

2018-02-23

# A comparison of life-history traits in calcifying Spirorbinae polychaetes living along natural pH gradients

Lucey, NM

<http://hdl.handle.net/10026.1/11565>

---

10.3354/meps12453

Marine Ecology Progress Series

Inter Research

---

*All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.*

1 **A comparison of life-history traits in calcifying Spirorbinae polychaetes living along**  
2 **natural pH gradients**

3 **Running head: Identifying traits tolerant to low pH**

4 Noelle M. Lucey \*<sup>1,2,3</sup>, Chiara Lombardi<sup>2</sup>, Maurizio Florio<sup>1,2</sup>, Simon D. Rundle<sup>3</sup>, Piero  
5 Calosi<sup>4</sup>, Maria Cristina Gambi<sup>5</sup>

6 <sup>1</sup> University of Pavia, Department of Earth and Environmental Sciences, Pavia 27100,  
7 Italy; [luceynm@si.edu](mailto:luceynm@si.edu)

8 <sup>2</sup> Marine Environment Research Centre ENEA, Pozzuolo di Lerici, La Spezia 19032,  
9 Italy; [chiara.lombardi@enea.it](mailto:chiara.lombardi@enea.it), [maustailish@hotmail.com](mailto:maustailish@hotmail.com)

10 <sup>3</sup> Plymouth University, Marine Biology and Ecology Research Centre, Plymouth PL4  
11 8AA, UK; [S. Rundle@plymouth.ac.uk](mailto:S.Rundle@plymouth.ac.uk)

12 <sup>4</sup> Université du Québec à Rimouski, Département de Biologie, Chimie et Géographie,  
13 Rimouski QC G5L 3A1, Quebec, Canada; [piero\\_calosi@uqar.ca](mailto:piero_calosi@uqar.ca)

14

15 <sup>5</sup> Stazione Zoologica “Anton Dohrn”, Dept. Integrative Marine Ecology, Villa Dohrn-  
16 Benthic Ecology Center, Ischia, Napoli 80121, Italy; [gambimc@szn.it](mailto:gambimc@szn.it)

17

18 **Corresponding author (\*):** Noelle M. Lucey; [luceynm@si.edu](mailto:luceynm@si.edu)

19 **Abstract**

20       Low pH vent systems are ideal natural laboratories to study the consequences of  
21 long-term low pH exposure on marine species, and thus identify life-history traits  
22 associated with low pH tolerance. This knowledge can help to inform predictions on  
23 which types of species may be less vulnerable in future ocean acidification (OA)  
24 scenarios. Accordingly, we investigated how traits of calcifying polychaete species  
25 (Serpulidae, Spirorbinae) varied with pH using a functional trait analysis at two natural  
26 pH gradients around Castello Islet in Ischia, Italy. We first observed the distribution and  
27 abundance patterns of all calcifying polychaete epiphytes in the canopy of *Posidonia*  
28 *oceanica* seagrass across these gradients. We then used laboratory trials to compare  
29 fecundity, settlement success, and juvenile survival in the dominant species from a  
30 control (*Pileolaria militaris* Claparède, 1870) and a low pH site (*Simplaria* sp.,  
31 (Serpulidae, Spirorbinae). We found significantly higher reproductive output, juvenile  
32 settlement rates, and juvenile survival in *Simplaria* sp. individuals from the low pH site,  
33 compared to *P. militaris* individuals from control pH sites, when observed in their  
34 respective *in situ* pH conditions. Our results suggest that tolerance to low pH may result,  
35 in part, from traits associated with successful reproduction and rapid settlement under  
36 low pH conditions. This finding implies that other species with similar life history traits  
37 may cope better in future OA scenarios, and should be targeted for future OA tolerance  
38 research.

39 **Keywords:** Ocean Acidification, Calcifiers, Settlement Success, Fecundity, Early-life  
40 Survival, Serpulidae, Population Resilience

## 41 **1 Introduction**

42 Anthropogenically-driven global changes may reduce or alter marine biodiversity  
43 (Raven et al. 2005, Widdicombe & Spicer 2008). One such change, ocean acidification  
44 (OA), occurs when surface seawater absorbs increasing atmospheric carbon dioxide  
45 (CO<sub>2</sub>), resulting in lowered pH and reduced availability of the carbonate ions many  
46 marine organisms require to build skeletal structures (IPCC, 2014). Despite confidence in  
47 forecasts of the chemical impacts from this process into the next century (Bopp et al.  
48 2013, IPCC 2014), uncertainty surrounds the corresponding biological and ecological  
49 impacts (Harley 2011, Gaylord et al. 2014). Determining sensitivities and tolerances to  
50 future OA conditions represents a necessary first step in improved understanding of how  
51 marine biodiversity will change in the next decades (Stockwell et al. 2003, van Oppen et  
52 al. 2015).

53 Functional trait analyses (FTA) can help determine sensitivity or tolerance of  
54 different species to low pH conditions. These analyses link environmental gradient  
55 survey data (e.g. species abundance patterns), to specific phenotypic trait changes in two  
56 or more species along the same gradients (e.g. body size, reproductive habit, fecundity)  
57 (McGill et al. 2006). The assumption is that by comparing a specific trait among  
58 phylogenetically similar species (i.e. sister species, or species derived from a common  
59 ancestral node), differences can be attributed to specific environmental effects. In the  
60 context of future OA, application of specific functional trait analyses along natural pH  
61 gradients provides a relatively straightforward tool to evaluate which types of species will  
62 play pivotal roles in reorganizing the biodiversity landscape (McGill et al. 2006).

63 Abundance surveys performed in volcanic vent systems, which emit CO<sub>2</sub>, have  
64 recently proven useful in identifying traits associated with invertebrate species  
65 sensitivities and tolerances to future OA change (Lucey et al. 2015, Gambi et al. 2016).  
66 For example, marine invertebrates without a pelagic life stage (i.e. brooders or direct  
67 developers) are more abundant in low pH sections of CO<sub>2</sub> vent gradients (Lucey et al.  
68 2015). Identification of a specific brooding oyster species with increased survivability in  
69 a low pH upwelling environment (Waldbusser et al. 2016) reinforced this pattern  
70 Unfortunately, natural system assessments generally lack trait data regarding fecundity,  
71 larval survival, settlement and recruitment (however, see Padilla-Gamino et al. (2016) for  
72 coralline algae assessment). This gap partly exists because field observations cannot  
73 provide detailed data involving a temporal element (e.g. settlement rates), or observations  
74 requiring thorough quantification of small-scale processes (e.g. egg production,  
75 fecundity). Collecting and observing such traits in a laboratory setting that mimics the  
76 individuals' *in situ* conditions can help to fill this gap. Combining such laboratory trials  
77 with natural system-based assessments may offer one mechanism to strengthen analyses  
78 of some functional traits.

79 The sensitivity of early developmental stages underscores the need to understand  
80 marine invertebrate life histories within future OA scenarios (Kurihara 2008, Albright  
81 2011, Byrne 2011, Crook et al. 2016). As such, our study aims to identify fecundity and  
82 early life history traits associated with low pH tolerance in marine invertebrates using  
83 calcifying serpulid polychaetes (Spirorbidae, Annelida) along two natural pH gradients.  
84 These gradients border the Castello Aragonese Islet off the coast of Ischia (Naples, Italy),  
85 and are a formed when volcanically derived CO<sub>2</sub> gas bubbling up through the seafloor

86 mixes with seawater, decreasing the pH from an ambient value of ~8.17 to as low as 6.57  
87 (Tedesco 1996, Hall-Spencer et al. 2008, Kroeker et al. 2011, Garrard et al. 2014), well  
88 representing business-as-usual IPCC pH projections for 2100 (IPCC, 2014) .

89 We selected calcareous polychaetes of the sub-family Spirorbinae (Annelida,  
90 Serpulidae), which inhabit *Posidonia oceanica* seagrass leaves as epiphytes along the  
91 Castello pH gradients, as our study focus. Generally, Spirorbinae polychaetes encompass  
92 a diverse suite of life history traits that can vary among closely related species  
93 (Macdonald 2003). Their dual life stages, with both free-swimming larval phases and  
94 sessile adult stages (Kupriyanova et al. 2001, 2006), offer another beneficial attribute.

95 First, we assess the distribution and abundance patterns of the calcifying polychaete  
96 assemblage on *Posidonia oceanica* leaves across two natural pH gradients bordering the  
97 Castello Islet: – one along the north (mean pH range 7.39 - 8.03), and one along the south  
98 (mean pH range 6.99 -8.03). Second, we use laboratory trials comparing two closely  
99 related species - one from a low pH site and the other from a control pH site - to  
100 investigate possible links between fecundity, juvenile survival, settlement traits, and low  
101 pH tolerance. Knowing which of these life history traits are associated with low pH  
102 tolerance will help inform our predictions on the types of species that may be less  
103 vulnerable in future ocean acidification scenarios

## 104 **2 Materials & Methods**

### 105 2.1 Field survey

---

106           The pH gradients used in this study occur along the north and south sides of the  
107   Castello Aragonese islet on Ischia, an island off the coast of Naples, Italy (Tyrrhenian  
108   Sea) (Fig. 1). To represent three pH levels, six sampling sites were chosen within  
109   *Posidonia oceanica* seagrass meadows, at depths of approximately  $3 \pm 0.5$  m. Sites are  
110   referred to as N3, N2, NC and S3, S2, SC, where “3” represents the extreme-low pH, “2”  
111   the low pH conditions, and “C” the control pH. The “N” and “S” represent north and  
112   south gradients (Fig. 1). Sites were chosen to be comparable in depth and *Posidonia*  
113   cover (Donnarumma et al. 2014). The site names are listed with their corresponding  
114   carbonate seawater parameters in Table 1. Seawater parameters represent a synthesis of  
115   all available data in the last six years to convey the most comprehensive and realistic  
116   time-series data for these study sites (Ricevuto et al. 2014). For additional water  
117   parameters and GPS coordinates, see Supplementary Material: *Site Details*.

118           To determine which calcifying polychaete species settle on *Posidonia* leaves in  
119   low pH environments, and how their abundance and distribution varies along the pH  
120   gradients, sampling by SCUBA diving was performed on September 29<sup>th</sup> - 30<sup>th</sup>, 2014.  
121   Four quadrats (replicates) of 40 x 40 cm were haphazardly placed at least 2 m apart on  
122   the seagrass canopy in each pH site. Within each quadrat, leaves of ten *Posidonia* shoots  
123   were randomly cut at the base of the rhizome and put in separate plastic bags. In the two  
124   extreme low pH sites (N3 and S3), initial visual inspection showed a highly reduced  
125   number of worms on leaves. Consequently, the number of sampled shoots was increased  
126   by cutting only the external leaf (oldest leaf) of 30 shoots within each of the four quadrats  
127   in both N3 and S3. This provided a more reliable estimate of worm abundance and helped  
128   preserve the seagrass from impact due to sampling. Samples were transferred in bags

129 containing seawater to the Villa Dohrn-Benthic Ecology Center of Ischia (Stazione  
130 Zoologica Anton Dohrn) within 1 h of sampling, and preserved in 4 % neutralized  
131 formalin for 24 h. They were then rinsed with fresh water and transferred into 70 %  
132 EtOH for long-term preservation.

133         The number of calcifying polychaetes on the *Posidonia* leaves of each shoot was  
134 determined by viewing each leaf from each replicate/quadrat under a dissecting  
135 microscope (AZ100, Nikon, Milan, Italy; magnification 1- 50x). Species were identified  
136 from their tube orientation, operculum and chaetae morphology. Due to the loss of some  
137 opercula, some specimens remained unidentified. These were included in the counts by  
138 determining the ratio between the number of specimens identified for a given species and  
139 the total number of specimens found at each site. This ratio was used to calculate the total  
140 number of each species for each site replicate (Supplementary Materials: *Table S.1*).

141         The average number of polychaetes at each site accounting for differences in the  
142 available settlement area was calculated by multiplying the shoot density to the  
143 settlement area (percentage of *Posidonia* shoots colonized by spirorbids \* average  
144 number of spirorbids *per* shoot), with shoot density data from Donnarumma et al. (2014).  
145 Only leaves longer than 5 cm were considered. In the extreme low pH sites (S3 and N3),  
146 where sampling included only external leaves, the estimation followed the same  
147 procedure.

## 148 2.2 Laboratory trials

---

149         One day after the field survey, live individuals were collected for the laboratory  
150 trial by SCUBA diving. This entailed cutting *Posidonia* leaves with visibly attached



151 polychaete tubes from the S2 (low pH) and NC (control pH) sites. Leaves were placed in  
152 fabric bags, keeping the individuals from both sites separated and in their original  
153 seawater conditions.

154 All material was transported to the ENEA Laboratory in La Spezia, Italy where  
155 specimens were sorted, identified and prepared for the trial (Supplementary Material:  
156 *Transport Details*). Specimens were held at the pH conditions from their respective field  
157 sites (control or low pH) during the sorting process (2 – 4 d). For the trial, we identified  
158 18 *Pileolaria militaris* adults from the control pH site (NC), and 12 *Simplaria* sp. adults  
159 from the low pH site (S2).

160 These sites were chosen because they have the greatest average pH difference  
161 (Table 1), and specific species were chosen because the low pH site's sample was  
162 dominated by *Simplaria* sp. and the control pH site's sample was dominated by *P.*  
163 *militaris*. Additionally, the lack of *Simplaria* sp. individuals found in the control pH sites,  
164 and of *Pileolaria militaris* individuals found in the low pH sites, precluded a reciprocal  
165 laboratory transplant experiment. This in itself demonstrates that different species have  
166 different levels of sensitivity to low pH conditions, and thus the comparison in life  
167 history traits between the two selected species represents the best avenue to identify traits  
168 that help defining species' successful colonization in low pH. As in most classical  
169 functional trait analyses, this between-species comparison is able to capture valuable  
170 information.

171 At the start of the trial, each adult was placed in a separate Petri dish  
172 preconditioned with a biofilm from a 2 d non-filtered seawater soak and filled with 3 mL

173 of pH-conditioned filtered seawater (1 adult *per* Petri dish). The pH in the dishes was set  
174 at 7.61 for *Simplaria* sp. adults, representing the average value found in the S2 field site  
175 considering time-series data (Table 1). Similarly, dishes with *P. militaris* adults were  
176 maintained at the control pH value, 8.1. All other seawater parameters matched the field  
177 values for both species (Table 2). In this setup, eight covered aquaria were filled with 20  
178 mL of seawater. This water served as a bath for the uncovered Petri dishes (6-8 dishes  
179 *per* aquaria). Half of the aquaria were maintained at the lowered pH level by bubbling  
180 enriched (elevated  $p\text{CO}_2$  air) into the seawater, while the other half was maintained at the  
181 control pH level by bubbling normal air into the seawater. The pH inside each Petri dish  
182 was attained through surface  $\text{CO}_2$  diffusion within the covered aquaria (Gattuso 2011).  
183 The  $p\text{CO}_2$  going to these aquaria was measured continuously throughout the exposure  
184 period using a  $\text{CO}_2$  gas analyzer (Li-820, Li-Cor Biosciences, Lincoln, NE, USA). All  
185 aquaria were held in a thermal water bath that maintained stable thermal conditions. Petri  
186 dishes were randomly moved between the aquaria every two days.

187         Seawater pH, temperature, and salinity were measured in each Petri dish daily  
188 with an integrated pH and temperature meter (SG2, Italy), and refractometer (V2, TMC,  
189 São Julião do Tojal, Portugal). The pH meter was calibrated daily with pH buffer  
190 standards (4.01, 7.0, 9.21; Mettler-Toledo, Leicester, UK). Seawater samples (250 mL)  
191 were taken at the beginning and end of the trial from the stock seawater prepared for each  
192 treatment. Samples were fixed with  $\text{HgCl}_2$  (0.02 %) to eliminate microbial activity, stored  
193 in borosilicate flasks (250 mL), and maintained in dark, dry conditions until total  
194 alkalinity ( $A_T$ ) was determined using gran titration method (Dickson et al. 2007).  
195 Carbonate-system parameters of  $p\text{CO}_2$  ( $\mu\text{atm}$ ), total carbon dioxide ( $\text{TCO}_2$ ,  $\text{mol kg}^{-1}$ ),

196 bicarbonate concentration ( $\text{HCO}_3^-$  mol  $\text{kg}^{-1}$ ), calcite saturation ( $\Omega_{\text{ca}}$ ), and aragonite  
197 saturation ( $\Omega_{\text{ara}}$ ) were calculated from  $A_{\text{T}}$ ,  $\text{pH}_{\text{T}}$  (total scale), temperature and salinity  
198 using the package SeaCarb v.2.4.8 in software R (Lavigne & Gattuso 2013). Water-  
199 chemistry parameters for each dish during the 14 d experimental phase, as well as  
200 discreet field data from each pH site are presented in Table 2.

201           Seawater in each Petri dish was changed every other day by removing water with  
202 a syringe and replacing it with new seawater. This water was collected from La Spezia  
203 bay (La Spezia, Italy), and cleaned with a  $0.1\mu\text{m}$  filter and UV sterilization system  
204 (V2ecton 600, TMC, São Julião do Tojal, Portugal) for 5 d before being transferred to  
205 sterile 2 L flasks. One flask was prepared for each treatment and placed in the  
206 temperature bath described above with bubbling elevated  $\text{pCO}_2$  air, or normal air,  
207 depending on the treatment. Additionally, a diet of rotifers, *Artemia* sp. and microalgae  
208 was added to seawater before each water change at 3 mL feed *per* 300 L seawater  
209 (Gamma Nutraplus Reef Feed, TMC, São Julião do Tojal, Portugal). Petri dishes were  
210 mixed three times *per* day by gently tilting aquaria to promote feeding. The density of  
211 worms in each Petri dish was purposefully low to avoid potential indirect effects caused  
212 by animal respiration (approx.  $< 0.092 \text{ mg}^{-1}$ ).

213           Under these laboratory conditions, all adults were monitored once a day with a  
214 light microscope (AZ100, Nikon; magnification ranges of 25x up to 50x) for the presence  
215 of embryos in the opercular brooding chamber. After adults released their first brood,  
216 both adults and offspring were monitored daily for the following 14 d. The number of  
217 offspring from each parent (brood size) was counted after the first day of brood release.  
218 The number of settled larvae was counted daily, along with any deaths or additional

219 broods. The parent tubes were photographed with a digital camera (Nikon Sight DS-U1,  
220 Nikon, Milan, Italy) mounted on a light microscope (AZ100, Nikon), and tested as a trait  
221 covariate to account for any bias between parental size and offspring traits (i.e. brood size,  
222 mortality, brood survival). Photographs were analyzed with ImageJ software (Rasband  
223 WS, US National Institutes of Health, Bethesda, MD, USA) to obtain tube area (mm<sup>2</sup>)  
224 (Abràmoff et al. 2004).

## 225 2.3 Data analysis

---

226

### 227 2.3.1 Field survey data

---

228 Two data sets generated from the field survey were analyzed: (a) the abundance  
229 of all calcifying polychaete species along the north and south pH gradients (distribution);  
230 (b) the abundance of the two dominant species, *Simplaria* sp. and *P. militaris* adults  
231 along the pH gradients. Initial data exploration using Cleveland dot- and boxplots  
232 revealed no outliers in either dataset. Conditional boxplots revealed heteroscedasticity of  
233 the variances among the pH sites for both datasets, and histograms indicated violation of  
234 normality (Züür et al. 2010a). Non-linear patterns within the species-level dataset also  
235 existed (Züür et al. 2010a). As a consequence, a Welch's ANOVA with a Games –  
236 Howell post-hoc test was used for both datasets to assess how the number of calcifying  
237 polychaetes varied along pH gradient, with 'gradient side' (north/south) and 'pH site' as  
238 fixed factors. This test is robust to non-parametric distribution of count data and  
239 heteroscedasticity of the variances.

240 Additionally, dataset (b) was analyzed by employing generalized additive models  
241 (GAMs) (Wood, 2006, 2011, 2014; Züür, 2010b) to describe the abundance of each  
242 species with respect to nominal 'pH' and to compare the abundance of each species along  
243 both gradient 'sides', GAMs accounted for the non-linear patterns in both the *Simplaria*  
244 sp. and *P. militaris* datasets, and were built using the mgcv (Wood, 2011) and nlme  
245 (Pinheiro et al. 2015) packages in R. For both species, gradient 'side' (factor: north or  
246 south) and 'pH' (fitted as a smoother) were set as the explanatory variables. Nominal  
247 mean water pH for each gradient side and site was based on the one-month average of  
248 September data from (Kroeker et al. 2011) to accurately represent seasonal pH values  
249 during the survey. The appropriate degrees of freedom of the smoothers were selected  
250 automatically using cross validation (Wood 2006, 2011). For *Simplaria* sp. only, the  
251 interaction between gradient side and pH was included using the 'by' command in the  
252 mgcv package (Wood, 2011). Both models were optimized by initially looking for the  
253 optimal random structure, followed by the optimal fixed structure (Züür et al. 2007).  
254 Akaike information criteria was used to compare models and residual plots were used to  
255 assess the mean-variance relationships; models for both species indicated no violation of  
256 the assumption for homogeneity of the variances. Over-dispersion was also calculated for  
257 each model (sum of Pearson residuals<sup>2</sup>/ residual d.f.). High over-dispersion, particularly  
258 in *Simplaria* sp., required the use of negative binomial distribution with a log link  
259 (Pinheiro et al. 2015; Züür 2010b; Züür et al. 2007). The optimization function of the  
260 models (k parameter) was adjusted for this study's specific dataset at six. All statements  
261 about abundance change are based on the significance of the main effect gradient side,  
262 and not on the interaction between gradient side and pH.

### 263 2.3.2 Laboratory trials

---

264 In order to assess how fecundity and early life history traits differed in response to  
265 differing pH conditions, we compared responses of the low pH– originating *Simplaria* sp.  
266 adults (from, and dominant in S2) – under low pH conditions, to the control pH–  
267 originating *P. militaris* adults (from, and dominant in NC) – under control pH conditions  
268 with one-way ANOVA tests. Traits analyzed included: brood size of each parent; time of  
269 larval release to settlement (d); and percentage brood mortality *per* parent on d 7 and d 14.  
270 Data were tested for normality of distribution and homogeneity of variance using  
271 Cleveland dot- and boxplots. Boxplots indicated homogeneity of the variances among the  
272 pH species groups, and histograms indicated no violation of normality of distribution  
273 (Züür et al. 2010a).

274 All statistical analyses were performed by using the statistical software R (v.3.1.3; R  
275 Core Team 2015).

## 276 3 Results

### 277 3.1 Field survey

#### 278 3.1.1 Species identity

---

279 All of the taxa found belonged to the Spirorbinae sub-family, within the  
280 Serpulidae family. The four main species were *Pileolaria militaris* Claparde, 1870,  
281 *Simplaria* sp., *Janua heterostropha* (Montagu, 1803) (= *J. (Dexiospira) pagenstecheri*),  
282 and *Neodexiospira pseudocorrugata* (Bush, 1905). A total of forty-eight undetermined  
283 Serpulinae were also encountered.

284 The taxonomy of the *Simplaria* sp. did not exactly match known records, but our  
285 primary prediction is that it is a putatively novel morphotype of *Simplaria*  
286 *pseudomilitaris* (Thiriot-Quievreux, 1965) – having more abundant, longer, and more  
287 pronounced distally projecting calcareous spines covering its operculum plate. Without  
288 further taxonomic analysis we reservedly designate these individuals as *Simplaria* sp.;  
289 and this inconsistency is further discussed in the Supplementary Materials: *Taxonomy*  
290 *Details*.

### 291 3.1.2 Species abundance and distribution

---

292 Total polychaete abundances on the *Posidonia* leaves along the pH gradient from  
293 the Castello CO<sub>2</sub> vents ranged from 0 to 224 individuals *per Posidonia* shoot. There was  
294 a decrease in the mean abundance from the control pH sites (SC and NC) to the extreme  
295 low pH sites (S3 and N3) along both the north and the south gradients, with a decline  
296 from 341 to 13 individuals in the south (SC to S3), and from 1,183 to 14 individuals in  
297 the north (NC to N3) ( $F_{5, 92.97} = 75.11, p < 0.001$ , Fig. 2A). The means in both the  
298 northern and southern extreme-low pH sites (N3 and S3) were comparable ( $p > 0.05$ , Fig.  
299 2A). However, overall mean abundance was three times lower in the southern gradient  
300 compared to the north ( $p < 0.05$ , Fig. 2A). Additionally, in the north, there was a strong  
301 linear relationship between abundance and pH conditions. This differed from the south  
302 gradient, as mean abundance in the low pH site was highest (S2: 144 individuals),  
303 compared to the control pH site (SC: 124 individuals;  $p > 0.05$ , Fig. 2A). Higher shoot  
304 densities in the low pH sites compared to the control pH sites (Donnarumma et al. 2014)  
305 did not change the overall abundance patterns observed on both the north and south  
306 gradients (Fig. 3). For example, polychaete densities remained very scarce in the extreme

307 low pH sites despite a mean 1,000 shoots *per m*<sup>2</sup> in S3 compared to the mean 467 shoots  
308 *per m*<sup>2</sup> in SC, and 719 to 380 shoots *per m*<sup>2</sup> in N3 vs. NC.

309         The results also indicate that the two dominant species are *Simplaria* sp. and *P.*  
310 *militaris* within all the sites. These two species are also closely related to each other  
311 taxonomically, compared to the other species identified. This prompted separate analyses  
312 of the distributions of both *Simplaria* sp. and *P. militaris*. The results show that the  
313 distribution pattern seen along the southern gradient of the total species analysis is due to  
314 *Simplaria* sp. (Fig. 2B). Furthermore, in the species-specific analysis, the total abundance  
315 along the pH sites ranged from 0 to 498 individuals in *P. militaris* and from 48 to 532  
316 individuals in *Simplaria* sp. While the overall number of individuals for both species was  
317 comparable, their distribution differed. As in the total species analysis, abundances  
318 significantly declined with decreasing pH when considering all sample sites (*P. militaris*:  
319  $F_{4, 11} = 9.37, p = 0.006$ , *Simplaria* sp.:  $F_{5, 78} = 24.27, p < 0.001$  (Fig. 2B & 2C). The  
320 mean abundance of *P. militaris* was highest in the north compared to the south gradient  
321 (52 vs. 7, respectively), and decreased from the control pH to extreme low pH in the  
322 north (NC to N3), and low to extreme low in the south (S2 to S3). *Simplaria* sp. mean  
323 abundance was higher in the south than in the north gradient. The *Simplaria* sp.  
324 abundance in the low pH south site (S2) was not significantly different to the mean  
325 abundance in the south control pH site (SC), but was different in the north between the  
326 NC control and N3 extreme low pH site. Additionally, *Simplaria* sp. was the only  
327 spirorbid species found in the site with the lowest mean pH of the Castello vent system  
328 (S3 pH:  $6.99 \pm 0.34$ ) (Hoffmann et al. 2011).



329 Comparisons of the smoothers (non-parametric curves) generated by the additive  
330 mixed models for the two gradients of both species confirmed that abundance decreases  
331 in both species with decreasing nominal pH across each gradient ( $p < 0.001$  for both *P.*  
332 *militaris* and *Simplaria* sp., Fig. 4). For *P. militaris*, there were significant declines in  
333 abundance with decreasing pH along both north and south gradients, however the  
334 northern gradient had significantly more individuals compared to the southern gradient.  
335 In contrast, *Simplaria* sp. abundances in the north and south were not significantly  
336 different when pH values were greater than 7.9 within the gradient (Fig. 4B).

### 337 3.1.3 Laboratory trials

---

338 The laboratory trials revealed that life history trait values vary significantly along  
339 the pH gradients. The average number of offspring *per* brood from low pH– originating  
340 *Simplaria* sp. parents was significantly higher than from control pH– originating *P.*  
341 *militaris* parents: means 8.08 ( $\pm 1.54$ ) vs. 3.61 ( $\pm 0.44$ ) ( $F_{1, 28} = 10.80$ ,  $p = 0.003$ , Fig. 5).  
342 Also, settlement success was significantly higher in *Simplaria* sp. compared to *P.*  
343 *militaris*: 86.5 % ( $\pm 6.8$ ) compared to 13.4 % ( $\pm 6.3$ ), respectively ( $F_{1, 28} = 58.80$ ,  $p <$   
344 0.001, Fig. 5). Additionally, all offspring from the *Simplaria* sp. parents metamorphosed  
345 and settled within 1 h in low pH seawater, whereas less than 13 % of the offspring from *P.*  
346 *militaris* parents settled in the first 24 h in control conditions.

347 Juvenile mortality rates 7 d after the first brood release was 4.2 % ( $\pm 2.9$ ) in  
348 *Simplaria* sp. and 48.8 % ( $\pm 8.6$ ) in *P. militaris* ( $F_{1, 28} = 16.77$ ,  $p < 0.001$ , Fig. 5). Net  
349 survival after 14 d, including additional offspring from subsequent broods, was  
350 significantly higher (6.3 times) in *Simplaria* sp. offspring with respect to *P. militaris*  
351 offspring: means 9.5 ( $\pm 1.7$ ) vs. 1.5 ( $\pm 0.4$ ) offspring *per* parent, respectively ( $F_{1, 28} =$

352 26.90,  $p < 0.001$ , Fig. 5). Furthermore, between day 7 and day 14, 10 out of 12 parents  
353 released a second brood in the *Simplaria* sp. group, but only 4 out of 18 parents from the  
354 *P. militaris* group produced a second brood. No influence of parental tube size was found  
355 on brood sizes ( $p \geq 0.05$ ), and no parental mortality occurred during the 14 d trial.

#### 356 **4 Discussion**

357 This study aimed to identify specific life history traits that offer species potential  
358 advantages to tolerating future OA conditions. We identified two primary species along  
359 the Castello pH gradients with a close phylogenetic relationship, *Pileolaria militaris* and  
360 *Simplaria* sp., and found that the higher abundances of *Simplaria* sp. in low pH (S2) were  
361 associated with the ability to produce more viable offspring able to quickly  
362 metamorphose and settle in low pH conditions, compared to that of its close relative  
363 dominant at a control pH site (NC), *P. militaris*, observed under control pH conditions.  
364 Below we discuss potential physiological and ecological explanations for differences in  
365 traits underling species' sensitivity to low pH.

##### 366 *Physiology considerations*

367 The most noticeable finding was that rapid offspring development accompanied  
368 OA tolerance, as demonstrated by the production of larvae that metamorphose in minutes  
369 in the low pH–originating *Simplaria* sp. under low pH conditions, compared to the  
370 multiple days required for control pH–originating *P. militaris* individuals in control  
371 conditions. This is noteworthy because the challenges of calcification associated with  
372 metamorphosis and initial juvenile tube growth in many marine invertebrates exposed to  
373 OA conditions have been well documented, with demonstrated altered metamorphosis,

374 slowed juvenile growth, weakened juveniles' tubes, and tube dissolution under pH levels  
375 comparable to those used here (Dupont et al. 2009, Byrne 2011, Lane et al. 2012). In  
376 contrast, our results indicate that the low pH–originating *Simplaria* sp. appears to have  
377 overcome these challenges. We hypothesize that this may be, in part, due to specialized  
378 larval glands that are able to expedite the secretion of a primary tube, resulting in  
379 successful metamorphosis. These specialized larval glands are commonly found in  
380 Serpulidae species with lecithotrophic (non-feeding) larvae (Kupriyanova et al. 2001),  
381 but while both of the species here have primary larval glands, there were noticeable  
382 physiological differences between them. The *Simplaria* sp. embryos and larvae had highly defined,  
383 large glands compared to *P. militaris* (see Fig. 6; white spots in the *Simplaria* sp.  
384 embryos). Moreover, in *Simplaria* spp. the contents of the primary shell gland are  
385 extruded *via* the anus and the calcareous secretion is molded by the movements of the  
386 larva into a tube capable of housing the entire settled larva in less than 5 min (Knight-  
387 Jones, 1978) (Nott 1973, Potswald 1978, Beckwitt 1980, Qian 1999).

388         Another interesting finding was the increased adult fecundity of low pH–  
389 originating *Simplaria* sp., compared to control pH–originating *P. militaris*. This was  
390 despite no significant difference in opercular brood chamber size between the two species  
391 (chamber size is directly proportional to the adult's overall size, and thus the number of  
392 offspring produced *per* brood) (Kupriyanova et al. 2001). In general, both species  
393 fertilize and incubate their eggs and embryos similarly: in a single chamber that provides  
394 aeration and physical protection from the outside environment (Thorp 1975). When ready,  
395 competent larvae exit these chambers through a pore at its base (Macdonald 2003).  
396 Explaining the fecundity differences may therefore involve testing for improved internal

397 fertilization, and/or accelerated embryo incubation in the low pH-originating *Simplaria*  
398 sp., comparatively (Chaparro et al. 2008, Segura et al. 2010).

399         The fecundity differences may also be an outcome of plasticity from multi-  
400 generational exposure in the low pH–originating *Simplaria* sp. population (Rodríguez-  
401 Romero et al. 2015, Chakravarti et al. 2016). The possibility that plasticity may be the  
402 coping mechanism for species dealing with rapid changes has recently been revitalized,  
403 yet evidence of plasticity’s role in promoting persistence is not consistent (Merilä 2015,  
404 Calosi et al. 2016). For example, a field-based reciprocal transplant experiment using  
405 *Simplaria* sp. collected from the same low pH site (S2) found that plasticity was not  
406 attributed to fecundity differences (Lucey et al. 2016). They also presented  
407 contradictory evidence that the low-pH originating *Simplaria* sp. were able to reproduce  
408 multiple times, in comparison to a population of control pH–originating *Simplaria* sp, yet  
409 inadequate sample sizes preventing statistical confirmation (Lucey et al. 2016). This  
410 alludes to the possibility that higher fecundity could be the consequence of modulating  
411 (i.e. plasticity) the ‘number of broods over time.’

412         Rapid metamorphosis and increased fecundity was also coupled with lower  
413 offspring mortality during the first two weeks of offspring life in the low pH–originating  
414 *Simplaria* sp. group. This suggests that these *Simplaria* sp. will have a higher likelihood  
415 of recruitment success and overall population persistence, compared to the control pH–  
416 originating *P. militaris* group (Hunt & Scheibling 1997). The field survey supports this  
417 idea: *Simplaria* sp. adults with embryos were found at every site along the gradient  
418 regardless of pH.

419           The overall decline of *Simplaria* sp. individuals at extreme low pH alludes to a  
420 pH threshold. This in partial agreement with Saderne and Wahl, (2013), where growth  
421 rates and recruitment of spirorbid *Spirorbis spirorbis* individuals at extreme low pH/ high  
422  $p\text{CO}_2$  levels ( $3150 \pm 446 \mu\text{atm}$ ) were significantly reduced, whereas at more realistic pH  
423 levels for end of the century projections, individuals did not show any adverse effects  
424 (Saderne & Wahl 2013). These pH values closely correspond to the low (S2) and extreme  
425 low (S3) pH values in this study and corroborate the idea that each species has specific  
426 pH ‘tipping’ points, as demonstrated in the larval mussels’ development, *Mytilus edulis*  
427 (Ventura et al. 2016). This theory that physiological tipping points may limit populations’  
428 pH tolerance complements that of Lucey et al. (2016), where abnormally low pH values  
429 at the low pH site (S2) may have confounded a potential local adaptation signature.

#### 430 *Ecological considerations*

431           Predation may also be playing a role in the distribution of spirorbid species  
432 around the  $\text{CO}_2$  vents, and their pH tolerance traits. Increases in spirorbids predation are  
433 likely as there have been documented increases in amphipod and copepod abundance in  
434 the low pH sites, known spirorbid predators (Knight-Jones et al. 1975, Kupriyanova et al.  
435 2001 p.60). This helps explain the decrease in spirorbids at the extreme low pH sites.  
436 Furthermore, it suggests a potential correlation between increased predation and the novel  
437 opercular spine morphology observed in the low pH– originating *Simplaria* sp., where  
438 rows of long, slender calcareous spines project from the top of the operculum and guard  
439 the tube opening. Further investigation is necessary to prove this theory (e.g. Harris, 1968,  
440 Knight-Jones et al. 1974, Bianchi 1981; also see Supplemental Materials: *Taxonomy*  
441 *Details*). There are also indirect predation threats that may be influencing the spirorbid

442 distributions: the very prominent reduction in overall *Posidonia* canopy height at the low  
443 pH sites as a consequence of intense grazing from the fish *Sarpa salpa* (Deudero et al.  
444 2008), compared to lower density long-leaved shoots in the control pH sites  
445 (Donnarumma et al. 2014, Scartazza et al. 2017). The increased grazing pressure under  
446 highly acidified conditions could explain the decreased spirorbid abundance, as fish  
447 grazing removes epiphytic invertebrates (Deudero et al. 2008). Additionally, this  
448 variation in the *Posidonia* canopy may indirectly be related to the observed low pH–  
449 originating *Simplaria* sp.’s fast juvenile growth. As Spirorbinae are small filter feeders  
450 that spend the majority of their lives inside tubes permanently attached to a substrate  
451 (Gee 1964, Potswald 1968, Tanur et al. 2010), the organisms in low pH/intense grazing  
452 may be rapidly maturing as a response to host plant phenology, a feature that has been  
453 highlighted for other *Posidonia* epiphytes (Piazzi et al. 2015).

454         In addition to predation, it is possible that there are biological interactions  
455 between the two species, *Simplaria* sp. and *P. militaris*, which are responsible for their  
456 distributions. They may be competing with each other for available space or food, or may  
457 have different water movement requirements (Beckwitt 1980; Terlizzi et al. 2000). These  
458 factors may be contributing to the relative success of *P. militaris* in the north, compared  
459 to its limited southern abundance. The northern sites are more exposed to open water and  
460 dominant winds (from north and north-west), whereas the southern sites are within a  
461 small bay with less water movement (Rodolfo-Metalpa et al. 2010). This could mean that  
462 *Simplaria* sp. populations are better suited to live in more sheltered conditions, or that  
463 they are able to fill a niche where conditions are less stable due to pH. A parallel example  
464 is provided by the differential occurrence of two non-calcifying polychaete sister species,

465 *Platynereis dumerilii* and *Platynereis massiliensis*, around the Ischia CO<sub>2</sub> vents, where  
466 the ecological exclusion of *P. dumerilii* in the high CO<sub>2</sub> areas appears to be explained by  
467 differences in physiological and life history traits (Lucey et al. 2015). For the spirorbids,  
468 a more complete trait analysis able to encompass the full relevant trait space (i.e. testing  
469 population samples of each species from all sites and a broader array of traits) would be  
470 useful to elucidate which factors are most relevant to explain OA resistance phenotypes  
471 (Laughlin & Messier, 2015).

## 472 **5 Conclusions**

473 This study aimed to identify if and how fecundity, settlement, and juvenile  
474 survival were associated with low pH in order to better understand which life-history  
475 traits may have an advantage in future marine environments. We found that traits  
476 associated with low pH tolerance included increased reproductive output, rapid larval  
477 settlement, and high juvenile survival rates. By association, we infer that species with  
478 similar life history traits may be better suited to live in future OA inflicted environments,  
479 potentially driving future biodiversity patterns. Overall, this study shows how it is  
480 possible to guide future research and better our predictive ability of future marine life  
481 under increasing ocean acidification by incorporating aspects of community ecology with  
482 trait biology.

## 483 **6 Acknowledgements**

484 We wish to thank A.V. Rzhavsky for his taxonomic advice about *Simplaria* and other  
485 Spirorbinae. We also thank B. Iacono and Capt. V. Rando (Villa Dohrn-Benthic Ecology  
486 Center at Ischia) for their fieldwork assistance, as well as S. Cocito, G. Cerrati and A.

487 Bordone for providing microscopy use, seawater collection and analysis at the ENEA  
488 Research Center. This manuscript has not been submitted elsewhere. NML was supported  
489 by a MARES PhD scholarship (FPA 2011-0016) awarded to CL and PC. PC is supported  
490 by a NSERC Discovery Grant and an FRQ-NT New University Researchers Start Up  
491 Program. The research was also supported by the Stazione Zoologica Anton Dohrn,  
492 Napoli (Italy).

### 493 **References**

- 494 Abràmoff MD, Magalhães PJ, Ram SJ (2004) Image Processing with ImageJ.  
495 *Biophotonics International* 11:36–42
- 496 Albright R (2011) Reviewing the Effects of Ocean Acidification on Sexual Reproduction  
497 and Early Life History Stages of Reef-Building Corals. *Journal of Marine Biology*  
498 2011:1–14
- 499 Bailey JH (1970) Spirobinae (Polychaeta) from the West Indies. *Studies of the Fauna of*  
500 *Curacao and other Caribbean Islands* 118:58–81
- 501 Bailey JH, Harris MP (1968) Spirorbinae (Polychaeta: Serpulidae) of the Galapagos  
502 Islands. *Journal of Zoology, London* 155:161–184
- 503 Beckwitt R (1980) Genetic structure of *Pileolaria pseudomilitaris* (Polychaeta:  
504 Spirorbidae). *Genetics* 96:711–726
- 505 Beckwitt R (1981) The interitance of morphological variation in *Pileolaria*  
506 *pseudomilitaris* (Polychaeta: Spirorbidae). *Zoological Journal of the Linnean*  
507 *Society* 71:237–247



- 508 Bianchi CN (1981) Guide per il riconoscimento delle specie animali delle acque lagunari  
509 e costiere italiane. Policheti Serpuloidei. Consiglio Nazionale delle Ricerche AQ  
510 1/96, 5: 1-187
- 511 Bopp L, Resplandy L, Orr JC, Doney SC, Dunne JP, Gehlen M, Halloran P, Heinze C,  
512 Ilyina T, Séférian R, Tjiputra J, Vichi M (2013) Multiple stressors of ocean  
513 ecosystems in the 21<sup>st</sup> century: projections with CMIP5 models. *Biogeosciences*  
514 10:6225–6245
- 515 Byrne M (2011) Global change ecotoxicology: Identification of early life history  
516 bottlenecks in marine invertebrates, variable species responses and variable  
517 experimental approaches. *Marine Environmental Research* 76: 3-15
- 518 Calosi P, Rastrick S, Lombardi C, de Guzman HJ, Davidson L, Jahnke M, Giangrande  
519 A, Hardege JD, Schulze A, Spicer JI, Gambi MC (2013) Adaptation and  
520 acclimatization to ocean acidification in marine ectotherms: an *in situ* transplant  
521 experiment with polychaetes at a shallow CO<sub>2</sub> vent system. *Philosophical*  
522 *Transactions of the Royal Society of London Series B, Biological Sciences* 368:  
523 1627
- 524 Calosi, P, De Wit, P, Thor, P, & Dupont, S (2016). Will life find a way? Evolution of  
525 marine species under global change. *Evolutionary Applications* 9(9): 1035–1042.
- 526 Campbell AL, Levitan DR, Hosken DJ, Lewis C (2016) Ocean acidification changes the  
527 male fitness landscape. *Scientific Reports* 6:31250
- 528 Casola E, Scardi M, Mazzella L, Fresi E (1987) Structure of the epiphytic community of

- 529 *Posidonia oceanica* leaves in a shallow meadow. *Marine Ecology* 8: 285–296.
- 530 Chakravarti LJ, Jarrold MD, Gibbin EM, Christen F, Massamba-N’Siala G, Blier PU,  
531 Calosi P (2016) Can trans-generational experiments be used to enhance species  
532 resilience to ocean warming and acidification? *Evolutionary Applications* 9:1133–  
533 1146
- 534 Chaparro OR, Montiel YA, Segura CJ, Cubillos VM, Thompson RJ, Navarro JM (2008)  
535 The effect of salinity on clearance rate in the suspension-feeding estuarine gastropod  
536 *Crepidatella dilatata* under natural and controlled conditions. *Estuarine, Coastal and*  
537 *Shelf Science* 76:861–868
- 538 Crook ED, Kroeker KJ, Potts DC, Rebolledo-Vieyra M, Hernandez-Terrones LM, Paytan  
539 A (2016) Recruitment and succession in a tropical benthic community in response to  
540 *in situ* ocean acidification. *Plos One* 11:e0146707
- 541 Deudero S, Morey G, Frau A, Moranta J, Moreno I (2008) Temporal trends of littoral  
542 fishes at deep *Posidonia oceanica* seagrass meadows in a temperate coastal zone.  
543 *Journal of Marine Systems* 70:182–195
- 544 Dickson AG, Sabine CL, Christian JR (2007) Guide to Best Practices for Ocean CO<sub>2</sub>  
545 Measurements. PICES Special Publication 3:191
- 546 Donnarumma L, Lombardi C, Cocito S, Gambi MC (2014) Settlement pattern of  
547 *Posidonia oceanica* epibionts along a gradient of ocean acidification: an approach  
548 with mimics. *Mediterranean Marine Science* 15(3):498-509
- 549 Dupont S, Lundve B, Thorndyke M (2010) Near future ocean acidification increases

- 550 growth rate of the lecithotrophic larvae and juveniles of the sea star *Crossaster*  
551 *papposus*. *Journal of Experimental Zoology (Mol Dev Evol)* 314B:382–389
- 552 Dupont S, Thorndyke MC, Havenhand J (2009) Impact of CO<sub>2</sub>–driven ocean  
553 acidification on invertebrates early life-history – What we know, what we need to  
554 know and what we can do. *Biogeosciences Discussions* 6:3109–3131
- 555 Gambi MC, Musco L, Giangrande A, Badalamenti F, Micheli F, Kroeker KJ (2016)  
556 Distribution and functional traits of polychaetes in a CO<sub>2</sub> vent system: Winners and  
557 losers among closely related species. *Marine Ecology Progress Series* 550:121–134
- 558 Garrard SL, Gambi MC, Scipione MB, Patti FP, Lorenti M, Zupo V, Paterson DM, Buia  
559 MC (2014) Indirect effects may buffer negative responses of seagrass invertebrate  
560 communities to ocean acidification. *Journal of Experimental Marine Biology and*  
561 *Ecology* 461:31–38
- 562 Gattuso JP, Hansson L (2011) *Guide to best practices for ocean acidification*. OUP  
563 Oxford, 2011
- 564 Gaylord B, Kroeker K, Sunday J (2014) Ocean acidification through the lens of  
565 ecological theory. *Ecology* 96:3–15
- 566 Gee JM (1964) The British Spirorbinae (Polychaeta, Serpulidae) with description of  
567 *Spirobis cuneatus* sp. nov. and review of the genus *Spirobis*. *Proceedings of the*  
568 *Zoological Society of London* 143:405–441
- 569 Hall-Spencer JM, Rodolfo-Metalpa R, Martin S, Ransome E, Fine M, Turner SM,  
570 Rowley SJ, Tedesco D, Buia MC (2008) Volcanic carbon dioxide vents show

- 571 ecosystem effects of ocean acidification. *Nature* 454:96–99
- 572 Harley CDG (2011) Climate Change, Keystone Predation, and Biodiversity Loss. *Science*  
573 334:1124–1127
- 574 Hendriks IE, Olsen YS, Ramajo L, Basso L, Steckbauer A, Moore TS, Howard J, Duarte  
575 CM (2014) Photosynthetic activity buffers ocean acidification in seagrass meadows.  
576 *Biogeosciences* 11:333–346
- 577 Hofmann GE, Smith JE, Johnson KS, Send U, Levin LA, Micheli F, Paytan A, Price NN,  
578 Peterson B, Takeshita Y, Matson PG, Derse Crook E, Kroeker KJ, Gambi MC,  
579 Rivest EB, Frieder CA, Yu PC, Martz TR (2011) High-frequency dynamics of ocean  
580 pH: a multi-ecosystem comparison. *PLoS ONE*. DOI 10.1371/journal.pone.0028983
- 581 Hunt HL, Scheibling RE (1997) Role of early post-settlement mortality in recruitment of  
582 benthic marine invertebrates. *Marine Ecology Progress Series* 155:269–301
- 583 Knight-Jones P (1984) A new species of *Protoleodora* (Spirorbidae: Polychaeta) from  
584 Eastern U.S.S.R., with a brief revision of related genera. *Zoological Journal of the*  
585 *Linnean Society* 80:109–120
- 586 Knight-Jones P, Fordy RM (1979) Setal structure, functions and interrelationships in  
587 Spirorbidae Serpulidae (Polychaeta, Sedentaria). *Zoologica Scripta* 82:119–138
- 588 Knight-Jones EW, Knight-Jones P, Llewellyn LC (1974) Spirorbinae (Polychaeta:  
589 Serpulidae) from southeastern Australia. Notes on their taxonomy, ecology, and  
590 distribution. *Records of the Australian Museum* 29:106–151
- 591 Kroeker KJ, Micheli F, Gambi MC, Martz TR (2011) Divergent ecosystem responses

- 592 within a benthic marine community to ocean acidification. Proceedings of the  
593 National Academy of Sciences of the United States of America 108:14515–14520
- 594 Kupriyanova EK, Macdonald TA, Rouse GW (2006) Phylogenetic relationships within  
595 Serpulidae (Sabellida, Annelida) inferred from molecular and morphological data.  
596 Zoologica Scripta 35:421–439
- 597 Kupriyanova EK, Nishi E, Ten Hove HA, Rzhavsky AV (2001) Life-history patterns in  
598 serpulimorph polychaetes: ecological and evolutionary perspectives. Oceanography  
599 and Marine Biology an Annual Review 39
- 600 Kurihara H (2008) Effects of CO<sub>2</sub>-driven ocean acidification on the early developmental  
601 stages of invertebrates. Marine Ecology Progress Series 373:275–284
- 602 Lane AC, Mukherjee J, Chan VBS, Thiyagarajan V (2012) Decreased pH does not alter  
603 metamorphosis but compromises juvenile calcification of the tube worm *Hydroides*  
604 *elegans*. Marine Biology:1983–1993
- 605 Laughlin, DC., Messier, J (2015) Fitness of multidimensional phenotypes in dynamic  
606 adaptive landscapes. Trends in Ecology & Evolution 30.8: 487-496
- 607 Lavigne H, Gattuso JP (2013) seacarb: seawater carbonate chemistry with R. R package  
608 version 2.4. <http://CRAN.R-project.org/package=seacar>
- 609 Lucey NM, Lombardi C, DeMarchi L, Schulze A, Gambi MC, Calosi P (2015) To brood  
610 or not to brood: Are marine invertebrates that protect their offspring more resilient  
611 to ocean acidification? Scientific Reports 5:12009
- 612 Lucey NM, Lombardi C, Florio M, DeMarchi L, Nannini M, Rundle S, Gambi MC,

- 613 Calosi P (2016) An *in situ* assessment of local adaptation in a calcifying polychaete  
614 from a shallow CO<sub>2</sub> vent system. *Evolutionary Applications* 9:1054-1071
- 615 Macdonald TA (2003) Phylogenetic relations among spirorbid subgenera and the  
616 evolution of opercular brooding. *Hydrobiologia* 496:125–143
- 617 McGill BJ, Enquist BJ, Weiher E, Westoby M (2006) Rebuilding community ecology  
618 from functional traits. *Trends in Ecology and Evolution* 21:178–185
- 619 Merilä J (2015) Perplexing effects of phenotypic plasticity. *Nature*:4–6
- 620 Nott JA (1973) Settlement of the Larvae of *Spirorbis spirorbis* L. *Journal of the Marine*  
621 *Biological Association of the United Kingdom* 53:437–453
- 622 Oppen MJH van, Oliver JK, Putnam HM, Gates RD (2015) Building coral reef resilience  
623 through assisted evolution. *Proceedings of the National Academy of Sciences*  
624 112:1–7
- 625 Padilla-Gamino JL, Gaitan-Espitia JD, Kelly M, Hofmann G (2016) Physiological  
626 plasticity and local adaptation to ocean acidification in the calcareous algae  
627 *Corallina vancouveriensis*: An ontogenetic and geographic approach. *Evolutionary*  
628 *Applications* 56:168–168
- 629 Piazzì L, Balata D, Ceccherelli G. (2015) Epiphyte assemblages of the Mediterranean  
630 seagrass *Posidonia oceanica*: an overview. *Marine Ecology* 37(1): 3-41
- 631 Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2015) {nlme}: Linear and  
632 Nonlinear Mixed Effects Models.  
633 <https://cran.rproject.org/web/packages/nlme/nlme.pdf>

- 634 Potswald HE (1968) The biology of fertilization and brood protection in *Spirorbis*  
635 (*Laeospira*) *morchi*. The Biological Bulletin 135:208–222
- 636 Potswald HE (1978) Metamorphosis in *Spirorbis* (Polychaeta). Settlement and  
637 metamorphosis of marine invertebrate larvae Elsevier, New York:127–143
- 638 Qian P (1999) Larval settlement of polychaetes. Hydrobiologia 402:239–253
- 639 R Core Team (2015) R: A Language and Environment for Statistical Computing.
- 640 Raven J, Caldera K, Elderfield H, Hoegh-Guldberg O, Liss P, Riebesell U, Shepherd J,  
641 Turley C, Watson A, Heap R, Banes R, Quinn R (2005) Ocean acidification due to  
642 increasing carbon dioxide. The Royal Society. Policy document 12/05 June 2005  
643 ISBN 0 85403 617 2
- 644 Ricevuto E, Kroeker KJ, Ferrigno F, Micheli F, Gambi MC (2014) Spatio-temporal  
645 variability of polychaete colonization at volcanic CO<sub>2</sub> vents indicates high tolerance  
646 to ocean acidification. Marine Biology 161(12):2909-2919
- 647 Rodríguez-Romero A, Jarrold MD, Massamba-N’ Siala G, Spicer JJ, Calosi P (2015)  
648 Multi-generational responses of a marine polychaete to a rapid change in seawater *p*  
649 CO<sub>2</sub>. Evolutionary Applications 9:1082–1095
- 650 Saderne V, Wahl M (2013) Differential responses of calcifying and non-calcifying  
651 epibionts of a brown macroalga to present-day and future upwelling *p*CO<sub>2</sub>. PloS one  
652 8:e70455
- 653 Scartazza A, Moscatello S, Gavrichkoca O, Buia MC, Lauteri M, Battistelli A, Lorenti M,  
654 Garrard SL, Calfapietra C, Brugnoli E (2017) Carbon and nitrogen allocation

- 655 strategy in *Posidonia oceanica* is altered by seawater acidification. *Science of the*  
656 *Total Environment* 607-608: 954-964.
- 657 Schaum C-E, Rost B, Collins S (2015) Environmental stability affects phenotypic  
658 evolution in a globally distributed marine picoplankton. *The ISME Journal*: 1–10
- 659 Schaum E, Rost B, Millar AJ, Collins S (2012) Variation in plastic responses of a  
660 globally distributed picoplankton species to ocean acidification. *Nature Climate*  
661 *Change* 3:298–302
- 662 Segura CJ, Chaparro OR, Paschke KA, Pechenik JA (2010) Capsule walls as barriers to  
663 oxygen availability: Implications for the development of brooded embryos by the  
664 estuarine gastropod *Crepidatella dilatata* (Calyptraeidae). *Journal of Experimental*  
665 *Marine Biology and Ecology* 390:49–57
- 666 Stockwell CA, Hendry AP, Kinnison MT (2003) Contemporary evolution meets  
667 conservation biology. *Trends in Ecology & Evolution* 18:94–101
- 668 Tanur AE, Gunari N, Sullan RMA, Kavanagh CJ, Walker GC (2010) Insights into the  
669 composition, morphology, and formation of the calcareous shell of the serpulid  
670 *Hydroides dianthus*. *Journal of Structural Biology* 169:145–60
- 671 Tedesco D (1996) Chemical and isotopic investigations of fumarolic gases from Ischia  
672 island (southern Italy): Evidence of magmatic and crustal contribution. *Journal of*  
673 *Volcanology and Geothermal Research* 74: 233–242
- 674 Terlizzi A, Conte E, Giangrande A (2000) Settlement patterns of two Spirorbidae  
675 (Annelida, Polychaeta) species in the harbour of Ischia (Gulf of Naples,



- 676 Mediterranean Sea). Italian Journal of Zoology 67:303–306
- 677 Thorp CH (1975) The structure of the operculum in *Pileolaria (Pileolaria) granulata* (L.)  
678 (Polychaeta, Serpulidae) and related species. Journal of Experimental Marine  
679 Biology and Ecology 20: 215–235
- 680 Thorson G (1950) Reproductive and larval ecology of marine bottom invertebrates.  
681 Biological Reviews 25:1–45
- 682 Ventura A, Schulz S, Dupont S (2016) Maintained larval growth in mussel larvae  
683 exposed to acidified under-saturated seawater. Scientific Reports 6:23728
- 684 Vine J, Julie H, Straughan D (1972) Spirorbinae (Polychaeta, Serpulidae) of the  
685 Hawaiian Chain Part 2, Hawaiian Spirorbinae. Pacific Science 2:150-182
- 686 Waldbusser GG, Gray MW, Hales B, Langdon CJ, Haley BA, Gimenez I, Smith SR,  
687 Brunner EL, Hutchinson G (2016) Slow shell building, a possible trait for resistance  
688 to the effects of acute ocean acidification. Limnology and Oceanography 61(6):  
689 1969-1983
- 690 Widdicombe S, Spicer JI (2008) Predicting the impact of ocean acidification on benthic  
691 biodiversity: What can animal physiology tell us? Journal of Experimental Marine  
692 Biology and Ecology 366:187-197
- 693 Wood SN (2006) Generalized additive models: An introduction with R. Chapman and  
694 Hall/CRC
- 695 Wood SN (2011) Fast stable restricted maximum likelihood and marginal likelihood

- 696 estimation of semiparametric generalized linear models. *Journal of the Royal*  
697 *Statistical Society (B)* 73(1):3-36
- 698 Wood SN (2014) Mixed GAM Computation Vehicle with GCV/AIC/REML smoothness  
699 estimation: mgcv package
- 700 Zuur AF, Ieno EN, Elphick CS (2010a) A protocol for data exploration to avoid common  
701 statistical problems. *Methods in Ecology and Evolution* 1:3–14
- 702 Zuur AF, Ieno EN, Walker N, Saveliev A, Smith GM (2010b) Mixed effects models and  
703 extensions in ecology with R. *Statistics for Biology and Health* 549
- 704 Zuur AF, Ieno EN, Smith GM (2007) *Analyzing Ecological Data*. Springer Science &  
705 Business Media

706 **7 Figure Captions**

707 Figure 1- Map of sampling sites (black dots) along the two pH gradients of the Castello  
708 Aragonese on Ischia Island (Naples, Italy), with southern and northern sites depicted by  
709 'S' and 'N', respectively, and with 'C' indicating control pH, '2' low pH and '3' extreme  
710 low pH. Corresponding seawater carbonate data for each site is in Table 1 and all sites  
711 are in 3 m *Posidonia* seagrass meadows.

712

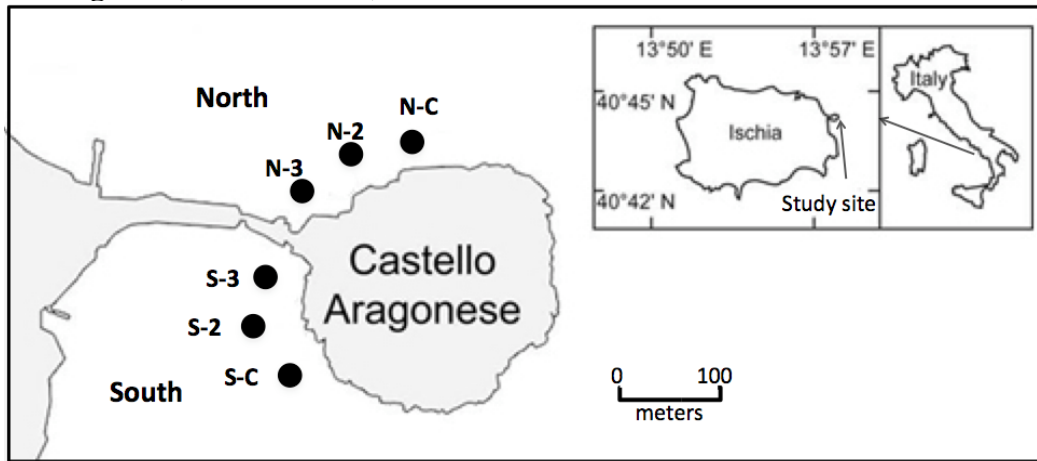
713 Figure 2 - Mean abundance of spirorbids sampled from south sites (SC, S2, S3) and north  
714 sites (NC, N1, N2), colored in red and gray respectively, and with 'C' indicating control  
715 pH, '2' low pH and '3' extreme low pH: (A) Total spirorbid abundance (all species  
716 combined) (B) *Simplaria* sp. abundance and (C) *P. militaris* abundance, with non-  
717 matching lowercase letters indicating significant differences among sites and S.E. as error  
718 bars.

719 Figure 3 – Total abundance of all spirorbids as they are related to *Posidonia* shoot  
720 density: mean number of spirorbids calculated as total species sampled *per* replicate plot  
721 area, multiplied by shoot density ( $m^2$ ), with S.D. as error bars.

722 Figure 4 - Trends in spirorbid species mean abundance (A) *P. militaris* and (B) *Simplaria*  
723 sp. Black dots: mean number of individuals found in each replicate along the northern  
724 gradient. Red dots: mean number of individuals found in each replicate along the  
725 southern gradient. Black lines are the smoothers for each gradient side; red and gray  
726 bands along smoother lines are 95 % CIs.

727 Figure 5- Fecundity traits and offspring survival from *Simplaria* sp. and *P. militaris*  
728 parents cultured in low and control pH conditions respectively, to match their field-  
729 originating pH values (7.6 and 8.1); purple and blue bars respectively. (A) Brood size is  
730 expressed as the mean number of offspring in the first brood release, (B) mortality as a  
731 percent of the beginning brood dead 7 d after initial brood release, and (C) settlement  
732 success as the percent of metamorphosed living offspring from each brood 1 day after  
733 brood release, (D) total survival as the mean number of offspring living 14 d after the  
734 initial brood release, plus any additional offspring released during the 14 d of exposure.  
735 Error bars show S.E.; each trait had significantly different means ( $p < 0.05$ ) between  
736 species groups.

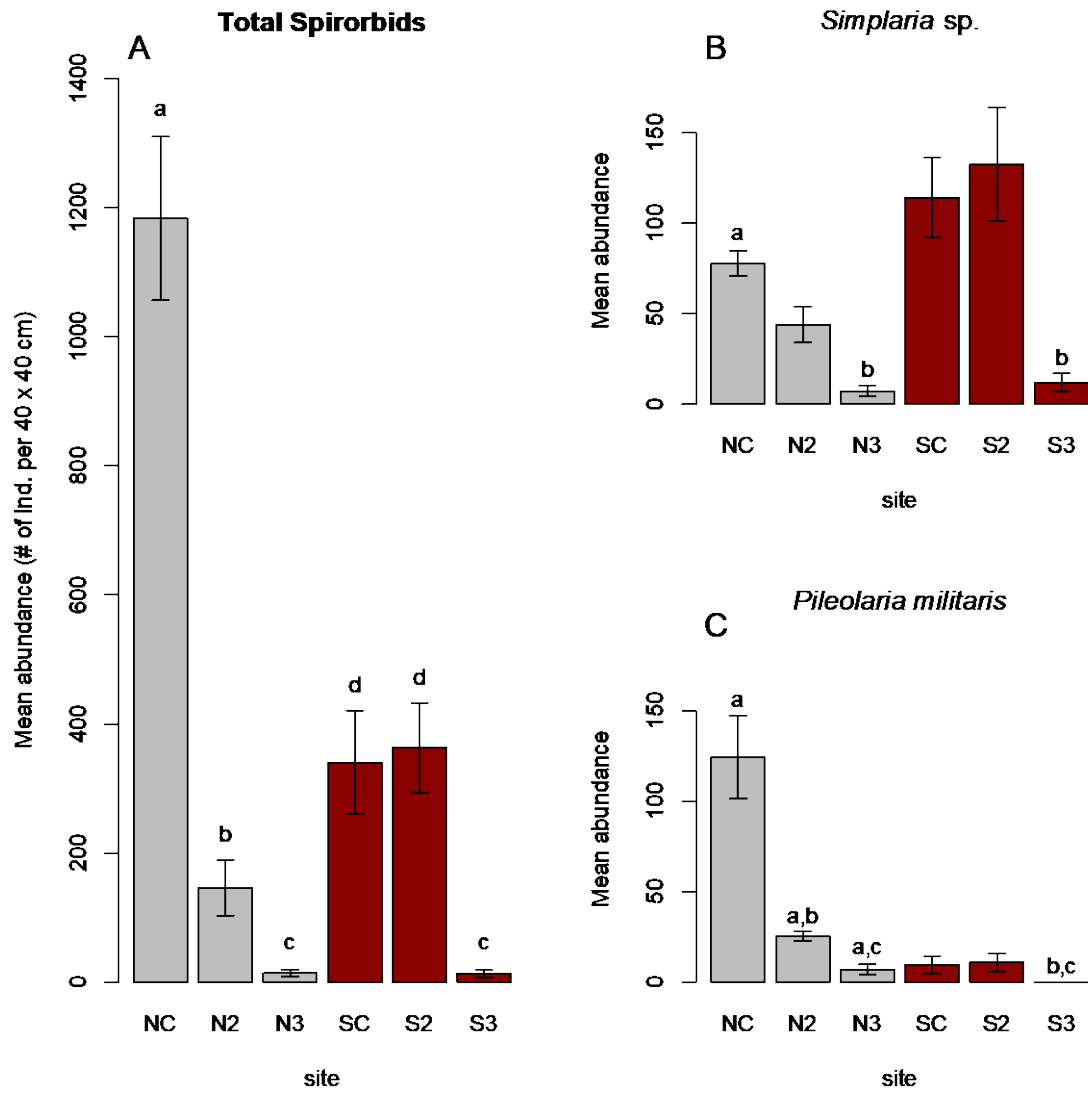
737 Figure 6 - (A) *Simplaria* sp. operculum containing embryos: embryonic calcified glands  
738 are indicated by white arrows (scale 0.5 mm), (B) a competent trochophore larvae from a  
739 *Simplaria* sp. mother (scale 0.1 mm)

740 **8 Figures (low definition)**

741

742 Figure 1

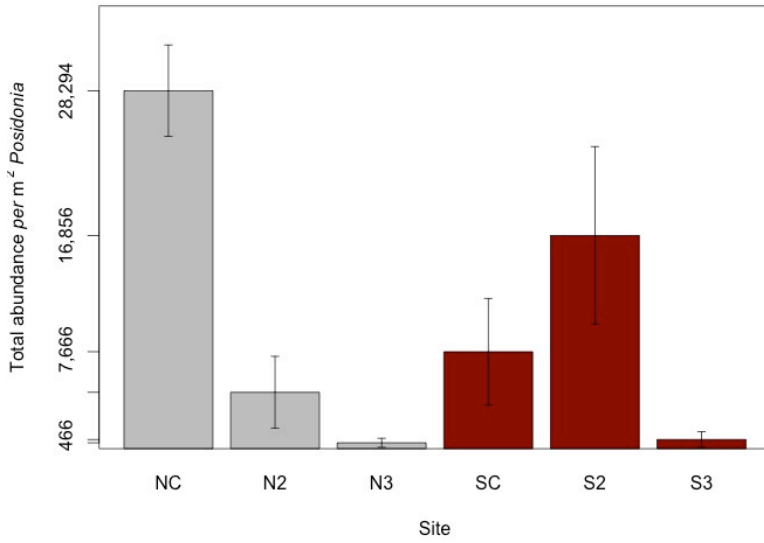
743



744

745 Figure 2

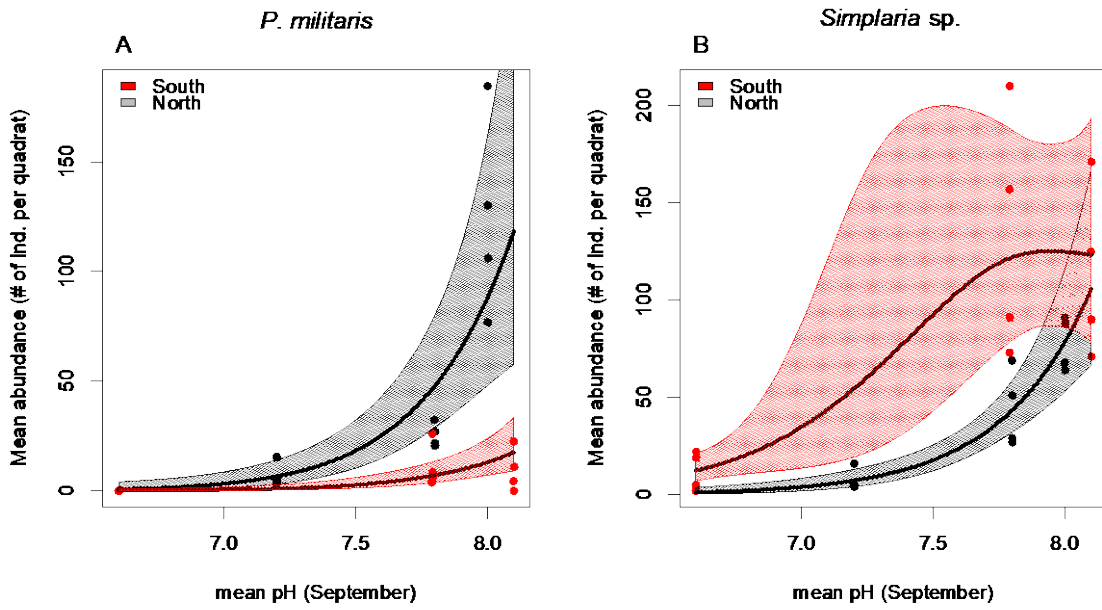
746



747

748 Figure 3

749

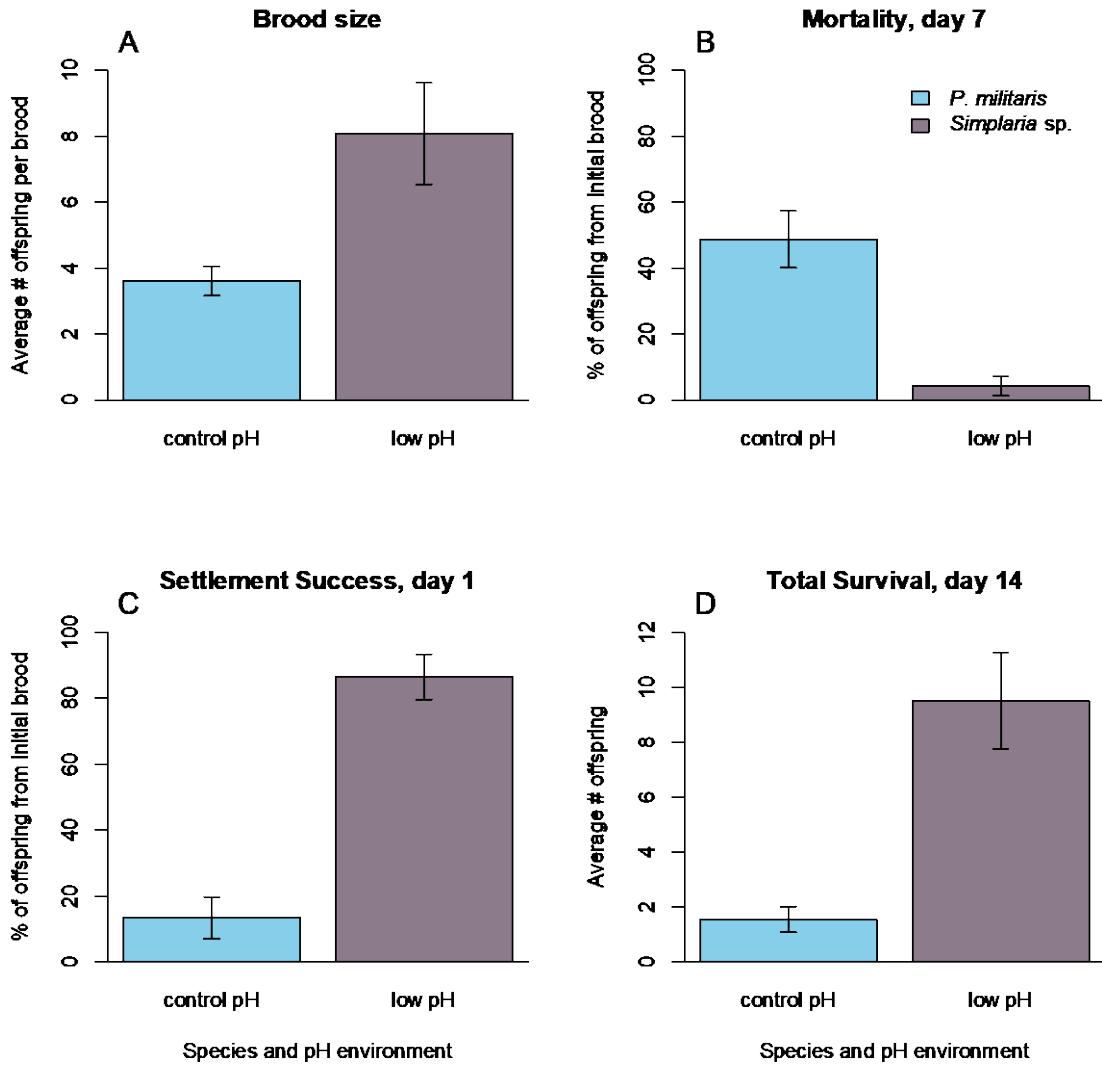


750

751 Figure 4

752

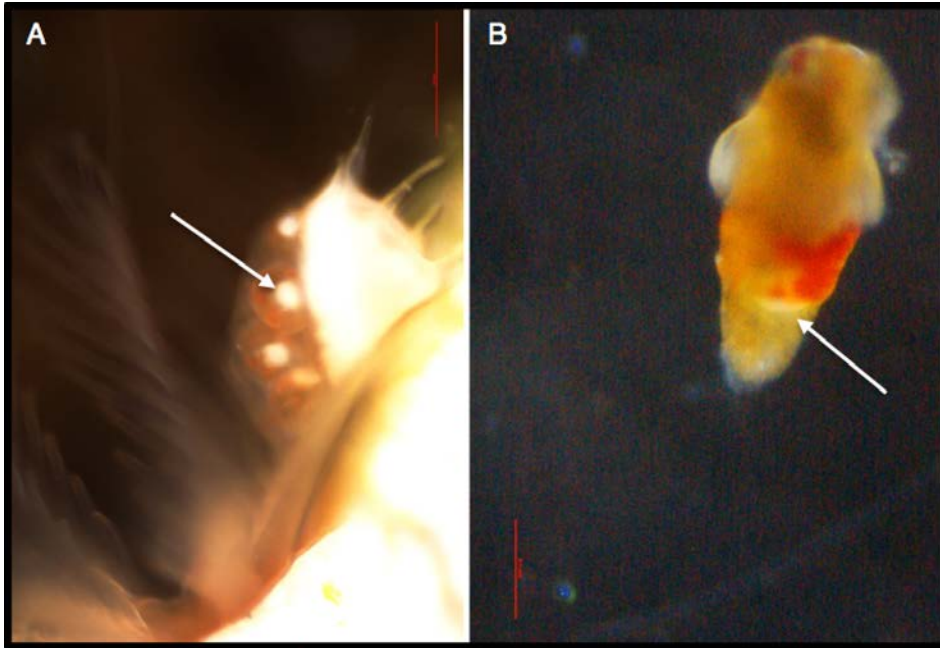
753



754  
755

Figure 5





756

757 Figure 6

758 **9 Tables**

759

760 Table 1 Seawater physico-chemical parameters from each pH site (mean  $\pm$  SD); averaged  
 761 from a published compilation of six time-series datasets between 2008-2015 in Ricevuto  
 762 et al. (2014).

763

Station	mean pH	$p\text{CO}_2(\mu\text{atm})$	$\Omega$ aragonite	$\Omega$ calcite	$A_T$ (equival $\text{kg}^{-1}$ )
Extreme low, S3	$6.99 \pm 0.34$	$8830.87 \pm 1942.55$	$0.75 \pm 0.50$	$0.99 \pm 0.65$	$2499.83 \pm 23.99$
Low, S2	$7.61 \pm 0.26$	$2031.19 \pm 1,411.65$	$1.49 \pm 0.61$	$2.52 \pm 0.95$	$2523.68 \pm 9.66$
Control, SC	$8.03 \pm 0.08$	$455.61 \pm 94.01$	$3.36 \pm 0.34$	$5.17 \pm 0.47$	$2499.35 \pm 6.94$
Extreme low, N3	$7.39 \pm 0.25$	$4302.71 \pm 5769.22$	$1.41 \pm 0.71$	$1.94 \pm 0.96$	$2549.45 \pm 25.26$
Low, N2	$7.65 \pm 0.29$	$2639.82 \pm 7993.29$	$2.07 \pm 0.70$	$2.91 \pm 1.23$	$2514.49 \pm 7.76$
Control, NC	$8.03 \pm 0.05$	$468.21 \pm 63.85$	$3.41 \pm 0.20$	$5.20 \pm 0.28$	$2499.67 \pm 4.68$

764

765 Table 2. Seawater physico-chemical parameters (a) at the field collection sites, and (b)  
 766 corresponding laboratory trial pH treatments (mean + SD), measured (in bold) or  
 767 calculated using the SeaCarb program\* over the total trial period for each habitat, either  
 768 daily (d) or monthly (m). pH is reported using the total scale.

	Control pH (SC)	Low pH (S2)
<i>(a) Field site data</i>		
<b>pH<sub>T</sub></b>	8.04 ± 0.09	7.84 ± 0.24
<b>Temperature</b> (°C)	23.4 ± 0.7	23.8 ± 0.7
<b>Salinity</b>	37.9 ± 0.3	37.9 ± 0.3
<b>A<sub>T</sub></b> (μmol kg <sup>-1</sup> )	2563 ± 3	2560 ± 7
<b>pCO<sub>2</sub></b> (μatm)	567 ± 100	1075 ± 943
<b>C<sub>T</sub></b> (mol kg <sup>-1</sup> )	0.002 ± 1.02E-04	0.002 ± 1.72E-04
<b>Ω calcite</b>	4.75 ± 0.53	3.52 ± 1.11
<b>Ω aragonite</b>	3.13 ± 0.35	2.32 ± 0.73
<i>(b) Laboratory trials</i>		
<b>pH<sub>T</sub></b> ( <i>days</i> )	8.08 ± 0.47	7.54 ± 0.53
<b>Temperature</b> (°C) ( <i>d</i> )	22.31 ± 0.57	22.17 ± 0.83
<b>Salinity</b> ( <i>d</i> )	36.38 ± 2.11	36.67 ± 2.87
<b>A<sub>T</sub></b> (μmol kg <sup>-1</sup> ) ( <i>m</i> )	2350.71 ± 53.70	2291.53 ± 122.55
<b>[CO<sub>2</sub>]</b> (mol kg <sup>-1</sup> )	9.65E-06 ± 3.10E-06	2.11E-05 ± 6.62E-06
<b>pCO<sub>2</sub></b> (μatm)	327.88 ± 108.21	721.73 ± 228.33
<b>[HCO<sub>3</sub><sup>-</sup>]</b> (mol kg <sup>-1</sup> )	0.002 ± 8.38E-05	0.002 ± 1.57E-04
<b>[CO<sub>3</sub><sup>2-</sup>]</b> (mol kg <sup>-1</sup> )	2.49E-04 ± 4.75E-05	1.42E-04 ± 2.55E-05
<b>C<sub>T</sub></b> (mol kg <sup>-1</sup> )	0.002 ± 4.601E-05	0.002 ± 1.47E-04
<b>Ω calcite</b>	5.82 ± 1.07	3.33 ± 0.60
<b>Ω aragonite</b>	3.82 ± 0.70	2.19 ± 0.39

\* Note: Lavigne & Gattuso 2013.

769  
770  
771

772 **10 Supplementary materials**

773

774 *Site Details:*

775 The north is relatively exposed to the dominant northwestern winds, and the south  
 776 is a bay-protected area. The venting area in the south is approximately 3000 m<sup>2</sup> and gases  
 777 are emitted at a rate of 1.4 x 10<sup>6</sup> L d<sup>-1</sup>. In the north, the area is only 2000 m<sup>2</sup> and the  
 778 venting rate is slightly decreased at 0.7 x 10<sup>6</sup> L d<sup>-1</sup> (Hall-Spencer et al. 2008). Specific  
 779 quantities of the emitted gases are comprised of the following: 90-95 % CO<sub>2</sub>, 3-6 % N<sub>2</sub>,  
 780 0.6-0.8 % O<sub>2</sub>, 0.2 - 0.08 % CH<sub>4</sub>, and 0.08-0.1 % Ar. No sulfur is present. Although  
 781 neither seasonal, tidal nor diurnal variation in gas flows have been recorded, the pH does  
 782 not stay static due to variable bubbling intensity, and shows quite variable values in  
 783 relatively short, hourly time frames (Kroeker et al. 2011).

<b>Site name and description:</b>	<b>Site GPS Coordinates:</b>
<b>SC: South Control</b>	40.729467, 13.964260
<b>S2: South Low pH</b>	40.730075, 13.963651
<b>S3: South Extreme Low pH</b>	40.731148, 13.963211
<b>NC: North Control</b>	40.732777, 13.965218
<b>N2: North Low pH</b>	40.732316, 13.964464
<b>N3: North Extreme Low pH</b>	40.732000, 13.963716

784

785 *Laboratory Trial Transport Details:*

786           Samples were transported from field sites by boat to the Villa Dohrn-Benthic  
787 Ecology Center and maintained inside 10 L coolers with fresh seawater from each of the  
788 collection sites. Samples were kept in seawater matching the pH level of their respective  
789 field origin; leaves were inspected to select for living spirobids, and cut in smaller  
790 portions to facilitate transport to the ENEA Laboratory in La Spezia, Italy. Transport  
791 containers were prepared with spirobids and unfiltered seawater (volume = 1300 mL; T  
792 =  $21.96 \pm 1.29$  °C; pH: control =  $8.03 \pm 0.08$ , low =  $7.61 \pm 0.26$ ; S = 36; density = approx.  
793 100 individuals *per* container) and kept in styrofoam coolers packed with ice to maintain  
794 a consistent water temperature. During the 8 h transport to ENEA, temperature and pH  
795 were recorded twice using a pH meter with integrated thermometer (SG2, Mettler-Toledo  
796 Analytical, Milan, Italy). The mean pH in the containers remained at 8.03 (control  
797 samples), or increased from 7.61 to 8.01 (low pH samples). The temperature decreased  
798 from 21.96 to 19.00 °C for 1 h in all containers. On arrival at the ENEA laboratory,  
799 containers were immediately placed in pre-conditioned temperature baths (T = 22.00 °C,  
800 S = 36). Temperature was controlled *via* two thermal baths connected to a temperature  
801 conditioner (TR 15, TECO, Naples, Italy) with heaters (V2-Them 300, São Julião do  
802 Tojal, Portugal). To enhance a homogeneous mixing of the water, and thus thermal  
803 stability of the system, submersible circulation pumps (Aquapump HJ-311, Mondial  
804 fauna, Milan, Italy) were also used. Containers were aerated with either ambient (control)  
805 air ( $p\text{CO}_2 \sim 380$   $\mu\text{atm}$ , for pH = 8.22), or CO<sub>2</sub>-enriched air ( $p\text{CO}_2 \sim 1000$   $\mu\text{atm}$ , for pH =  
806 7.70). CO<sub>2</sub> gas was slowly released into a Buchner flask to enable mixing using a CO<sub>2</sub>  
807 regulator (6000 CO<sub>2</sub>, BOC, La Spezia, Italy).

808 *Taxonomy Details:*

809           The tubes and operculum of both the adults and juveniles of the *Simplaria* sp.  
810 specimens found in this study closely resemble that of *Simplaria pseudomilitaris*  
811 (Thiriot-Quiévreux, 1965), a taxon first described in Villefrance sur Mer, France, and  
812 later identified in the Gulf of Naples by Harris (1968), although reported as *Spirorbis*  
813 *berkeleyana* (Rioja, 1942) (Knight-Jones et al. 1974), and in the fouling inside Port of  
814 Ischia, Italy (Terlizzi et al. 2000). Morphological similarities of the two are their sinistral  
815 coiled (clockwise) tube orientation, similar tube diameter (between 1.5-2 mm), latitudinal  
816 tube ridges, and 2-3 indistinct longitudinal tube ridges. The operculum also has a single  
817 opercular plate with ornamentation (protuberances, or spines, projecting from top of  
818 operculum). The operculum has been described having an elliptical cap with a partially  
819 encircling distal papillated rim that is absent on the substratum side (see also Bianchi  
820 1981), yet this feature is not in agreement with this study's specimens, where the rim  
821 completely surrounds the distal papillated rim. This feature is, however, in better  
822 agreement with a description of *S. pseudomilitaris* from the west coast of the USA made  
823 by Beckwitt (1981), who further noted the high variation in operculum morphology in the  
824 species. The primary trait that is found in the *Simplaria* sp. specimens of this study that is  
825 not in agreement with the *S. pseudomilitaris* descriptions from the literature is the extent  
826 of 'ornamentation' on the operculum plate (e.g. Bianchi 1981; Fig. 6).

827           The morphology of the *Simplaria* sp. here also closely matches *Pileolaria*  
828 *quasimilitaris* with respect to larval and operculum morphology, a taxon first described in  
829 the Caribbean Sea (Bailey 1970). In particular, *P. quasimilitaris* has distally projecting  
830 calcareous spines on the operculum that form a complete crown. However, it still does

831 not completely agree as there are up to three indeterminate rows of long, slender spines  
832 observed in the operculum crown center in this study's specimens *versus* the two rows of  
833 spines originally described for *P. quasimilitaris*. Two other difference between these two  
834 species are in the tubes and chaetae: this study's specimens have latitudinal ridges and 2-  
835 3 indistinct longitudinal ridges, and no sickle chaetae on the third thoracic fascicles,  
836 *versus* the many longitudinal ridges and knobs of *P. quasimilitaris* and presence of these  
837 sickle chaetae.

838         The key taxonomic feature for the genus *Pileolaria* is the presence of sickle  
839 chaetae on the third thoracic fascicles (Knight-Jones et al. 1974). In the sister genus  
840 *Simplaria* erected by Knight-Jones (1984), all of the characters of the genus *Pileolaria*  
841 are found, except the sickle chaetae in the third thoracic fascicles. After examining over  
842 40 *Simplaria* sp. specimens from this study, no sickle chaetae were found. In the original  
843 description of *P. quasimilitaris* by Bailey (1970) the chaetae of the third thoracic fascicle  
844 are defined as "hooked" chaetae. The morphology of sickle chaetae, is, however, quite  
845 variable (Knight-Jones & Fordy 1979) and "hooked" chaetae, *sensu* Bailey (1970) can be  
846 considered as sickle chaetae. Regardless, the specimens of this study also lacked hooked  
847 chaetae in the third thoracic fascicles. Therefore this relevant character, sickle chaetae  
848 absence, leads us to exclude the attribution to our specimens to any other *Pileolaria*  
849 species with spines on the operculum (e.g., as *P. semimilitaris*, Vine et al. 1972), and  
850 consider our taxon as a member of the *Simplaria* genus, and be considered or a  
851 morphotype/ecotype of *S. pseudomilitaris* having more abundant, longer, pronounced  
852 distally projecting calcareous spines covering the operculum plate, or a new species from  
853 the genus of *Simplaria*. The opercular morphology is a character quite variable in this

854 species, which has lead also to confusion of *S. pseudomilitaris* with other species (e.g.  
 855 *Spirorbis regalis* in Bailey and Harris (1968)). Analysis of additional *Simplaria*  
 856 *pseudomilitaris* material from both type locality and other areas could help to account for  
 857 the possible variability in opercular morphology, however only a genetic analysis would  
 858 help to determine the correct species status. Yet this is beyond the scope of this study.  
 859 Therefore, the specimens of this study are designated as *Simplaria* sp.

### 860 *Supplementary Tables*

861 Table S.1 Number of spirorbids identified in each site replicate, and the ratio of  
 862 specimens identified (ID'ed) at the species level to the total number of specimens found.  
 863 These ratios were used to calculate the number of *Simplaria* and *P. militaris* in each  
 864 replicate, as complete identification of each specimen was not possible due to lost  
 865 taxonomic features.

Site	Repli- cate	Total spp.	<i>P.</i> <i>militaris</i>	<i>Simplaria</i> sp.	Other spp.	ID'ed	Ratio ID'ed	<i>Simplaria</i> sp. %
<b>NC</b>	A	146	62	48	13	123	0.84	39%
	B	178	68	54	12	134	0.75	40%
	C	161	26	32	12	59	0.37	54%
	D	243	78	29	5	112	0.46	26%
<b>N2</b>	A	50	12	16	0	28	0.56	57%
	B	80	19	33	2	54	0.68	61%
	C	54	25	35	1	61	1.13	57%
	D	94	20	54	1	75	0.8	72%
<b>N3</b>	A-D	46	5	5	0	10	0.22	50%
<b>SC</b>	A	113	4	28	3	35	0.31	80%
	B	71	1	25	0	25	0.35	100%
	C	132	0	57	2	59	0.45	97%
	D	180	5	78	5	83	0.46	94%
<b>S2</b>	A	75	2	39	1	42	0.56	93%
	B	106	9	42	3	54	0.51	78%
	C	234	1	99	3	103	0.44	96%
	D	158	0	41	1	42	0.27	98%
<b>S3</b>	A-D	47	0	13	0	13	0.28	100%

866