Ocean acidification affects calcareous tube growth in adult stage and reared offspring of serpulid polychaetes

V. Díaz-Castañeda[1*], T. E. Cox[2,3*], F. Gazeau[3], S. Fitzer[4], J. Delille[3], S. Alliouane[3], J.-P. Gattuso[3,5]

* corresponding authors who contributed equally to this work

[1]Centro de Investigación Científica y Educación Superior de Ensenada, Departmento de Ecología Marina. Carret. Tij. - Ensenada 3918, C.P. 22860 Ensenada, Baja California, México.

[2] University of New Orleans, Department of Biological Sciences 2000 Lakeshore Drive New Orleans, LA, 70148 USA

[3] Sorbonne Université, CNRS, Laboratoire d'Océanographie de Villefranche, 181 chemin du Lazaret, F-06230 Villefranche-sur-mer, France

[4] Institute of Aquaculture, University of Stirling, FK9 4LA, Scotland, UK

[5] Institute for Sustainable Development and International Relations, Sciences Po, 27 rue Saint Guillaume, F-75007 Paris, France

Published in Journal of Experimental Biology 2019 222: jeb196543 by Company of Biologists.
The original publication is available at: https://doi.org/10.1242/jeb.196543

ABSTRACT

The energetically costly transition from free-swimming larvae to benthic life stage and maintenance of a calcareous structure can make calcifying marine invertebrates vulnerable to ocean acidification. The first goal of this study was to evaluate the impacts of ocean acidification on calcified tube growth for two Serpulidae polychaete worms. *Spirorbis* sp. and *Spirobranchus triqueter* were collected at 11 m depth from the Northwest Mediterranean Sea and maintained for 30 and 90 d, at three mean pH_T levels (total scale) of 8.1 (ambient), 7.7, and 7.4. Moderately decreased tube elongation rates were observed in both species at a pH_T of 7.7 while severe reductions occurred at pH_T 7.4. There was visual evidence of dissolution and tubes were more fragile at lower pH but, fragility was not attributed to changes in fracture toughness. Instead, it appeared to be due to the presence of larger alveoli covered in a thinner calcareous layer. The second objective of the study was to test for effects in offspring development of the species *S. triqueter*. Spawning was induced, and offspring were reared in the same pH conditions the parents experienced. Trochophore size was reduced at the lowest pH level but settlement success was similar across pH conditions. Post-settlement tube growth was most affected. At 38 d post-settlement, juvenile tubes at pH_T of 7.7 and 7.4 were half the size of those at pH_T 8.1. Results suggest future carbonate chemistry will negatively affect initiation and persistence of both biofouling and epiphytic polychaete tube worms.

Key words: pH, *Spirorbis* sp., *Spirobranchus triqueter*, trans-generational effects, tube worms

INTRODUCTION

The uptake of anthropogenic carbon dioxide (CO_2) by the ocean generates changes in the carbonate chemistry of seawater. Dissolved inorganic carbon increases whereas the concentration of carbonate ions (CO_3^{2-}) and pH decrease. This process is known as ocean acidification and it can have deleterious effects on some species, particularly calcifiers. The impact of ocean acidification on invertebrates has been studied in the past two decades (Byrne, 2011; Kroeker et al., 2013; reviewed by: Kurihara, 2008). Many prior studies have shown that adult invertebrate calcifiers exposed to elevated CO_2 partial pressure (pCO_2) exhibited physiological stress and reduced calcification rates (Gazeau et al., 2007; Miles et al., 2007; Pörtner et al., 2004).

Serpulidae is a large family of sedentary benthic polychaete worms that have a worldwide distribution and secrete and live within calcareous tubes. The family includes members from the subfamilies Spiborbinae, Filograninae and Serpulinae (Ten Hove and Kupriyanova, 2009). They live in varied marine habitats and are economically relevant as one of the most significant groups of marine fouling invertebrates. For example, many have undesirable effects such as clogging of seawater intake pipes and fouling of ship hulls (Bastida-Zavala et al., 2017; Hoagland and Turner, 1980). Many serpulids are epiphytes on marine plants or live attached to rocks and shells and a few species aggregate to form calcified reefs (Kupriyanova et al., 2001; Smith et al., 2005). They are also often major ecological components of marine communities; they occur in great numerical abundance or biomass, add structural complexity to the habitat, and link pelagic and benthic food webs by filter feeding (Kupriyanova et al., 2001).

The mineralogy and structure of serpulid polychaete tubes could make them vulnerable to ocean acidification (Smith et al., 2013). The calcareous tube is formed by secretions from a pair of exocrine glands in the ventral part of the peristomium; these glands are in contact with the surrounding seawater and discharge a mucopolysaccharide matrix (Neff, 1971) that induces the precipitation of calcium carbonate ($CaCO_3$) from seawater. The mixture of $CaCO_3$ and mucopolysaccharides is then deposited to the leading edge of the tube by the collar. The timing and the details of the formation can be species-specific but, it tends to occur rapidly (within hours) and appears necessary for survival (Rouse and Pleijel, 2001). Furthermore, unlike many other groups in which calcification is impacted by ocean acidification such as corals, crustaceans, and molluscs, most serpulid tubes lack an external organic layer which would isolate their calcified structures from the surrounding seawater and protect them from negative effects (Ries et al., 2009). Serpulid tubes vary in skeletal mineralogy from aragonite, a form of $CaCO_3$ highly soluble at low pH, to moderate to high-Mg calcite and many tubes are a mixture of both forms (Smith et al., 2013; Vinn et al., 2008). The few studies which have investigated low pH effects on serpulid tube growth have found a range of responses from no effect at elevated temperature (Chan et al. 2013) to reduced growth rates and changes in mineralogy. Mineralogical changes often result in subsequent losses in fracture hardness and elasticity (Chan et al., 2012; Lane et al., 2013; Lane et al., 2015; Li et al., 2014).

Early life stages of invertebrates are often the most sensitive to environmental changes (Byrne, 2011; Kurihara, 2008). The transition from a pelagic to a benthic life history stage is irreversible, energetically costly, and it is when most of the mortality is thought to occur. Thus, effects at the early benthic stages could cause a major bottleneck in population dynamics (Gosselin and Qian, 1997). Indeed, several bioassays with invertebrate larvae reared under elevated pCO_2 have shown deformed larvae, reduced sizes at metamorphosis, and smaller juveniles (Dupont et al., 2008; Kroeker et al., 2013; Kurihara, 2008).

The consequences of ocean acidification on the offspring of serpulid polychaetes are not yet fully understood. The success of larvae may depend on reproductive investment by parental generation, direct effects of lower pH conditions on larval and juvenile physiology, and their ability to adapt to rapid changes over generations. Lane et al. (2013) studied inter-generational adaptation in the serpulid polychaete *Hydroides elegans* to find that both paternal and maternal low pH exposure affects offspring growth rate with evidence for trans-generational plasticity. Rodríguez-Romero et al. (2016) worked for six generations with the dorvilleid polychaete *Ophryotrocha labronica*, which does not produce calcium carbonate tubes. They found that in early generations (F1 and F2), fecundity was significantly lower at low pCO_2 but from F3 onwards there were no significant differences between pCO_2 treatments, indicating trans-generational adaptation. This highlighted the usefulness of trans-generational experiments to understand the response to ocean acidification.

The objective of the present study is to test for the effects of ocean acidification on the serpulid polychaetes, *Spirobranchus triqueter* and *Spirorbis* sp. These species have differing ecologies and belong to different serpulid phylogenetic clades (see Smith et al., 2013). *Spirobranchus triqueter* is a biofouling species, a broadcast spawner, and has a calcified

operculum while *Spirorbis* sp. broods eggs, lacks an operculum, and lives epiphytically on seagrass and algae (Kupriyanova et al., 2001). These differing ecologies and evolutionary lineages may have consequences on their sensitivity to ocean acidification (Lucey et al., 2015; Smith et al., 2013). The tube growth of these polychaetes was measured after being maintained under three pH_T (total scale) conditions (8.1, 7.7, 7.4) for 30 to 90 days. Additionally, we tested for effects on the offspring of *S. triqueter* under the same control and pH_T conditions. We hypothesize: (1) that calcareous tube growth is negatively impacted at low pH and (2) the early life stage development is also negatively affected from lowered parental investment and/or from direct effects of pH on development and growth.

MATERIALS & METHODS

Serpulid collection and calcein staining

Thirty *Posidonia oceanica* seagrass shoots (33 cm maximum leaf length) colonised by *Spirorbis* sp. were collected on 26 March 2014 at 11 m depth in a seagrass meadow within the Bay of Villefranche (NW Mediterranean Sea; 43°40.73′N, 07°19.39′E).

Spirobranchus triqueter (Linnaeus, 1758) was collected eight days later on 3 April 2014 on 2 x 1 m Perspex© sheets. Sheets were deployed on 6 March 2014 in the same meadow and at the same depth where *Spirorbis* sp. was collected and, ~ 4 weeks later, had a single *S. triqueter* recruitment event colonising its surface. At the time of collection, all tubes were of similar size (about 1.5 cm in length). The sheets were then cut into 20 x 10 cm plates.

Posidonia shoots with *Spirorbis* sp. and the plates harboring S. *triqueter* were immediately brought to the laboratory and placed in a tank with buffered calcein (60 mg L^{-1}) for 48 to 72 h, in a temperature-controlled room (14 °C). The purpose was to stain the tubes in order to have a mark for future growth measurements. The polychaetes were fed *ad libitum* with the microalgae *Isochrysis galbana* during staining. The plates and seagrass shoots were then placed into the experimental setup (Fig. 1).

Experimental setup and pH control – *Spirorbis* sp. and *S. triqueter* parental generation

After staining, plates and seagrass shoots with attached serpulids were placed into nine transparent (Perspex) open-top cylinder tanks of 8.4 L (Fig. 1). There were five shoots per tank. The nine tanks were randomly assigned to three pH treatments (three tanks per pH level) referred to as ambient, treatments #1 and #2 with targeted pH_T levels of 8.1, 7.7 and 7.4 respectively. Tanks initially contained *Spirorbis* sp. on leaves and 8 d later, after collection, also contained *S. triqueter* on plates. Both species were kept in a flow-through system in a temperature-controlled room (14 - 15 °C) under a 9.5:14.5 light:dark photoperiod (to mimic day length at depth in March) provided by six 39 W Solar Nature Ultra light (JBL) tubes for up to 30 or 90 d, for *Spirorbis* sp. and *S. triqueter*, respectively. During this time, polychaetes were fed twice per week (75 mL, 60,000 cells mL of a culture *I. galbana*). The flow through was interrupted for 1 h during feeding.

Seawater, pumped at 8 m depth 100 m from the coast, was continuously supplied to three 200 L header tanks at a minimal rate of 50 L h^{-1}. From each header tank, seawater was delivered continuously by gravity to the three experimental tanks at each pH level. The flow into experimental tanks was adjusted to reach a renewal time of about 15 min, ensuring good water quality without large fluctuations of pH, nutrients, dissolved oxygen, and temperature. A pump was located in each header tank to ensure mixing.

pH was controlled by bubbling pure CO_2 in the corresponding header tanks using a pH-stat system (IKS, Karlsbad, Aquastar). The pH electrodes of the pH-stat system were inter-calibrated every two days using a glass combination electrode (Metrohm Ecotrode Plus) calibrated on the total scale using TRIS buffer with a salinity of 38 corresponding to the salinity of the seawater pumped from the bay.

Carbonate chemistry measurements

Every two or three days, during both the experiments on adults and *S. triqueter* offspring, samples were taken for dissolved inorganic carbon (C_T) in each header tank and for total alkalinity (A_T) in the ambient header tank. Furthermore, during the experiment on offspring, approximately every 2-3 d, samples for C_T and A_T were taken prior to each seawater renewal (see thereafter). Samples for C_T (60 mL) were immediately poisoned with 100 µL of a saturated solution of mercuric chloride ($HgCl_2$) and analysed within two days using an AIRICA (automated infra-red inorganic carbon analyzer, Marianda, Germany) coupled to a LI-COR infrared gas analyser (LI-6262), on triplicate 1.2 mL subsamples at 22 °C. The instrument was calibrated before every set of measurements using certified reference material (CRM) from A. Dickson (Scripps University, San Diego, USA; batch 132). Samples for A_T (300 mL) were filtered on 47 mm diameter GF/F membranes, immediately poisoned with 100 µL of saturated

$HgCl_2$ and analysed within two days. A_T was determined at 22 °C on triplicate 50 mL subsamples by potentiometric titration on a Metrohm Titrando 880 titrator coupled to a glass combination electrode (Metrohm, Ecotrode Plus) calibrated daily on the total scale using TRIS buffers of salinity 38. A_T was calculated as described by Dickson et al. (2007). During the experiment, standards provided by A. Dickson (batch 132) were used to check precision and accuracy (n = 11; 1.3 and 1.3 µmol kg^{-1}, respectively). The pH_T levels and temperature were also measured approximately every two days in each cylinder using a glass combination electrode (Metrohm, Ecotrode Plus) calibrated as described above. At the conclusion of study for *Spirorbis* sp. (at 40 d in the experiment for *S. triqueter* adults), we considered we had successfully shown the pH_T and temperature in experimental cylinders reflected the pH of the header tanks and the temperature of the room. Thereafter, pH and temperature measurements in the cylinders were interrupted and only C_T and A_T were measured ever two or three days in the header tanks for the remainder of the experiment with *S. triqueter*. The carbonate chemistry was assessed using C_T and A_T (flag 15) at salinity of 37.5 with the corresponding room temperature in R package seacarb (Lavigne et al. 2014).

Tube elongation-*Spirorbis* sp. and *S. triqueter* parental generation

Tube elongation rates were determined for *Spirorbis* on day 30 and for *S. triqueter* throughout the experiment at 36 and 90 d for the parental population. Polychaetes were photographed under a fluorescence Zeiss microscope at 515 nm, a wavelength under which calcein fluoresces yellow-green. Growth measurements were performed using the Image J analysis software. New tube growth for *Spirorbis* sp. was estimated from the external arc of the coil to the stained line of the tube edge, while for *S. triqueter* it was estimated as the length from the staining line to the distal end of the tube.

Culturing of *S. triqueter* offspring and measures of growth and development

Spawning

After *S. triqueter* had been maintained for 45 d, four females and one to two males were randomly selected from each treatment. Gametes were obtained by breaking the posterior part of tubes; this mechanical perturbation caused the release of eggs and sperm. Once they began spawning they were immediately placed into a beaker (three in total, one for each pH level), at a seawater pH_T of 8.1, where fertilisation took place. They were left for 30 min to allow embryos to develop. Offspring were cultured and maintained in the same temperature-controlled room as the parents.

Trochophore density and size

Embryos were then transferred to 2 L beakers (three in total, one for each pH level) containing filtered seawater at the respective pH level taken from the header tanks and closed with plastic wrap to avoid CO_2 exchange with the atmosphere. Fifteen hours after fertilisation, the density of developed trochophore was estimated for each treatment in 1 mL samples which were placed under a microscope and counted with a Sedgewick grid.

Trochophore larvae from each treatment were maintained in these 2 L beakers at the respective pH levels (Fig. 1). During development from embryo to competent larvae, seawater was changed twice a day and replaced with water from the respective header tanks. Approximately every 2 d for a month, an attempt was made to collect seawater from beakers and measure carbonate chemistry using the methods described earlier. Larvae were fed daily with 10 mL of a culture of *Isochrysis galbana* at a density of 50,000 cells mL^{-1}.

Two and 10 d after the appearance of trochophore larvae, 20 larvae from each treatment were placed into separate Petri dishes. The dishes were then placed for 3 min in a refrigerator to reduce mobility. Larval size was measured using a stereoscopic microscope with a micrometer previously calibrated using a scaled slide. The larvae not measured remained in culture until they reached metamorphic competence (three segments).

Settlement

After 20 d of larval culture, when the metatrochophore stage had been reached, a batch of 25-30 competent larvae from each of the three pH treatments were pipetted into 18 Petri dishes (six per treatment) filled with seawater from respective header tanks. In each Petri dish, larvae were provided a microscope slide covered in biofilm to induce settlement. Biofilm was obtained by placing the slides in running seawater for 8 to 10 d prior to induce settlement. After 48 h, settled larvae were counted and percent settled calculated.

Offspring tube elongation

Slides with settled *S. triqueter* were stained for 40 h in a calcein (100 mg L^{-1}) bath that had been buffered to a pH_T of 8.1. The 18 slides were then placed into (three in total) 2 L glass beakers filled with seawater at the respective pH level. At 15, 22, and 38 d after the appearance of first settlement the new tube length of juveniles was assessed using the same method as described for their parental generation.

Tube hardness and fracture toughness- *S. triqueter* parental generation

At the conclusion of the experiment, *S. triqueter* tubes from the parental generation were oven-dried at 60 ºC for 48 h, stored in air tight containers, and shipped to the University of Glasgow, Scotland. New growth of *S. triqueter* tubes (three per treatment, nine in total) were embedded in epoxy resin (EpoxyCure, Buehler) blocks and sliced transversely using a diamond

trim saw blade. Resin blocks were ultra-polished using aluminium oxide (0.3 and 1 µm) and colloidal silica (0.6 µm). Fracture toughness was determined using Vickers Hardness microindentation (Micro Vickers 401, MVA, Wilson Wolpert Co., Ltd). Indents were made on the calcite material surrounding the porous structures of the tubes midway between the pores and the resin (indents per tube n = 6 - 15). A load of 0.2 N was applied for 10 s, the lengths of the diagonals of the indent were measured to calculate the Vicker's hardness (*H*) and the length of the cracks developed from the corners of the indent measured to determine fracture (KIC; Fitzer et al., 2015).

Statistical Analysis

Replication was considered at the individual level with the exception of settlement success when each slide was considered a replicate. Pooling of individuals was done to increase sample size for statistical analyses. Replicate containers were thus only considered as sources of variability. Subsequently, analyses were subjected to potential container effects. However, obvious differences in measures between containers were not observed, all containers were a source of individual measures, and sampling efforts (environment monitored, treated similarly, transferred between container type with development) were taken to minimize this effect. In instances where multiple measurement intervals were collected from the same pH treatment, data were treated separately by day. This data treatment was done because when data were pooled for statistical analyses in a two-factor test with pH treatment and time interval included as factors, data did not meet parametric requirements and could not be successfully transformed to meet parametric requirements. Therefore, all data were tested with separate one-way analysis of variance (ANOVA). Prior to testing, data were checked for normality and homogeneity of variance and transformed when necessary. Measures of tube elongation at 90 d in *S. triqueter*

(parental generation) and measures at 38 d in the offspring still did not meet parametric requirements and in these instances a Kruskal-Wallis test was used (see Table 3) . Additionally, rates of tube elongation by *Spirorbis* sp. were square root transformed prior to testing. Tukey's or Dunn's (when non-parametric) post hoc tests were used to examine for pairwise differences when significant main results were found. Unless otherwise noted, data are reported as mean ± standard error (SE) throughout.

RESULTS

Carbonate chemistry and environmental measures

Spirorbis sp. *and S. triqueter parental generation*

Environmental conditions in the header tanks are shown in Table 1 and Supplementary Table 1. Under ambient conditions, pH_T averaged 8.11 ± 0.04 (SD) during the culture of *Spirorbis* sp. and 8.13 ± 0.06 (SD) during the culturing of the adult S. *triqueter*, while in the two low pH conditions, it was, on average, 7.73 ± 0.05 and 7.72 ± 0.07 in treatment #1 and 7.40 ± 0.07 and 7.38 ± 0.14 in treatment #2 for the two species, respectively. Serpulid worms cultured at pH_T 8.1 and 7.7 were exposed to seawater always supersaturated with respect to both calcite and aragonite. In contrast, at the lowest pH condition, seawater was mostly undersaturated with respect to aragonite (Ω_a = 0.4 to 1.1) and close to saturation for calcite (Ω_c = 0.6 to 1.5). pH_T measured in the cylinders (from 31 March to 15 May) was very close to values determined in the header tanks (Fig. 2).

S. triqueter offspring

Environmental and carbonate chemistry parameters in header tanks and in the beakers in which offspring were reared (before water replacement) are shown in Table 1 and 2. Under ambient conditions, pH_T averaged 8.10 ± 0.05 (SD) in the beakers. In the two low pH conditions, pH_T increased by 0.1 to 0.2 units between water changes but the pH gradient between treatment levels was maintained (ambient > #1 > #2). For example, the mean pH_T in header tanks for treatment #1 and treatment #2 was 7.7 and 7.4, respectively, whereas the mean (± SD) pH_T in beakers at time of water change for treatment #1 was 7.82 ± 0.06 and for pH treatment #2, 7.57 ± 0.06. Similarly, the aragonite and calcite saturation was proportionally increased from values measured in the header tanks at time of water change.

Tube elongation – *Spirorbis* sp. and *S. triqueter* parental generation

Spirorbis sp. tube elongation (measured once at the end of study) was affected by pH (Table 3, Fig. 3). Rates were relatively reduced at the two lower pH levels and tubes grown at the lowest pH were thinner and broke easily.

Tube elongation by *S. triqueter* (parental generation) was also impacted by lowered pH conditions and rates differed over the duration of the experiment (Table 3, Fig. 3). Over 36 d and 90 d, individuals maintained at ambient pH had the fastest rates of tube elongation: 0.23 (± 0.02) mm d^{-1} over 36 d. This rate reduced to 0.17 (± 0.01) mm d^{-1} over 90 d. During the 36 d when ambient treated serpulids had the fastest rates of tube elongation, the *S. triqueter* exposed to the lower pH levels (#1 and #2) had reduced elongation rates that significantly differed from the rates measured under ambient pH, those at pH_T level of 7.7 having greater rates than those at pH_T level of 7.4 (Table 3, ambient > #1 > #2). After a longer period of growth (90 d), individuals of *S. triqueter* kept at pH_T level of 7.7 (treatment #1) and 7.4 (#3 at pH_T 7.4) had further reduced

elongation rates (mean of 0.06 ± 0.01 and 0.03 ± 0.02 mm d^{-1}) that did not differ from each other (Table 3, ambient > 1 = 2).

S. triqueter trochophore density and size

Data are most congruent with the hypothesis that lowered pH conditions affected trochophore density and growth (Table 3, Fig. 4). Indeed, the mean density in treatment #2 (57 ± 11.8 trochophore ml^{-1}) was substantially lower than the mean density at ambient (101 ± 12). Initial size estimates of larvae at day 2 were similar and all larvae increased in size from day 2 to day 10. However, at day 10 post-fertilisation, the larvae at the lowest pH level were significantly smaller (Table 3, 376 ± 7.9, 369.4 ± 5.5 and 361.2 ± 6.7 µm for ambient, treatment #1 and #2, respectively).

S. triqueter settlement

Most larvae attained metamorphic competence but at the lowest pH level (treatment #2) five abnormal larvae were observed (out of 25 - 30 larvae). All five of them had one side of the body more developed than the other making the larvae swim in an unusual way. Settlement of metatrochophores took place first (21 d after fertilization) in treatment #2 (mean pH_T of 7.4 to 7.6), then 24 to 48 h later (22 to 23 d after fertilization) for those maintained in ambient and pH treatment #1. Settlement success of competent larvae ranged between 89 to 93% and no differences between treatments were detected (Table 3).

Tube elongation – *S. triqueter* offspring

One hour after settlement larvae began to secrete a transparent primary tube, and 24 h later they were cemented permanently in place, building the secondary calcified white tube. Initially, the calcified tubes in the lowest pH level were significantly smaller (ambient = 1.67 ± 0.03, #1 = 1.67 ± 0.02 and #2 = 1.64 ± 0.02 mm), but a week later (22 d) juveniles in pH treatment #1 and #2

were elongating tubes at a similar rate to those in ambient (Table 3, Fig. 4). Then, 38 d post-settlement, *S. triqueter* juveniles in ambient conditions had nearly doubled rates from previous days while the juveniles at the lower pH levels (treatment #1 and #2) continued to elongate tubes at a similar rate, slightly above the rate recorded at 22 d.

Fracture toughness and hardness - *S. triqueter* parental generation

Fracture toughness (KIC) was greatest for tubes maintained at the intermediate pH level (#1) yet remained similar for worms measured from all three treatment levels (Table 3, Fig. 5). Likewise, tubes maintained at the mid-pH level (treatment #1) were significantly harder compared with the significantly reduced hardness at the pH treatment #2 (Table 3, Fig. 5). Alveoli size visually differed among treatments (Fig. 6). Alveoli were present inside the two lateral tube flanges. Alveoli form in tube growth and house the notopodial chaetae (Hedley, 1958). As the tube elongates the chaetae move forward and prevent calcareous deposition in a new location. In cross section, the tube section formed at the lowest pH (treatment #2) had the largest alveoli with a thinner layer of calcareous covering corresponding to the significantly reduced hardness of the tube. However, the tubes grown in treatment #1 had very small alveoli; noticeably smaller than those in tubes at ambient pH_T.

DISCUSSION

Our results support the hypothesis that ocean acidification will affect the calcification of serpulid tubes. The lowered pH and the subsequent lowered saturation states of calcite and aragonite reduced tube growth and appeared to alter tube integrity in both species.

Tube growth

According to Waldbusser et al. (2015), the effects that ocean acidification has on shell formation of bivalve larvae are modulated by a combination of dissolution of the tube as well as physiological changes to the internal acid-base balance, affecting the rate of new $CaCO_3$ deposition. Dissolution and reduced growth under acidified conditions have also been reported in other serpulid species. A well-studied tropical serpulid, *Hydroides elegans*, raised under lowered pH (7.8 - 7.4 NBS) produced smaller, thinner, less dense and weaker tubes (Chan et al., 2012; Li et al., 2014; Li et al., 2016). In one report, most of the *H. elegans* larvae raised at pH conditions below 7.7 (NBS scale) were unable to produce calcified tubes at the time of metamorphosis and juvenile calcification was also negatively affected (Lane et al., 2013). In the Baltic Sea, tube growth of *Spirorbis spirorbis*, an epiphytic species on algae, was decreased by 25% and 40% at 1200 and 3150 µatm CO_2, respectively (Saderne and Wahl, 2013). Adult and juvenile tubes exhibited dissolution that in some cases exposed the worms and their embryo bags (Saderne and Wahl, 2013).

Mineral composition

The fact that most serpulid tubes lack an outer organic protective layer and are composed of aragonite, calcite and, for some, high Mg-calcite makes them sensitive to acidified conditions (Chan et al., 2012; Smith et al., 2013; Vinn, 2013; Vinn and Kupriyanova, 2011). Mineral composition of serpulid tubes shows a strong phylogenetic signal, but it can be somewhat plastic (Smith et al., 2013). *Spirorbis spirorbis* (as *S. borealis*) have been reported to be 10 to 14% aragonite with the rest being moderate to high Mg calcite depending on environmental factors such as temperature (Bornhold and Milliman, 1973). This bimineralic composition is typical for *Spirorbis* spp. and both mineral types are highly soluble. Therefore, this serpulid group is

suspected to be highly sensitive to dissolution (Smith et al., 2013); results in the present study support this hypothesis. *Spirobranchus triqueter* tubes have been reported to be composed of 98.4% moderate to high calcite and 1.6% aragonite (Vinn et al., 2008). In contrast, polychaete species belonging to the genus *Hydroides* are reported to be almost entirely aragonitic (Smith et al., 2013). However, biomineralogical plasticity (increased the ratio of magnesium to calcite) in response to CO_2-induced ocean acidification was observed in the biofouling species *Hydroides crucigera* (Ries, 2011). Ontogenetic mineral changes have also been noted; juvenile *H. elegans* tubes contained more amorphous calcium carbonate and were predominantly aragonitic whereas adult tubes were bimineralic with considerably more calcite (Chan et al., 2012). Probably early stages of *S. triqueter* are less aragonitic than *H. elegans* and this gives them more resistance to changes in seawater chemistry, as we did not observe polychaetes unable to build tubes. However, lowered pH conditions had the greatest impact post-settlement on *S. triqueter* juvenile tube elongation. Thus, ontogenetic change in mineral composition is a possibility in this species but more studies are needed on the topic. Also, the present study may underestimate effects on *S. triqueter*, because the calcified opercula, known to be almost entirely aragonitic in this group (see Smith et al., 2013), were not investigated.

Tube hardness and elasticity

Alterations in tube mineralogy can result in deterioration of the tube hardness and elasticity which are two factors that can have important ecological consequences on survival (predation, withstanding wave force; Chan et al., 2012; Li et al., 2014). However, for S. triqueter, in the present study, the change in alveolus size, reduced hardness and not the calcified fracture toughness (KIC) presumably accounts for the fragility of the tube at pH_T 7.4. Spirobranchus triqueter may have some ability to compensate to conditions at a pH_T of 7.7, as evidenced by the

greater hardness and smaller alveoli. Perhaps, *S. triqueter* allocated energy to maintain structural tube integrity at the expense of growth. The price of compensating for lowered pH has an associated energy cost. Such changes in the energy budget have been observed in several calcifiers such as molluscs and foraminifera (Pan et al., 2015; Wood et al., 2008). When additional energy is channelled to calcification in order to repair dissolved zones of the tube or shell, it cannot be used for other processes, restricting growth and reproductive output. Furthermore, the ability to change tube morphology in response to environmental conditions has been observed. For example, *H. elegans* subjected to both elevated temperature and lowered pH conditions produced longitudinal keels that increased the volume of tubes and mitigated the weakness observed under acidified conditions at lower temperature (Li et al., 2016).

Larval development

Few studies have noted ocean acidification effects on serpulid larval size or metamorphosis (Lane et al., 2013; Lane et al., 2015). We observed reduced larvae density and size but no impact on metamorphosis when cultured at a pH_T 7.4 (Fig. 4). Some authors (Kurihara, 2008; Parker et al., 2010) have reported that effects at adult stages may have "carry-over" consequences from one life history stage to the next. In the present study and in many studies on the topic to date (e.g. Chan et al. 2012, Li et al. 2014, Li et al. 2016), fertilization of tubeworm gametes has occurred at a pH_T of 8.1. It has been reported that pH, salinity, and temperature do not directly alter fertilization in tubeworms such as H. elegans (Li et al. 2014). However, without an acclimation period or some examination to rule out carry over effects, fertilization at ambient prior to treatment conditions may mitigate or, alternatively cause cellular stress and exacerbate low pH impacts on early development. Future efforts should ensure that all life stages are kept at treatment

conditions to better determine ocean acidification effects. Nevertheless, decreased size in early developmental stages of marine organisms has been shown to affect juvenile fitness by reducing competitive ability and increasing post settlement mortality (Anil et al., 2001).

The negative effect observed at early development (Fig. 4) could be explained by the lower pH_T (7.4) used and exposure of both parents to lowered pH conditions; as differential gender effects have been noted (see Lane et al., 2015). The more extreme ocean acidification scenario could cause great stress on the parental generation or more drastic direct effects on acid-base balance or ion regulation in trochophore larvae. For example, from a 5 d transplant experiment, the polychaete *Sabella spallanzanii* was estimated to only be able to survive in the short-term under low pH conditions because ocean acidification causes a significant decrease in carbonic anhydrase concentration and an increase in energy metabolism (Turner et al., 2015). While the mechanism resulting in smaller trochophores at 10 d (not at 2 d) post-fertilisation is unclear (Fig. 4). There are several accounts of reduced larval size in invertebrates under acidified conditions (Kroeker et al., 2010). Echinoderm larvae of the sea urchins *Hemicentrotus pulcherrimus* and *Paracentrotus lividus* subjected to elevated CO_2 presented delayed development and smaller size (Byrne et al., 2013; Dupont et al., 2008). In the present study, however, lowered pH conditions did not delay settlement.

Additionally, invertebrate larvae with short life cycles have the potential for short-term evolution in response to ocean acidification; this capacity of adaptation can reduce biological impacts of climate change (Hoffmann and Sgrò, 2011). Polychaete worms transplanted to CO_2 vents in Ischia (Italy) have shown acclimation, adaptation, and plasticity to elevated pCO_2 environments (Calosi et al., 2013). No acclimation was observed for *S. triqueter* in the present study (Fig. 3,4); experiments with several generations may be needed in order to evaluate

adaptive capability of this species. If there was trans-generational plasticity it was not apparent with our study design. For instance, Lane et al. (2015) concluded *H. elegans* exhibited trans-generation plasticity evident only when the first-generation offspring were cultured in ambient pH (NBS, 8.1; i.e. mismatch between parental and offspring exposure).

Ecological relevance

Reduced tube growth and the lower number of trochophores produced at the lowered pH conditions (Fig. 4) are likely to affect serpulid recruitment. Examinations of serpulid establishment in natural settings support this conclusion but, also highlight the importance of including ecological interactions and environmental variability when predicting population responses. Observational studies along CO_2 vents have found a reduction in numbers of serpulid individuals nearer to the vent source; citing both direct effects on calcification and decreased competitive abilities with more tolerant species (Cigliano et al., 2010; Donnarumma et al., 2014; Martin et al., 2008). Species of *Spirorbis* that settle on photosynthesising organisms can be somewhat buffered from ocean acidification effects by the chemically mediated environment provided by their host (Cox et al., 2017; Hendriks et al., 2014; Saderne and Wahl, 2013). However, to be buffered, the benefits during the day need to be great enough to offset the negative effects of respiration at night. In addition, the tube worm response can be driven by temperature (Campbell and Fourqurean, 2014; Ni et al., 2017). For example, *Spirorbis spirorbis*, in the laboratory, had reduced recruitment on a fucoid alga at extreme pCO_2 (3150 µatm) but not at moderate levels (1200 µatm, Saderne and Wahl, 2013). An *in situ* benthocosm study, using the same species, concluded that early life stages are promoted by moderately warm temperatures. However, high temperatures exacerbate tube dissolution and do not alter growth, when water is undersaturated with respect to $CaCO_3$ (Ni et al., 2017). In the Northwest Mediterranean Sea,

Spirobis sp. recruited less on tiles placed inside an *in situ* acidified enclosure than on tiles placed in the control enclosure at ambient pH. Also, the density of epiphytic *Spirorbis* sp. on *Posidonia oceanica* seagrass leaves appeared unaffected in both enclosures, leading to the conclusion that the chemically mediated boundary layer around the leaf provided an ocean acidification refuge (Cox et al., 2017). In contrast though, *Spirorbis* sp. had fewer individuals on leaves of the subtropical seagrass *Thalassia testudinum* exposed to lowered pH and the effect was greater during winter months (Campbell and Fourqurean, 2014). Moreover, food availability is a critical factor in larval development and juvenile growth (Olson and Olson, 1989). In the present study worms were fed adequately and that could have allowed for some resistance to more negative effects.

Future implications under global climate change

Ocean warming will shape the response to ocean acidification. It is predicted that tropical tube worms will shift towards the poles in distributions with continued global climate change (Faroni-Perez, 2017). In the predictive model, which considered both warming and acidification, elevated temperature was the most influential driver of distributions, although Faroni-Perez (2017) points to the need for more information on the effects of ocean acidification on tube worms to inform models. This shift in distributions should cause alarm because serpulid reefs allow for greater biodiversity and their loss can have implications on temperate shallow-water ecosystem function. The composition of biofouling communities will also probably change significantly with a decrease in invertebrate calcifiers, particularly Serpulidae.

ACKNOWLEDGEMENTS

We are grateful to S. Schenone and L. Urbini for their assistance with the experiment. We also thank P. Mahacek and A. Le Fur for their assistance in construction of settlement tiles and tanks. We thank C. Rouvière for assisting with the imaging of calcein markers and S. Marro who provided the algal cultures. D. Luquet, G. de Liège, and D. Robin kindly assisted in diving for organism collections.

AUTHOR CONTRIBUTIONS

V.D.-C., F.G., and J.-P. G. designed the study. J.-P. G. and F.G. obtained the financial support. V.D.-C. cultured the tube worms and measured development and growth with some assistance from J.D. The setup and maintenance of the mesocosm system and calcein baths was done by S.A., J.D., E.C. with supervision of F.G., advice of J.-P. G., and assistance from V.D.-C. S.A. determined C_T and A_T with assistance from J.D., E.C, F.G. and V.D.-C. Fracture toughness and hardness was determined by S.F. E.C. compiled data, analysed it, and wrote the manuscript with V.D.-C. All authors made additional edits to the manuscript.

COMPETING INTERESTS

No competing interests to report.

FUNDING

This work was funded by the "European Free Ocean Carbon Enrichment" (eFOCE; BNP Paribas Foundation), the European Commission through the project "Mediterranean Sea Acidification in a changing climate" (MedSeA; grant agreement 265103) and the MISTRALS-MERMEX (INSU, CNRS) program.

REFERENCES

Anil, A. C., Desai, D. and Khandeparker, L. (2001). Larval development and metamorphosis in *Balanus amphitrite* Darwin (Cirripedia; Thoracica): significance of food concentration, temperature and nucleic acids. *J. Exp. Mar. Biol. Ecol.* **263**, 125–141.

Bastida-Zavala, J. R., McCann, L. D., Keppel, E. and Ruiz, G. M. (2017). The fouling serpulids (Polychaeta: Serpulidae) from United States coastal waters: an overview. *Eur. J. Taxon.*

Bornhold, B. D. and Milliman, J. D. (1973). Generic and environmental control of carbonate mineralogy in serpulid (polychaete) tubes. *J. Geol.* **81**, 363–373.

Byrne, M. (2011). Impact of ocean warming and ocean acidification on marine invertebrate life history stages: vulnerabilities and potential for persistence in a changing ocean. In *Oceanogr. Mar. Biol.* pp. 1–42.

Byrne, M., Lamare, M., Winter, D., Dworjanyn, S. A. and Uthicke, S. (2013). The stunting effect of a high CO_2 ocean on calcification and development in sea urchin larvae, a synthesis from the tropics to the poles. *Philos. Trans. R. Soc. B Biol. Sci.* **368**, 20120439–20120439.

Calosi, P., Rastrick, S. P. S., Lombardi, C., de Guzman, H. J., Davidson, L., Jahnke, M., Giangrande, A., Hardege, J. D., Schulze, A., Spicer, J. I., et al. (2013). Adaptation and acclimatization to ocean acidification in marine ectotherms: an *in situ* transplant experiment with polychaetes at a shallow CO_2 vent system. *Philos. Trans. R. Soc. B Biol. Sci.* **368**, 20120444–20120444.

Campbell, J. E. and Fourqurean, J. W. (2014). Ocean acidification outweighs nutrient effects in structuring seagrass epiphyte communities. *J. Ecol.* **102**, 730–737.

Chan, V. B. S., Li, C., Lane, A. C., Wang, Y., Lu, X., Shih, K., Zhang, T. and Thiyagarajan, V. (2012). CO_2-driven ocean acidification alters and weakens integrity of the calcareous tubes produced by the serpulid tubeworm, *Hydroides elegans*. *PLoS ONE* **7**, e42718.

Cigliano, M., Gambi, M. C., Rodolfo-Metalpa, R., Patti, F. P. and Hall-Spencer, J. M. (2010). Effects of ocean acidification on invertebrate settlement at volcanic CO_2 vents. *Mar. Biol.* **157**, 2489–2502.

Cox, T. E., Díaz-Castañeda, V., Martin, S., Alliouane, S., Mahacek, P., Le Fur, A., Gattuso, J.-P. and Gazeau, F. (2017). Effects of in situ CO_2 enrichment on epibiont settlement on artificial substrata within a *Posidonia oceanica* meadow. *J. Exp. Mar. Biol. Ecol.* **497**, 197–211.

Donnarumma, L., Lombardi, C., Cocito, S. and Gambi, M. C. (2014). Settlement pattern of *Posidonia oceanica* epibionts along a gradient of ocean acidification : an approach with mimics. *Mediterr. Mar. Sci.* **15**, 498–509.

Dupont, S., Havenhand, J., Thorndyke, W., Peck, L. and Thorndyke, M. (2008). Near-future level of CO_2-driven ocean acidification radically affects larval survival and development in the brittlestar *Ophiothrix fragilis*. *Mar. Ecol. Prog. Ser.* **373**, 285–294.

Faroni-Perez, L. (2017). Climate and environmental changes driving idiosyncratic shifts in the distribution of tropical and temperate worm reefs. *J. Mar. Biol. Assoc. U. K.* **97**, 1023–1035.

Fitzer, S. C., Zhu, W., Tanner, K. E., Phoenix, V. R., Kamenos, N. A. and Cusack, M. (2015). Ocean acidification alters the material properties of *Mytilus edulis* shells. *J. R. Soc. Interface* **12**, 20141227–20141227.

Gazeau, F., Quiblier, C., Jansen, J. M., Gattuso, J.-P., Middelburg, J. J. and Heip, C. H. R. (2007). Impact of elevated CO_2 on shellfish calcification. *Geophys. Res. Lett.* **34**, LO7603.

Gosselin, L. and Qian, P. (1997). Juvenile mortality in benthic marine invertebrates. *Mar. Ecol. Prog. Ser.* **146**, 265–282.

Hedley, R. H. (1958). Tube formation by *Pomatoceras triqueter* (Polychaeta). *J. Mar. Biol. Assoc. UK* **37**, 315–322.

Hendriks, I. E., Olsen, Y. S., Ramajo, L., Basso, L., Steckbauer, A., Moore, T. S., Howard, J. and Duarte, C. M. (2014). Photosynthetic activity buffers ocean acidification in seagrass meadows. *Biogeosciences* **11**, 333–346.

Hoagland, K. E. and Turner, R. D. (1980). Range extensions of teredinids (shipworms) and polychaetes in the vicinity of a temperate-zone nuclear generating station. *Mar. Biol.* **58**, 55–64.

Hoffmann, A. A. and Sgrò, C. M. (2011). Climate change and evolutionary adaptation. *Nature* **470**, 479–485.

Kroeker, K. J., Kordas, R. L., Crim, R. N. and Singh, G. G. (2010). Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms: Biological responses to ocean acidification. *Ecol. Lett.* **13**, 1419–1434.

Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., Duarte, C. M. and Gattuso, J.-P. (2013). Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Glob. Change Biol.* **19**, 1884–1896.

Kupriyanova, E., Nishi, E., Ten Hove, H. A. and Rzhavsky, A. (2001). Life history patterns in serpulimorph polychaetes: ecological and evolutionary perspectives. *Ocean. Mar Biol Annu Rev* **39**, 1–101.

Kurihara, H. (2008). Effects of CO_2-driven ocean acidification on the early developmental stages of invertebrates. *Mar. Ecol. Prog. Ser.* **373**, 275–284.

Lane, A. C., Mukherjee, J., Chan, V. B. S. and Thiyagarajan, V. (2013). Decreased pH does not alter metamorphosis but compromises juvenile calcification of the tube worm *Hydroides elegans*. *Mar. Biol.* **160**, 1983–1993.

Lane, A., Campanati, C., Dupont, S. and Thiyagarajan, V. (2015). Trans-generational responses to low pH depend on parental gender in a calcifying tubeworm. *Sci. Rep.* **5**,10847.

Li, C., Chan, V. B. S., He, C., Meng, Y., Yao, H., Shih, K. and Thiyagarajan, V. (2014). Weakening mechanisms of the serpulid tube in a High-CO_2 world. *Environ. Sci. Technol.* **48**, 14158–14167.

Li, C., Meng, Y., He, C., Chan, V. B. S., Yao, H. and Thiyagarajan, V. (2016). Mechanical robustness of the calcareous tubeworm *Hydroides elegans* : warming mitigates the adverse effects of ocean acidification. *Biofouling* **32**, 191–204.

Lucey, N. M., Lombardi, C., DeMarchi, L., Schulze, A., Gambi, M. C. and Calosi, P. (2015). To brood or not to brood: Are marine invertebrates that protect their offspring more resilient to ocean acidification? *Sci. Rep.* **5**, 10029.

Martin, S., Rodolfo-Metalpa, R., Ransome, E., Rowley, S., Buia, M.-C. C., Gattuso, J.-P. and Hall-Spencer, J. (2008). Effects of naturally acidified seawater on seagrass calcareous epibionts. *Biol. Lett.* **4**, 689–692.

Miles, H., Widdicombe, S., Spicer, J. I. and Hall-Spencer, J. (2007). Effects of anthropogenic seawater acidification on acid–base balance in the sea urchin *Psammechinus miliaris*. *Mar. Pollut. Bull.* **54**, 89–96.

Neff, J. (1971). Ultrastructural studies of the secretion of calcium carbonate by the serpulid polychaete worm, *Pomatoceros caeruleus* . *Z. Fu¨r Zellforsch.* **120**, 160–186.

Ni, S., Taubner, I., Böhm, F., Winde, V. and Böttcher, M. E. (2017). Effect of ocean acidification and elevated temperature on growth of calcifying tubeworm shells *Spirorbis spirorbis*: An *in-situ* benthocosm approach. *Biogeosciences Discuss.* 1–44.

Olson, R. R. and Olson, M. H. (1989). Food limitation of planktotrophic marine invertebrate larvae: does it control recruitment success? *Annu. Rev. Ecol. Syst.* **20**, 225–247.

Pan, T.-C. F., Applebaum, S. L. and Manahan, D. T. (2015). Experimental ocean acidification alters the allocation of metabolic energy. *Proc. Natl. Acad. Sci.* **112**, 4696–4701.

Parker, L. M., Ross, P. M. and O'Connor, W. A. (2010). Comparing the effect of elevated pCO_2 and temperature on the fertilization and early development of two species of oysters. *Mar. Biol.* **157**, 2435–2452.

Pörtner, H. O., Langenbuch, M. and Reipschläger, A. (2004). Biological impact of elevated ocean CO_2 concentrations: Lessons from animal physiology and earth history. *J. Oceanogr.* **60**, 705–718.

Ries, J. B. (2011). Skeletal mineralogy in a high-CO_2 world. *J. Exp. Mar. Biol. Ecol.* **403**, 54–64.

Ries, J. B., Cohen, A. L. and McCorkle, D. C. (2009). Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. *Geology* **37**, 1131–1134.

Rodríguez-Romero, A., Jarrold, M. D., Massamba-N'Siala, G., Spicer, J. I. and Calosi, P. (2016). Multi-generational responses of a marine polychaete to a rapid change in seawater pCO_2. *Evol. Appl.* **9**, 1082–1095.

Rouse, G. W. and Pleijel, F. (2001). *Polychaetes.* Oxford; New York: Oxford University Press.

Saderne, V. and Wahl, M. (2013). Differential responses of calcifying and non- calcifying epibionts of a brown macroalga to present-day and future upwelling pCO_2. *PLoS ONE* **8**, e70455.

Smith, A. M., McGourty, C. R., Kregting, L. and Elliot, A. (2005). Subtidal *Galeolaria hystrix* (Polychaeta: Serpulidae) reefs in Paterson Inlet, Stewart Island, New Zealand. *N. Z. J. Mar. Freshw. Res.* **39**, 1297–1304.

Smith, A. M., Riedi, M. A. and Winter, D. J. (2013). Temperate reefs in a changing ocean: skeletal carbonate mineralogy of serpulids. *Mar. Biol.* **160**, 2281–2294.

Ten Hove, H. A. and Kupriyanova, E. (2009). *Zootaxa, Taxonomy of Serpulidae (Annelida, Polychaeta): The state of affairs*. Auckland, New Zealand: Magnolia Press.

Turner, L. M., Ricevuto, E., Massa-Gallucci, A., Gambi, M.-C. and Calosi, P. (2015). Energy metabolism and cellular homeostasis trade-offs provide the basis for a new type of sensitivity to ocean acidification in a marine polychaete at a high-CO_2 vent: adenylate and phosphagen energy pools versus carbonic anhydrase. *J. Exp. Biol.* **218**, 2148–2151.

Vinn, O. (2013). Occurrence, formation and function of organic sheets in the mineral tube structures of Serpulidae (Polychaeta, Annelida). *PLoS ONE* **8**, e75330.

Vinn, O. and Kupriyanova, E. K. (2011). Evolution of a dense outer protective tube layer in serpulids (Polychaeta, Annelida). *Carnets Géologie Noteb. Geol.* **(CG2011_L05),** 137-147.

Vinn, O., Ten Hove, H. A., Mutvei, H. and KirsimäE, K. (2008). Ultrastructure and mineral composition of serpulid tubes (Polychaeta, Annelida). *Zool. J. Linn. Soc.* **154**, 633–650.

Waldbusser, G. G., Hales, B., Langdon, C. J., Haley, B. A., Schrader, P., Brunner, E. L., Gray, M. W., Miller, C. A. and Gimenez, I. (2015). Saturation-state sensitivity of marine bivalve larvae to ocean acidification. *Nat. Clim. Change* **5**, 273–280.

Wood, H. L., Spicer, J. I. and Widdicombe, S. (2008). Ocean acidification may increase calcification rates, but at a cost. *Proc. R. Soc. B Biol. Sci.* **275**, 1767–1773.

Table 1. Carbonate chemistry calculated from determined A_T and C_T with a salinity of 37.5 and measured temperature (T, °C) within ambient, treatment #1, and treatment #2 header tanks over the experimental duration for *Spirorbis* sp. (30 d), and *Spirobranchus triqueter* collected from the field (90 d) and reared in tanks (offspring, 43 d): total alkalinity (A_T, μmol kg^{-1}), pH (on the total scale; pH_T), partial pressure of carbon dioxide (pCO_2, μatm), total dissolved inorganic carbon (C_T, μmol kg^{-1}) and saturation states with respect to aragonite (Ω_a) and calcite (Ω_c).

				Ambient					**Treatment #1**					**Treatment #2**				
		T	**A_T**	**pH_T**	**pCO_2**	**C_T**	**Ω_A**	**Ω_C**	**pH_T**	**pCO_2**	**C_T**	**Ω_a**	**Ω_c**	**pH_T**	**pCO_2**	**C_T**	**Ω_a**	**Ω_c**
Spirorbis sp.	Mean	16.2	2551	8.11	367	2251	3.3	5.1	7.73	1011	2439	1.5	2.4	7.40	2268	2557	0.8	1.2
	SD	0.8	6	0.04	37	24	0.2	0.4	0.05	128	12	0.1	0.2	0.07	401	23	0.1	0.2
S. triqueter	Mean	15.9	2546	8.13	347	2242	3.3	5.1	7.72	1047	2440	1.5	2.3	7.38	2510	2565	0.8	1.2
	SD	1.2	10	0.06	49	20	0.2	0.3	0.07	177	26	0.2	0.3	0.14	848	46	0.2	0.3
S. triqueter	Mean	14.9	2546	8.15	328	2235	3.4	5.2	7.73	1019	2438	1.5	2.3	7.41	2381	2560	0.8	1.2
Offspring	SD	0.4	13	0.03	29	17	0.2	0.3	0.10	247	39	0.3	0.5	0.18	1038	62	0.3	0.4

Table 2. Carbonate chemistry calculated from determined A_T and C_T with a salinity of 37.5 and measured temperature (T,℃) within ambient, treatment #1, and treatment #2 culture beakers just prior to water changes over 28 d of experimental duration for *Spirobranchus triqueter (S. t.)* offspring: total alkalinity (A_T, μmol kg^{-1}), pH (on the total scale; pH_T), partial pressure of carbon dioxide (pCO_2, μatm), total dissolved inorganic carbon (C_T, μmol kg^{-1}) and saturation states with respect to aragonite (Ω_a) and calcite (Ω_c). * signifies measures for juveniles (post-settlement 1-2 d).

				Ambient					Treatment #1					Treatment #2				
Date	**Day**	**T**	A_T	pH_T	pCO_2	C_T	Ω_a	Ω_c	pH_T	pCO_2	C_T	Ω_a	Ω_c	pH_T	pCO_2	C_T	Ω_a	Ω_c
5/23/2014	2	18.1	2546	8.06	416	2258	3.2	4.9	-	-	-	-	-	-	-	-	-	-
5/24/2014	3	14.7	2510	8.03	454	2275	2.6	4.0	-	-	-	-	-	-	-	-	-	-
5/25/2014	4	15.4	2521	8.06	422	2264	2.8	4.4	7.74	966	2411	1.5	2.3	-	-	-	-	-
5/26/2014	5	14.2	2537	8.14	339	2242	3.2	4.9	7.83	779	2399	1.7	2.7	-	-	-	-	-
5/28/2014	8	14.4	2535	8.14	340	2239	3.2	5.0	7.85	738	2387	1.8	2.8	7.56	1529	2497	1.0	1.5
5/31/2014	11	15.2	2543	8.15	325	2228	3.4	5.3	7.75	958	2431	1.5	2.4	7.59	1414	2489	1.1	1.7
6/5/2014	16	15.5	2546	-	-	-	-	-	-	-	-	-	-	7.53	1647	2512	1.0	1.5
6/6/2014	17	14.6	2542	-	-	-	-	-	7.78	866	2418	1.6	2.5	-	-	-	-	-
6/7/2014	18	14.8	2544	-	-	-	-	-	7.89	667	2375	2.0	3.1	7.54	1614	2511	1.0	1.5
6/10/2014	21*	15.0	2549	-	-	-	-	-	7.91	638	2370	2.1	3.3	7.54	1607	2514	1.0	1.5
6/13/2014	24*	15.0	2537	8.16	320	2222	3.4	5.3	7.85	735	2384	1.9	2.9	7.68	1124	2451	1.3	2.1
6/17/2014	28*	15.0	2539	8.08	395	2269	3.0	4.6	-	-	-	-	-	-	-	-	-	-
Mean		**15.1**	**2540**	**8.10**	**380**	**2250**	**3.1**	**4.8**	**7.82**	**793**	**2397**	**1.8**	**2.8**	**7.57**	**1489**	**2496**	**1.1**	**1.6**
SD		**1.2**	**12**	**0.05**	**47**	**18**	**0.3**	**0.4**	**0.06**	**125**	**22**	**0.2**	**0.3**	**0.06**	**198**	**24**	**0.1**	**0.2**

Table 3. Results of one-way ANOVA or Kruskal-Wallis (specified below) tests used to test for differences in *Spirorbis* sp. and *S. triqueter* tube elongation, development, and tube fracture hardness and toughness when exposed to three pH treatments (ambient, treatment #1 and treatment #2). Significant differences are in bold text with the results of pairwise poc hoc tests. * $p<0.05$, ** $p<0.01$

Parameters	n	df	MS	F-stat (or H)	p-value	pairwise post hoc
Tube elongation rates						
Spirorbis sp. (square-root transformed)	14-15	2	0.315	16.30	**<0.001**	ambient >1*>2**
S. triqueter parental generation						
36 d	13	2	0.084	92.55	**<0.001**	ambient > 1**>2**
90 d (Kruskal-Wallis)	23-28	2		28.45	**<0.001**	ambient > 1 = 2*
S. triqueter offspring						
Trochophore size						
2 d post-fertilization	10	2	9.733	2.39	0.111	N/A
10 d post-fertilization	10	2	564.133	12.35	**<0.001**	ambient = 1 > 2**
Settlement success	6	2	41.8	0.93	0.415	N/A
Juvenile tube growth						
15 d post-settlement	11-13	2	0.0000207	9.83	**<0.001**	ambient = 1 > 2**
22 d post-settlement	11-13	2	0.00000451	4.13	**0.025**	ambient = 1, ambient = 2, 1 > 2*
38 d post-settlement (Kruskal-Wallis)	18	2		32.82	**<0.001**	ambient >1 = 2*
Hardness (H)	3	2	35203	16.47	**<0.001**	ambient < 1 and > 2, 2 > 1**
Fracture toughness (K_{IC})	3	2	5982627612	0.50	0.606	N/A

Figures

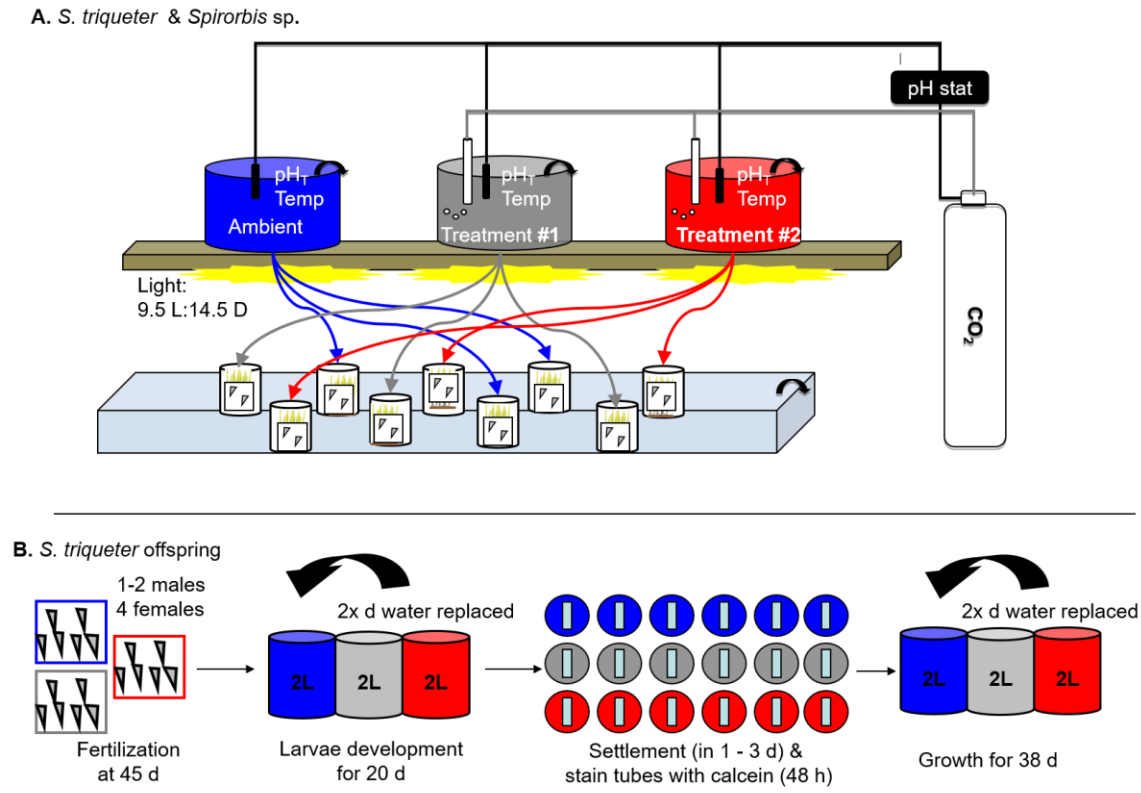

Fig. 1 Experimental design: *Spirorbis* sp. on *Posidonia oceanica* shoots *and Spirobranchus triqueter* on tiles were collected from the field and exposed to three pH treatments: ambient, treatment #1, and treatment #2 for 30 and 90 days, respectively. Header tanks were used to feed three replicate cylinder tanks containing tube worms. A pH-stat system was used to bubble pure CO_2 into header tanks to maintain pH. After 45 d, one to two males and four females of *S. triqueter* from each treatment were induced to spawn. Larvae were allowed to develop for 20 d in 2 L beakers and transferred to Petri dishes that contained a slide covered with biofilm for settlement. Worms settled within 1-3 d and were placed into a calcein bath for 48 h. Then juveniles were placed into 2 L beaks and allowed to grow for 38 d. Offspring were exposed to experimental conditions a total of 43 d. The water was changed in culture beakers twice per day.

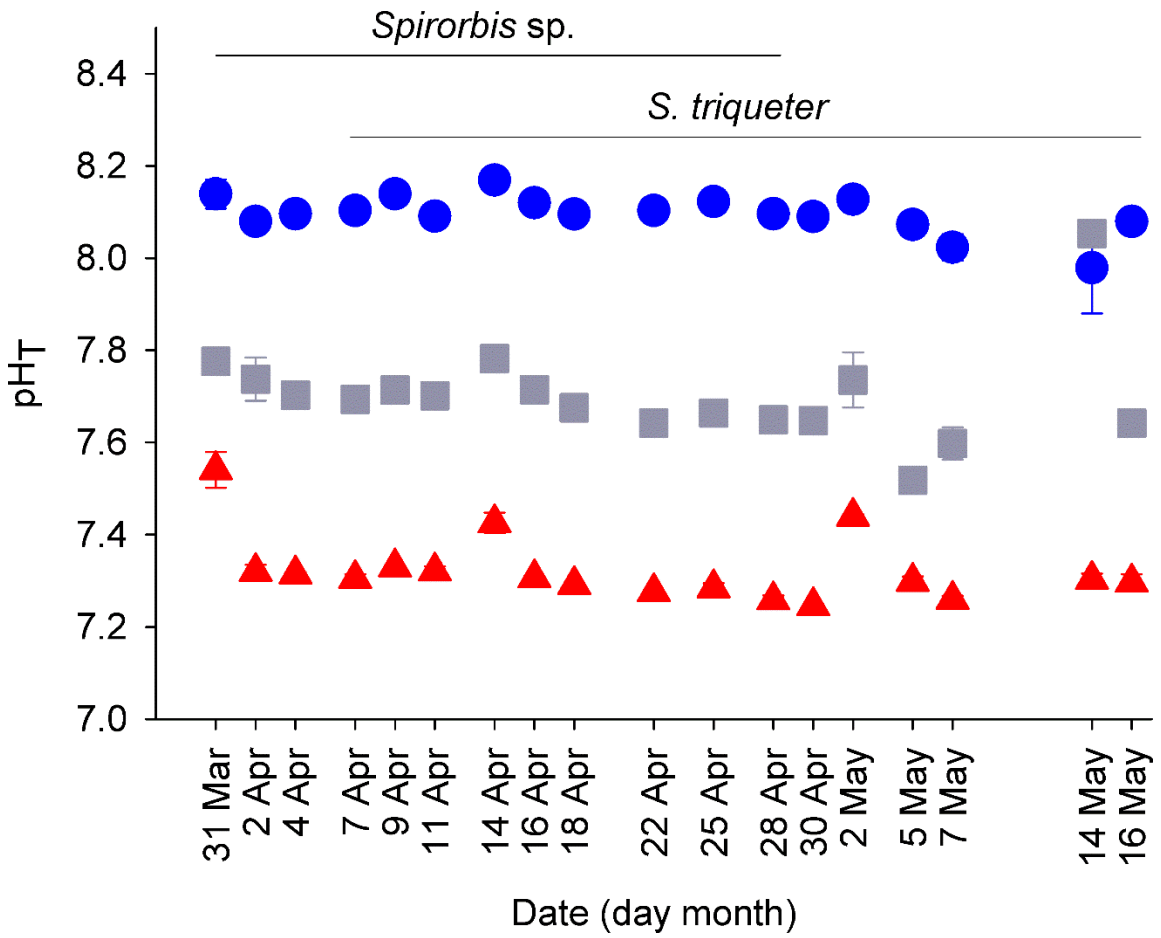

Fig. 2 pH_T measured in experimental cylinders in March (Mar), April (Apr), and May with *Spirorbis* sp. and *S. triqueter* (parental generation).

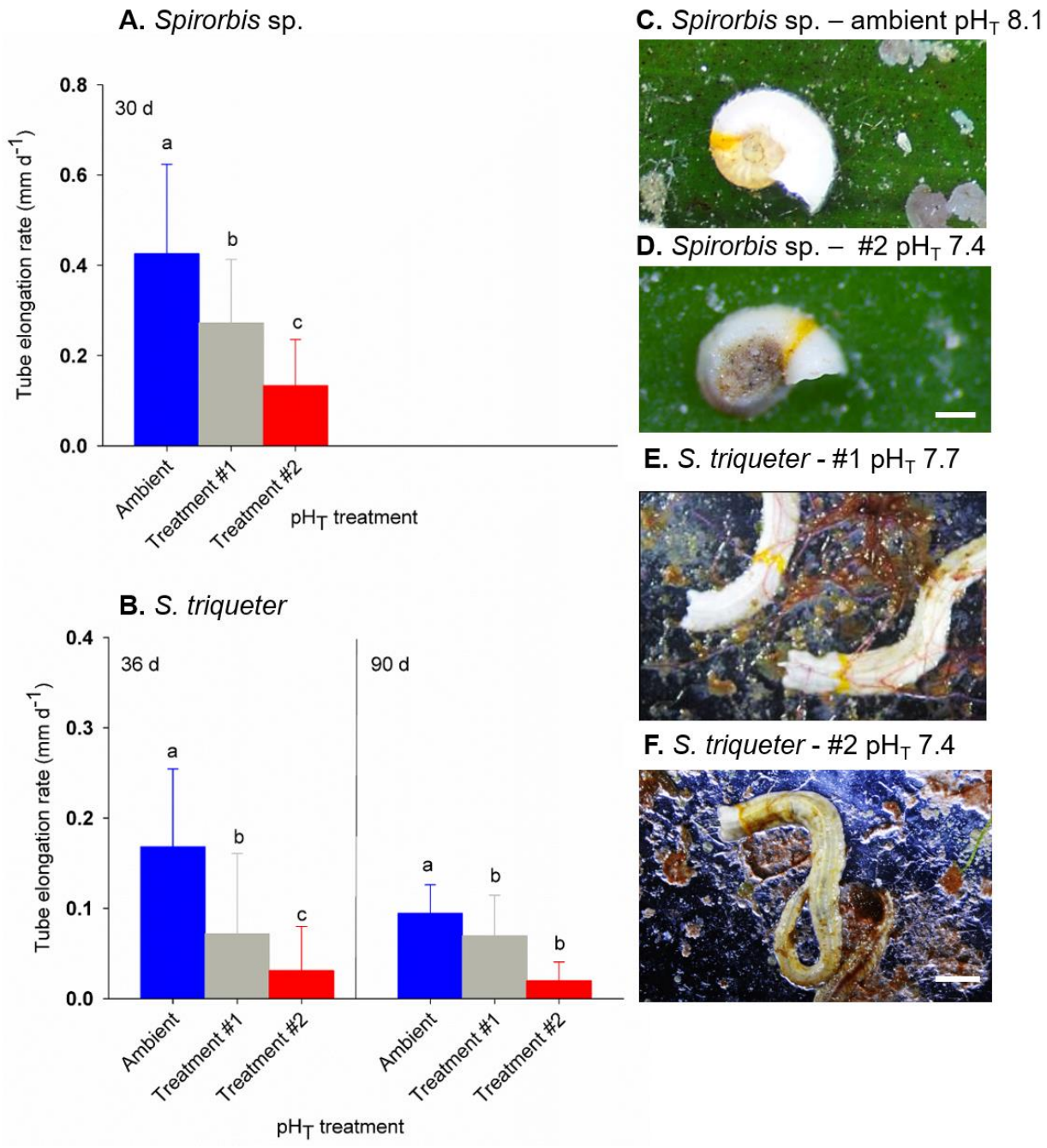

Fig. 3 Tube elongation rates (mean + SE in mm d^{-1}) for *Spirorbis* sp. at 30 d and *S. triqueter* (parental generation, B) at 36 and 90 d of experimental exposure to ambient, treatment #1, and treatment #2 conditions. Sample sizes were as follows *S. triqueter* at 36 d all treatments had an n = 13; for *Spirorbis* n = 15, 14, 15 and for *S. triqueter* at 90 d: n = 23, 28, 25 for ambient,

treatment #1 and #2 conditions, respectively. Lines within graph note the distinct measurement interval which was tested separately. Letters above bars represent statistical results from pairwise comparisons when main effects were found in one-way ANOVA or Kruskal-Wallis test (see Table 3). Images C-F capture the visual differences in new growth, after the yellow calcein mark, between treatments for *Spirorbis* sp. (C, D) at 30 d and *S. triqueter* at 90 d (E,F). Scale bar represents 1 and 9 mm for *Spirorbis* sp. and *S.triqueter*, respectively.

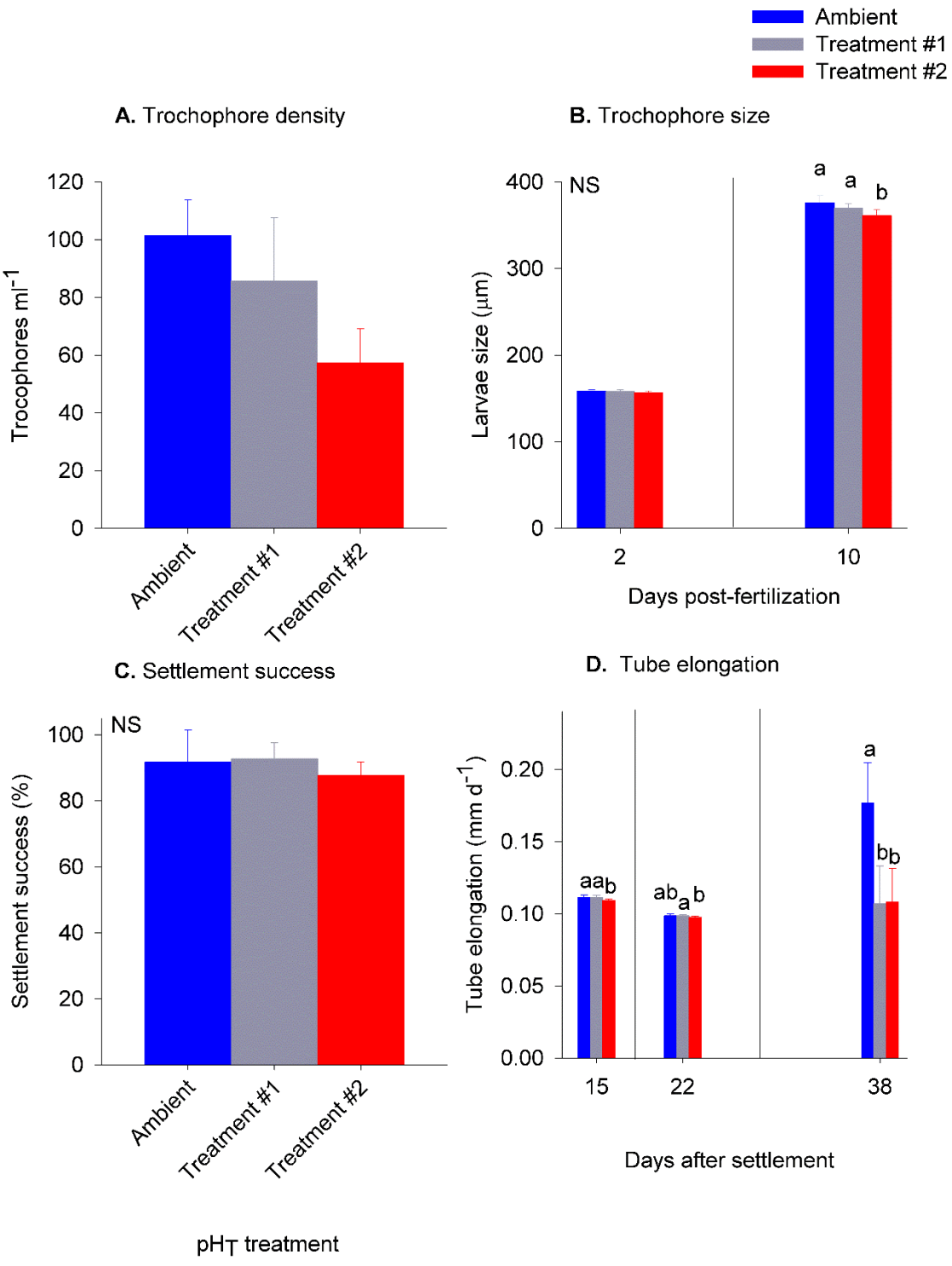

Fig. 4 *Spirobranchus triqueter* early life history stage development (A, B,C) and juvenile tube elongation rates (D, mean + SE) when cultured under ambient, treatment #1, treatment #2. Sample sizes were as follows: n = 10 for trocophore density and size at all treatment conditions, n = 6 for settlement success at all treatment conditions but not statistically tested, at 15 and 22 d n = 11, 12, 13 for ambient, treatment #1 and #2 conditions, respectively and at 38 d n = 18 for all treatment conditions. Lines within graph note the distinct measurement intervals which were tested separately. Letters above bars represent statistical results from pairwise comparisons when main effects were found in one-way ANOVA or Kruskal-Wallis test (NS = no significant differences found, see Table 3).

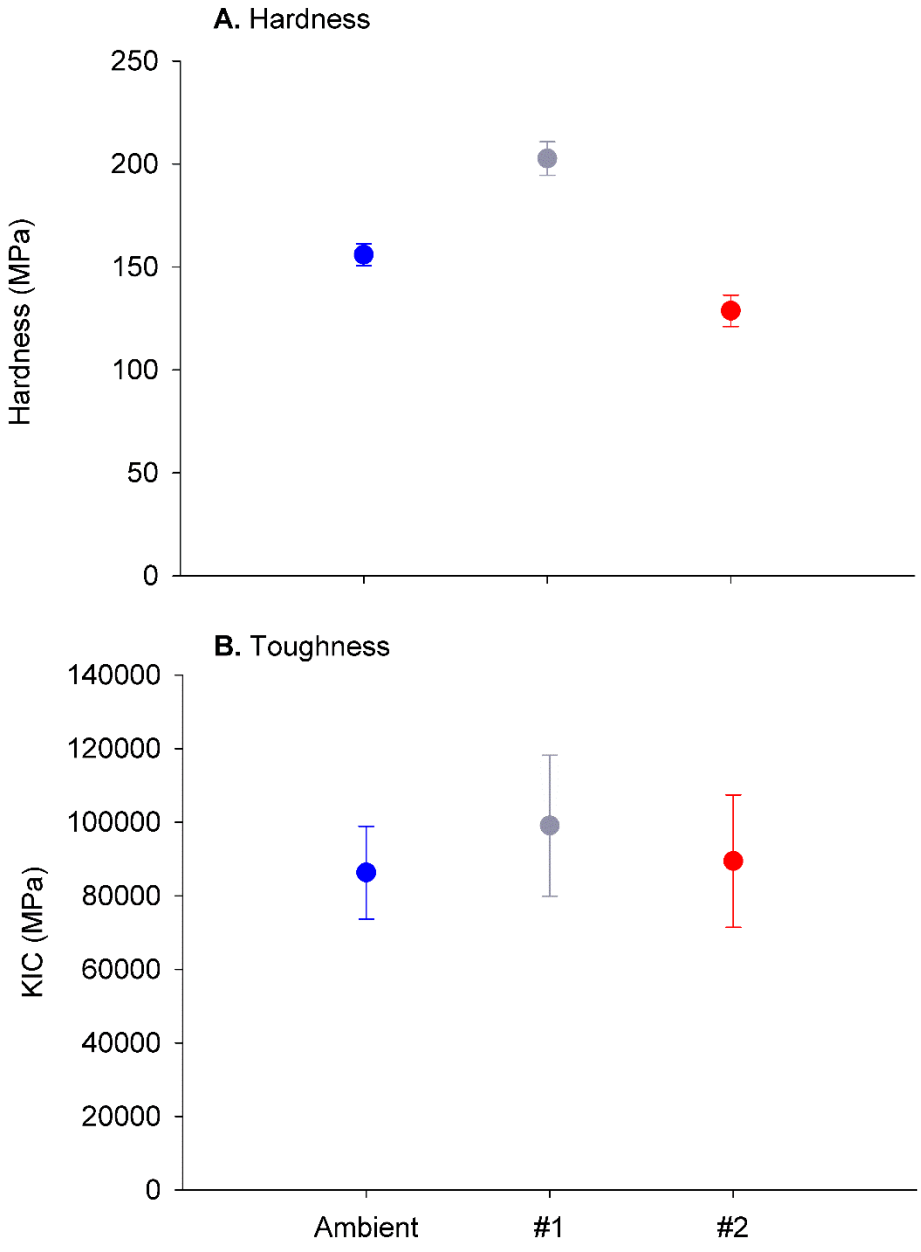

Fig. 5 Fracture hardness (A) and toughness (B, mean ± SE in Megapascals, MPa) of new growth of *S. triqueter* (parental generation) after 90 d of exposure to ambient (8.1), treatment #1 (7.7), and treatment #2 (7.4) pH_T (total scale) conditions. n = 3, see Table 3 for statistical results.

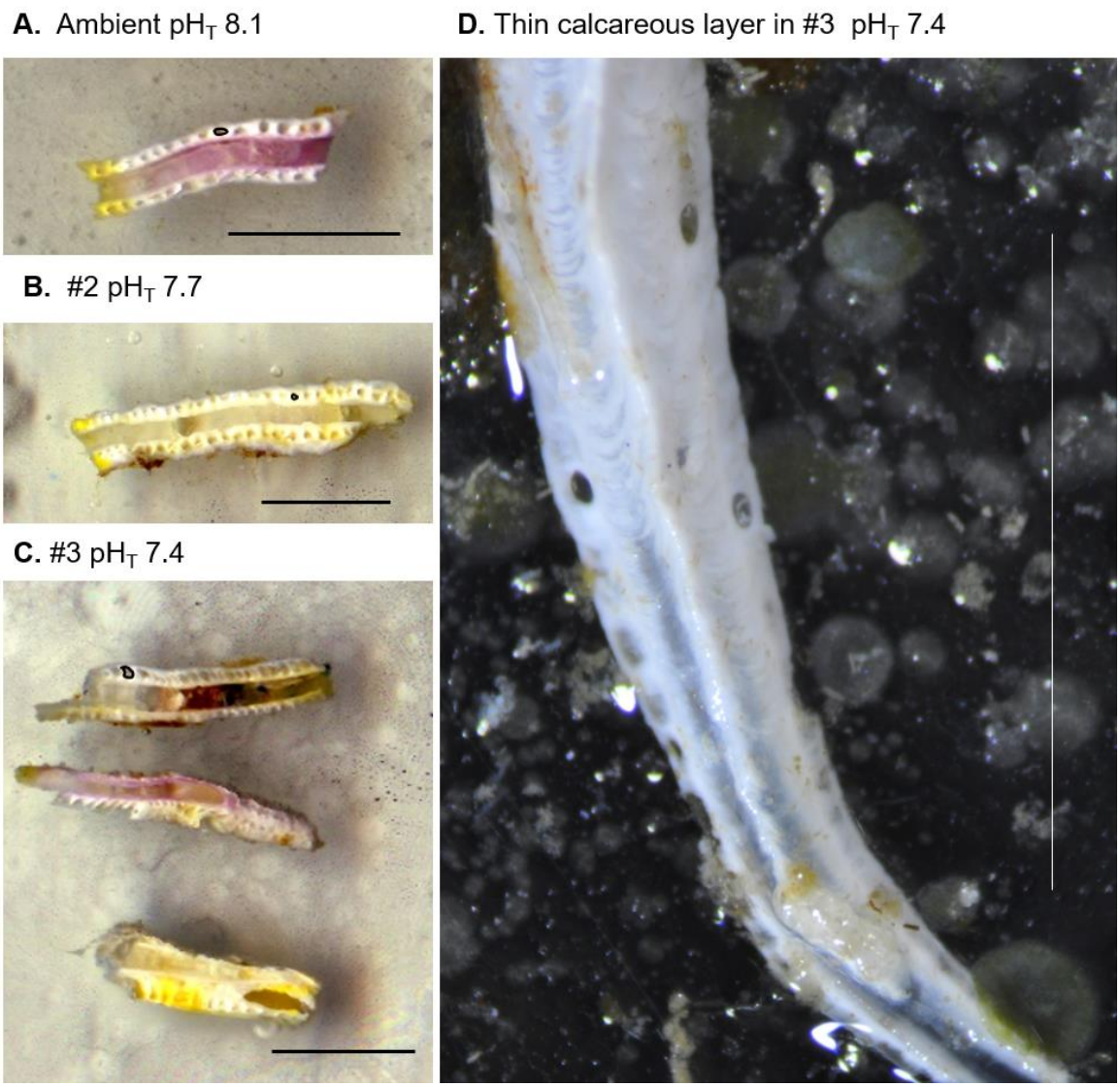

Fig. 6 Images that show the fragility of *S. triqueter* (parental generation) tubes in treatment #2 after 90 d of exposure. In A,B,C tubes have been sectioned and prepared for measures of fracture toughness and hardness. An alveolus in each treatment is outlined in black to note the difference in size. Note the small alveoli after exposure to pH$_T$ 7.7 and the large alveoli covered in a thin calcareous layer after 90 d at pH$_T$ 7.4. On the right (D) is a close-up (~4x) image of an intact worm tube at pH$_T$ (total scale) 7.4 showing the exposed alveoli and the thin calcareous layer. Scale bar is adjusted in all images for direct comparison.

Table S1. Carbonate chemistry calculated from determined A_T and C_T with a salinity of 37.5 and measured temperature (T, °C) within ambient, treatment #1, and treatment #2 header tanks over the experimental duration for *Spirorbis* sp. (30 d), and *Spirobranchus triqueter* collected from the field (90 d) and reared in tanks (offspring, 43 d): total alkalinity (A_T, μmol kg^{-1}), pH (on the total scale; pH_T), partial pressure of carbon dioxide (pCO_2, μatm), total dissolved inorganic carbon (C_T, μmol kg^{-1}) and saturation states with respect to aragonite (Ω_a) and calcite (Ω_c).

					Ambient					Treatment #1					Treatment #2				
Date	Day # *S.* sp.	Day # *S. t*	T	A_T	pH_T	pCO_2	C_T	Ω_a	Ω_c	pH_T	pCO_2	C_T	Ω_a	Ω_c	pH_T	pCO_2	C_T	Ω_a	Ω_c
3/28/2014	1	-	14.9	2555	8.12	354	2260	3.2	5.0	7.78	878	2430	1.6	2.5	7.47	1899	2544	0.8	1.3
3/31/2014	3	-	15.1	2556	8.10	375	2271	3.1	4.8	7.80	838	2422	1.7	2.7	7.34	2601	2590	0.6	1.0
4/2/2014	5	-	15.5	2554	8.11	373	2265	3.2	4.9	7.78	882	2426	1.7	2.6	7.40	2244	2564	0.7	1.2
4/4/2014	7	-	15.4	2550	8.16	321	2229	3.5	5.4	7.74	988	2441	1.5	2.3	7.46	1931	2539	0.8	1.3
4/7/2014	10	1	15.8	2555	8.12	357	2253	3.3	5.1	7.79	872	2423	1.7	2.6	7.46	1935	2542	0.9	1.3
4/9/2014	12	3	16.1	2549	8.12	363	2249	3.3	5.1	7.71	1042	2444	1.5	2.3	7.52	1674	2514	1.0	1.5
4/11/2014	14	5	16.8	2554	8.07	407	2272	3.1	4.8	7.73	995	2437	1.6	2.5	7.43	2105	2548	0.8	1.3
4/14/2014	17	8	17.1	2556	8.13	351	2238	3.5	5.3	7.73	1014	2440	1.6	2.5	7.45	2029	2543	0.9	1.4
4/16/2014	19	10	17.0	2551	8.10	382	2254	3.2	5.0	7.71	1047	2441	1.5	2.4	7.34	2613	2576	0.7	1.1
4/18/2014	21	12	16.7	2549	8.07	410	2270	3.1	4.7	7.68	1153	2456	1.4	2.2	7.43	2123	2545	0.8	1.3
4/22/2014	25	14	16.4	2535	8.06	416	2264	3.0	4.6	7.63	1294	2462	1.2	1.9	7.28	3037	2587	0.6	0.9
4/24/2014	27	16	16.4	2547	8.10	379	2254	3.2	5.0	7.69	1102	2449	1.4	2.2	7.33	2678	2579	0.7	1.0
4/25/2014	28	19	17.0	2548	8.20	283	2183	3.9	6.1	7.72	1038	2437	1.5	2.4	7.34	2611	2573	0.7	1.1
4/28/2014		22	16.9	2551	8.11	364	2244	3.3	5.2	7.72	1042	2441	1.5	2.4	7.42	2140	2547	0.8	1.3
4/30/2014	-	24	17.3	2546	8.09	391	2252	3.2	5.0	7.69	1119	2445	1.5	2.3	7.33	2689	2574	0.7	1.0
5/2/2014	-	26	17.1	2545	8.12	360	2235	3.4	5.2	7.73	1008	2429	1.6	2.4	7.20	3626	2622	0.5	0.8
5/5/2014	-	29	17.4	2548	8.10	374	2243	3.3	5.1	7.70	1082	2441	1.5	2.3	7.38	2410	2559	0.8	1.2
5/7/2014	-	31	17.7	2542	8.11	368	2232	3.4	5.2	7.67	1156	2444	1.4	2.2	7.31	2851	2577	0.7	1.0
5/14/2014	-	38	17.4	2539	-	-	-	-	-	-	-	-	-	-	7.11	4581	2657	0.4	0.6
5/16/2014	-	40	18.1	2539	-	-	-	-	-	-	-	-	-	-	7.36	2479	2551	0.8	1.2
5/23/2014	-	47-O2	15.0	2546	8.35	193	2258	3.0	4.8	-	-	-	-	-	-	-	-	-	-
5/24/2014	-	48-O3	14.7	2536	8.13	343	2239	3.2	5.0	7.62	1308	2474	1.1	1.8	7.63	1290	2472	1.2	1.8
5/26/2014	-	50-O5	15.4	2537	8.12	357	2242	3.2	5.0	7.74	969	2426	1.5	2.4	-	-	-	-	-
5/28/2014	-	61-O8	14.2	2551	8.16	317	2239	3.4	5.2	-	-	-	-	-	7.60	1366	2497	1.1	1.7
5/31/2014	-	64-O11	14.4	2539	8.16	319	2228	3.3	5.2	-	-	-	-	-	7.59	1408	2489	1.1	1.7
6/3/2014	-	67-O14	15.2	2543	-	-	-	-	-	7.70	1063	2447	1.4	2.2	-	-	-	-	-
6/5/2014	-	69-O16	14.1	2539	-	-	-	-	-	7.66	1170	2464	1.2	1.9	-	-	-	-	-
6/7/2014	-	71-O18	15.5	2544	-	-	-	-	-	7.85	736	2387	1.9	3.0	-	-	-	-	-

6/10/2014	-	74-O21	14.6	2550	-	-	-	-	-	7.80	833	2419	1.7	2.6	7.55	1573	2514	1.0	1.5
6/13/2014	-	77-O24	14.8	2553	8.18	300	2222	3.6	5.5	-	-	-	-	-	7.23	3346	2629	0.5	0.8
6/17/2014	-	81-O28	15.0	2559	8.12	360	2266	3.2	4.9	7.86	716	2399	1.9	3.0	7.47	1896	2547	0.9	1.3
6/20/2014	-	84-O31	15.0	2521	8.10	377	2244	3.0	4.7	7.68	1122	2436	1.3	2.0	7.12	4322	2643	0.4	0.6
6/23/2014	-	87-O34	14.9	2545	8.17	315	2225	3.4	5.3	7.86	709	2385	1.9	3.0	7.18	3752	2640	0.4	0.7
6/27/2014	-	O38	15.1	2581	8.18	304	2246	3.6	5.6	7.68	1150	2495	1.3	2.1	7.43	2119	2584	0.8	1.2
6/30/2014	-	O41	15.2	2538	8.19	292	2202	3.6	5.6	7.58	1430	2487	1.1	1.7	7.34	2597	2573	0.6	1.0
7/2/2014	-	O43	15.0	2547	-	-	-	-	-	-	-	-	-	-	7.35	2518	2577	0.7	1.0
***Spirorbis* sp.**		**Mean**	**16.2**	**2551**	**8.11**	**367**	**2251**	**3.3**	**5.1**	**7.73**	**1011**	**2439**	**1.5**	**2.4**	**7.40**	**2268**	**2557**	**0.8**	**1.2**
		SD	**0.8**	**6**	**0.04**	**37**	**24**	**0.2**	**0.4**	**0.05**	**128**	**12**	**0.1**	**0.2**	**0.07**	**401**	**23**	**0.1**	**0.2**
S. triqueter		**Mean**	**15.9**	**2546**	**8.13**	**347**	**2242**	**3.3**	**5.1**	**7.72**	**1047**	**2440**	**1.5**	**2.3**	**7.38**	**2510**	**2565**	**0.8**	**1.2**
		SD	**1.2**	**10**	**0.06**	**49**	**20**	**0.2**	**0.3**	**0.07**	**177**	**26**	**0.2**	**0.3**	**0.14**	**848**	**46**	**0.2**	**0.3**
***S. triqueter* offspring**		**Mean**	**14.9**	**2546**	**8.15**	**328**	**2235**	**3.4**	**5.2**	**7.73**	**1019**	**2438**	**1.5**	**2.3**	**7.41**	**2381**	**2560**	**0.8**	**1.2**
		SD	**0.4**	**13**	**0.03**	**29**	**17**	**0.2**	**0.3**	**0.10**	**247**	**39**	**0.3**	**0.5**	**0.18**	**1038**	**62**	**0.3**	**0.4**