiferans, echinoderms (Wood et al. 2008), molluscs (Ries et al. 2009), corals (Ries et al. 2009; Jury et al. 2009, Rodolfo-Metalpa et al. 2010a), bryozoans (Rodolfo-Metalpa et al. 2010b), and crustaceans (Ries et al. 2009) either increased or maintained the same levels of calcification under moderate elevation in $p\text{CO}_2$ (400–1,000 ppm $p\text{CO}_2$). Finally, a few calcareous organisms, such as the barnacle *Chthamalus stellatus*, may survive and grow at extremely low mean pH 6.6 as shown in our study area (Hall-Spencer et al. 2008).

At intermediate and low pH stations, the species present were in relatively high abundances perhaps indicating a decrease in species competition favouring the abundance of the few survivors or that the organism's survival is compatible

Fig. 4 Trend of abundance of some of the most abundant and relevant species collected in the studied stations. In parentheses are mean values of $\Omega_{\text{aragonite}}$



with elevated DIC and HCO_3^- levels that characterize the low pH zones.

The majority of the organisms settled on the scouring pads were juveniles or adult stages of small mesoherbivore species typically associated with *Posidonia oceanica* meadows and infralittoral macroalgal assemblages. Such habitats are very common around the study area and act as source for larvae, as well as juvenile and small adult dispersion stages, some of which were also bearing eggs (e.g. the polychaete *Exogone naidina*). This was particularly evident for peracarid crustaceans (amphipods, isopods, tanaids) which are active swimmers, and for gastropods, which together with peracarids also undergo strong daily migration in seagrasses and macroalgal habitats (Russo et al. 1984b; Lorenti and Scipione 1990; Cozzolino et al. 1992). It is likely that peracarids migrated to the collectors from the macroalgal and *Posidonia* habitats that surround the study area on both sides of the Castello. The active behaviour of peracarids and the pelagic habits of copepods and ostracods may explain why crustaceans were so evenly distributed in all stations at both sides, relative to other less mobile organisms (e.g. polychaetes).

Most of the species collected have been previously recorded on such vegetated habitats (shallow infralittoral macroalgae and *Posidonia* meadows) and are common around the island of Ischia (Gambi et al. 2003), as well as around the Castello at normal pH (Russo et al. 1984a; Scipione 1999). However, a few of the most abundant polychaete species, *Platynereis dumerilii*, *Syllis prolifera* and *Amphiglena mediterranea*, were previously observed

also on the intermediate and low pH zones (Gambi et al. 1997, 2000; Rouse and Gambi 1997). Their relatively high occurrence on the collectors is a confirmation of their ability to thrive in such variable, and low pH conditions.

There are no studies on the physiological effects of $p\text{CO}_2$ and pH variability on the species recorded in our study. From an ecological view point, *P. dumerilii* is particularly tolerant to organic pollution, being considered as a “polluted waters biological detector” (Bellan 1980).

Among the species listed for the area in the study of Hall-Spencer et al. (2008), only the gastropod *Osilinus turbinatus* was present also in our samples. This is surely due to the different methods of faunal collection among the two studies.

Reduction in the overall biodiversity of the invertebrates along with the reduction in pH is consistent also with data on epiphytes of *P. oceanica* leaves (Martin et al. 2008) and on macroalgae (Porzio et al. 2008) observed in the same area, building on evidence for biodiversity loss and altered community composition in waters acidified by CO_2 (Fabry et al. 2008; Barry et al. 2010).

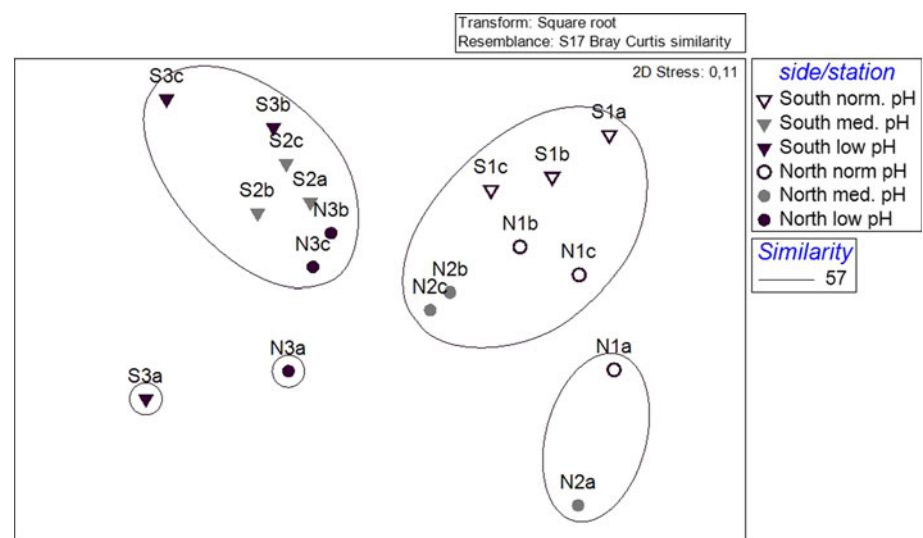
Vent systems are not perfect predictors of future ocean ecology owing to temporal variability in pH, spatial proximity of populations unaffected by acidification and the unknown effects of other global changes in parameters such as temperature, currents and sea level (Reibeseell 2008). However, such vents acidify sea water on sufficiently large spatial and temporal scales to integrate ecosystem processes such as production, competition and predation (Hall-Spencer et al. 2008). A further step in this study will be to test if the

Table 4 ANOVA pair-wise analyses among the stations of the main taxa and species

Taxa	Variable	Pairwise comparisons among stations					
		S1–S2	S1–S3	S2–S3	N1–N2	N1–N3	N2–N3
Foraminifera	Individuals	n.s.	n.s.	n.s.	*	*	***
	Species	**	*	n.s.	n.s.	***	*
Polychaeta	Individuals	**	***	n.s.	*	**	n.s.
	Species	**	n.s.	n.s.	***	***	n.s.
Gasteropoda	Individuals	**	***	n.s.	*	**	n.s.
	Species	**	**	n.s.	n.s.	n.s.	n.s.
Bivalvia	Individuals	**	**	n.s.	n.s.	n.s.	n.s.
	Individuals	*	n.s.	n.s.	n.s.	*	**
Amphipoda	Individuals	*	n.s.	n.s.	n.s.	n.s.	n.s.
	Species	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Tanaidacea	Individuals	***	n.s.	***	n.s.	n.s.	n.s.
Total Crustacea	Individuals	**	n.s.	n.s.	n.s.	n.s.	n.s.
	Species	n.s.	n.s.	n.s.	n.s.	*	n.s.
Abundant species							
<i>Syllis prolifera</i>	Individuals	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
<i>Amphiglena mediterranea</i>	Individuals	*	n.s.	*	n.s.	n.s.	n.s.
<i>Leptochelia dubia</i>	Individuals	***	n.s.	***	n.s.	n.s.	n.s.
<i>Caprella acanthifera</i>	Individuals	*	n.s.	n.s.	n.s.	n.s.	n.s.
	Total n. taxa	***	***	n.s.	*	***	n.s.
	Total n. individuals	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Diversity (H')	n.s.	n.s.	n.s.	*	***	n.s.
	Evenness (J')	n.s.	n.s.	n.s.	n.s.	*	n.s.

* $P < 0.05$; ** $P < 0.01$;*** $P < 0.001$;

n.s., Not significant

Fig. 5 nMDS ordination model (based on Bray-Curtis similarity) of the studied stations. The circles group sample points clustering together at Bray-Curtis similarity 57% and significant using the SIMPROF test ($P < 0.05$)

pattern observed in our samples is consistent also in other periods of the year and to look at individual species responses and adaptation to fluctuating and low pH conditions along the vents. Our understanding of the processes that underlie observed effects of ocean acidification on ecosystems and biogeochemistry is still rudimentary, as is our

ability to forecast its impacts. There is an urgent need therefore to develop tools to assess and quantify such impacts across the entire range of biological responses, from sub-cellular regulation to ecosystem reorganization and from short-term physiological acclimation to evolutionary adaptation.

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