

# Distribution and functional traits of polychaetes in a CO<sub>2</sub> vent system: winners and losers among closely related species

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**ABSTRACT:** We report on fine taxonomic and functional analyses of polychaetes associated with rocky reefs along a gradient of ocean acidification (OA) at the volcanic CO<sub>2</sub> vent system off the Castello Aragonese (Ischia Island, Italy). Percent cover of algae and sessile invertebrates (a determinant of polychaete distribution) was classified into functional groups to disentangle the direct effects of low pH on polychaete abundance from the indirect effects of pH on habitat and other species associations. A total of 6459 polychaete specimens belonging to 83 taxa were collected. Polychaete species richness and abundance dramatically dropped under the extreme low pH conditions due to the disappearance of both calcifying and non-calcifying species. Differences in distribution patterns indicate that the decreasing pH modified the structure and biological traits of polychaete assemblages independent of changes in habitat. A detailed taxonomic analysis highlighted species-specific responses to OA, with closely related species having opposing responses to decreasing pH. This resulted in an increase in the abundance of filter feeders and herbivores with decreasing pH, while sessile polychaetes disappeared in the extreme low pH zones, and were replaced by discretely motile forms. Reproductive traits of the polychaete assemblages changed as well, with brooding species dominating the most acidified zones. The few taxa that were abundant in extreme low pH conditions showed high tolerance to OA (e.g. *Amphiglena mediterranea*, *Syllis prolifera*, *Platynereis* cf. *dumerilii*, *Parafabricia mazzellae*, *Brifacia aragonensis*), and are promising models for further studies on the responses of benthic organisms to the effects of reduced pH.

**KEY WORDS:** Annelida · Algal cover · Ocean acidification · Hard bottoms · pH gradient · Covariation · Mediterranean Sea · Functional trait analysis

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## INTRODUCTION

Increased anthropogenic CO<sub>2</sub> emissions are predicted to be among the major drivers of global change in the coming century in both terrestrial and marine ecosystems (Gattuso & Buddemeier 2000, IPCC 2014). Large among-species variation in bio-

logical responses to CO<sub>2</sub>-induced ocean acidification (OA) is evident in the literature, but it is biased towards calcifying organisms (Ries et al. 2009, Kroeker et al. 2010, 2013a), with most available data derived from laboratory or mesocosm studies (Fabry et al. 2008, Feely et al. 2009, Kroeker et al. 2010, 2013a). However, recent studies performed in natu-

rally acidified areas (especially CO<sub>2</sub> vent systems) have led to increasing knowledge about population and community responses, as well as the identification of 'loser' and 'winner' organisms (Hall-Spencer et al. 2008, Vizzini et al. 2010, Fabricius et al. 2011, Kroeker et al. 2011, Boatta et al. 2013, Inoue et al. 2013, Baggini et al. 2014, Garrard et al. 2014); the latter being robust, able to thrive under high pCO<sub>2</sub> conditions, and occasionally even being more competitive under OA conditions (Kroeker et al. 2013a). Nevertheless, given the relatively limited number of naturally acidified systems available to study, field data on multispecies or assemblage responses to OA are still relatively scarce.

The natural volcanic CO<sub>2</sub> vents surrounding the rocky reefs of Castello Aragonese (Ischia Island, Italy, Mediterranean Sea) serve as a primary example of a naturally acidified marine ecosystem where variability in pH and pCO<sub>2</sub> is not confounded by other environmental factors such as salinity, temperature or oxygen content (Hall-Spencer et al. 2008, Kroeker et al. 2011). Venting is caused by a subterranean source of volcanic CO<sub>2</sub> and other trace gases (no sulphur; Tedesco 1996) that are bubbled into a shallow stretch of coast consisting of sloping, rocky reefs. Shallow rocky reef communities that are exposed to increased levels of acidification near the highest venting activity suffer natural temporal fluctuations (Kroeker et al. 2011). This variability in pCO<sub>2</sub> and carbonate chemistry allows for an examination of the ecosystem effects of decreasing pH—including the mean pH of 7.7 to 7.8 that is predicted for the end of this century (Caldeira & Wickett 2003).

Previous studies at the Ischia CO<sub>2</sub> vents found decreased abundance of conspicuous calcifying taxa in the most acidified zones, with different species responding similarly to OA (Martin et al. 2008, Kroeker et al. 2011, 2013b, Donnarumma et al. 2014). Macroalgae diversity decreased as well (Porzio et al. 2011), with the resulting communities dominated by filamentous and fleshy erect algae (such as *Dictyota* spp.) (Kroeker et al. 2013b,c). The abundance of calcified taxa dropped due to a decrease in their competitive ability (Kroeker et al. 2013b), while other species (including weak calcifiers) were able to thrive under extreme low pH conditions, such as amphipods (Kroeker et al. 2011, Scipione 2013) and polychaetes (Cigliano et al. 2010, Kroeker et al. 2011, Calosi et al. 2013, Ricevuto et al. 2012, 2014). Despite the high variability in species' responses, the diversity, biomass, and trophic complexity of rocky reef benthic marine communities generally decreases with acidification, although abundance is typically

not highly influenced. Detrimental effects are mainly observed as decreases in large mollusks and, as previously mentioned, other calcifying organisms such as serpulid polychaetes. In turn, the increased abundance of small bodied, non-calcifying invertebrates, including other polychaetes, results in constant invertebrate densities, which partly buffer the general decrease in assemblage biomass (i.e. community compensation) (Kroeker et al. 2011).

Polychaetes are often used as indicators of taxonomic richness, as well as suitable surrogates depicting community patterns in the marine benthos (Fresi et al. 1984, Sparks-McConkey & Watling 2001, Ols-gard et al. 2003, Van Hoey et al. 2004, Giangrande et al. 2005). The multi-faceted response of polychaetes to environmental disturbance are probably a consequence of their high morpho-functional diversity (Wilson 1991, Giangrande 1997, Jumars et al. 2015), which allows different species to occupy a gradient from pristine to disturbed habitats (see Giangrande et al. 2005 and references therein). The degree of tolerance/sensitivity to environmental disturbance or stress appears to be highly species-specific, since congeneric species can show opposite responses to the same source of environmental stress (Musco et al. 2009). These marine worms are abundant on rocky reefs (e.g. Musco 2012 and references therein), with variation in their distributions determined by substrate type as well as the quality and quantity of algal and sessile invertebrate cover (e.g. Dorgham et al. 2014). Macroalgae and other sessile invertebrates influence the diversity and abundance patterns of the associated zoobenthic communities by structuring rocky reef habitats at small scales (Jacobi & Langevin 1996, Parker et al. 2001, Chemello & Milazzo 2002) and polychaete assemblages (Abbiati et al. 1987, Giangrande 1988, Sardá 1991, Tena et al. 2000, Frascchetti et al. 2002, Giangrande et al. 2003, Antoniadou et al. 2004, Serrano et al. 2006, Dorgham et al. 2014).

Previous analyses of polychaete distributions at the Ischia CO<sub>2</sub> vents highlighted differences in tolerance among species. Polychaetes in the family Fabriciidae were highly abundant in at very low pH areas, suggesting some tolerance to OA (Giangrande et al. 2014). Polychaetes were also present in artificial settlement collectors placed along the pH gradient during different periods of the year, including at the lowest pH conditions (Cigliano et al. 2010, Ricevuto et al. 2014), suggesting some species may be robust and resilient to OA due to their high physiological plasticity and local adaptation (Calosi et al. 2013). As a whole, polychaetes appear to have a relatively simple re-

response to OA compared to their multi-faceted responses to other stressors (e.g. Giangrande et al. 2005, Musco et al. 2009). In fact, decreasing pH seems to impair calcifying taxa such as Serpulinae and Spirorbinae (Donnarumma et al. 2014), while non-calcifying taxa appear to be relatively tolerant and able to thrive under OA conditions (Ricevuto et al. 2014). However, studies focusing on the reproduction and physiology of particular polychaete species suggest that their response to OA might not be that obvious. In fact, polychaetes species that demonstrate parental care appear to be more protected under OA compared to their free spawning relatives (Lucey et al. 2015). Moreover, some non-calcifying species appear tolerant when exposed to OA, whilst others show evident physiological stress (Calosi et al. 2013). These different individual responses of apparently related polychaete species suggest that the effects of OA on natural assemblages might be more complex than expected. Given the importance of algae and sessile macrobenthos for polychaete ecology (Giangrande 1988, Giangrande et al. 2003, Antoniadou & Chintiroglou 2006, Dorgham et al. 2014), understanding how polychaete assemblages vary with other important habitat-forming species along a decreasing pH gradient is crucial to disentangle the direct effects of OA from indirect effects via habitat modification.

The present work complements previous analyses of rocky reefs benthic assemblages performed by Kroeker et al. (2011) around the Ischia CO<sub>2</sub> vent system. We used species-level taxonomic resolution to study the polychaete assemblages associated with these vents, which allowed us to examine variation in species' responses and identify 'winners' and 'losers'. In addition, the complexity of the rocky reef habitat in which polychaetes live requires us to account for potential covariation with algae and sessile invertebrates. To address important species associations, this work includes an analysis of the main functional and ecological traits of the polychaete assemblages along the OA gradients. Biological functional traits, including reproductive and trophic ones, are often species-specific (Giangrande 1997, 2000, Jumars et al. 2015); therefore, scoring taxa using fine taxonomy allows for a more precise view of functional changes occurring at the assem-

blage level. Moreover, analysis at coarser taxonomic levels has often proved to be inadequate in explaining patterns of assemblage distributions, especially on hard bottom environments (Musco et al. 2009, 2011).

## MATERIALS AND METHODS

### Study area and data collection

The sub-marine CO<sub>2</sub> vents system at the Castello Aragonese off Ischia Island (Tyrrhenian Sea, Italy) has been intensively studied in recent years (Hall-Spencer et al. 2008). To date, it is one of the best-known naturally acidified systems in the world (Gambi 2014). The vents occur between 0.5 and 3.0 m depth, directly adjacent to steep rocky reefs located on the north and south sides of the small Castello islet. According to Kroeker et al. (2011), 3 pH zones can be identified along a continuous 150 m stretch of rocky reef on both sides of the islet (Fig. 1), moving from areas of high vent activity (>10 bubble columns m<sup>-2</sup>; defined as extreme low pH), to moderate vent activity (<10 bubble columns m<sup>-2</sup>; low pH), to no visible vent activity (ambient pH). Each zone is ca. 20 m in length and is separated from the next zone by at least 20 to 25 m.

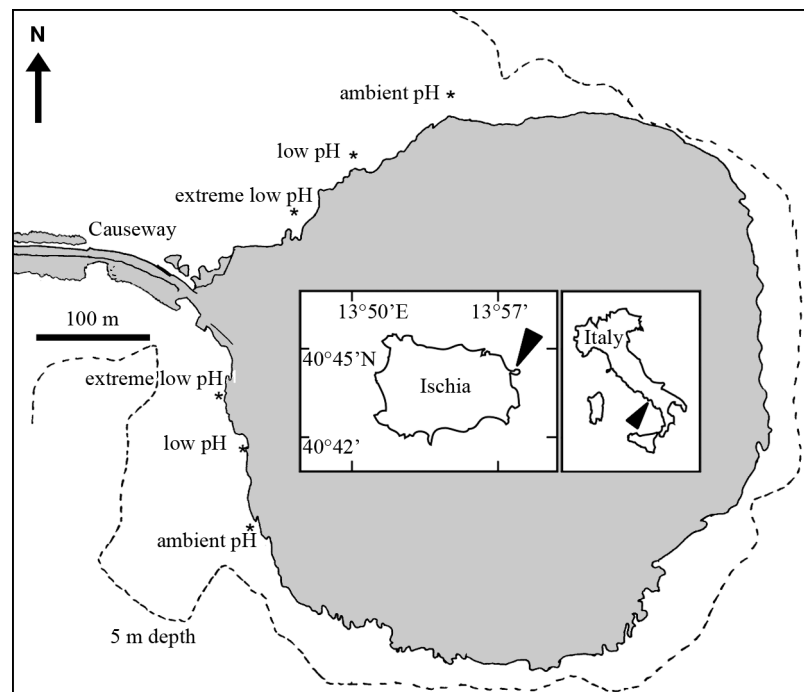


Fig. 1. Study area of Castello Aragonese (Island of Ischia, Italy, Tyrrhenian Sea), where the vent system is located, with indication of the pH zones on the north and south sides of the islet

We recorded pH and temperature hourly *in situ* at these 3 zones with a modified Honeywell Durafet pH sensor, for consecutive periods of 1 to 4 wk. Discrete water samples were collected during pH-meter deployment near the instrument to measure total alkalinity (TA) and dissolved inorganic carbon (DIC), and to calibrate the sensors. On the north side of the islet, mean ( $\pm$ SD) pH was  $8.0 \pm 0.1$  in ambient pH,  $7.8 \pm 0.2$  in low pH and  $7.2 \pm 0.4$  in very low pH zones; mean  $p\text{CO}_2$  was  $567 \pm 100$ ,  $1075 \pm 943$  and  $6558 \pm 21347 \mu\text{atm}$ ; mean TA was  $2563 \pm 3$ ,  $2560 \pm 7$  and  $2559 \pm 13 \mu\text{mol kg}^{-1}$ ; mean DIC was  $1768 \pm 96$ ,  $2395 \pm 66$  and  $2579 \pm 207 \mu\text{mol kg}^{-1}$ . On the south side, mean pH was  $8.1 \pm 0.1$ ,  $7.8 \pm 0.3$  and  $6.6 \pm 0.5$  in ambient, low and very low pH zones, respectively; mean  $p\text{CO}_2$  was  $440 \pm 192$ ,  $1581 \pm 2711$  and  $23989 \pm 16638 \mu\text{atm}$ ; mean TA was  $2563 \pm 2$ ,  $2560 \pm 7$  and  $2563 \pm 13 \mu\text{mol kg}^{-1}$ ; and mean DIC was  $2281 \pm 73$ ,  $2318 \pm 99$  and  $3849 \pm 790 \mu\text{mol kg}^{-1}$  (see Hofmann et al. 2011, Kroeker et al. 2011 for details).

Benthic invertebrates were collected in November 2011 from 400 cm<sup>2</sup> plots (20 × 20 cm quadrats) haphazardly distributed within each pH zone ( $n = 4 \text{ zone}^{-1}$ ). In each pH zone, 2 plots were set out within the first 5 m and 2 within the last 5 m, with each plot being at least 3 m from the paired one. Plots were deployed at 1 to 1.5 m depth on continuous rocky substrate with slopes ranging from 50 to 85°. Motile invertebrates (<5 cm) were collected using an airlift suction sampler with a fine mesh bag (150  $\mu\text{m}$  mesh size) that operated over the 20 × 20 cm plot for 30 s. The remaining benthos was scraped off the substrate with a hammer and chisel. Samples were fixed with 4% buffered formalin for 24 h and then rinsed and preserved in 70% ethanol. A dissecting microscope

was used to separate the invertebrates from the algae and sessile benthos. Polychaetes were identified to the lowest possible taxonomic level (typically species; see Supplement 1 at [www.int-res.com/articles/suppl/m550p121\\_supp.pdf](http://www.int-res.com/articles/suppl/m550p121_supp.pdf)) and counted.

Based on the most recent literature (Wilson 1991, Giangrande 1997, 2000, Jumars et al. 2015) and personal observations by polychaete specialists (M.C.G., L.M., A.G., F.B.), each polychaete taxon was assigned to a morpho-functional category accounting for relevant biological and ecological traits. In particular, we considered feeding behavior (5 categories: carnivore, herbivore, omnivore, detritus/deposit-feeder, suspension feeder; following Giangrande et al. 2000, Jumars et al. 2015), motility (4 categories: motile, discretely motile, motile/discretely motile, sessile; following Jumars et al. 2015); structural features, including size (3 length categories: <5 mm [interstitial], 5–20 mm, and >20 mm), tube features (3 categories: calcareous tube [serpulids], other tube [including both mucous and membranaceous], and no tube). Reproductive strategy traits, including development mode, were based on relevant literature (Wilson 1991, Giangrande 1997, Lucey et al. 2015) as well as original descriptions, taxonomic reviews, and personal observations (2 categories for development: direct or indirect; 2 categories for embryo protection: broadcaster or brooder).

Following Kroeker et al. (2013b), we estimated the percent cover of 9 key functional macrobenthic groups (algae and sessile invertebrates) from photos of the original rocky plots. Morpho-functional categories included biofilm and filamentous algae (BFA), fleshy turf algae (FTA), calcareous turf algae (CTA), erect fleshy algae (EFA), erect calcareous algae (ECA), calcified filter feeders (CFF; mainly barnacles of the genus *Balanus*), encrusting fleshy algae (EnFA), encrusting calcareous algae (EnCA) (including crustose coralline algae), and sponges (Spo) (see Supplement 2).

Table 1. Distance-based multivariate analysis for a linear model (DISTLM) analysis. Multivariate regression was based on Bray-Curtis dissimilarities, with covers of morpho-functional categories of the sessile macrobenthos as predictor variables of the polychaete distribution in a linear regression model with tests by permutation (9999). Results are of the marginal tests (i.e. fitting each variable one at a time). Polychaete abundance data were square root transformed. Significant p-values in **bold**. Prop: proportion of explained variation

Variable	SS (trace)	Pseudo- <i>F</i>	p	Prop.
CTA (Calcareous turf algae)	1460.5	0.7429	0.7053	0.0327
CFF (Calcified filter feeders)	1386.4	0.7040	0.7428	0.0310
EnCA (Encrusting calcareous algae)	3402.2	1.8119	0.0714	0.0761
EnFA (Encrusting fleshy algae)	1306.6	0.6622	0.7567	0.0292
ECA (Erect calcareous algae)	2043.0	1.0534	0.3220	0.0457
BFA (Biofilm-filamentous algae)	1988.3	1.0238	0.3753	0.0445
FTA (Fleshy turf algae)	7025.7	4.1013	<b>0.0011</b>	0.1571
Spo (Sponges)	1993.9	1.0268	0.3744	0.0446
EFA (Erect fleshy algae)	6713.4	3.8868	<b>0.0015</b>	0.1501

### Experimental design and data analyses

Variations in the structure of assemblages and the abundance of species were analyzed in a model consisting of 2 orthogonal factors: 'side' (2 fixed levels: north and south side of the Castello islet) and 'pH' (3 fixed levels: ambient, low, and extreme low pH), which

allowed us to analyze variation in assemblage distributions under different pH conditions on both sides of the pH gradient, as well as their interaction effects (side  $\times$  pH) (Kroeker et al. 2011). Multivariate analysis of co-variance MANCOVA, based on Bray-Curtis similarity, was used to test for differences in polychaete assemblages and their functional/ecological traits among pH zones and between sides. Macroalgae and sessile invertebrate cover were used as covariates in the analyses to account for the potential indirect effects of changes in habitat-forming species along the pH gradient. The variation in number of individuals of the most abundant species (i.e. those species comprising the 1<sup>st</sup> percentile of total polychaete abundance) was tested by analysis of co-variance (ANCOVA) based on Euclidean dissimilarity distance, with the percent cover of macrobenthic functional groups as covariates. Distance-based multivariate analysis for a linear model (DISTLM) was used to select the covariates for MANCOVAs and ANCOVAs. In particular, by DISTLM marginal tests we selected those macrobenthic functional groups (% cover) significantly explaining part of the variation of the assemblage and the abundance of species. Each term in the MANCOVA and ANCOVA was tested using 9999 random permutations of the appropriate units (Anderson & ter Braak 2003). Outputs of MANOVAs and ANOVAs were used to verify the effectiveness of the above-mentioned MANCOVAs and ANCOVAs, with respect to disentangling the effects of habitat formers on assemblages/species distributions from those of side and pH gradient.

A constrained analysis of principal coordinates (CAP; Anderson & Willis 2003) was used to assess the relative influence of pH on the distribution patterns of polychaete assemblages, and correlations of polychaete taxa and functional macrobenthic groups with the canonical axes.

All analyses were performed using PRIMER v.6 software, including the add-on package PERMANOVA+ (Anderson et al. 2008).

## RESULTS

We collected a total of 6459 polychaete specimens belonging to 83 taxa (most identified to species; see Supplement 1 at [www.int-res.com/articles/suppl/m550p121\\_supp.pdf](http://www.int-res.com/articles/suppl/m550p121_supp.pdf)). Fifteen taxa accounted for 89% of the collected individuals. *Amphiglena mediterranea* (Sabellidae) represented ~55% of all individuals, followed by the Syllidae species *Haplosyllis granulosa*, *Syllis prolifera*, *S. gerlachi* and *Exogone dispar*,

and the Nereididae *Platynereis* cf. *dumerilii*. The Syllidae included the highest number of taxa (36), while the Fabriciidae (usually relatively rare on Mediterranean rocky reefs) were unusually speciose and abundant. We identified 6 species of Fabriciidae, including the recently described *Parafabricia mazzellae* and *Brifacia aragonensis* (Giangrande et al. 2014).

Both number of taxa and number of individuals were lowest in the extreme low pH zones, especially on the north side, while the highest number of species and individuals occurred in the ambient pH zone on the south side (Fig. 2). The number of taxa decreased from 73 (ambient zones) and 56 (low pH zones), with 44 taxa in common, to only 23 taxa in the extreme low pH zones (22 occurring along the whole gradient, and a single taxon in common between extreme low and low pH samples) (Supplement 1). The richness of taxa was reduced by 68% in the most acidified areas, and no species were limited to the extreme low pH zones. The species most tolerant to low pH were members of Nereididae, Syllidae,

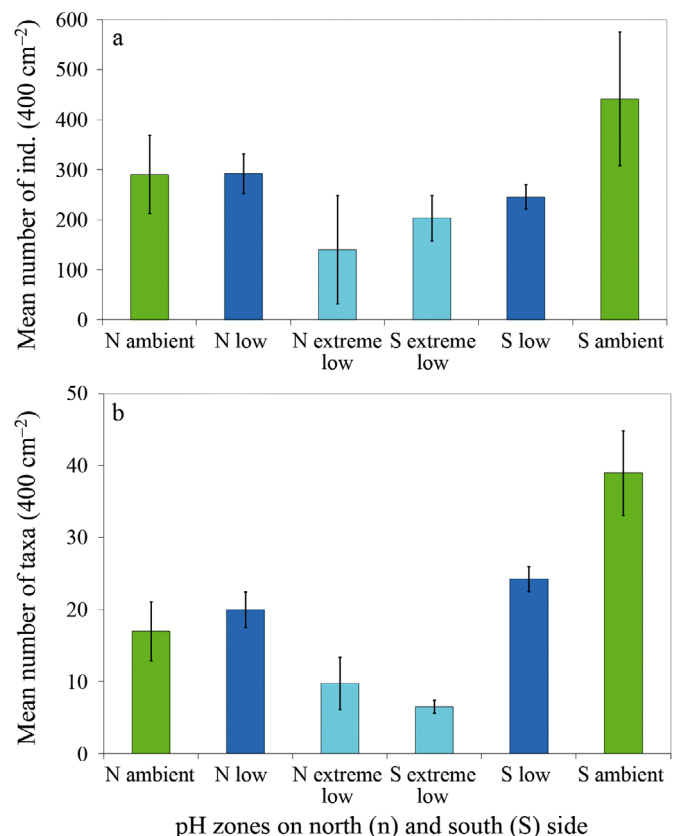


Fig. 2. (a) Abundance as mean ( $\pm$ SE) number of individuals and (b) diversity as mean ( $\pm$ SE) number of taxa of polychaetes in the 3 pH zones (ambient, low, extreme low) along the southern (S) and northern (N) sides of the vent system

Sabellidae and Fabriciidae. Even the most tolerant species had limited abundance in low pH conditions, except for *P. cf. dumerilii*, *A. mediterranea*, *P. mazzellae*, and *S. prolifera*, which were highly abundant. The 6 species of the calcareous tubicolous Serpulidae that were abundant in the ambient zones were not observed in the extremely low pH areas.

Among the 9 functional categories of macroalgae and sessile invertebrates (see Supplement 2), only FTA and EFA explained part (15.7 and 15.0%, respectively) of the observed variation in polychaete assemblages (Table 2). FTA percent cover was positively correlated with the number of polychaete taxa ( $R^2 = 0.48$ ;  $p = 0.001$ , while the percent cover of EFA was negatively correlated with the number of polychaete taxa ( $R^2 = 0.31$ ;  $p = 0.006$ ). There was a positive trend in the percent cover of FTA and the total number of individuals ( $R^2 = 0.15$ ;  $p = 0.07$ ) and a negative trend in the percent cover of EFA and the total number of individuals ( $R^2 = 0.08$ ;  $p = 0.175$ ).

Significant differences in polychaete assemblages between sides, as well as among pH zones were observed when the FTA and EFA functional groups were included as covariates. These results indicate that changes in pH modified the structure of polychaete assemblages independent of both the side and the changes in the associated habitat-forming taxa (MANCOVA; Table 2). The preliminary MANOVA performed on the same dataset (i.e. MANOVA of polychaete assemblages not considering FTA and EFA as covariates) revealed a significant interaction between side and pH zone (pseudo- $F_{2,18} = 2.1117$ ;  $p < 0.01$ ).

The polychaetes collected in extreme low, low and ambient pH conditions were clearly separated in the CAP plot, with those collected in extreme low pH being the most distinct (Fig. 3; allocation success 87.5%). The 2 axes had high canonical correlations with the polychaete assemblages (CAP1- $\delta^2 = 0.8984$ ; CAP2- $\delta^2 = 0.6711$ ), supporting the separation of 3 groups in the bi-plot (Fig. 3). Several polychaete taxa were correlated with the canonical axes (Pearson correlation, 0.3 cut-off), with the largest number of species either characterizing the ambient zone (e.g. the serpulids *Serpula vermicularis* and *Hydroides pseudouncinatus*) or being in an intermediate position between ambient and low pH (Fig. 3a). Only *Salvatoria clavata* and *P. cf. dumerilii* characterized the intermediate position between low and extreme low pH, while only *B. aragonensis* characterized the extreme low pH zone. After overlaying the functional categories of macroalgae and sessile invertebrates on the same CAP plot (Fig. 3b), FTA and EFA showed the highest correlation to the canonical axes (Pearson

Table 2. Two-way MANCOVAs testing for differences in the multivariate polychaete assemblages with selected algal covers (FTA: fleshy turf algae; EFA: erect fleshy algae) introduced in the model as co-variables. Analysis was based on Bray-Curtis similarity, data were fourth root transformed, with 9999 permutations. MS: mean square; df: degrees of freedom. pH: areas at very low, low, and ambient pH; Si: side; Res: residuals. Significant p-values are in **bold**

Source	df	MS	Pseudo- <i>F</i>	p(perm)	Unique perms
FTA	1	7025.7	5.663	<b>0.0001</b>	9930
EFA	1	2934.7	2.366	<b>0.0114</b>	9914
pH	2	4591.8	3.701	<b>0.0001</b>	9901
Si	1	2499.6	2.015	<b>0.0368</b>	9915
pH×Si	2	1609.9	1.298	0.1661	9913
Res	16	1240.6			

correlation, 0.4 cut-off), with EFA associated with the very low pH polychaete assemblages and FTA associated with the ambient pH assemblages (Fig. 3b).

Among the 15 most abundant species (>1% total abundance), there were varying and complex responses to OA. Six species, including the Syllidae *S. gerlachi*, *H. granulosa*, *E. dispar* and *Exogone naidina* and the Serpulidae *Spirobranchus polytrema* and *S. vermicularis* showed significant differences in distribution along the pH gradient, being more abundant in ambient pH samples (ANCOVA; Table 3). In contrast, we did not detect any differences in abundance among pH zones for 8 species, including the dominant *A. mediterranea*, 2 Fabriciidae (*Fabricia stellaris* and *P. mazzellae*), 2 Syllidae (*S. prolifera* and *Brania pusilla*), 1 Nereididae (*Perinereis cultrifera*) and 1 Opheliidae (*Polyophthalmus pictus*). Finally, in the case of *Nereis zonata* (Nereididae), the abundance varied among pH zones depending on the side, without a clear pattern (i.e. significant side × pH). Preliminary ANOVAs performed on the same datasets (i.e. ANOVA of species abundances not considering covariates) revealed an interaction between side and pH for the analysis of *S. polytrema* (pseudo- $F_{2,18} = 3.5181$ ;  $p < 0.05$ ), *S. gerlachi* (pseudo- $F_{2,18} = 5.1002$ ;  $p < 0.05$ ), *E. naidina* (pseudo- $F_{2,18} = 9.7845$ ;  $p < 0.01$ ), *P. pictus* (pseudo- $F_{2,18} = 7.5243$ ;  $p < 0.01$ ), *B. pusilla* (pseudo- $F_{2,18} = 5.0585$ ;  $p < 0.05$ ).

Analyses of the polychaete functional/ecological traits testing for differences in the distribution patterns of selected morpho-functional categories (MANCOVAs) revealed different responses depending on the typology of the considered trait (ecological, structural, reproductive) (Fig. 4, Table 4). DISTLM analyses performed for each of the functional/ecological traits revealed that only 1 or 2 of the 9 functional cat-

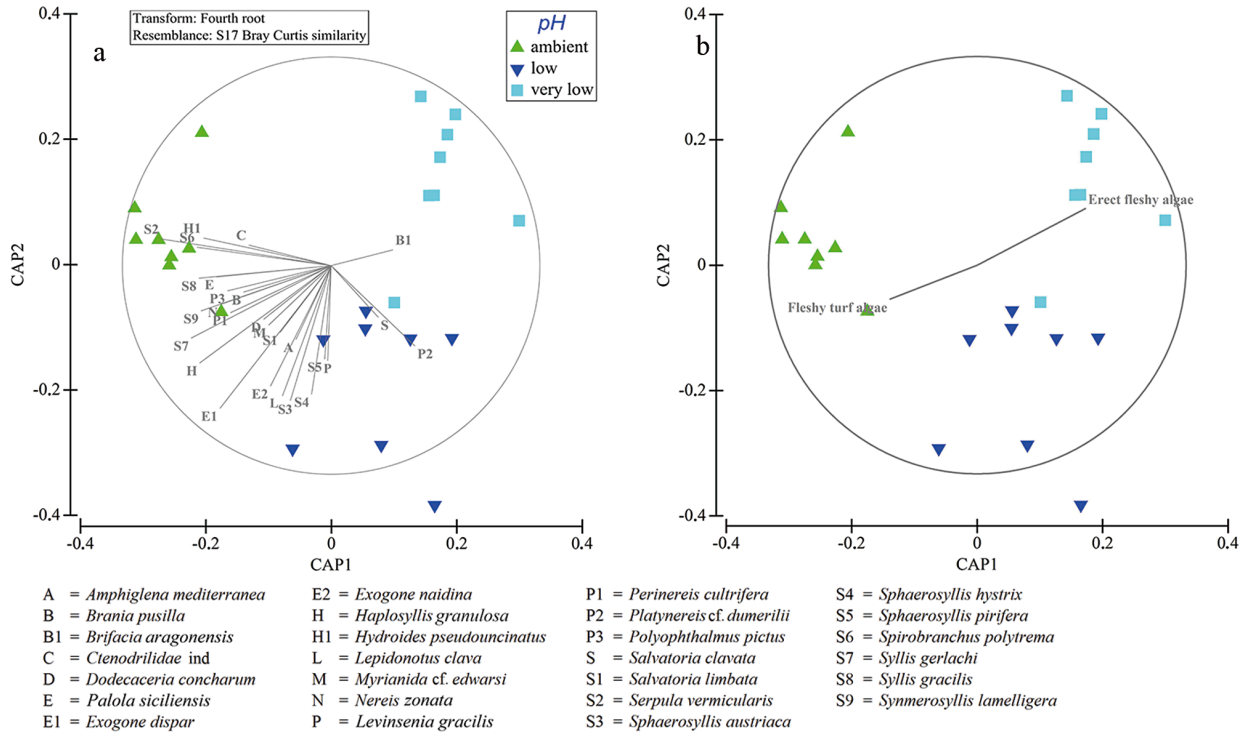


Fig. 3. Canonical analysis of the principal coordinates (CAP) plot showing canonical axes that best discriminate polychaete assemblages according to the 3 pH zones and correlations of (a) original polychaete taxa and (b) functional macrobenthic groups (habitat formers)

egories of macroalgae and sessile invertebrates explained variation in the polychaete assemblages (data not shown). In particular, percent cover of FTA explained variation in all polychaete functional traits (feeding behavior, motility, embryo protection, de-

velopment, worm size, tube features) and was thus used as a covariate in the successive MANCOVAs (Table 4). The percent cover of ECA explained variation in polychaete size categories, and was used as a covariate with FTA in the MANCOVA (Table 4e).

Table 3. Two-way ANCOVA outcomes testing for differences in abundance (no. of ind.) of the most abundant polychaete species (cut-off: 1% of total abundance) with algal covers (selected by distance-based multivariate analysis for a linear model [DISTLM] marginal tests) introduced in the model as co-variables. Analyses based on Euclidean distance, data fourth root transformed, 9999 permutations. Significant *F*-values are given in italics, significant *F*-values of ecological interest are given in **bold italics**. \**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.001. See Table 1 for co-variable abbreviations

Family	Species	Total abundance	Co-variables	Side × pH	Side	pH
Sabellidae	<i>Amphiglena mediterranea</i>	3543	EnCA	0.9857	2.3744	0.7376
Syllidae	<i>Syllis gerlachi</i>	346	FTA, EFA	2.1613	0.0012	<b>3.594*</b>
Syllidae	<i>Exogone dispar</i>	313	FTA, EFA	1.4579	0.0208	<b>9.7581**</b>
Syllidae	<i>Haplosyllis granulosa</i>	241	FTA, EFA	1.0573	<b>10.905**</b>	<b>4.2406*</b>
Syllidae	<i>Syllis prolifera</i>	216	EnCA	1.2613	2.8344	0.3664
Nereididae	<i>Platynereis cf. dumerilii</i>	202	ECA, Spo	0.3780	2.9317	0.9586
Nereididae	<i>Nereis zonata</i>	189	FTA	<b>11.917***</b>	<b>7.6709**</b>	<b>4.4578*</b>
Syllidae	<i>Exogone naidina</i>	142	FTA, EFA	3.2061	0.7217	<b>4.5567*</b>
Fabriciidae	<i>Parafabricia mazzellae</i>	110	-	0.0613	3.7165	0.5106
Opheliidae	<i>Polyopthalmus pictus</i>	93	FTA, EFA	2.0463	0.2524	1.1770
Serpulidae	<i>Spirobranchus polytrema</i>	78	FTA	1.6803	1.4739	<b>4.9304*</b>
Syllidae	<i>Brania pusilla</i>	77	FTA, EFA	0.1029	1.0116	0.3144
Fabriciidae	<i>Fabricia stellaris</i>	72	EnCA	0.2928	2.8916	0.0135
Serpulidae	<i>Serpula vermicularis</i>	72	FTA, EFA	1.3495	1.3507	<b>13.716***</b>
Nereididae	<i>Perinereis cultrifera</i>	66	FTA	1.2404	0.3580	2.4182

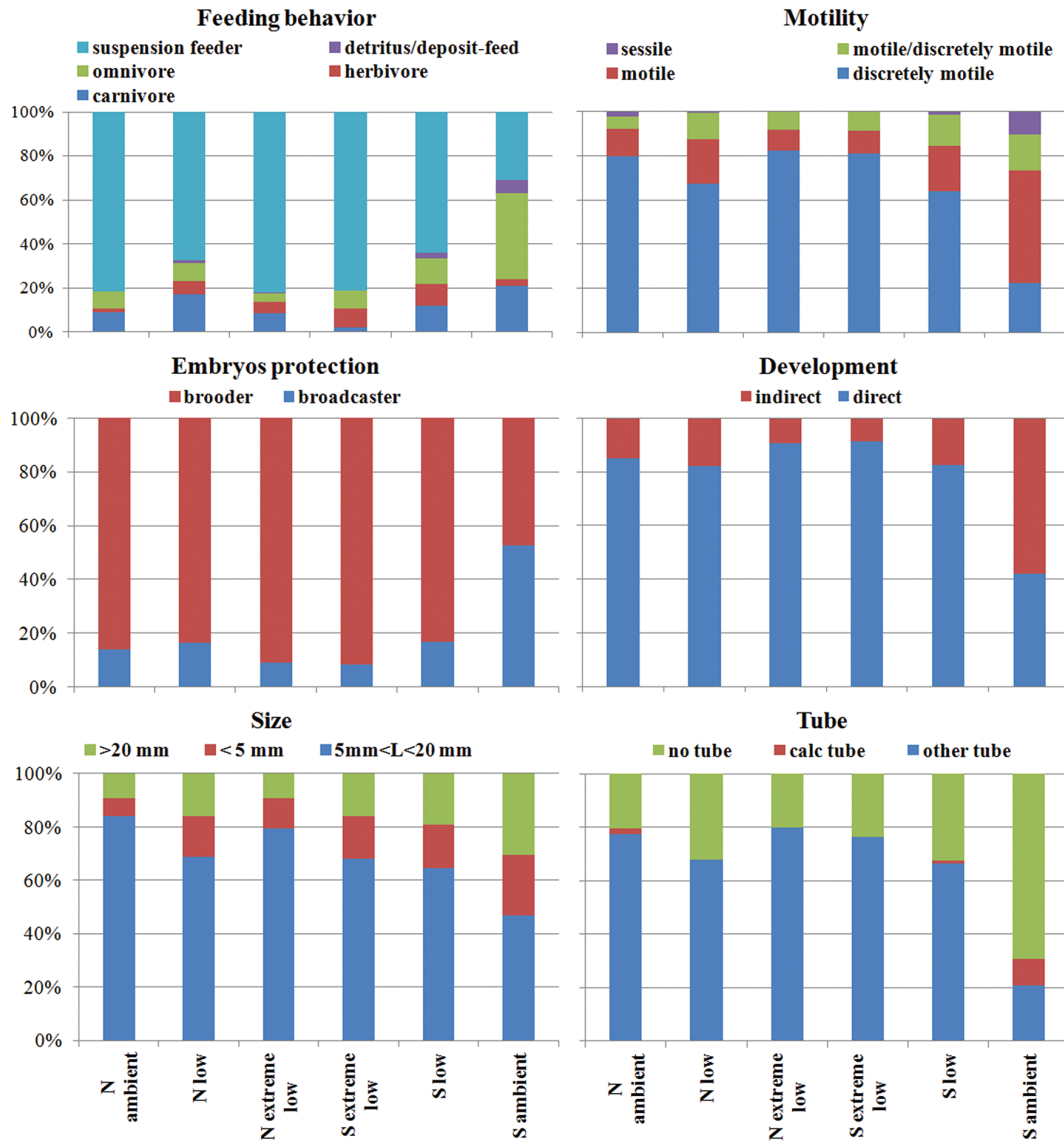


Fig. 4. Polychaete functional/ecological traits. Percentage of individuals distributed among the different categories of feeding behavior, motility, embryo protection, development, size and tube features in the 3 pH zones (ambient, low, extreme low) along the southern (S) and northern (N) sides of the Castello vent system

### Polychaete ecological traits

Our results illustrate how pH modified both the trophic structure of polychaete assemblages and the pattern of motility independently from both the side of the islet and the variation attributed to changes in habitat-forming species (FTA) in the study area (Table 4a,b). Notwithstanding differences between the north and south sides of the study area, the general trend indicated that the percentage of filter feeders and herbivores tended to increase with decreasing pH, whilst detritus/deposit feeders, omnivores and carnivores showed an opposite trend (Fig. 4). The percentage of discretely motile individuals

increased with decreasing pH in comparison to the other motility categories which showed an opposite trend—particularly the sessile polychaetes, which disappeared in the very low pH zones (Fig. 4).

### Polychaete reproductive traits

The 2 MANCOVAs revealed no difference between sides and significant changes in reproductive traits among pH zones both for embryo protection (Table 4c) and development strategies (Table 4d). These results indicate that decreased pH modified the reproductive features of the polychaete assemblages inde-



Table 4. Two-way MANCOVAs testing for differences in morpho-functional categories of polychaetes with selected algal covers (FTA: fleshy turf algae; ECA: erect calcareous algae) introduced in the model as co-variables. (a) Feeding categories, (b) motility, (c) embryo protection, (d) development, (e) size, (f) tube features. Analysis based on Bray-Curtis similarity, percentages of the categories transformed by the formula  $\arcsin[\sqrt{(\%/100)}]$ , 9999 permutations. MS: mean square; df: degrees of freedom; pH: areas at very low, low, and ambient pH; Si: side; Res: residuals. Significant p-values are given in **bold**

		df	MS	Pseudo- <i>F</i>	p(perm)	Unique perms
(a)	FTA	1	2742.1	13.050	<b>0.0003</b>	9963
	pH	2	942.6	4.486	<b>0.0080</b>	9948
	Si	1	862.5	4.105	<b>0.0403</b>	9964
	pH×Si	2	371.1	1.766	0.1705	9948
	Res	17	210.1			
(b)	FTA	1	2908.8	16.925	<b>0.0002</b>	9954
	pH	2	948.7	5.520	<b>0.0062</b>	9951
	Si	1	1080.4	6.286	<b>0.0159</b>	9954
	pH×Si	2	406.3	2.364	0.1082	9962
	Res	17	171.9			
(c)	FTA	1	1941.6	19.400	<b>0.0010</b>	9859
	pH	2	501.0	5.005	<b>0.0210</b>	9949
	Si	1	274.5	2.743	0.1151	9815
	pH×Si	2	250.3	2.500	0.1107	9954
	Res	17	100.1			
(d)	FTA	1	2127.0	20.271	<b>0.0006</b>	9832
	pH	2	547.1	5.215	<b>0.0180</b>	9956
	Si	1	319.4	3.044	0.1046	9833
	pH×Si	2	322.2	3.071	0.0685	9949
	Res	17	104.9			
(e)	ECA	1	913.1	6.284	<b>0.0225</b>	9945
	FTA	1	393.3	2.706	0.0963	9964
	pH	2	125.8	0.866	0.4854	9939
	Si	1	202.3	1.392	0.2737	9959
	pH×Si	2	188.6	1.298	0.2982	9958
	Res	16	145.3			
(f)	FTA	1	3074.5	21.035	<b>0.0003</b>	9948
	pH	2	355.2	2.430	0.1041	9959
	Si	1	329.2	2.252	0.1546	9943
	pH×Si	2	505.8	3.461	0.0643	9947
	Res	17	146.2			

pendent of variation in the associated habitat former (FTA). There was a clear increase of the percentage of brooders compared to broadcasters with decreasing pH, with an increase of polychaetes characterized by direct development compared to forms producing early stage larvae (Fig. 4).

### Polychaete structural traits

We detected no difference in size (body length) (Table 4e) or tube features (Table 4f) among pH zones, although size co-varied with ECA (this last

factor being poorly represented or absent in low pH/very low pH zones; see Supplement 2) and with FTA in the case of tube features. Visual differences among the percentages of categories roughly indicating an increase in polychaetes of intermediate size (between 5 and 20 mm in length), and an increase of polychaetes building membranaceous/mucous tubes (Fig. 4) are not large enough to be considered direct effects of OA. However, the ineffectiveness of the MANCOVAs in disentangling the direct effects of OA from indirect effects due to side and variation of habitat formers cannot be excluded.

All preliminary MANOVAs performed to test differences in polychaete structural, ecological and reproductive traits, not considering the effect of covariables, revealed the significance of the side × pH interaction term (data not shown).

## DISCUSSION

The results of this paper contribute to an improved understanding of changes in the rocky reef benthic communities at the Ischia CO<sub>2</sub> vent system (Kroeker et al. 2011) by highlighting the sensitivity and resilience to OA among polychaete species. The distribution and structure of the polychaete assemblages across the different pH conditions examined showed a dramatic reduction in the number of taxa with decreasing mean pH. This was caused by the progressive disappearance of non-tolerant taxa that were not replaced by any other, and resulted in the majority of the species being linked to ambient and (to a lesser extent) low pH zones. Accordingly, the pH zones studied showed consistent differences and were clearly separated in the CAP analysis.

Our results suggest that variability in the polychaete assemblage and species distributions among pH zones is influenced by concurrent changes in the algal assemblages. In fact, 15.7 and 15.0% of the observed variation in the polychaete assemblage was explained by the percent cover of FTA and EFA, respectively. FTA and EFA are the most common functional groups in the studied area (Porzio et al. 2011, Kroeker et al. 2013b), and were more abundant and evenly distributed in the most acidified areas (see Supplement 2 at [www.int-res.com/articles/suppl/m550p121\\_supp.pdf](http://www.int-res.com/articles/suppl/m550p121_supp.pdf)). It should be noted that this pattern could be biased by the methods used to estimate the algal assemblages (i.e. photo analysis), because erect algae could potentially 'hide' other forms. In addition to its influence on polychaete assemblages, FTA appears to play a pivotal role in

this ecosystem. FTA appears to outcompete coralline algae in the low pH zones during succession and changes habitat features at a landscape scale (Kroeker et al. 2013b). Taking into account that both FTA and EFA include branching taxa (e.g. *Dictyota* spp., *Sargassum vulgare*) and have a relatively complex architectural structure (i.e. with small interstices and micro-habitats), these algal forms could favor small-sized, interstitial species, such as many of the syllid, sabellid and fabriciid polychaetes we found in our samples.

Other than the occurrence of specific algal forms, whose effects are still poorly known (Katzmann 1971, Sanchez-Moyano et al. 2002, Çinar & Gönlügür-Demirci 2005), the overall algal cover and presence of epiphytes of the main algae may also influence polychaete abundance and diversity (Giangrande 1988, Sardá 1991, Frascchetti et al. 2002). However, the highest species and feeding guild diversity of polychaetes seems to occur among filamentous bushy algae (Antoniadou et al. 2004, Antoniadou & Chintiroglou 2006), which roughly correspond to our EFA guild.

Most taxa were represented in the study area with few individuals, and only 15 of the 83 taxa exceeded 1% of the total abundance. The Serpulidae, having calcified tubes, were less abundant in low pH and absent in the extreme low pH zones, mimicking the pattern of other calcified groups such as mollusks and coralline algae (Kroeker et al. 2011, Porzio et al. 2011). Six of these dominant polychaetes appeared to be sensitive to acidification, since their abundance was lower in areas of increasing water acidification. Conversely, some of the remaining dominant taxa showed relatively high, uniform abundances along the pH gradient, including the most acidified areas, thus demonstrating some degree of tolerance to OA. Overall, abundances were also lower in extreme low pH zones, although this trend was less marked than that of species richness. This is because some taxa responded differentially (e.g. the dominant *Amphiglena mediterranea*, which was abundant everywhere, or *Parafabricia mazzellae*, whose patchy distribution peaked in one sample from the extreme low pH zone), likely due to an increased competitive ability under these extreme conditions in comparison to less tolerant taxa such as the Serpulidae, which often share habitat and feeding modes with Sabellidae and Fabriciidae under normal pH conditions (Jumars et al. 2015). A shift in dominance by non-calcified taxa and a decrease in calcified taxa is a well-documented effect of OA in CO<sub>2</sub> vent sys-

tems (Martin et al. 2008, Fabricius et al. 2011, Kroeker et al. 2011, 2013, Inoue et al. 2013, Donnarumma et al. 2014).

Our study provides field evidence that OA may have different effects on species that are phylogenetically closely related. For instance, among Sabellida, the dominant Sabellidae (*A. mediterranea*) and Fabriciidae (*P. mazzellae*, *Fabricia stellaris*) are OA-tolerant, while the Serpulidae (*Spirobranchus polytrema*, *S. vermicularis*) are OA-sensitive. These differential responses appeared primarily dependent upon calcification. Within the Nereididae, *Platynereis* cf. *dumerilii* and *Perinereis cultrifera* appeared to be tolerant, while *Nereis zonata* did not show a clear pattern. Results for the motile non-calcifying Syllidae are intriguing. Syllids deserve special attention when analyzing biodiversity patterns of hard-bottom benthic communities due to their impressive diversity and abundance (Musco 2012). This is also the case for the rocky reefs in our study area: almost 50% of the dominant species belonged to the syllid subfamilies Exogoninae and Syllinae. Among the Exogoninae the co-generic *Exogone naidina* and *E. dispar* appeared sensitive, while *Brania pusilla* appeared tolerant. However, the irregular patchy distribution of the latter suggests caution in interpretation. These results differ from those reported for assemblages characterized through artificial collectors, in which *E. naidina* was relatively abundant in very acidic conditions (Cigliano et al. 2010). However, the artificial collectors were exposed *in situ* for a single month, while our data reflect the long-term exposure of the natural assemblage and, therefore, long-term responses of these species to the stressor. Within the Syllinae, the abundance of *Haplosyllis granulosa* was significantly reduced under low pH, suggesting this may be a sensitive species. *Syllis gerlachi* also appeared to be sensitive, while the co-generic *S. prolifera* was tolerant, confirming previous observations (Calosi et al. 2013, Ricevuto et al. 2014). These 2 species of *Syllis* are among the most abundant and common macrofaunal organisms in Mediterranean shallow rocky bottom habitat, representing 12.6 and 14.8% of the syllid individuals, respectively, among 233 samples collected at pristine locations over a large geographic area at a Mediterranean regional scale (including the Adriatic, Ionian, Tyrrhenian, Sicilian, and Sardinian Seas). In fact, *S. prolifera* reached an average density of  $406.7 \pm 730.1$  ind. m<sup>-2</sup> and a frequency of presence of 78.5%, while for *S. gerlachi* these same measures were  $346.9 \pm 500.2$  ind. m<sup>-2</sup> and 90.1%, respectively (L. Musco unpubl. data), an impressive

density considering that Syllidae may represent over 20% of the macrofaunal abundance in pristine conditions (Musco 2012). These 2 species are omnivorous and reportedly feed on similar items (Giangrande et al. 2000), suggesting they may interact or even compete. Consequently, these interactions are susceptible to modification by OA.

As a whole, analyses of feeding guilds and motility indicated an increase in filter feeders, herbivores and discretely motile forms, and a complete disappearance of sessile species at low pH. We can hypothesize that sessile species cannot react to temporary and localized extreme low pH values, whereas motile and discretely motile species can move back and forward. Among the discretely motile species, filter feeders may be facilitated by an increase in microbial photosynthetic rate and cyanobacterial blooms under OA (Das & Mangwani 2015). This would be the case for *A. mediterranea*, a discretely motile filter feeder, and could explain the success of this species: the quality and abundance of food might compensate for the low quality of the water (extreme low pH). Although our analyses of polychaete size and tube features indicated no significant differences associated with pH, the observed increase in intermediate-sized polychaetes as well as forms having non-calcareous tubes suggests that these structural traits might represent 'winner' features under OA.

Some of the dominant species in our acidified areas (e.g. *S. prolifera*, *A. mediterranea*, *P. cf. dumerilii*) also dominated in artificial settlement collectors deployed in extremely low pH conditions (Cigliano et al. 2010). This dominance has been shown to have a physiological basis (Calosi et al. 2013). *A. mediterranea* showed a marked physiological plasticity, but no genetic differentiation—indicating that adaptation or acclimatization are both successful colonization strategies in acidified environments (Calosi et al. 2013). Similar studies focusing on some of the species analyzed in the present study (particularly syllids) are lacking. Such studies will be needed to better understand the processes underlying changes in community patterns.

The vent population of *P. cf. dumerilii* was physiologically and genetically different from nearby populations outside the vents (Calosi et al. 2013), despite being morphologically identical. However, a sibling species, morphologically indistinguishable from *P. dumerilii*—*P. massiliensis* (Moquin-Tandon, 1869)—has been recently found in the highly acidified areas around Ischia Island. These sibling species show different life and reproductive strategies and can be distinguished only by analyzing mature adult speci-

mens, since *P. massiliensis* broods eggs inside a tube while *P. dumerilii* is a free spawner, with epitokous transformation and swarming in open water (Lucey et al. 2015, Valvassori et al. 2015). The clear increase (in percentage) of brooders and polychaetes characterized by direct development (2 traits that often co-occur) in low pH zones highlighted here is in accordance with Lucey et al. (2015), who also discussed the evolutionary implications of this pattern and suggested that the combination of these 2 traits would represent a winning reproductive strategy in the future oceans.

The present paper provides results on detailed taxonomic and functional analysis of the polychaetes (one of the most abundant benthic groups); a novel approach considering that the match between species composition and functional traits has been rarely performed in CO<sub>2</sub> vents systems (Kroeker et al. 2013), and is limited to observation of reproductive habits (Lucey et al. 2015). In addition, the community level approach considering polychaetes as part of the complex community inhabiting Castello's vent system is among the main and novel aspect of the present study. In fact, the inclusion of the habitat formers as determinants in the analysis allowed us to disentangle the effects of the different factors influencing polychaete ecology and distribution in the study area. This allowed us to account for the idiosyncratic variation of the algal cover between sides and among pH zones (see Supplement 1), resulting in the less than obvious correlation between polychaete distribution and some functional categories of macroalgae, which would have hidden the detected effects of OA on the analyzed assemblages (i.e. the meaning of the significant side × pH interaction in the preliminary MANOVAs and ANOVAs).

In conclusion, the detailed taxonomic and structural analyses of the polychaete assemblages of the rocky reefs at the CO<sub>2</sub> vent system highlighted a strong reduction in species diversity in the most acidified sites. Under harsh pH conditions, the community was not composed of new species found only in these conditions, but rather by a few taxa also found in other conditions, which appeared tolerant of OA and remained quite abundant (*A. mediterranea*, *S. prolifera*, *P. cf. dumerilii*, *P. mazzellae*, *B. aragonensis*, *F. stellaris*, *P. pictus*, *P. cultrifera*), whilst others were affected and sensitive to OA and disappeared or underwent drastic decreases in abundance (*S. gerralchi*, *H. granulosa*, *E. dispar*, *E. naidina*, *N. zonata*, *S. polytrema*, *S. vermicularis*). These 2 groups of species can be considered winners and losers, respectively, in the face of the OA challenge. Further studies are

required to understand the genetic, physiological and ecological processes favoring or impairing their fitness in a changing environment. Interestingly, most of the winners were discretely motile, herbivores or filter feeders, and brooders with direct development, highlighting these traits as possible winning ecological and life-history traits to be tested more broadly with in-depth experimental studies and among other marine groups. Many of the species reported here could be interesting models with which to study the complex and multi-faceted responses of marine organisms to rising levels of  $p\text{CO}_2$  and carbonate chemistry alterations in the near future oceans. These models could cover a wide array of conditions, since our results suggested that polychaete tolerance (or vulnerability) to OA appears to be highly species-specific.

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