

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**

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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 (CO₂), 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 CO₂, 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 CO₂ 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 CO₂ 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 ± 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 29th - 30th, 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 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 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 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 $p\text{CO}_2$ (μatm), total carbon dioxide (TCO_2 , mol kg^{-1}),

196 bicarbonate concentration (HCO_3^- mol kg^{-1}), calcite saturation (Ω_{ca}), and aragonite
197 saturation (Ω_{ara}) were calculated from A_T , pH_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 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 (mm²)
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 residuals² / 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 CO₂ 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*² in S3 compared to the mean 467 shoots
308 *per m*² in SC, and 719 to 380 shoots *per m*² 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 ± 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 (± 1.54) vs. 3.61 (± 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 % (± 6.8) compared to 13.4 % (± 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 % (± 2.9) in
348 *Simplaria* sp. and 48.8 % (± 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 (± 1.7) vs. 1.5 (± 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 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 CO₂ vents, where
466 the ecological exclusion of *P. dumerilii* in the high CO₂ 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.

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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

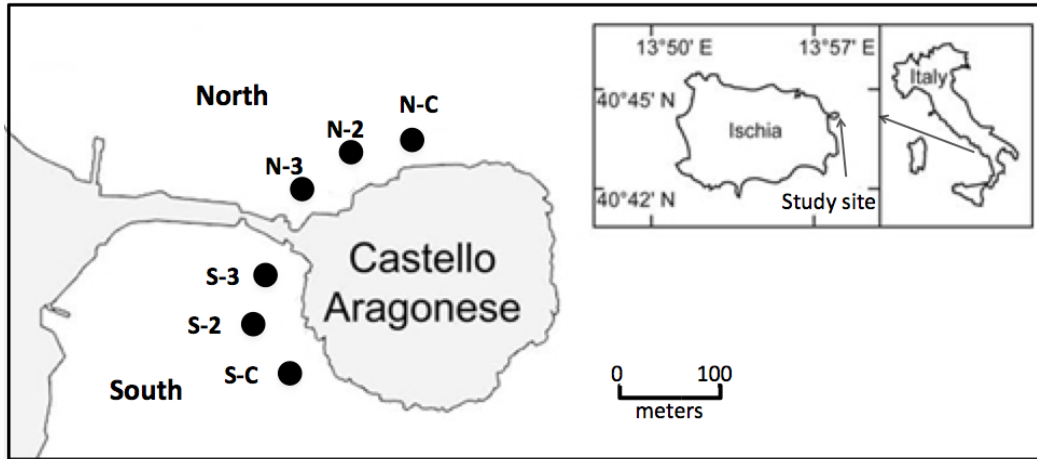
713 Figure 2 - Mean abundance of spirorbids sampled from south sites (SC, S2, S3) and north
714 sites (NC, N1, N2), colored in red and gray respectively, and with 'C' indicating control
715 pH, '2' low pH and '3' extreme low pH: (A) Total spirorbid abundance (all species
716 combined) (B) *Simplaria* 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 (m²), 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.

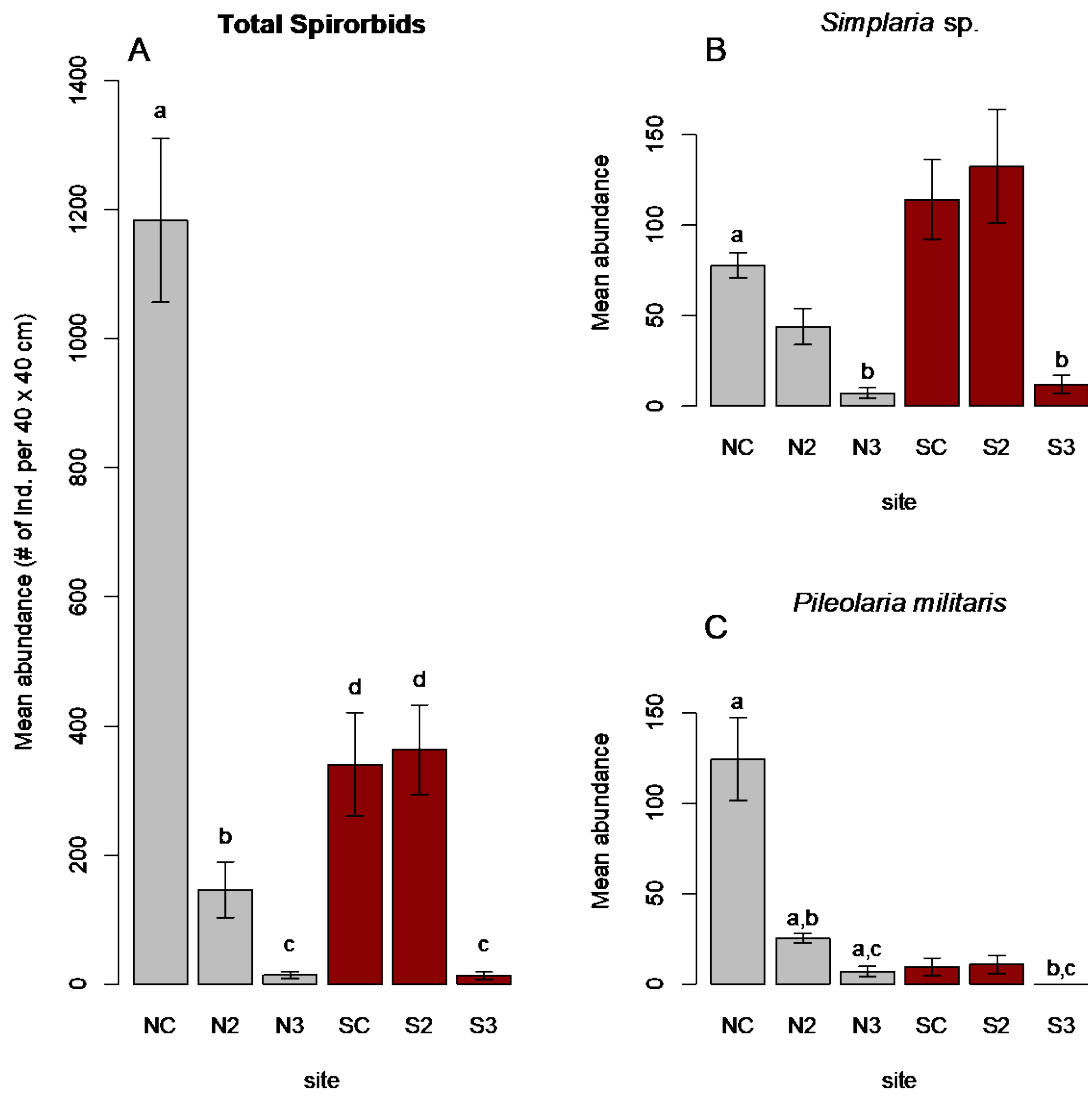
737 Figure 6 - (A) *Simplaria* sp. operculum containing embryos: embryonic calcified glands
738 are indicated by white arrows (scale 0.5 mm), (B) a competent trochophore larvae from a
739 *Simplaria* sp. mother (scale 0.1 mm)

740 **8 Figures (low definition)**

741

742 Figure 1

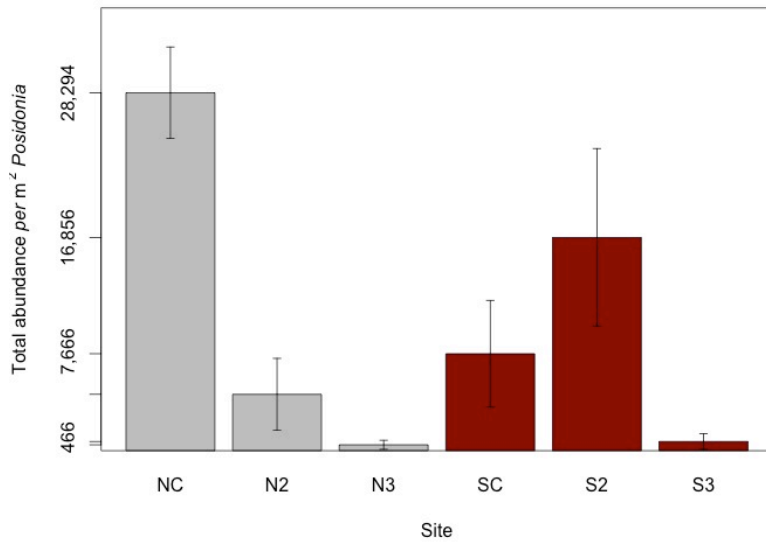
743



744

745 Figure 2

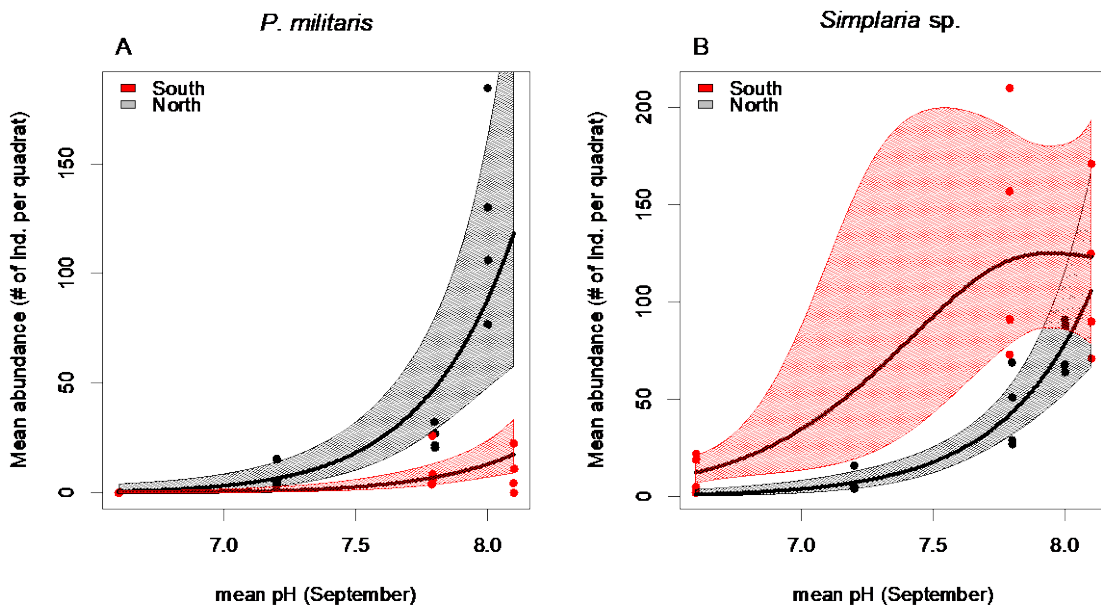
746



747

748 Figure 3

749

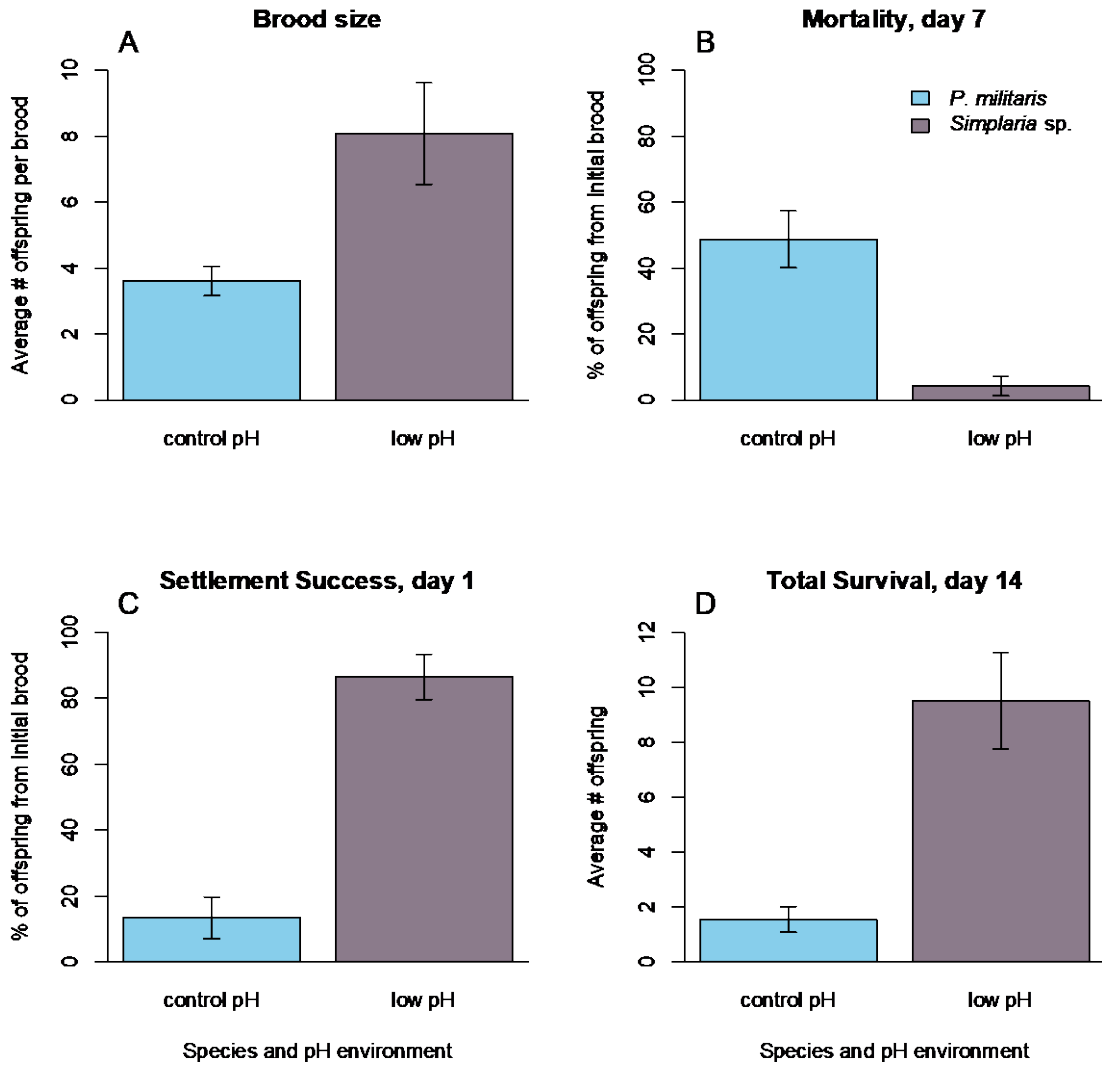


750

751 Figure 4

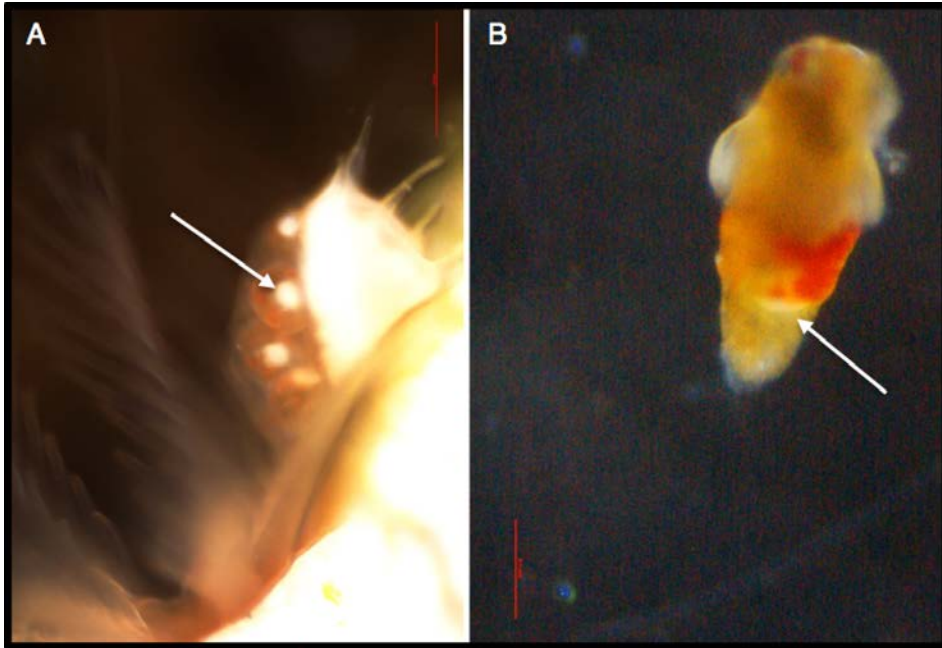
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Figure 5



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	$p\text{CO}_2(\mu\text{atm})$	Ω aragonite	Ω calcite	A_T (equival kg^{-1})
Extreme low, S3	6.99 ± 0.34	8830.87 ± 1942.55	0.75 ± 0.50	0.99 ± 0.65	2499.83 ± 23.99
Low, S2	7.61 ± 0.26	$2031.19 \pm 1,411.65$	1.49 ± 0.61	2.52 ± 0.95	2523.68 ± 9.66
Control, SC	8.03 ± 0.08	455.61 ± 94.01	3.36 ± 0.34	5.17 ± 0.47	2499.35 ± 6.94
Extreme low, N3	7.39 ± 0.25	4302.71 ± 5769.22	1.41 ± 0.71	1.94 ± 0.96	2549.45 ± 25.26
Low, N2	7.65 ± 0.29	2639.82 ± 7993.29	2.07 ± 0.70	2.91 ± 1.23	2514.49 ± 7.76
Control, NC	8.03 ± 0.05	468.21 ± 63.85	3.41 ± 0.20	5.20 ± 0.28	2499.67 ± 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>		
pH_T	8.04 ± 0.09	7.84 ± 0.24
Temperature (°C)	23.4 ± 0.7	23.8 ± 0.7
Salinity	37.9 ± 0.3	37.9 ± 0.3
A_T (μmol kg ⁻¹)	2563 ± 3	2560 ± 7
pCO₂ (μatm)	567 ± 100	1075 ± 943
C_T (mol kg ⁻¹)	0.002 ± 1.02E-04	0.002 ± 1.72E-04
Ω calcite	4.75 ± 0.53	3.52 ± 1.11
Ω aragonite	3.13 ± 0.35	2.32 ± 0.73
<i>(b) Laboratory trials</i>		
pH_T (<i>days</i>)	8.08 ± 0.47	7.54 ± 0.53
Temperature (°C) (<i>d</i>)	22.31 ± 0.57	22.17 ± 0.83
Salinity (<i>d</i>)	36.38 ± 2.11	36.67 ± 2.87
A_T (μmol kg ⁻¹) (<i>m</i>)	2350.71 ± 53.70	2291.53 ± 122.55
[CO₂] (mol kg ⁻¹)	9.65E-06 ± 3.10E-06	2.11E-05 ± 6.62E-06
pCO₂ (μatm)	327.88 ± 108.21	721.73 ± 228.33
[HCO₃⁻] (mol kg ⁻¹)	0.002 ± 8.38E-05	0.002 ± 1.57E-04
[CO₃²⁻] (mol kg ⁻¹)	2.49E-04 ± 4.75E-05	1.42E-04 ± 2.55E-05
C_T (mol kg ⁻¹)	0.002 ± 4.601E-05	0.002 ± 1.47E-04
Ω calcite	5.82 ± 1.07	3.33 ± 0.60
Ω aragonite	3.82 ± 0.70	2.19 ± 0.39

769 * Note: Lavigne & Gattuso 2013.

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 m² and gases
 777 are emitted at a rate of 1.4 x 10⁶ L d⁻¹. In the north, the area is only 2000 m² and the
 778 venting rate is slightly decreased at 0.7 x 10⁶ L d⁻¹ (Hall-Spencer et al. 2008). Specific
 779 quantities of the emitted gases are comprised of the following: 90-95 % CO₂, 3-6 % N₂,
 780 0.6-0.8 % O₂, 0.2 - 0.08 % CH₄, 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).

Site name and description:	Site GPS Coordinates:
SC: South Control	40.729467, 13.964260
S2: South Low pH	40.730075, 13.963651
S3: South Extreme Low pH	40.731148, 13.963211
NC: North Control	40.732777, 13.965218
N2: North Low pH	40.732316, 13.964464
N3: North Extreme Low pH	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 ± 1.29 °C; pH: control = 8.03 ± 0.08 , low = 7.61 ± 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 ($p\text{CO}_2 \sim 380$ μatm , for pH = 8.22), or CO₂-enriched air ($p\text{CO}_2 \sim 1000$ μatm , for pH =
806 7.70). CO₂ gas was slowly released into a Buchner flask to enable mixing using a CO₂
807 regulator (6000 CO₂, 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-Quévieux, 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. %
NC	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%
N2	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%
N3	A-D	46	5	5	0	10	0.22	50%
SC	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%
S2	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%
S3	A-D	47	0	13	0	13	0.28	100%

866