

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

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ABSTRACT: Low-pH vent systems are ideal natural laboratories to study the consequences of long-term low-pH exposure on marine species and thus identify life-history traits associated with low-pH tolerance. This knowledge can help to inform predictions on which types of species may be less vulnerable in future ocean acidification (OA) scenarios. Accordingly, we investigated how traits of calcifying polychaete species (Serpulidae, Spirorbinae) varied with pH using a functional trait analysis at 2 natural pH gradients around the Castello Aragonese islet off Ischia, Italy. We first observed the distribution and abundance patterns of all calcifying polychaete epiphytes in the canopy of *Posidonia oceanica* seagrass across these gradients. We then used laboratory trials to compare fecundity, settlement success, and juvenile survival in the dominant species from a control (*Pileolaria militaris* Claparède, 1870) and a low-pH site (*Simplaria* sp.). We found significantly higher reproductive output, juvenile settlement rates, and juvenile survival in *Simplaria* sp. individuals from the low-pH site, compared to *P. militaris* individuals from control pH sites, when observed in their respective *in situ* pH conditions. Our results suggest that tolerance to low pH may result, in part, from traits associated with successful reproduction and rapid settlement under low-pH conditions. This finding implies that other species with similar life-history traits may respond similarly, and should be targeted for future OA tolerance research.

KEY WORDS: Ocean acidification · Calcifiers · Settlement success · Fecundity · Early-life survival · Serpulidae · Population resilience

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INTRODUCTION

Anthropogenically driven global changes may reduce or alter marine biodiversity (Raven et al. 2005, Widdicombe & Spicer 2008). One such change, ocean acidification (OA), occurs when surface seawater absorbs increasing atmospheric CO₂, resulting in lower

pH and reduced availability of the carbonate ions many marine organisms require to build skeletal structures (IPCC 2014). Despite confidence in forecasts of the chemical impacts from this process into the next century (Bopp et al. 2013, IPCC 2014), uncertainty surrounds the corresponding biological and ecological impacts (Harley 2011, Gaylord et al. 2015).

Determining sensitivities and tolerances to future OA conditions represents a necessary first step in improved understanding of how marine biodiversity will change in the next decades (Stockwell et al. 2003, van Oppen et al. 2015).

Functional trait analyses (FTA) can help determine sensitivity or tolerance of different species to low pH conditions. These analyses link environmental gradient survey data (e.g. species abundance patterns) to specific phenotypic trait changes (e.g. body size, reproductive habit, fecundity) in 2 or more species along the same gradients (McGill et al. 2006). The assumption is that by comparing a specific trait among phylogenetically similar species (i.e. sister species, or species derived from a common ancestral node), differences can be attributed to specific environmental effects. In the context of future OA, application of specific functional trait analyses along natural pH gradients provides a relatively straightforward tool to evaluate which types of species will play pivotal roles in reorganizing the biodiversity landscape (McGill et al. 2006).

Abundance surveys performed in volcanic vent systems, which emit CO₂, have recently proven useful in identifying traits associated with invertebrate species sensitivities and tolerances to future OA change (Lucey et al. 2015, Gambi et al. 2016). For example, marine invertebrates without a pelagic life stage (i.e. brooders or direct developers) are more abundant in low-pH sections of CO₂ vent gradients (Lucey et al. 2015). Identification of a specific brooding oyster species with increased survivability in a low-pH upwelling environment (Waldbusser et al. 2016) reinforced this pattern. Unfortunately, natural system assessments generally lack trait data regarding fecundity, larval survival, settlement, and recruitment (however, see Padilla-Gamiño et al. [2016] for coralline algae assessment). This gap partly exists because field observations cannot provide detailed data involving a temporal element (e.g. settlement rates) or observations requiring thorough quantification of small-scale processes (e.g. egg production or fecundity). Collecting and observing such traits in a laboratory setting that mimics the individuals' *in situ* conditions can help to fill this gap. Combining such laboratory trials with natural system-based assessments may offer one mechanism to strengthen analyses of some functional traits.

The sensitivity of early developmental stages underscores the need to understand marine invertebrate life histories within future OA scenarios (Kurihara 2008, Albright 2011, Byrne 2012, Crook et al. 2016). As such, our study aims to identify fecundity and

early life-history traits associated with low-pH tolerance in marine invertebrates using calcifying serpulid polychaetes (Spirorbinae) along 2 natural pH gradients. These gradients border the Castello Aragonese islet off the coast of Ischia (Naples, Italy) and are formed when volcanically derived CO₂ gas bubbling up through the seafloor mixes with seawater, decreasing the pH from an ambient value of ~8.17 to as low as 6.57 (Tedesco 1996, Hall-Spencer et al. 2008, Kroeker et al. 2011, Garrard et al. 2014), well representing business-as-usual IPCC pH projections for 2100 (IPCC 2014).

We focus on calcareous polychaetes of the subfamily Spirorbinae, which inhabit *Posidonia oceanica* seagrass leaves as epiphytes along the Castello pH gradients. Generally, Spirorbinae polychaetes encompass a diverse suite of life-history traits that can vary among closely related species in the free-swimming larval phases and sessile adult stages (Macdonald 2003, Kupriyanova et al. 2001, 2006).

First, we assess the distribution and abundance patterns of the calcifying polychaete assemblage on *P. oceanica* leaves across 2 natural pH gradients bordering the Castello Aragonese islet: one along the north (mean pH range 7.39–8.03), and one along the south (mean pH range 6.99–8.03). Second, we use laboratory trials comparing 2 closely related species—one from a low pH site and the other from a control pH site—to investigate possible links between fecundity, juvenile survival, settlement traits, and low-pH tolerance. Knowing which of these life-history traits are associated with low-pH tolerance will help inform our predictions on the types of species that may be less vulnerable in future ocean acidification scenarios.

MATERIALS & METHODS

Field survey

The pH gradients used in this study occur along the north and south sides of the Castello Aragonese islet off Ischia (Fig. 1). To represent 3 pH levels, 6 sampling sites were chosen within *Posidonia oceanica* seagrass meadows at depths of approximately 3 ± 0.5 m. Sites are referred to as N3, N2, NC and S3, S2, SC, where '3' represents extremely low pH, '2' low-pH conditions, and 'C' the control pH. The 'N' and 'S' represent north and south gradients (Fig. 1). Sites were chosen to be comparable in depth and *Posidonia* cover (Donnarumma et al. 2014). The site names are listed with their corresponding carbonate

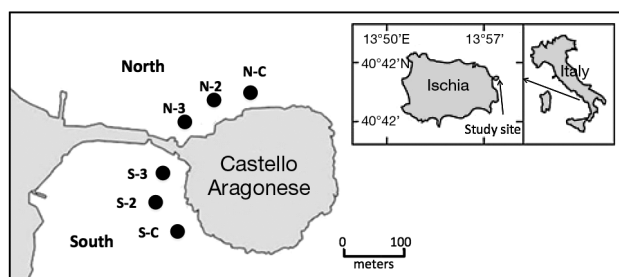


Fig. 1. Sampling sites (black dots) along the 2 pH gradients with southern and northern sites depicted by 'S' and 'N', respectively, and with 'C' indicating control pH, '2' low pH and '3' extremely low pH. Corresponding seawater carbonate data for each site is given in Table 1. All sites were located at 3 m depth in *Posidonia* seagrass meadows

seawater parameters in Table 1. Seawater parameters represent a synthesis of all available data in the last 6 yr to convey the most comprehensive and realistic time-series data for these study sites (Ricevuto et al. 2014). For additional water parameters and GPS coordinates, see Supplement 1 at www.int-res.com/articles/suppl/m589p141_supp.pdf.

To determine which calcifying polychaete species settle on *Posidonia* leaves in low-pH environments, and how their abundance and distribution varies along the pH gradients, sampling by SCUBA diving was performed on 29 to 30 September 2014. Four quadrats (replicates) of 40 × 40 cm were haphazardly placed at least 2 m apart on the seagrass canopy in each pH site. Within each quadrat, leaves of 10 *Posidonia* shoots were randomly cut at the base of the rhizome and put in separate plastic bags. In the 2 extremely low pH sites (N3 and S3), initial visual inspection showed a reduced number of worms on leaves. Consequently, the number of sampled shoots was increased by cutting only the external leaf (oldest leaf) of 30 shoots within each of the 4 quadrats in both N3 and S3. This provided a more reliable estimate of worm abundance and helped preserve the seagrass from impact due to sampling. Samples were

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

The number of calcifying polychaetes on the *Posidonia* leaves of each shoot was determined by viewing each leaf from each replicate/quadrat under a dissecting microscope (AZ100, Nikon, Milan, Italy; magnification 1–50×). Species were identified from their tube orientation, operculum, and chaetae morphology. Due to the loss of some opercula, some specimens remained unidentified. These were included in the counts by determining the ratio between the number of specimens identified for a given species and the total number of specimens found at each site. This ratio was used to calculate the total number of each species for each site replicate (see Supplement 2).

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

Laboratory trials

One day after the field survey, live individuals were collected for the laboratory trial by SCUBA diving. This entailed cutting *Posidonia* leaves with visibly attached polychaete tubes from the S2 and NC sites. Leaves were placed in fabric bags, keeping the leaves from both sites separated and in their original seawater conditions.

Table 1. Seawater physico-chemical parameters from each site (mean ± SD), averaged from a published compilation of 6 time-series datasets between 2008 and 2015 in Ricevuto et al. (2014), including saturation state (Ω) of aragonite and calcite, and total alkalinity (A_T)

Station	pH	pCO_2 (μatm)	Ω aragonite	Ω calcite	A_T (equival. kg^{-1})
Extremely 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 ± 1411.65	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
Extremely 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

All material was transported to the ENEA Laboratory in La Spezia, Italy, where specimens were sorted, identified, and prepared for the trial (Supplement 3). Specimens were held at the pH conditions from their respective field sites (control or low pH) during the sorting process (2 to 4 d). For the trial, we identified 18 *Pileolaria militaris* adults from NC and 12 *Simplaria* sp. adults S2.

These sites were chosen because they have the greatest average pH difference, and also have adequate numbers of adults. Species were chosen based on the finding that S2 was dominated by *Simplaria* sp. and NC was dominated by *P. militaris*. Additionally, the lack of *Simplaria* sp. individuals from NC, and lack of *Pileolaria militaris* individuals from S2 precluded a reciprocal laboratory transplant experiment. This in itself demonstrates that different species have different levels of sensitivity to low-pH conditions, and thus, the comparison in life-history traits between the 2 selected species represents the best avenue to identify traits that help define species' successful colonization in low pH conditions. As in most classical functional trait analyses, this between-species comparison is able to capture valuable information.

At the start of the trial, each adult was placed in a separate Petri dish preconditioned with a biofilm from a 2 d non-filtered seawater soak and filled with 3 ml of pH-conditioned filtered seawater (1 adult per Petri dish). The pH in the dishes was set at 7.61 for *Simplaria* sp. adults, representing the average value found in the S2 field site considering time-series data (Table 1). Similarly, dishes with *P. militaris* adults were maintained at the control pH value of 8.1. All other seawater parameters matched the field values for both species (Table 2). In this setup, 8 covered aquaria were filled with 20 ml of seawater. This water served as a bath for the uncovered Petri dishes (6 to 8 dishes per aquaria). Half of the aquaria were maintained at the low pH level by bubbling enriched (elevated $p\text{CO}_2$) air into the seawater, while the other half was maintained at the control pH level by bubbling normal air into the seawater. The pH inside each Petri dish was attained through surface CO_2 diffusion within the covered aquaria (Gattuso & Hansson 2011). The $p\text{CO}_2$ bubbled into the aquaria was measured continuously throughout the exposure period using a CO_2 gas analyzer (Li-820, Li-Cor Biosciences). All

Table 2. Seawater physico-chemical parameters (a) at the field collection sites and (b) corresponding laboratory trial pH treatments (mean \pm SD), measured (in **bold**) or calculated using the SeaCarb program (Lavigne & Gattuso 2013) over the total trial period for each habitat. pH is reported using the total scale

	Control pH (SC)	Low pH (S2)
(a) Field site data		
pH_T	8.04 \pm 0.09	7.84 \pm 0.24
Temperature (°C)	23.4 \pm 0.7	23.8 \pm 0.7
Salinity	37.9 \pm 0.3	37.9 \pm 0.3
A_T ($\mu\text{mol kg}^{-1}$)	2563 \pm 3	2560 \pm 7
pCO₂ (μatm)	567 \pm 100	1075 \pm 943
C_T (mol kg^{-1})	0.002 \pm 1.02E-04	0.002 \pm 1.72E-04
Ω calcite	4.75 \pm 0.53	3.52 \pm 1.11
Ω aragonite	3.13 \pm 0.35	2.32 \pm 0.73
(b) Laboratory trials		
pH_T	8.08 \pm 0.47	7.54 \pm 0.53
Temperature (°C)	22.31 \pm 0.57	22.17 \pm 0.83
Salinity	36.38 \pm 2.11	36.67 \pm 2.87
A_T ($\mu\text{mol kg}^{-1}$)	2350.71 \pm 53.70	2291.53 \pm 122.55
[CO ₂] (mol kg^{-1})	9.65E-06 \pm 3.10E-06	2.11E-05 \pm 6.62E-06
pCO₂ (μatm)	327.88 \pm 108.21	721.73 \pm 228.33
[HCO ₃ ⁻] (mol kg^{-1})	0.002 \pm 8.38E-05	0.002 \pm 1.57E-04
[CO ₃ ²⁻] (mol kg^{-1})	2.49E-04 \pm 4.75E-05	1.42E-04 \pm 2.55E-05
C_T (mol kg^{-1})	0.002 \pm 4.601E-05	0.002 \pm 1.47E-04
Ω calcite	5.82 \pm 1.07	3.33 \pm 0.60
Ω aragonite	3.82 \pm 0.70	2.19 \pm 0.39

aquaria were held in a thermal water bath that maintained stable thermal conditions. Petri dishes were randomly moved between the aquaria every 2 d.

Seawater pH, temperature, and salinity were measured in each Petri dish daily with an integrated pH and temperature meter (SG2), and refractometer (V2, TMC). The pH meter was calibrated daily with pH buffer standards (4.01, 7.0, 9.21; Mettler-Toledo). Seawater samples (250 ml) were taken at the beginning and end of the trial from the stock seawater prepared for each treatment. Samples were fixed with HgCl_2 (0.02%) to eliminate microbial activity, stored in borosilicate flasks (250 ml), and maintained in dark, dry conditions until total alkalinity (A_T) was determined using gran titration method (Dickson et al. 2007). Carbonate-system parameters of $p\text{CO}_2$ (μatm), total carbon dioxide (TCO_2 , mol kg^{-1}), bicarbonate concentration (HCO_3^- , mol kg^{-1}), calcite saturation (Ω_{ca}), and aragonite saturation (Ω_{ara}) were calculated from A_T , pH_T (total scale), temperature, and salinity using the package SeaCarb v.2.4.8 in software R (Lavigne & Gattuso 2013). Water-chemistry parameters for each dish during the 14 d experimental phase as well as discreet field data from each pH site are presented in Table 2.

Seawater in each Petri dish was changed every other day by removing water with a syringe and re-

placing it with new seawater. This water was collected from La Spezia bay (La Spezia, Italy) and cleaned with a 0.1 μm filter and UV sterilization system (V2ecton 600, TMC) for 5 d before being transferred to sterile 2 l flasks. One flask was prepared for each treatment and placed in the temperature bath described above with bubbling elevated $p\text{CO}_2$ air, or normal air, depending on the treatment. Additionally, a diet of rotifers, *Artemia* sp., and microalgae was added to seawater before each water change at 3 ml feed per 300 l seawater (Gamma Nutraplus Reef Feed, TMC). Petri dishes were mixed 3 times per day by gently tilting aquaria to promote feeding. The density of worms in each Petri dish was purposefully low to avoid potential indirect effects caused by animal respiration (approx. $<0.092 \text{ mg}^{-1}$).

Under these laboratory conditions, all adults were monitored once a day with a light microscope (AZ100, Nikon; magnification ranges of 25 \times up to 50 \times) for the presence of embryos in the opercular brooding chamber. After adults released their first brood, both adults and offspring were monitored daily for the following 14 d. The number of offspring from each parent (brood size) was counted after the first day of brood release. The number of settled larvae was counted daily, along with any deaths or additional broods. The parent tubes were photographed with a digital camera (Nikon Sight DS-U1, Nikon) mounted on a light microscope (AZ100, Nikon) and tested as a trait covariate to account for any bias between parental size and offspring traits (i.e. brood size, mortality, and brood survival). Photographs were analyzed with ImageJ software (Rasband WS, US National Institutes of Health) to obtain tube area (mm^2) (Abràmoff et al. 2004).

Data analysis

Field survey data

Two data sets generated from the field survey were analyzed: (a) the abundance of all calcifying polychaete species along the north and south pH gradients (distribution); (b) the abundance of the 2 dominant species, *Simplaria* sp. and *P. militaris*, adults along the pH gradients. Initial data exploration using Cleveland dot- and boxplots revealed no outliers in either dataset. Conditional boxplots revealed heteroscedasticity of the variances among the pH sites for both datasets, and histograms indicated violation of normality (Züür et al. 2010a). Non-linear patterns within the species-level dataset also existed (Züür et al. 2010a). As a consequence, a Welch's ANOVA with

a Games-Howell post-hoc test was used for both datasets to assess how the number of calcifying polychaetes varied along pH gradient, with 'gradient side' (north/south) and 'pH site' as fixed factors. This test is robust to non-parametric distribution of count data and heteroscedasticity of the variances.

Additionally, dataset (b) was analyzed by employing generalized additive models (GAMs) (Wood 2006, 2011, 2014, Züür et al. 2010b) to describe the abundance of each species with respect to nominal 'pH' and to compare the abundance of each species along both gradient 'sides'. GAMs accounted for the non-linear patterns in both the *Simplaria* sp. and *P. militaris* datasets and were built using the *mgcv* (Wood 2011) and *nlme* (Pinheiro et al. 2015) packages in R. For both species, gradient 'side' (factor: north or south) and 'pH' (fitted as a smoother) were set as the explanatory variables. Nominal mean water pH for each gradient side and site was based on the 1 mo average of September data from Kroeker et al. (2011) to accurately represent seasonal pH values during the survey. The appropriate degrees of freedom (df) of the smoothers were selected automatically using cross validation (Wood 2006, 2011). For *Simplaria* sp. only, the interaction between gradient side and pH was included using the 'by' command in the *mgcv* package (Wood 2011). Both models were optimized by initially looking for the optimal random structure, followed by the optimal fixed structure (Züür et al. 2007). Akaike information criteria were used to compare models, and residual plots were used to assess the mean-variance relationships; models for both species indicated no violation of the assumption for homogeneity of the variances. Overdispersion was also calculated for each model (sum of Pearson residuals² / residual df). High overdispersion, particularly in *Simplaria* sp., required the use of negative binomial distribution with a log link (Pinheiro et al. 2015, Züür et al. 2007, 2010b). The optimization function of the models (k parameter) was adjusted for this study's specific dataset at 6. All statements about abundance change are based on the significance of the main effect gradient side and not on the interaction between gradient side and pH.

Laboratory trials

To assess how fecundity and early life-history traits differed in response to differing pH conditions, we compared responses of the low-pH-originating *Simplaria* sp. adults (from and dominant in S2) under low pH conditions, to the control-pH-originating *P. mili-*

taris adults (from and dominant in NC) under control pH conditions with 1-way ANOVA tests. Traits analyzed included: brood size of each parent, time of larval release to settlement (d), and percentage brood mortality per parent on Days 7 and 14. Data were tested for normality of distribution and homogeneity of variance using Cleveland dot- and boxplots. Boxplots indicated homogeneity of the variances among the pH species groups, and histograms indicated no violation of normality of distribution (Zür et al. 2010a).

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

RESULTS

Field survey

Species identity

All of the taxa found belonged to the Spirorbinae sub-family, within the Serpulidae family. The 4 main species were *Pileolaria militaris* Claparde, 1870, *Simplaria* sp., *Janua heterostropha* (Montagu, 1803) (= *J. (Dexiospira) pagenstecheri*), and *Neodexiospira pseudocorrugata* (Bush, 1905). A total of 48 undetermined Serpulidae were also encountered.

The taxonomy of the *Simplaria* sp. did not exactly match known records, but our primary assumption is that it is a putatively novel morphotype of *Simplaria pseudomilitaris* (Thiriou-Quievreux, 1965); having more abundant, longer, and more pronounced distally projecting calcareous spines covering its operculum plate. Without further taxonomic analysis, we designated this species *Simplaria* sp. and this is further discussed in Supplement 4.

Species abundance and distribution

Total polychaete abundances on the *Posidonia* leaves along the pH gradient from the CO₂ vents ranged from 0 to 224 ind. shoot⁻¹. There was a decrease in mean abundance, as the the total number of individuals per quadrat replicate, from the control pH sites (SC and NC) to the extremely

low pH sites (S3 and N3) along both the north and the south gradients, with a decline from 341 to 13 individuals in the south (SC to S3) and from 1183 to 14 individuals in the north (NC to N3) ($F_{5, 92.97} = 75.11$, $p < 0.001$, Fig. 2A). The total number of individuals in each quadrat replicate, averaged by site, in both the northern and southern extremely low pH sites (N3 and S3) were comparable ($p > 0.05$, Fig. 2A). However, overall mean abundance was 3-fold lower in the southern gradient compared to the north ($p < 0.05$, Fig. 2A). Additionally, in the north, there was a strong linear relationship between abundance and pH conditions. This differed from the south gradient, where mean abundance in the low pH site was highest (S2: 144 ind.), compared to the control pH site (SC: 124 ind.; $p > 0.05$; Fig. 2A). Higher shoot densities in the low pH sites compared to the control pH sites (Donnarumma et al. 2014) did not change the overall abundance patterns observed on both the north and south gradients (Fig. 3). For example, polychaete densities remained very scarce in the extremely low pH sites despite a mean of 1000 shoots m⁻² in S3 compared to 467 shoots m⁻² in SC, and 719 to 380 shoots m⁻² in N3 vs. NC.

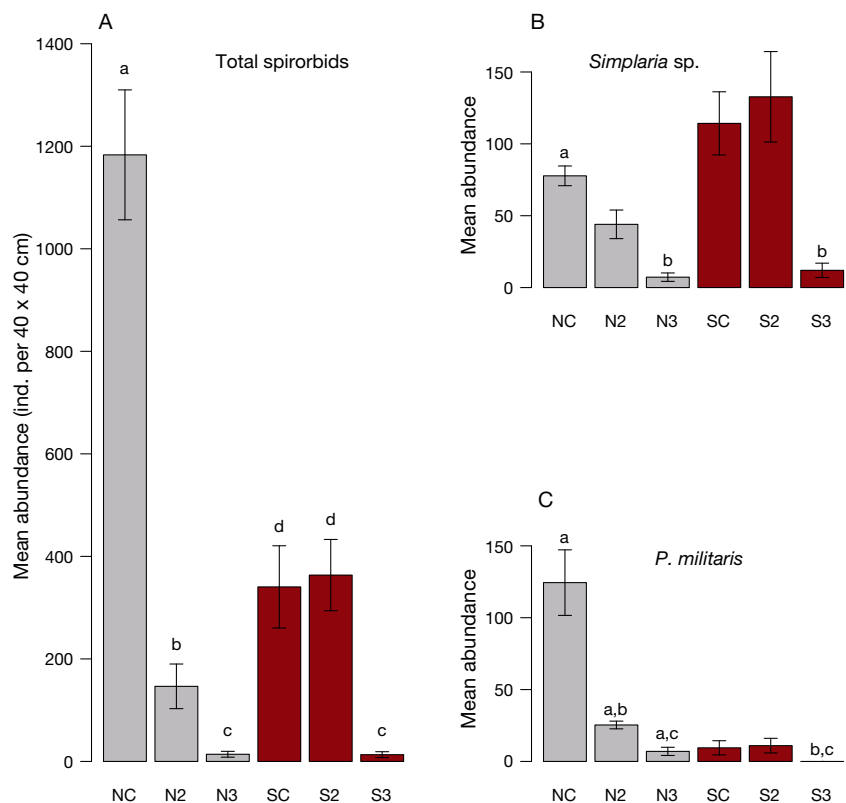


Fig. 2. Abundance of spirorbids sampled from southern (S) and northern (N) sites. (A) Total spirorbids (all species combined); (B) *Simplaria* sp.; (C) *Pileolaria militaris*. C: control pH; 2: low pH; 3: extremely low pH. Different lowercase letters indicate significant differences among sites. Data are mean \pm SE

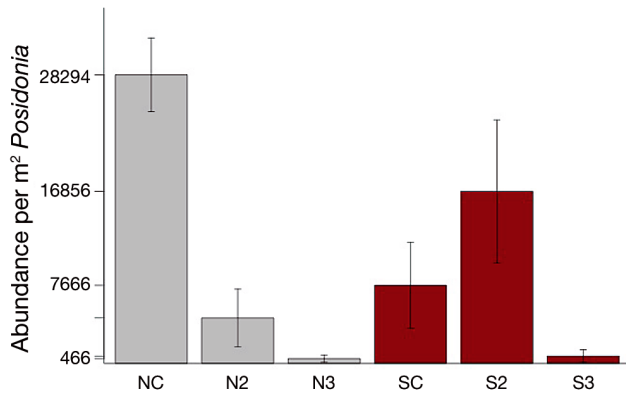


Fig. 3. Total abundance of all spirorbids accounting for site differences in *Posidonia* shoot density. Data are mean \pm SD

The results also indicate that the 2 dominant species were *Simplaria* sp. and *P. militaris* within all the sites. These 2 species are also closely related to each other taxonomically, compared to the other species identified. This prompted separate analyses of the distributions of both *Simplaria* sp. and *P. militaris*. In the species-specific analysis, the total abundance along the pH sites ranged from 0 to 498 individuals of *P. militaris* and from 48 to 532 individuals of *Simplaria* sp. While the overall number of individuals for both species was comparable, their distribution differed. As in the total species analysis, abundances significantly declined with decreasing pH when considering all sample sites (*P. militaris*: $F_{4,11} = 9.37$, $p = 0.006$, *Simplaria* sp.: $F_{5,78} = 24.27$, $p < 0.001$; Fig. 2B,C). The mean abundance of *P. militaris* regardless of site pH was highest in the north compared to the south gradient (49 vs. 7, respectively) and decreased from NC to N3, and from S2 to S3. *Simplaria*

sp. mean abundance was higher in the south than in the north gradient. The *Simplaria* sp. abundance in S2 was not significantly different from mean abundance at SC but was different in the north between the NC and N3 extremely low pH site. Additionally, *Simplaria* sp. was the only spirorbid species found at S3 (pH: 6.99 ± 0.34) (Hofmann et al. 2011).

Comparisons of the smoothers (non-parametric curves) generated by the additive mixed models for the 2 gradients of both species confirmed that abundance decreases in both species with decreasing nominal pH across each gradient ($p < 0.001$ for both *P. militaris* and *Simplaria* sp.; Fig. 4). For *P. militaris*, there were significant declines in abundance with decreasing pH along both north and south gradients; however, the northern gradient had significantly more individuals compared to the southern gradient. In contrast, *Simplaria* sp. abundances in the north and south were not significantly different when pH values were >7.9 within the gradient (Fig. 4B).

Laboratory trials

The laboratory trials revealed that life-history trait values varied significantly along the pH gradients. The average number (± 1 SE) of offspring per brood from low-pH-originating *Simplaria* sp. parents was significantly higher than from control-pH-originating *P. militaris* parents: (mean \pm SE) 8.08 ± 1.54 vs. 3.61 ± 0.44 offspring brood⁻¹ ($F_{1,28} = 10.80$, $p = 0.003$; Fig. 5). Also, settlement success was significantly higher in *Simplaria* sp. compared to *P. militaris*: (mean \pm SE) $86.5 \pm 6.8\%$ compared to 13.4 ± 6.3 ($F_{1,28} = 58.80$, $p < 0.001$; Fig. 5). Additionally, all offspring from the

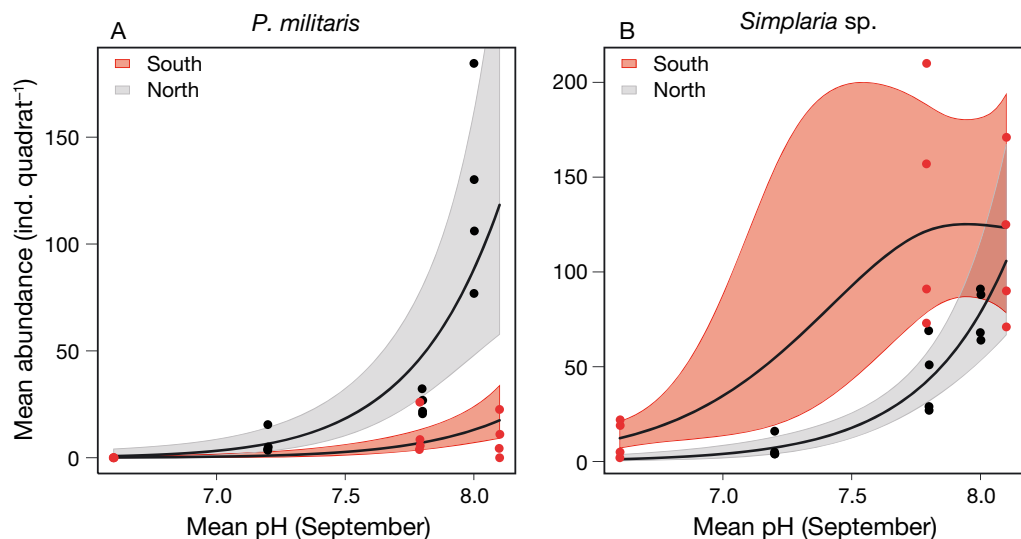


Fig. 4. Trends in spirorbid species mean abundance: (A) *Pileolaria militaris* and (B) *Simplaria* sp. Black/red dots: mean number of individuals found in each replicate along the northern/southern gradient. Black lines are the smoothers for each gradient side; red and gray bands along smoother lines are 95% CI

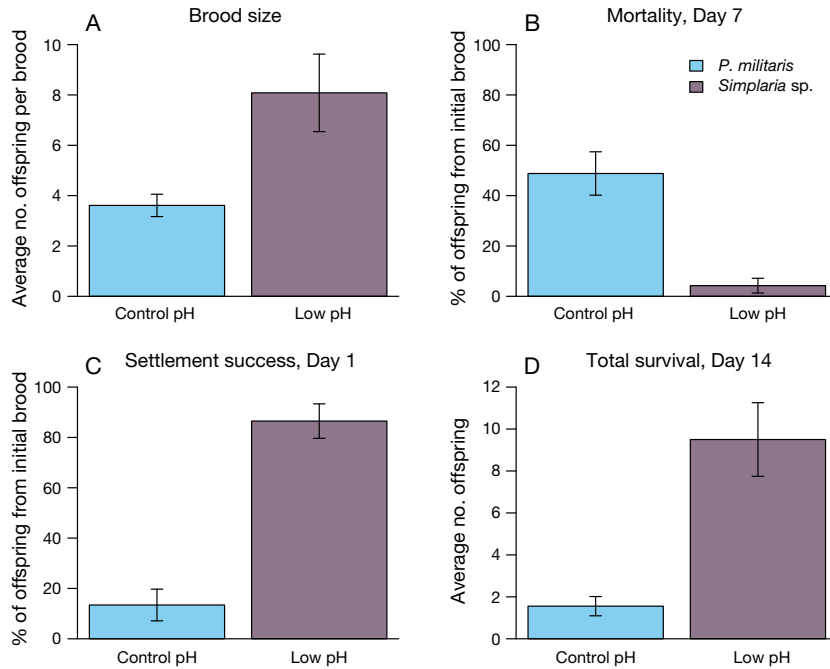


Fig. 5. Fecundity traits and offspring survival from *Simplaria* sp. and *P. militaris* parents cultured in low- and control pH conditions, respectively, to match their field-originating pH values (7.6 and 8.1). (A) Brood size is expressed as the mean number of offspring in the first brood release, (B)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

Simplaria sp. parents metamorphosed and settled within 1 h in low-pH seawater, whereas <13% of the offspring from *P. militaris* parents settled in the first 24 h in control conditions.

Juvenile mortality rates 7 d after the first brood release were (mean \pm SE) $4.2 \pm 2.9\%$ for *Simplaria* sp. and $48.8 \pm 8.6\%$ for *P. militaris* ($F_{1,28} = 16.77$, $p < 0.001$; Fig. 5). Net survival after 14 d, including additional offspring from subsequent broods, was significantly higher (6.3-fold) in *Simplaria* sp. offspring with respect to *P. militaris* offspring: (mean \pm SE) 9.5 ± 1.7 vs. 1.5 ± 0.4 offspring parent⁻¹, respectively ($F_{1,28} = 26.90$, $p < 0.001$; Fig. 5). Furthermore, between Day 7 and Day 14, 10 out of 12 parents released a second brood in the *Simplaria* sp. group, but only 4 out of 18 parents from the *P. militaris* group produced a second brood. No influence of parental tube size was found on brood sizes ($p \geq 0.05$), and no parental mortality occurred during the 14 d trial.

DISCUSSION

This study aimed to identify specific life-history traits that offer species potential advantages to tolerating future OA conditions. We identified 2 primary species along the 2 pH gradients with a close phylogenetic relationship, *Pileolaria militaris* and *Simplaria* sp., and found that the higher abundances of *Simplaria* sp. in low pH (S2) were associated with the ability to produce more viable offspring able to quickly metamorphose and settle in low pH conditions compared to that of its close relative dominant at a control pH site (NC), *P. militaris*, observed under control pH conditions. Below we discuss potential physiological and ecological explanations for differences in traits underlying species' sensitivity to low pH.

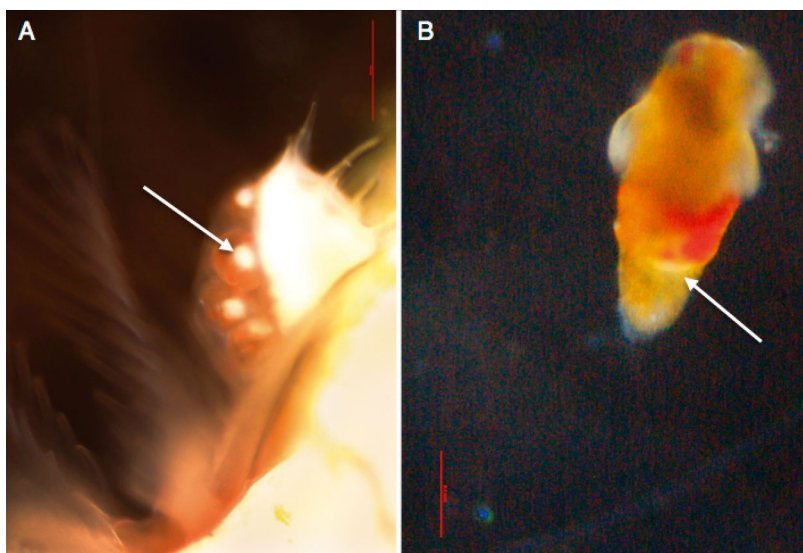


Fig. 6. (A) *Simplaria* sp. operculum containing embryos: embryonic calcified glands are indicated by white arrows (scale 0.5 mm), (B) a competent trochophore larvae from a *Simplaria* sp. mother (scale 0.1 mm)

Physiology considerations

The most noticeable finding was that rapid offspring development accompanied OA tolerance, as demonstrated by the production of larvae that metamorphose in minutes in the low-pH-originating *Simplaria* sp. under low-pH conditions, compared to the multiple days required for control-pH-originating *P. militaris* individuals in control conditions. This is noteworthy because the challenges of calcification associated with metamorphosis and initial juvenile tube growth in many marine invertebrates exposed to OA conditions have been well documented, with demonstrated altered metamorphosis, slowed juvenile growth, weakened juvenile tubes, and tube dissolution under pH levels comparable to those used here (Dupont et al. 2009, Byrne 2012, Lane et al. 2013). In contrast, our results indicate that the low-pH-originating *Simplaria* sp. appears to have overcome these challenges. We hypothesize that this may be, in part, due to specialized larval glands that are able to expedite the secretion of a primary tube, resulting in successful metamorphosis. These specialized larval glands are commonly found in Serpulidae species with lecithotrophic (non-feeding) larvae (Kupriyanova et al. 2001), but while both of the species here have primary larval glands, there were noticeable physiological differences. The *Simplaria* sp. embryos and larvae had highly defined, large glands compared to *P. militaris* (see Fig. 6; white spots in the *Simplaria* sp. embryos). Moreover, in *Simplaria* spp., the contents of the primary shell gland are extruded via the anus and the calcareous secretion is molded by the movements of the larva into a tube capable of housing the entire settled larva in <5 min (Nott 1973, Knight-Jones 1978, Potswald 1978, Beckwitt 1980, Qian 1999).

Another interesting finding was the increased adult fecundity of low-pH-originating *Simplaria* sp., compared to control-pH-originating *P. militaris*. This was despite no significant difference in opercular brood chamber size between the 2 species (chamber size is directly proportional to the adult's overall size, and thus the number of offspring produced per brood) (Kupriyanova et al. 2001). In general, both species fertilize and incubate their eggs and embryos similarly: in a single chamber that provides aeration and physical protection from the outside environment (Thorpe 1975). When ready, competent larvae exit these chambers through a pore at its base (Macdonald 2003). Explaining the fecundity differences may therefore involve testing for improved internal fertilization, and/or accelerated embryo incubation

in the low-pH-originating *Simplaria* sp., comparatively (Chaparro et al. 2008, Segura et al. 2010).

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

Rapid metamorphosis and increased fecundity was also coupled with lower offspring mortality during the first 2 wk of offspring life in the low-pH-originating *Simplaria* sp. group. This suggests that these *Simplaria* sp. will have a higher likelihood of recruitment success and overall population persistence compared to the control-pH-originating *P. militaris* group (Hunt & Scheibling 1997). The field survey supports this idea: *Simplaria* sp. adults with embryos were found at every site along the gradient regardless of pH.

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

Ecological considerations

Predation may also be playing a role in the distribution of spirorbid species around the CO₂ vents, and in their pH tolerance traits. Increases in spirorbids predation are likely as there have been documented increases in amphipod and copepod abundance in the low-pH sites, known spirorbid predators (Knight-Jones et al. 1974, Kupriyanova et al. 2001). This helps explain the decrease in spirorbids at the extreme low pH sites. Furthermore, it suggests a potential correlation between increased predation and the novel opercular spine morphology observed in the low-pH-originating *Simplaria* sp., where rows of long, slender calcareous spines project from the top of the operculum and guard the tube opening. Further investigation is necessary to prove this hypothesis (e.g. Harris 1968, Knight-Jones et al. 1974, Bianchi 1981; also see Supplement 4). There are also indirect predation threats that may be influencing the spirorbid distributions: the very prominent reduction in overall *Posidonia* canopy height at the low-pH sites as a consequence of intense grazing from the fish *Sarpa salpa* (Deudero et al. 2008), compared to lower density long-leaved shoots in the control-pH sites (Donnarumma et al. 2014, Scartazza et al. 2017). The increased grazing pressure under highly acidified conditions could explain the decreased spirorbid abundance, as fish grazing removes epiphytic invertebrates (Deudero et al. 2008). Additionally, this variation in the *Posidonia* canopy may indirectly be related to the observed low-pH-originating *Simplaria* sp.'s fast juvenile growth. Because Spirorbinae are small filter feeders that spend the majority of their lives inside tubes permanently attached to a substrate (Gee 1964, Potswald 1968, Tanur et al. 2010), the organisms in low pH/intense grazing may be rapidly maturing as a response to host plant phenology, a feature that has been highlighted for other *Posidonia* epiphytes (Piazzi et al. 2016).

In addition to predation, it is possible that there are biological interactions between the 2 species, *Simplaria* sp. and *P. militaris*, which are responsible for their distributions. They may be competing with each other for available space or food, or may have different water movement requirements (Beckwitt 1980, Terlizzi et al. 2000). These factors may be contributing to the relative success of *P. militaris* in the north, compared to its limited southern abundance. The northern sites are more exposed to open water and dominant winds (from north and north-west), whereas the southern sites are within a small bay with less water movement (Cigliano et al. 2010). This could

mean that *Simplaria* sp. populations are better suited to live in more sheltered conditions, or that they are able to fill a niche where conditions are less stable due to pH. A parallel example is provided by the differential occurrence of 2 non-calcifying polychaete sister species, *Platynereis dumerilii* and *P. massiliensis*, around the Ischia CO₂ vents, where the ecological exclusion of *P. dumerilii* in the high-CO₂ areas appears to be explained by differences in physiological and life-history traits (Lucey et al. 2015). For the spirorbids, a more complete trait analysis able to encompass the full relevant trait space (i.e. testing population samples of each species from all sites and a broader array of traits) would be useful to elucidate which factors are most relevant to explain OA-resistant phenotypes (Laughlin & Messier 2015).

CONCLUSIONS

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

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