Science of the Total Environment 693 (2019) 133476

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Ocean acidification and elevated temperature negatively affect recruitment, oxygen consumption and calcification of the reef-building *Dendropoma cristatum* early life stages: Evidence from a manipulative field study

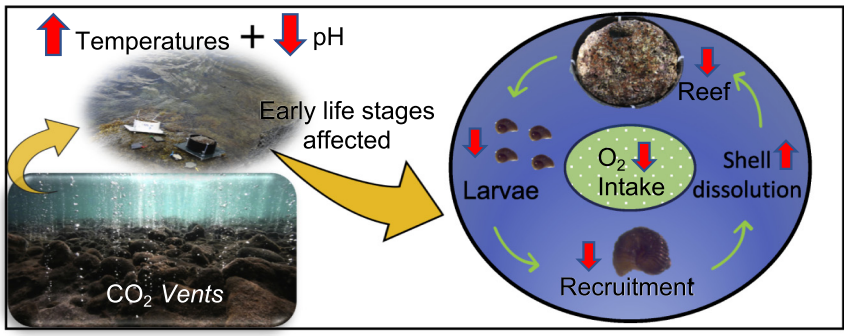
Cinzia Alessi [a,*], Folco Giomi [a], Francesco Furnari [a], Gianluca Sarà [a], Renato Chemello [a,b], Marco Milazzo [a,b]

[a] Department of Earth and Marine Sciences (DiSTeM), University of Palermo, via Archirafi 20-22, 90123 Palermo, Italy
[b] Consorzio Nazionale Interuniversitario per le Scienze del Mare (CoNISMa), Rome, Italy

HIGHLIGHTS

- Effects of pH and temperature in a Mediterranean intertidal reef-building species were tested for the first time;
- Portions of vermetid reefs have been transplanted in a natural CO_2 vent and air-temperature was manipulated;
- The combination of high temperature and low pH reduced the oxygen consumption of the vermetid *D. cristatum*;
- Number of embryos, recruitment, and calcification of early life stages of *D. cristatum* were impacted by temperature and pH;
- Shell of embryos and recruits exposed to high pCO_2 showed an increase in Mg concentration and a higher dissolution.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 May 2019
Received in revised form 10 July 2019
Accepted 17 July 2019
Available online 19 July 2019

Editor: Henner Hollert

Keywords:
Climate change
Intertidal species
Larval development
Larval settlement
Physiological traits

ABSTRACT

Expected temperature rise and seawater pH decrease may affect marine organism fitness. By a transplant experiment involving air-temperature manipulation along a natural CO_2 gradient, we investigated the effects of high pCO_2 (~1100 μatm) and elevated temperature (up to +2 °C than ambient conditions) on the reproductive success, recruitment, growth, shell chemical composition and oxygen consumption of the early life stages of the intertidal reef-building vermetid *Dendropoma cristatum*. Reproductive success was predominantly affected by temperature increase, with encapsulated embryos exhibiting higher survival in control than elevated temperature conditions, which were in turn unaffected by altered seawater pH levels. Decreasing pH (alone or in combination with temperature) significantly affected the shell growth and shell chemical composition of both embryos and recruits. Elevated temperatures along with lower pH led to decreases of ~30% oxygen consumption and ~60% recruitment. Our results suggest that the early life stages of the reef-builder *D. cristatum* are highly sensitive to expected environmental change, with major consequences on the intertidal vermetid reefs they build and indirectly on the high biodiversity levels they support.

* Corresponding author at: Dipartimento di Scienze della Terra e del Mare (DiSTeM), Università di Palermo, Via Archirafi 20, I-90123 Palermo, Italy.
E-mail address: cinzia.alessi01@community.unipa.it (C. Alessi).

https://doi.org/10.1016/j.scitotenv.2019.07.282
0048-9697/

1. Introduction

Increasing anthropogenic CO_2 is leading to ocean warming and ocean acidification (OA) (Caldeira, 2005), with model projections suggesting seawater temperature will further rise by 2.0–4.5 °C (Pörtner et al., 2014) and pH will decrease by 0.4 units by the end of this century (Caldeira and Wickett, 2003). Such phenomena are already changing the state of many marine ecosystems, affecting key processes such as reproduction, development, recruitment and growth of ecologically relevant reef-building species such as calcifying algae, corals and molluscs (Byrne, 2011; Kroeker et al., 2013; Parker et al., 2010). Early life stages are the most vulnerable to ongoing and expected environmental changes (Kroeker et al., 2011). Development success, settlement, and recruitment of many marine species can be impaired (Albright et al., 2010) either directly, affecting metabolic processes or their ability to recognize appropriate settlement substrata, or indirectly, by altering the community composition of the receiving habitats (Doropoulos et al., 2012; Fabricius et al., 2017; Milazzo et al., 2019).

Although marine intertidal species may exhibit high tolerance limits and thermal plasticity, acute exposure to heat stress at low tide may have detrimental effects on their metabolism and the ability to repair, replace and restructure thermally sensitive biochemical components of the cells (Somero, 2002). Consequently, a reduction in energy available for reproduction, growth, and behavior can occur over time (Michaelidis et al., 2005; Portner et al., 2005). Similarly, prolonged exposure to warming conditions can be detrimental for both embryonic and larval stages of intertidal tropical species (Stillman, 2003), causing a reduction in development time, faster growth and in some cases a decrease in larval size and viability (Byrne, 2011; Davis et al., 2013; Armstrong et al., 2017). Under these circumstances OA may increase the energy costs associated with the calcification process (Byrne and Przeslawski, 2013), with calcifying organisms exhibiting smaller body sizes, abnormal structures (Garilli et al., 2015; Kurihara, 2008; Parker et al., 2010) and altered chemical composition of their carbonate skeletons and shells (Bentov and Erez, 2006; Milazzo et al., 2014; Nash et al., 2013). For example, it has been well documented that the presence of Mg^{2+} in the calcifying fluid postpones crystallization of calcite and favors the formation of the metastable aragonite polymorph (Fernandez-Diaz, 1996), hence weakening skeleton and shell structures.

Many biogenic habitats, e.g. coral reefs, mussel beds and algal-vermetid reefs, are built up by calcifying organisms that can be affected by OA and warming, with cascading effects on their associated biodiversity (Milazzo et al., 2019; Sunday et al., 2017). Indeed, recent studies demonstrate that the combined effects of OA and warming could be more detrimental for marine organisms than the effect of the single stressors alone (Davis et al., 2013; Parker et al., 2010; Ries et al., 2016). However, little is known about how the interaction of OA and warming can affect marine habitat-forming species, particularly in temperate systems.

In this study, we exposed the intertidal reef-building vermetid *Dendropoma cristatum* (Biondi, 1859) (sin. *D. petraeum* (Monterosato, 1884)) in situ to manipulated air-temperature and seawater pH conditions along a volcanic CO_2 gradient in Levante bay (Vulcano Island, Italy). This location is characterized by numerous degassing zones located at shallow depths (1–8 m) along the SW shore of the bay (38°25.1′N, 14°57.6′E) (Boatta et al., 2013). At the venting sites seawater pH is acidic (e.g. 5.2–5.5 units) and carbon dioxide is the dominant gas emitted (>99%), while ambient conditions occur >500 m from the venting zone (Boatta et al., 2013). This creates a large gradient in carbonate chemistry that has been characterized by a number of recent studies (e.g., Arnold et al., 2012; Boatta et al., 2013; Calosi et al., 2013; Johnson et al., 2012; Lidbury et al., 2012; Milazzo et al., 2014).

Dendropoma cristatum is a gonochoric species with internal fertilization and intra-capsular larval development that occurs in the maternal mantel cavity (Calvo et al., 2009; Templado et al., 2015). The fertilized eggs develop within egg capsules for one month until the crawling larvae are released. The duration of the crawling larval stage is only a few hours and during this stage the larvae find a suitable spot for settlement in order to start a sessile life. Its peculiar life cycle and low larval dispersion make this species ideal for a transplant experiment in the field. In natural conditions, gregarious vermetids form a complex intertidal habitat which provides many ecological services, such as preventing coastal erosion, supporting high levels of biodiversity and regulating sediment deposition (Milazzo et al., 2017).

Specifically, here we tested the effects of pH and temperature on the reproductive/recruitment success, the metabolism and the calcification of *D. cristatum* early life stages. To achieve this, we coupled transplant experiments in natural CO_2 seeps with laboratory analyses on *D. cristatum* embryos and recruits. We expect that the alteration of air-temperature and pH, alone or in combination, may affect the early life stages of *D. cristatum*. Encapsulation has been suggested to limit the exchange of diffusive gases (Strathmann and Strathmann, 1995), whereby the intracapsular fluid buffer capacity may reduce the potential effect of the extracapsular elevated seawater pCO_2 (Noisette et al., 2014). Thus, we assume that the encapsulated development in this species will minimize gas exchange and we hypothesize that embryos will be affected by elevated temperature only, while pH will exert a bigger influence on the recruitment and survival of *D. cristatum* recruits. We also hypothesize that OA and warming may synergistically interact on the metabolic balance, predominantly affecting growth (i.e. shell formation or calcification) of recruits but also the chemical composition of their shells.

2. Materials and methods

2.1. Animal collection and transplant experimental set-up

In the Southern Tyrrhenian Sea (Western Mediterranean), *D. cristatum* larval incubation occurs in the month of May inside the maternal mantle cavity and the recruitment stage typically ranges from early June to late October (Franzitta et al., 2016; Templado et al., 2015). Twelve vermetid cores of 13 cm diameter were collected from the North Western coast of Sicily, Italy (Barcarello, 38°12′47.4"N 13°17′28.4″E). All cores had a similar abundance of *D. cristatum* adults (estimated by a visual count). Cores were collected in May 2014 during low tide using a pneumatic drill (Airtec 478 SN). Core surfaces were photographed to assess the living adult density (number of individuals ≥3 mm in 100 cm^2) following Milazzo et al. (2014). After accurate inspection under a binocular microscope (Leica MZ-APO), any individuals ≤1 mm were removed with soft forceps, and cores were then transplanted off Baia di Levante (Vulcano Island; NE Sicily, Italy) where a well-established natural pH/CO_2 gradient occurs (e.g., Arnold et al., 2012; Boatta et al., 2013; Calosi et al., 2013; Taylor et al., 2014; Cattano et al., 2016; Brown et al., 2017; Cornwall et al., 2017; Urbarova et al., 2019).

We used two sites, ~200 m apart, along the pCO_2 gradient; one with mean pH (OA pH site at ~7.8 pH) and one with ambient mean pH (~8.1 pH units). These sites correspond to sites 40–60 and R2 in Boatta et al. (2013) for low pH and control pH respectively.

Each vermetid core was fixed to a bakelite slab and enclosed within a 14 cm diameter PVC tube. Half of the slabs and tubes were colored white and half were colored black in order to manipulate temperature in situ (Fig. S1). Plastic bands and four steel screws (12 cm) were used to fix each structure at mean sea level in the intertidal rocky shore, with all cores exposed eastward. Tide level was calculated during the experimental period using WXTide software (http://wxtide32.com). Temperature was logged every 30 min with temperature sensors (TidbiT v2 Water Temperature Data Logger, ONSET, resolution 0.02 °C at 25 °C) placed underneath the vermetid cores to record temperature data during larval development (occurring in the tubular shells inside the cores) and recruitment events (on the surface of the cores). Temperature

sensors were placed in natural cavities within the mid-inner parts of the cores. Since intertidal organisms are more exposed to temperature stress during low tide and under maximum irradiance, we compared the temperature data for white- and black-framed cores between 10 am and 4 pm. Cores (three for each combination of black/white frames, Control/OA pH) were placed in Baia di Levante from May 28th to July 23rd 2014 (i.e. 57 days), encompassing the peak period of vermetid recruitment.

2.2. Experimental design

For the transplant experiment, twelve vermetid cores were randomly assigned to treatment conditions following a fully crossed 2-factor design consisting of control temperature and control pH (8.1 pH_Ta), elevated temperature and control pH (8.1 pH_T+), control temperature and low pH (7.8 pH_Ta), and a combination of elevated temperature and low pH (7.8 pH_T+), resulting in 3 replicates × 2 temperatures × 2 pHs = 12 cores.

2.3. Reproductive and recruitment success

At the end of the experiment, cores together with the egg capsules remaining within the maternal mantle were collected in order to define the reproductive success, measured as number of egg capsules and embryos produced by the adult population. To induce females to release their egg capsules (Fig. S2), each core was submerged in a solution of seawater and H_2O_2 (3 vol.%) for 30 s and subsequently submerged in seawater (3 °C warmer than ambient) for 20 min. All the egg capsules collected were preserved under 4% formalin, subsequently substituted with a solution of 70% ethanol and deionized water (DIW). The number of recruits on the surface of each core was counted by observation under a binocular microscope connected to a HD camera (Leica MC 170) (≤1 mm). Living recruits were separated from the dead recruits by presence/absence of the operculum (Milazzo et al., 2014), in order to establish the Recruitment Success (total living recruits) and the Percentage of Mortality (number of dead recruits /total recruits * 100).

2.4. Early stage shell growth and dissolution

To assess growth, i.e. shell formation, under the environmental conditions experienced by recruits in the study site for 2 months, we collected each individual with soft forceps to avoid damage to the shell during the collection process. Newly attached recruits exhibit an evident white protruding edge that marks the limit between the protoconch (built up during the embryonic stage) and the teleoconch (which starts to be produced after settlement). We measured teleoconch growth under the different environmental conditions (Fig. S3). After collection, recruits were placed on their right side on transparent wax close to a micrometric slide (1 mm scale) and photographed with an optical microscope connected to a camera (Leica MC 170 HD camera, 20× magnification). Photographs were analyzed using Image J to assess length (μm), width (μm) and teleoconch surface (μm^2).

2.5. Chemical composition of early stage shells

We made qualitative analyses of the calcification of both recruits and embryos using a Scanning Electron Microscope (S.E.M; Oxford Leo 440, 65–1500× magnification). Fifteen recruits and 4 mature (i.e. late-stage) embryos were randomly collected in each treatment combination of temperature and pH. Samples were rinsed twice with DIW, carefully placed upon 12.5 mm surface stubs in the same orientation and, after being dried in a lab stove, covered with graphite using an Imetek K900 evaporator. The same S.E.M. settings were used to visualize all samples (±65× magnification, Working Distance (WD) 10 mm, Extra-High-Tension (EHT) between 15 and 20 kV and 100 pA/Ampere I probe, tungsten filament). Images were analyzed using Image J to quantify dissolution of the organic periostracum (dissolved area/total area * 100) and measurements in mm were converted to μm.

Finally, we analyzed the mineralogical shell composition of recruits and larvae, determining Ca, Mg and Sr concentrations using an EDS - LINK ISIS integrated to the S.E.M. The scanning time of the EDS (Energy Dispersive Spectrometer) was set at 60 s per 5 μm area with the following settings: EHT 20 kV, I probe 600 pA and WD 25 mm. The results of the X-EDS analyses were obtained by comparison with certified international standards and the final concentration of Ca, Mg and Sr are expressed in percentage (W%). Observations were made on 16 recruits (randomly chosen from those previously collected) and 16 encapsulated embryos. We selected 4 specific points in the oldest part of the shell in the protoconch of larvae and recruits to analyze Ca, Mg and Sr concentration and 2 additional points in the teleoconch of recruits in order to investigate calcification in the new portion of the shell formed under in situ conditions (see Fig. S4). In the protoconch the 4 points were on the apex, the costa, the intern-costa and the white protruding edge between the protoconch and teleoconch. The two points on the teleoconch were at the start of the teleoconch and close to the end. We made three measurements at each of the 6 shell sites for each recruit and subsequently analyzed the average for each shell portion (protoconch and teleoconch).

2.6. Oxygen consumption

We investigated the effects of pH and temperature on the metabolism of settled recruits under lab conditions. The oxygen consumption ($\mu mol\ L^{-1}\ h^{-1}$) was determined using a Unisense respirometer. According with the temperature series recorded in the field we chose two temperature values (24 °C and 28 °C) for the laboratory experiment. pH (8.1 and 7.8) was maintained at the same level as the origin of the recruits and temperature (24 °C and 28 °C) levels were crossed with each pH. The oxygen consumption of a single organism was close to the instrumental lowest threshold, therefore we used groups of 3 individuals inside a 10 ml chamber for each respirometric measurement. Recruits were acclimated to field conditions for 48 h directly before starting chamber incubations.

2.7. Seawater carbonate chemistry

The biogeochemistry of the bay has been previously assessed to identify the most suitable areas for ocean acidification research, and the chosen sites for our manipulative experiment were outside of any high level metal contamination (Boatta et al., 2013; Vizzini et al., 2013). Measurements of the seawater carbonate system were made throughout the study duration in May/June and July 2014 (every ~10 days; i.e., three visits on late May and June and three visits on July). A 556 MPS YSI (Yellow Springs, USA) probe was used to measure seawater temperature and salinity. pH was measured in triplicate on each visit using a meter (Orion Star A216 pH/RDO/DO) and pH electrode (Orion 8107BNUMD – Ross Ultra pH/ATC triode), calibrated with pH 7.0 and 9.0 NBS buffers, cross referenced with Tris and Amp seawater buffers to convert to the total scale (Dickson et al., 2007). Water samples for total alkalinity (A_T) were taken in replicates ($n = 3$) in June and July from each transplant location and were filtered through 0.2 mm pore size filters, treated with 0.05 ml of 50% $HgCl_2$ to avoid biological alteration, and then stored in the dark at 4 °C. A_T was measured using an open-cell titration using a Metrohm 809 Titrando and Metrohm 800 Dosino. A_T measurements were made at 25 °C using a temperature bath and were measured against a CRM (see Dickson et al., 2007). The pCO_2 and the saturation state of aragonite were calculated from pH, A_T, temperature and salinity with the free-access CO_2 SYS package.

Table 1
Water Chemistry. Average values (±S.E.), range and medians of the temperature treatments (e.g., Ta: white-framed cores; T+: black-framed cores) both at high and low tide, and of the main parameters of the carbonate chemistry in the experimental sites off Levante Bay (Vulcano Island) during June and July 2014. Stats comparison for pH, pCO_2, Ω Calcite, Ω Aragonite are reported, with significant effects in bold. Comparisons between temperature treatments are reported in the text and in Table S1. n.a.: not available.

June		7.8_pH site	8.1_pH site	
Low tide ($n = 198$)				
Air-temperature	Mean ± S.E.	24.59 (±0.01)	n.a.	
Ta (°C)	Range	20.06–28.37		
	Median	25.04		
Air-temperature	Mean ± S.E.	25.12 (±0.01)	24.96 (±0.01)	
T+ (°C)	Range	19.67–30.24	19.91–30.26	
	Median	25.26	25.08	
High tide ($n = 218$)				
Seawater-temperature	Mean ± S.E.	23.60 (±0.009)	n.a.	
Ta (°C)	Range	20.03–26.72		
	Median	24.52		
Seawater-temperature	Mean ± S.E.	23.67 (±0.009)	23.46 (±0.009)	
T+ (°C)	Range	20.06–26.87	19.98–26.77	
	Median	24.57	24.34	
Seawater chemistry (n = 3)		**7.8_pH site**	**8.1_pH site**	**pseudoF** ***P* value**
Salinity (ppm)	Mean	38	38	–
pH_T	Mean ± S.E.	7.72 (±0.11)	8.08 (±0.01)	pF = 10.54
	Range	7.50–7.84	8.07–8.1	***p* = 0.0315**
	Median	7.81	8.07	
pCO_2 (μatm)	Mean ± S.E.	1134 (±351)	390 (±13)	pF = 4.48
	Range	753–1836	365–408	$p = 0.1016$
	Median	813	397	
Alkalinity (mmol kg^{-1})	Mean	2526.9	2519.7	–
Ω Calcite	Mean ± S.E.	3.07 (±0.61)	5.83 (±0.12)	pF = 19.72
	Range	1.86–3.78	5.67–6.06	***p* = 0.0113**
	Median	3.58	5.76	
Ω Aragonite	Mean ± S.E.	2.02 (±0.40)	3.84 (±0.08)	pF = 19.55
	Range	1.22–2.49	3.73–3.99	***p* = 0.0115**
	Median	2.36	3.80	
July		**7.8_pH site**	**8.1_pH site**	
Low tide ($n = 120$)				
Air-temperature	Mean ± S.E.	26.31 (±0.006)	n.a.	
Ta (°C)	Range	24.82–27.78		
	Median	26.30		
Air-temperature	Mean ± S.E.	26.42 (±0.006)	26.27 (±0.007)	
T+ (°C)	Range	24.63–28.15	24.94–28.52	
	Median	26.43	26.18	
High tide ($n = 153$)				
Seawater-temperature	Mean ± S.E.	26.24 (±0.005)	n.a.	
Ta (°C)	Range	24.77–28.10		
	Median	25.99		
Seawater-temperature	Mean ± S.E.	26.30 (±0.005)	26.05 (±0.005)	
T+ (°C)	Range	24.80–28.15	24.75–28.21	
	Median	26.04	25.79	
Seawater chemistry (n = 3)		**7.8_pH site**	**8.1_pH site**	**pseudoF** ***P* value**
Salinity (ppm)	Mean	38	38	–
pH_T	Mean ± S.E.	7.69 (±0.09)	8.09 (±0.03)	pF = 19.44
	Range	7.5–7.79	8.06–8.11	***p* = 0.0116**
	Median	7.77	8.10	
pCO_2 (μatm)	Mean ± S.E.	1188 (±298)	365.7 (±17.2)	pF = 21.28
	Range	868–1783	344.7–399.4	***p* = 0.0099**
	Median	914	353	
Alkalinity (mmol kg^{-1})	Mean	2501	2520.35	–
Ω Calcite	Mean ± S.E.	3.06 (±0.51)	6.38 (±0.19)	pF = 37.56
	Range	2.05–3.63	6.00–6.62	***p* = 0.0036**
	Median	3.50	6.51	
Ω Aragonite	Mean ± S.E.	1.58 (±0.45)	4.23 (±0.13)	pF = 37.54
	Range	1.06–1.90	3.98–4.39	***p* = 0.0036**
	Median	1.77	4.32	

2.8. Statistical analyses

Due to a violation of two of the assumptions of analysis of variance we used a linear mixed model (Zuur et al., 2009) to compare temperature differences between black and white-framed vermetid cores between tide levels. The spread of residuals changed for each level of tide, consequently we chose to incorporate this pattern by adding the "varIdent" variance function structure in the model allowing different values per each level (Factor = Tide; low tide ≤22 cm (mean sea level for the study area) and high tide >22 cm). Further, we used a random intercept model using "day" as a random component, assuming that the variation of temperature around the model intercept for each day is normally distributed with a certain variance. All other analyses were performed using PERMANOVA. The adult density, the reproductive and

recruitment success, and the dissolution state were analyzed through a fully crossed 2-factor experimental design with pH (two levels: 7.8 Vs 8.1) and temperature (two levels: Ta Vs T+) as fixed and orthogonal factors. For oxygen consumption we considered three orthogonal factors: pH (7.8 Vs 8.1), origin temperature (Ta Vs T+) and experimental temperature (T-24 Vs T-28). For the chemical composition of the shell we evaluated the effects of three factors, pH and T as fixed and orthogonal factors and "organism" as a random factor. Finally, to compare differences between levels of significant factors or their interactions we ran Pairwise *t*-tests. Primer software (PRIMER 6.1.10 & Permanova ß20 (University of Plymouth, UK) was used for all statistical analyses (Clarke and Gorley, 2006).

3. Results

3.1. Seawater carbonate chemistry and temperature conditions at low tide

The mean surface seawater pH_T, Ω Calcite and Ω Aragonite were consistently lower in the low pH site (7.8 pH) compared to the ambient site (8.1 pH) both in June and July (Table 1). pCO_2 levels did not significantly change in June between sites, while significantly differed on July (Table 1).

The comparison between Ta and T+ (Fig. 1) was only possible for sites with 7.8 pH since one temperature sensor from the 8.1 pH site was lost (Table 1). During the experimental period, air-temperatures on black-framed cores (T+), recording the lowest and highest temperatures, ranged between 19.67 °C to 30.26 °C. Air-temperatures on white-framed cores (Ta) ranged between 20.06 and 28.37 °C. At low tide, the difference between maximum temperatures (ΔT_{max}) recorded in the Ta and T+ cores were 1.89 °C, and the difference between minimum temperatures (ΔT_{min}) was −0.36 °C. The linear mixed model indicated that temperature difference (i.e., pairwise comparisons of T+ and Ta recorded every 30 m^{-1} for the entire experiment duration) differed significantly between tide levels, with temperature difference in low tide (on average 0.35 °C ± 0.03 S.E.) being clearly higher than in high tide (0.07 °C ± 0.01 S.E.; Wald chi square test = 53.001, $p < 0.001$). The standard deviation for the random part of the model was d = 0.13 while the residuals variance in the high tide level was 22% lower than in the low tide.

3.2. Reproduction and recruitment success

The number of brood-stocks (i.e. adults) present on each core for all experimental conditions of pH and temperature did not significantly differ between cores (pHxT interaction: Pseudo-F_{1-11} = 4.4132; p(perm) = 0.0622) (Table S1).

Analysis of reproductive success found no significant differences in the number of egg capsules between pH treatments (Pseudo-F_{1-11} = 0.3106; P(perm) = 0.626). Conversely, the number of embryos per egg capsule significantly increased in cores that experienced control temperature (Pseudo-F_{1-11} = 5.6074; p(perm) = 0.0429) (Fig. 2A; Table S1). We found a highly significant difference in the size of the embryos developed at different temperature and pH (pHxT interaction: Pseudo-F_{1-643} = 15,14; p(perm) = 0.0001). The largest embryos were found in the 7.8 pH_T+ condition (772.5 ± 7.4 in length and 584.41 ± 6.53 μm wide) while the smallest embryos were found in the 8.1 pH_Ta condition (723.77 ± 3.18 and 534.56 ± 2.67 μm respectively) (Fig. 2B; Table S1).

Seawater pH had a strong influence on the density of living recruits, with higher recruitment success in the 8.1 pH site (Fig. 3A) (591 in control pH and 260 in 7.8 pH) (Pseudo-$_{F1-11}$ = 12.932; p(perm) = 0.0097), with no effect of temperature on the number of living recruits (Pseudo-$_{F1-11}$ = 0.5298; p(perm) = 0.4867) (Table S1). No significant effect on the mortality of recruits among treatments was observed (Pseudo-F_{1-11} = 0.00195; p(perm) = 0.9044) (Table S1).

3.3. Shell growth, dissolution and chemical composition of the early stages

The growth of 481 recruits was assessed. Teleoconch surface was significantly affected by pH with maximum shell formation recorded at 8.1 pH (198,000 ± 11,432 μm^2) and a minimum at 7.8 pH (154,000 ± 13,129 μm^2 respectively) (Pseudo-F_{1-481} = 5.437; p(perm) =

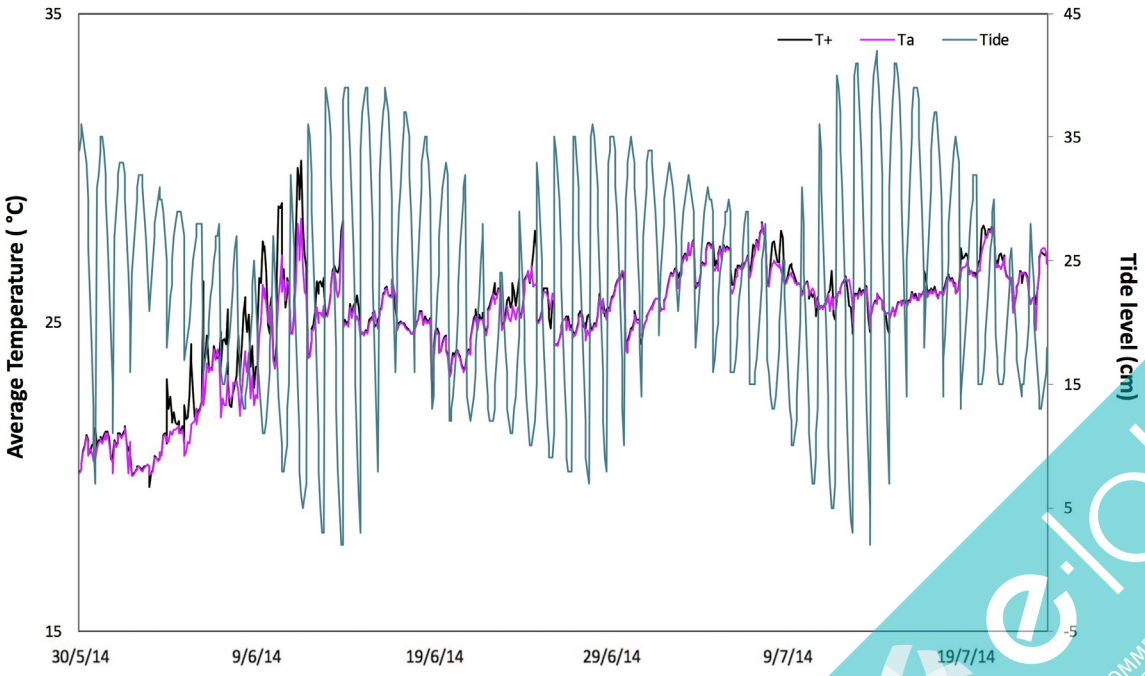


Fig. 1. Temperature Recorded. Temperature and tide values recorded between 10 am and 4 pm. The black line shows the temperature of black framed cores, the pink line shows the temperature recorded in the white framed cores. The blue line represents the tide. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

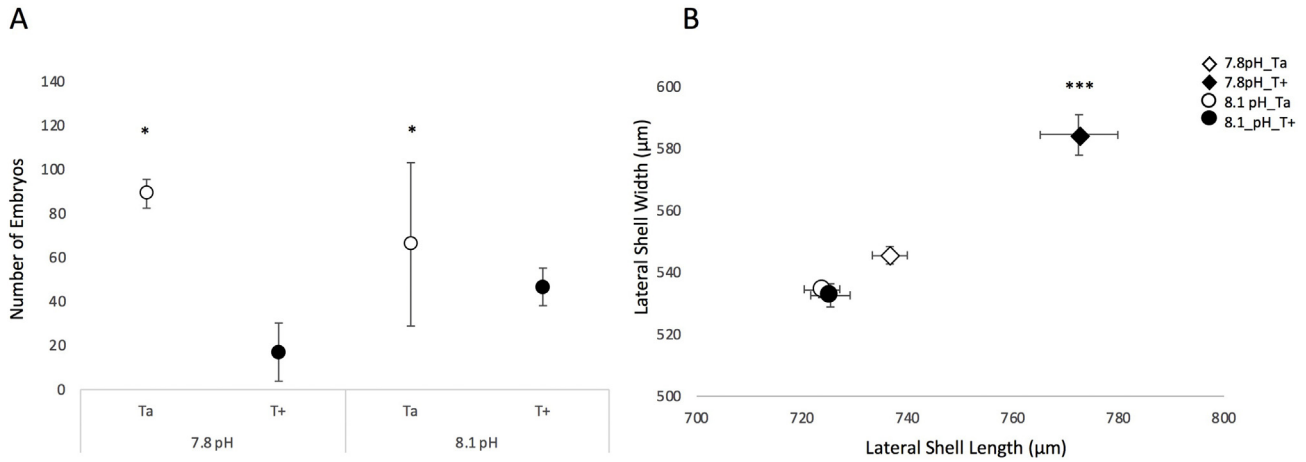


Fig. 2. Effects of pH and temperature on Embryos. A) Average number (±S.E.) of embryos di *D. cristatum* in different pH conditions (7.8 Vs 8.1) and temperature treatments (Ta Vs T+) from the transplanted vermetid cores. B) Average (±S.E.) lateral shell length and width (e.g. spiral height) of late stage embryos of *D. cristatum*.(p level: 0.05 "*"–0.001 "****").

0.021) (Table S1; Fig. S3). Data on length and the width of the recruits collected were coherent with the teleoconch surface. Mean maximum length of the recruits was found in 8.1 pH_T+ site (965.61 ± 11.92 µm) and the minimum length in average was recorded in 8.1 pH_Ta site (945.13 ± 8.21 µm).

Similarly, pH had a significant effect on the shell dissolution of the recruits that settled and grew at 7.8 pH sites (11.2% ± 4.1 of corroded surface) and almost absent shell corrosion was recorded at 8.1 pH (0.05% ± 0.08) (Pseudo-F_{1-59} = 14.391; p(perm) = 0.0001; Table S1; Fig. S5).

We found higher Mg concentration in the protoconch (1.36% ± 0.25) (Pseudo-F_{1-15} = 10.071; P(perm) = 0.0088) and the teleoconch (4.36% ± 0.75) (Pseudo- F_{1-15} = 32.143; P(perm) = 0.0001) of recruits collected from 7.8 pH sites compared to those collected at 8.1 pH sites (0.64% ± 0.07 and 0.8% ± 0.09 respectively) (Fig. 4). As expected, a significant increase in Ca^{2+} concentration was found both in the protoconch (95.72% ± 0.63) (Pseudo-F_{1-15} = 7.6315; P(perm) = 0.0193) and the teleoconch (92.52% ± 0.99) (Pseudo-F_{1-15} = 23.839; P(perm) = 0.009) in the 8.1 pH site (Table S1). No significant difference in Sr^{2+} concentration was observed both in the protoconch and teleoconch of recruits (Fig. 4; Table S1).

Analysis of Mg^{2+} concentration in the embryo shells (Fig. S6) showed a strong influence of pH and temperature factors in 7.8 pH_T + sites (0.38% ± 0.05) (Pseudo-F_{1-15} = 7.2125; P(perm) = 0.0204). The Ca^{2+} concentration exhibited the highest values at 8.1 pH (97.21% ± 0.15) (Pseudo-F_{1-15} = 7.1784; P(perm) = 0.0205) (Table S1). Again, no differences in Sr^{2+} concentration were recorded (Table S1).

3.4. Oxygen consumption

Oxygen consumption of the recruits after 1 h of chamber incubation at 24 °C did not differ between the 7.8 pH and 8.1 pH sites (average ± E.S.; 7.8 pH 13.29 ± 1.66 µmol l^{-1} h^{-1}; 8.1 pH 15.58 ± 1.33 µmol l^{-1} h^{-1}). However, a significant increase in the oxygen consumption was noticed after incubations at an experimental temperature of 28 °C (7.8 pH: 33.35 ± 2 µmol l^{-1} h^{-1}; 8.1 pH: 52.82 ± 2.84 µmol l^{-1} h^{-1}) (Fig. 5). Differences in oxygen consumption for the interaction pH x Te (Pseudo-F_{1-63} = 5.4941; p(perm) = 0.0222) suggest a synergistic effect between them, with pair-wise *t*-tests confirming that recruits consumed more oxygen at 28 °C than 24 °C, in both pH conditions (Low = 7.8 and Ctrl = 8.1), and exerted a significant decrease in the oxygen consumption at 28 °C in 7.8 pH conditions (Table S1).

4. Discussion and conclusions

This study represents the first in situ attempt to verify the effects of OA along a gradient while manipulating temperature conditions. To assess responses of the early life-stages of the Mediterranean reef-

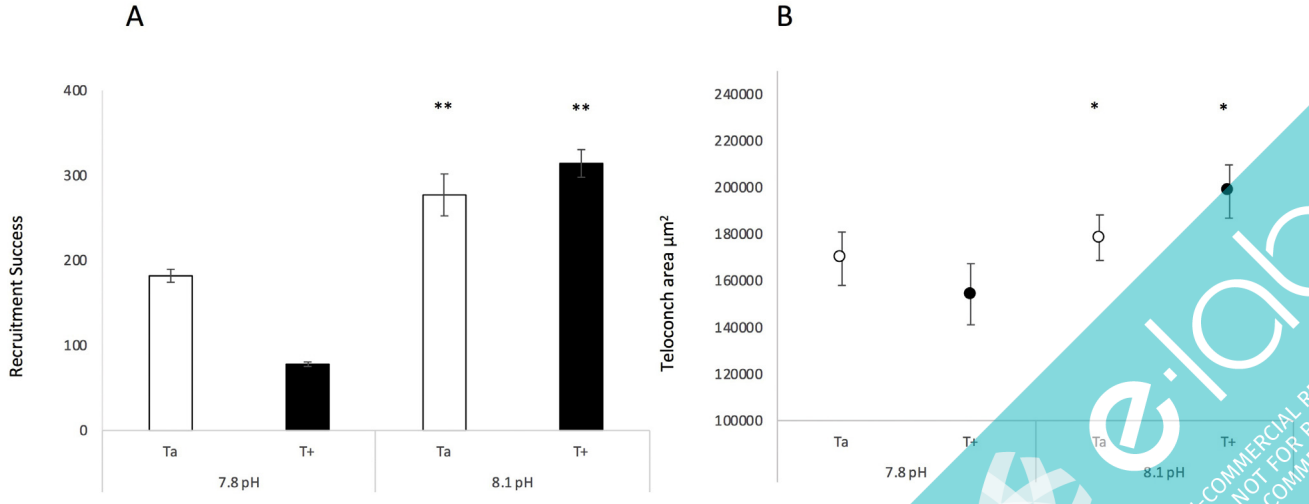


Fig. 3. Effects of pH and temperature on Recruits. A) Average number (±S.E.) of living *D. cristatum* recruits in each experimental condition. B) Average surface (±S.E.) of the recruit teloconch (µm²) in each experimental condition. (p level: 0.05 "*", 0.01"**", 0.001 "****").

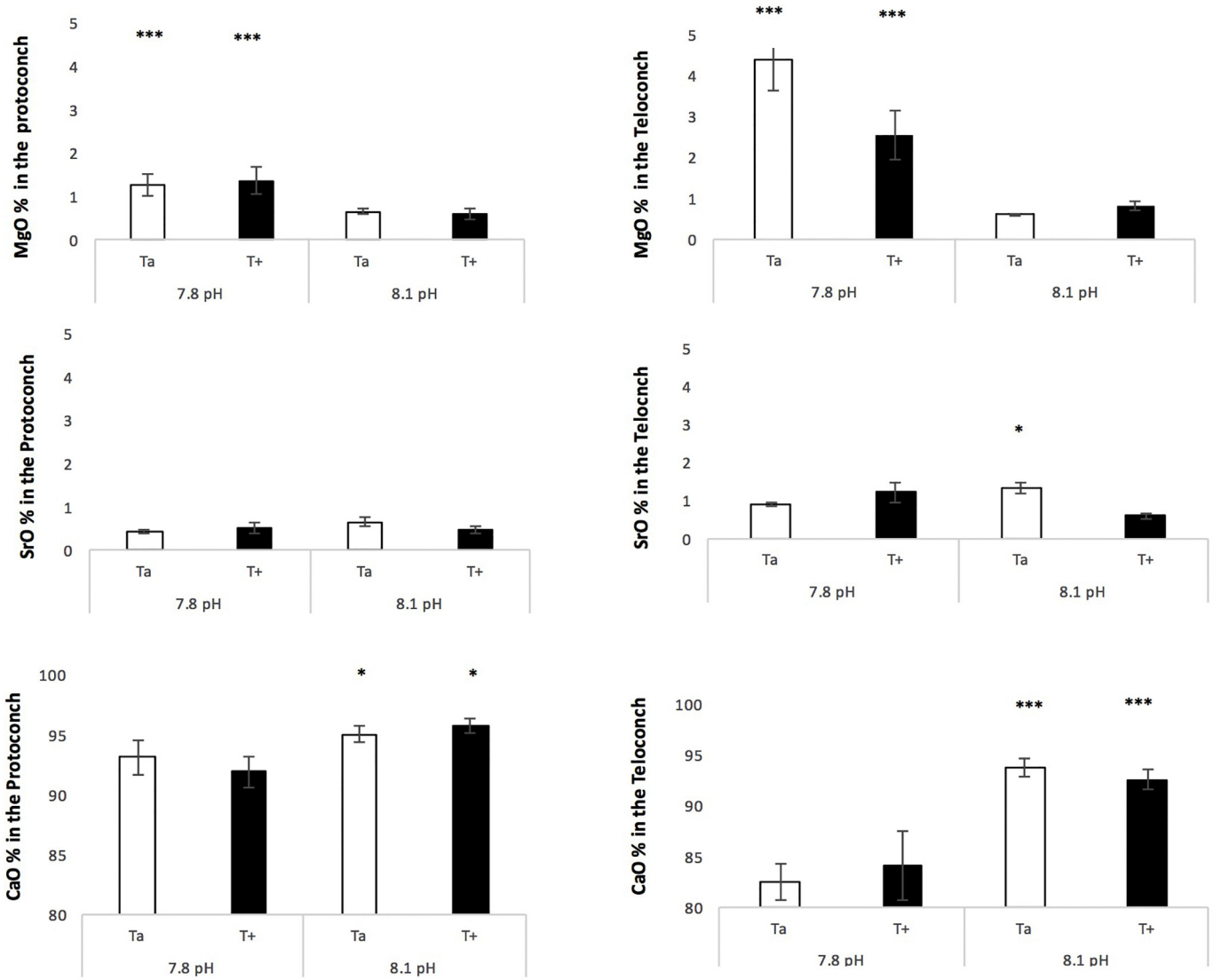


Fig. 4. Chemical composition of the recruit shells (Mg, Ca and Sr) in the protoconch (left) and teleoconch (right) of *D. cristatum*. Average (±S.E.) chemical composition (as percentages, see materials and methods) of the recruit shells (Mg, Ca and Sr) in the protoconch (left) and teloconch (right) of *D. cristatum* recruits. (p level: 0.05 "*", 0.01"**", 0.001 "***").

building species *Dendropoma cristatum*, we transplanted vermetid reef cores in two sites exposed to control and "end-of-century" carbonate chemistry (pH/pCO_2) conditions in addition to successfully manipulating air-temperature using white- and black-framed cores. Specifically, vermetids at low tide were exposed to both relevant temperature extremes (with a + 1.89 °C recorded in black-framed compared with white-framed) and temperature means (on average a + 0.35 °C in paired comparisons recorded every 30 min^{-1} between black and white cores), therefore well mimicking expected temperature rise.

In accordance with previous studies (Armstrong et al., 2017; Kupriyanova and Havenhand, 2005; Parker et al., 2010), we found a significantly lower number of embryos under elevated temperature conditions, suggesting that their development and/or the fertilization phase might be negatively affected by increasing temperature. However, embryos found under 7.8pH x T+ conditions were 30% larger than embryos that developed under control pH and control temperature conditions, which is likely a compensation mechanism to enhance early stage survival. Although many studies have found temperature to speed up larval growth (Davis et al., 2013; Hernández et al., 2010; Reitzel et al., 2004), we believe that the size of the offspring collected from 7.8 pH_T+ could be explained by the intracapsular nutrition of this species. Indeed, during intracapsular development embryos rely on nurse eggs, i.e. dead embryos, for nutrition (Templado et al., 2015).

Recruitment success of *D. cristatum* was more strongly affected by a pH decrease rather than change in temperature. These results corroborate existing evidence of the effects of ocean acidification on vermetid recruitment (Milazzo et al., 2014), highlighting the elevated heat tolerance of the recruit stages of this intertidal species. Espinel-Velasco et al. (2018) described how pH, as well as other stressors (i.e. temperature, salinity), might alter the microbial bio-film on the diffusive boundary layer of settlement substrate, consequently affecting settlement due to the influence of specific components of the microbial community that respond differently to warming and ocean acidification. Recruitment of vermetids is strictly linked to crustose coralline algae (CCA), and changes in the substrate-associated benthic microbial community could alter their settlement (La Marca et al., 2018), as already observed for different coral larvae (Russell et al., 2013; Webster et al., 2013). Moreover, the aragonitic disc that crawling larvae form for settlement is subjected to dissolution, causing the detachment of recruits and therefore increasing the mortality of early stages (Milazzo et al., 2014).

Growth rate and its control under changing environmental conditions are closely related to the capacity of a species to maintain its

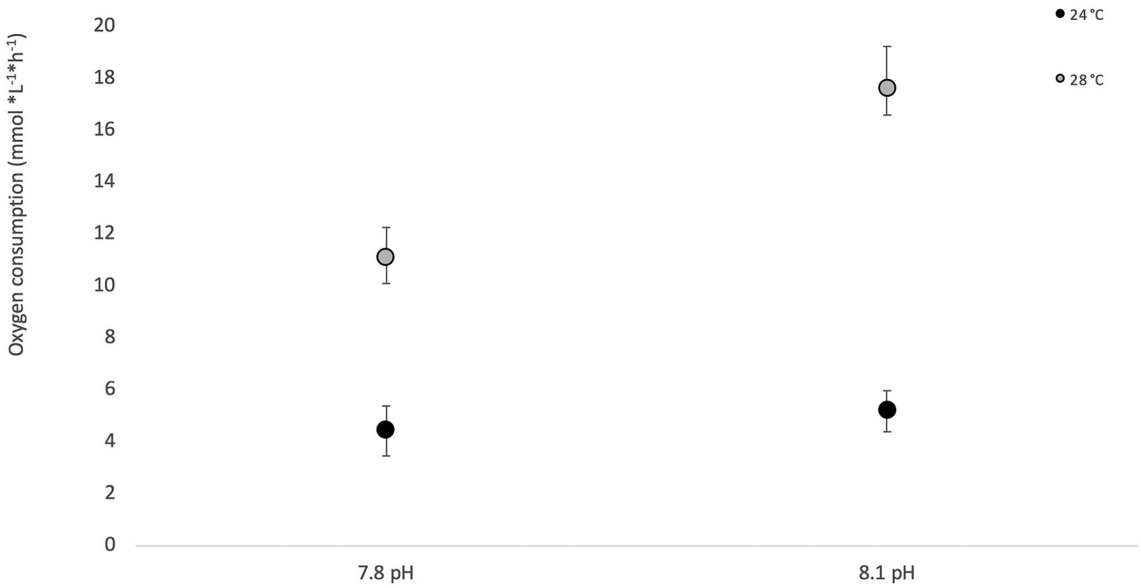


Fig. 5. Recruit Oxygen Consumption. Average (±S.E.) oxygen consumption of *D. cristatum* recruits recorded at two different temperature (24 °C e 28 °C) and pH (7.8 and 8.1 pH units) levels.

calcification rate. Indeed, those species that are able to maintain their growth rate in high pCO_2 have an efficient ion regulatory apparatus and the capacity to create optimal conditions at the sites of calcification (Gazeau et al., 2013). For example, mussels and some species of oysters are able to partially mitigate the effect of elevated pCO_2 conditions (7.7 pH unit) in the presence of high food availability (Langer et al., 2014; Ries et al., 2009). Similar to many other species of molluscs (Armstrong et al., 2017; Beniash et al., 2010; Noisette et al., 2014; Parker et al., 2010; Ries et al., 2009; Rodolfo-Metalpa et al., 2011), the growth of *D. cristatum* early stages was negatively affected by OA, specifically reduced shell size and an increase in shell dissolution. Costs for homeostatic regulation are usually altered under an acute abiotic stress regime, with repercussions on metabolic and growth performance (Melzner et al., 2009).

4.1. Effects of OA and air temperature on the chemical composition of early stage vermetid shells

Our analysis of the chemical composition of shells revealed interesting information regarding the percentage concentration of Mg and Ca. One assumption of this study was that encapsulated embryos would be protected by the intra-capsular fluid, which may buffer against the external environment during development (Chaparro et al., 2009; Przeslawski et al., 2015). However, altered shell Ca and Mg concentrations were found under different experimental conditions. Calcium concentration was significantly higher in the 8.1 pH sites in both embryos and recruits. On the contrary, magnesium concentration was significantly higher in shells that developed in 7.8 pH sites. Moreover, the shells of the recruits at 7.8 pH sites showed a significant increase in dissolution resulting in thinner and lighter shells, therefore encapsulation did not reduce the effect of acidification during development (Armstrong et al., 2017; Davis et al., 2013; Noisette et al., 2014). The results of Ca and Mg concentrations in the recruits affirm that the early stages of this species will be severely affected by the future pH and temperature conditions. This result may reflect an impaired ability of vermetids to remove Mg from hemolymph and extra-pallial fluids (Bentov and Erez, 2006; Milazzo et al., 2014). This inability to remove Mg from the calcification fluid may inhibit crystal nucleation and have serious future implications in this species. While no significant differences were recorded for Sr concentration in the embryo shells, a different pattern was observed in the recruit shells. EDS revealed a high concentration of Sr in the 8.1 pH_Ta treatment, underlining a strong influence of the combination of temperature and pH. Sr plays an important role in the stabilization of the orthorhombic aragonite, but its concentration might be correlated to several factors such as growth rate and/or metabolism (Gillikin et al., 2005). For these reasons, more studies on Mg/Ca and Sr/Ca ratios would allow better understanding of the biomineralization processes in *D. cristatum*.

4.2. Effects of OA and temperature on oxygen consumption

Our observations on the metabolic rate of *D. cristatum* recruits suggest that pH and temperature act synergistically. Metabolic responses are usually variable among species and a pH decrease can have diverse effects on marine organisms. A moderate increase of pCO_2 (<1200 μatm) has a small effect on the metabolic rate of bivalve molluscs, while high pCO_2 (at ~2000–3500 μatm) saturation can induce a rise in oxygen consumption (Dupont et al., 2010). In addition, high temperatures often cause a strong stress response in marine mollusc species inducing changes in metabolic energy production (Beniash et al., 2010). We found a high oxygen consumption at 28 °C, that decreased significantly in the 7.8 pH site, likely because elevated pCO_2 impairs the metabolic response and results in a sizeable narrowing of the larval thermal window (Pörtner, 2008). In conclusion, our results suggest that *D. cristatum*, although it is an intertidal species potentially adapted to a highly fluctuating environment, is sensitive to the combined effect of pH and temperature, especially in the early life stage.

In summary, this study suggests that the early stage of *D. cristatum* and the species persistence could be strongly compromised if the hypothesized pH reduction and temperature rise scenario for the end of the century occurs. The higher metabolic costs under these conditions demonstrates that *D. cristatum* is negatively affected by the synergistic interaction of multiple stressors, in this case temperature and pH, even though it is an intertidal species resilient to various types of environmental stress (i.e. exposure to dry conditions due to tidal cycles). Both stress factors compromise oxygen consumption and negatively affect the bio-mineralization processes of *D. cristatum* during development and recruitment.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.07.282.

Declaration of Competing Interest

The authors declare no competing financial interests.

Acknowledgements

The authors would like to thank Matteo Mercurio, responsible for the welfare of *D. cristatum* during the short period in the laboratory. The authors would also like to thank Federico Quattrocchi for performing some of the statistical analyses. We are grateful to Maarten Van Rouveroy for providing the picture of the CO_2 *vents* used in the graphical abstract. This project was funded by the EU-FP7 MedSeA project (grant agreement no. 265103) to M.M. and partially by the project PRIN TETRIS 2010 (Italian Ministry of University and Research, MIUR) to G.S.

Author contributions

CA and MM conceived and conducted the experiment. FG performed *D. cristatum* respirometric measurements. FF performed SEM and EDS analyses. CA and MM wrote the manuscript, with a contribution from FG. RC and GS contributed to the revision of the manuscript. All the authors commented on the first draft of the manuscript.

References

Albright, R., Mason, B., Miller, M., Langdon, C., 2010. Ocean acidification compromises recruitment success of the threatened Caribbean coral Acropora palmata. Proc. Natl. Acad. Sci. U. S. A. 107, 20400–20404. doi:10.1073/pnas.1007273107/−/DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1007273107

Armstrong, E.J., Allen, T.R., Beltrand, M., Dubousquet, V., Stillman, J.H., Mills, S.C., 2017. High pCO2 and elevated temperature reduce survival and alter development in early life stages of the tropical sea hare Stylocheilus striatus. Mar. Biol. 164. https://doi.org/10.1007/s00227-017-3133-x.

Arnold, T., Mealey, C., Leahey, H., Miller, A.W., Hall-Spencer, J.M., Milazzo, M., Maers, K., 2012. Ocean acidification and the loss of phenolic substances in marine plants. PLoS One 7, e35107. https://doi.org/10.1371/journal.pone.0035107.

Beniash, E., Ivanina, A., Lieb, N.S., Kurochkin, I., Sokolova, I.M., 2010. Elevated level of carbon dioxide affects metabolism and shell formation in oysters Crassostrea virginica. Mar. Ecol. Prog. Ser. 419, 95–108. https://doi.org/10.3354/meps08841.

Bentov, S., Erez, J., 2006. Impact of biomineralization processes on the Mg content of foraminiferal shells: a biological perspective. Geochemistry, Geophys. Geosystems 7. https://doi.org/10.1029/2005GC001015.

Boatta, F., D'Alessandro, W., Gagliano, A.L., Liotta, M., Milazzo, M., Rodolfo-Metalpa, R., Hall-Spencer, J.M., Parello, F., 2013. Geochemical survey of Levante Bay, Vulcano Island (Italy), a natural laboratory for the study of ocean acidification. Mar. Pollut. Bull. 73, 485–494. https://doi.org/10.1016/j.marpolbul.2013.01.029.

Brown, N.E.M., Milazzo, M., Rastrick, S.P.S., Hall-Spencer, J.M., Therriault, T.W., Harley, C.D.G., 2017. Natural acidification changes the timing and rate of succession, alters community structure, and increases homogeneity in marine biofouling communities. Glob. Chang. Biol. https://doi.org/10.1111/gcb.13856.

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. Oceanogr. Mar. Biol. An Annu. Rev. 49, 1–42. https://doi.org/10.1016/j.marenvres.2011.10.00.

Byrne, M., Przeslawski, R., 2013. Multistressor impacts of warming and acidification of the ocean on marine invertebrates' life histories. Integr. Comp. Biol. 53, 582–596. https://doi.org/10.1093/icb/ict049.

Caldeira, K., 2005. Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. J. Geophys. Res. 110, C09S04. https://doi.org/10.1029/2004JC002671.

Caldeira, K., Wickett, M.E., 2003. Anthropogenic carbon and ocean pH. Nature 425. https://doi.org/10.1038/425365a 365–365.

Calosi, P., Rastrick, S.P.S., Graziano, M., Thomas, S.C., Baggini, C., Carter, H.A., Hall-Spencer, J.M., Milazzo, M., Spicer, J.I., 2013. Distribution of sea urchins living near shallow water CO2 vents is dependent upon species acid–base and ion-regulatory abilities. Mar. Pollut. Bull. 73, 470–484. https://doi.org/10.1016/J.MARPOLBUL.2012.11.040.

Calvo, M., Templado, J., Oliverio, M., MacHordom, A., 2009. Hidden Mediterranean biodiversity: molecular evidence for a cryptic species complex within the reef building vermetid gastropod Dendropoma petraeum (Mollusca: Caenogastropoda). Biol. J. Linn. Soc. 96, 898–912. https://doi.org/10.1111/j.1095-8312.2008.01167.x.

Cattano, C., Giomi, F., Milazzo, M., 2016. Effects of ocean acidification on embryonic respiration and development of a temperate wrasse living along a natural CO_2 gradient. Conserv. Physiol. 4, cov073. https://doi.org/10.1093/conphys/cov073.

Chaparro, O.R., Segura, C.J., Montory, J.A., Navarro, J.M., Pechenik, J.A., 2009. Brood chamber isolation during salinity stress in two estuarine mollusk species: from a protective nursery to a dangerous prison. Mar. Ecol. Prog. Ser. 374, 145–155. https://doi.org/10.3354/meps07777.

Clarke, K.R., Gorley, R.N., 2006. PRIMER v6: User Manual/Tutorial. Prim, Plymouth UK https://doi.org/10.1111/j.1442-9993.1993.tb00438.x 192 p.

Cornwall, C.E., Revill, A.T., Hall-Spencer, J.M., Milazzo, M., Raven, J.A., Hurd, C.L., 2017. Inorganic carbon physiology underpins macroalgal responses to elevated CO_2. Sci. Rep. 7, 4297. https://doi.org/10.1038/srep46297.

Davis, A.R., Coleman, D., Broad, A., Byrne, M., Dworjanyn, S.A., Przeslawski, R., 2013. Complex responses of intertidal molluscan embryos to a warming and acidifying ocean in the presence of UV radiation. PLoS One 8, e55939. https://doi.org/10.1371/journal.pone.0055939.

Dickson, A.G., Sabine, C.L., Christian, J.R., 2007. Guide to Best Practices for Ocean CO 2 Measurements. North Pacific Marine Science Organization.

Doropoulos, C., Ward, S., Diaz-Pulido, G., Hoegh-Guldberg, O., Mumby, P.J., 2012. Ocean acidification reduces coral recruitment by disrupting intimate larval-algal settlement interactions. Ecol. Lett. 15, 338–346. https://doi.org/10.1111/j.1461-0248.2012.01743.x.

Dupont, S., Dorey, N., Thorndyke, M., 2010. What meta-analysis can tell us about vulnerability of marine biodiversity to ocean acidification? Estuar. Coast. Shelf Sci. 89, 182–185. https://doi.org/10.1016/j.ecss.2010.06.013.

Espinel-Velasco, N., Hoffmann, L., Agüera, A., Byrne, M., Dupont, S., Uthicke, S., Webster, N.S., Lamare, M., 2018. Effects of ocean acidification on the settlement and metamorphosis of marine invertebrate and fish larvae: A review. Mar. Ecol. Prog. Ser. 606, 237–257. https://doi.org/10.3354/meps12754.

Fabricius, K.E., Noonan, S.H.C., Abrego, D., Harrington, L., De'ath, G., 2017. Low recruitment due to altered settlement substrata as primary constraint for coral communities under ocean acidification. Proc. R. Soc. B Biol. Sci. 284, 20171536. https://doi.org/10.1098/rspb.2017.1536.

Lurdes Fernandez-Diaz (1), A.P., 1996. The role of magnesium in the crystallization of calcite and aragonite in a porous medium. SEPM J. Sediment. Res. Vol. 66, 482–491. doi:https://doi.org/10.1306/D4268388-2B26-11D7-8648000102C1865D.

Franzitta, G., Capruzzi, E., La Marca, E.C., Milazzo, M., Chemello, R., 2016. Recruitment patterns in an intertidal species with low dispersal ability: the reef-building Dendropoma cristatum (Biondi, 1859) (Mollusca: Gastropoda). Ital. J. Zool. 83, 400–407. https://doi.org/10.1080/11250003.2016.1205152.

Garilli, V., Rodolfo-Metalpa, R., Scuderi, D., Brusca, L., Parrinello, D., Rastrick, S.P.S., Foggo, A., Twitchett, R.J., Hall-Spencer, J.M., Milazzo, M., 2015. Physiological advantages of dwarfing in surviving extinctions in high-CO 2 oceans. Nat. Clim. Chang. 5, 678–682. https://doi.org/10.1038/nclimate2616.

Gazeau, F., Parker, L.M., Comeau, S., Gattuso, J.P., O'Connor, W.A., Martin, S., Pörtner, H.O., Ross, P.M., 2013. Impacts of ocean acidification on marine shelled molluscs. Mar. Biol. 160, 2207–2245. https://doi.org/10.1007/s00227-013-2219-3.

Gillikin, D.P., Lorrain, A., Navez, J., Taylor, J.W., Andr??, L., Keppens, E., Baeyens, W., Dehairs, F., 2005. Strong biological controls on Sr/Ca ratios in aragonitic marine bivalve shells. Geochemistry, Geophys. Geosystems 6, Q05009. https://doi.org/10.1029/2004GC000874.

Hernández, J.C., Clemente, S., Girard, D., Pérez-Ruzafa, Á., Brito, A., 2010. Effect of temperature on settlement and postsettlement survival in a barrens-forming sea urchin. Mar. Ecol. Prog. Ser. 413, 69–80. https://doi.org/10.3354/meps08684.

Johnson, V., Russell, B., Fabricius, K.E., Brownlee, C., Hall-Spencer, J.M., 2012. Temperate and tropical brown macroalgae thrive, despite decalcification, along natural CO_2 gradients. Glob. Chang. Biol. 18, 2792–2803.

Kroeker, K.J., Micheli, F., Gambi, M.C., Martz, T.R., 2011. Divergent ecosystem responses within a benthic marine community to ocean acidification. Proc. Natl. Acad. Sci. 108, 14515–14520. https://doi.org/10.1073/pnas.1107789108.

Kroeker, K.J., Kordas, R.L., Crim, R., Hendriks, I.E., Ramajo, L., Singh, G.S., Duarte, C.M., Gattuso, J.P., 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Glob. Chang. Biol. 19, 1884–1896. https://doi.org/10.1111/gcb.12179.

Kupriyanova, E.k., Havenhand, J.n., 2005. Effects of temperature on sperm swimming behaviour, respiration and fertilization success in the serpulid polychaete, galeolaria caespitosa (annelida: Serpulidae). Invertebr. Reprod. Dev. 48, 7–17. https://doi.org/10.1080/07924259.2005.9652166.

Kurihara, H., 2008. Effects of CO2-driven ocean acidification on the early developmental stages of invertebrates. Mar. Ecol. Prog. Ser. 373, 275–284. https://doi.org/10.3354/meps07802.

La Marca, E.C., Catania, V., Quatrini, P., Milazzo, M., Chemello, R., 2018. Settlement performance of the Mediterranean reef-builders Dendropoma cristatum (Biondi 1859) in response to natural bacterial films. Mar. Environ. Res. 137, 149–157. https://doi.org/10.1016/J.MARENVRES.2018.03.005.

Langer, G., Nehrke, G., Baggini, C., Rodolfo-Metalpa, R., Hall-Spencer, J.M., Bijma, J., 2014. Limpets counteract ocean acidification induced shell corrosion by thickening of aragonitic shell layers. Biogeosciences 11, 7363–7368. https://doi.org/10.5194/bg-11-7363-2014.

Lidbury, I., Johnson, V., Hall-Spencer, J.M., Munn, C., Cunliffe, M., 2012. Community-level response of coastal microbial biofilms to ocean acidification in a natural carbon dioxide vent ecosystem. Mar. Pollut. Bull. 64, 1063–1066.

Melzner, F., Gutowska, M.A., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M.C., Bleich, M., Pörtner, H.-O., 2009. Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? Biogeosci. Discuss. 6, 4693–4738. https://doi.org/10.5194/bgd-6-4693-2009.

Michaelidis, B., Ouzounis, C., Paleras, A., Portner, H.O., 2005. Effects of long-term moderate hypercapnia on acid-base balance and growth rate in marine mussels Mytilus galloprovincialis. Mar. Ecol. Ser. 293, 109–118.

Milazzo, M., Rodolfo-Metalpa, R., Chan, V.B.S., Fine, M., Alessi, C., Thiyagarajan, V., Hall-Spencer, J.M., Chemello, R., 2014. Ocean acidification impairs vermetid reef recruitment. Sci. Rep. 4, 1–7. https://doi.org/10.1038/srep04189.

Milazzo, M., Fine, M., La Marca, E.C., Alessi, C., Chemello, R., 2017. Drawing the Line at Neglected Marine Ecosystems: Ecology of Vermetid Reefs in a Changing Ocean. In: Rossi, S., Bramanti, L., Gori, A., Covadonga, O. (Eds.), Marine Animal Forests: The Ecology of Benthic Biodiversity Hotspots. Springer International Publishing, Cham, pp. 345–367 https://doi.org/10.1007/978-3-319-21012-4_9.

Milazzo, M., Alessi, C., Quattrocchi, F., Chemello, R., D'Agostaro, R., Gil, J., Vaccaro, A.M., Mirto, S., Gristina, M., Badalamenti, F., 2019. Biogenic habitat shifts under long-term ocean acidification show nonlinear community responses and unbalanced functions of associated invertebrates. Sci. Total Environ. 667, 41–48. https://doi.org/10.1016/J.SCITOTENV.2019.02.391.

Nash, M.C., Opdyke, B.N., Troitzsch, U., Russell, B.D., Adey, W.H., Kato, A., Diaz-Pulido, G., Brent, C., Gardner, M., Prichard, J., Kline, D.I., 2013. Dolomite-rich coralline algae in reefs resist dissolution in acidified conditions. Nat. Clim. Chang. 3, 268–272. https://doi.org/10.1038/nclimate1760.

Noisette, F., Comtet, T., Legrand, E., Bordeyne, F., Davoult, D., Martin, S., 2014. Does encapsulation protect embryos from the effects of ocean acidification? The example of Crepidula fornicata. PLoS One 9, 1–11. https://doi.org/10.1371/journal.pone.0093021.

Parker, L.M., Ross, P.M., 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. https://doi.org/10.1007/s00227-010-1508-3.

Pörtner, H.O., 2008. Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. Mar. Ecol. Prog. Ser. 373, 203–217. https://doi.org/10.3354/meps07768.

Portner, H.O., Langenbuch, M., Michaelidis, B., 2005. Synergistic effects of temperature extremes, hypoxia, and increases in CO2 on marine animals: from earth history to global change. J. Geophys. Res. 110, C09S10. https://doi.org/10.1029/2004JC002561.

Pörtner, H.O., et al., 2014. Coordinating Lead Authors: Lead Authors: Contributing Authors: Review Editors. vol. 2014. Cambridge Univ. Press, Cambridge, United Kingdom New York, NY, USA, pp. 411–484.

Przeslawski, R., Byrne, M., Mellin, C., 2015. A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae. Glob. Chang. Biol. 21, 2122–2140. https://doi.org/10.1111/gcb.12833.

Reitzel, A.M., Miner, B.G., McEdward, L.R., 2004. Relationships between spawning date and larval development time for benthic marine invertebrates: a modeling approach. Mar. Ecol. Prog. Ser. 280, 13–23. https://doi.org/10.3354/meps280013.

Ries, J.B., Cohen, A.L., Mccorkle, D.C., Ries, J.B., Cohen, A.L., Mccorkle, D.C., 2009. Marine calcifi ers exhibit mixed responses to CO_2-induced ocean acidifi cation. Geol. Soc. Am. 37, 1131–1134. https://doi.org/10.1130/G30210A.1.

Ries, J.B., Ghazaleh, M.N., Connolly, B., Westfield, I., Castillo, K.D., 2016. Impacts of seawater saturation state ($\Omega A = 0.4$–4.6) and temperature (10, 25 °C) on the dissolution kinetics of whole-shell biogenic carbonates. Geochim. Cosmochim. Acta 192, 318–337. https://doi.org/10.1016/j.gca.2016.07.001.

Rodolfo-Metalpa, R., Houlbrèque, F., Tambutté, É., Boisson, F., Baggini, C., Patti, F.P., Jeffree, R., Fine, M., Foggo, A., Gattuso, J.P., Hall-Spencer, J.M., 2011. Coral and mollusc resistance to ocean acidification adversely affected by warming. Nat. Clim. Chang. 1, 308–312. https://doi.org/10.1038/nclimate1200.

Russell, B.D., Connell, S.D., Findlay, H.S., Tait, K., Widdicombe, S., Mieszkowska, N., 2013. Ocean acidification and rising temperatures may increase biofilm primary productivity but decrease grazer consumption. Phil Trans 368, 20120438.

Somero, G.N., 2002. Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living. Integr. Comp. Biol. 42, 780–789. https://doi.org/10.1093/Icb/42.4.780.

Stillman, J.H., 2003. Acclimation capacity underlies susceptibility to climate change. Science 301, 65. https://doi.org/10.1126/science.1083073 80-..

Strathmann, R.R., Strathmann, M.F., 1995. Oxygen supply and limits on aggregation of embryos. J. Mar. Biol. Assoc. United Kingdom 75, 413–428. https://doi.org/10.1017/S0025315400018270.

Sunday, J.M., Fabricius, K.E., Kroeker, K.J., Anderson, K.M., Brown, N.E., Barry, J.P., Connell, S.D., Dupont, S., Gaylord, B., Hall-Spencer, J.M., Klinger, T., Milazzo, M., Munday, P.L., Russell, B.D., Sanford, E., Thiyagarajan, V., Vaughan, M.L.H., Widdicombe, S., Harley, C.D.G., 2017. Ocean acidification can mediate biodiversity shifts by changing biogenic habitat. Nat. Clim. Chang. 7, 81–85. https://doi.org/10.1038/nclimate3161.

Taylor, J.D., Ellis, R., Milazzo, M., Hall-spencer, J.M., Cunliffe, M., 2014. Occurring carbon dioxide and pH gradient. FEMS Microbiol. Ecol. 89, 670–678. https://doi.org/10.1111/1574-6941.12368.

Templado, J., Richter, A., Calvo, M., 2015. Reef building Mediterranean vermetid gastropods: disentangling the Dendropoma petraeum species complex. Mediterr. Mar. Sci. 17, 13–31. https://doi.org/10.12681/mms.1333.

Urbarova, I., Forêt, S., Dahl, M., Emblem, Å., Milazzo, M., Hall-Spencer, J.M., Johansen, S.D., 2019. Ocean acidification at a coastal CO_2 vent induces expression of stress-related transcripts and transposable elements in the sea anemone Anemonia viridis. PLoS One 14, 1–22. https://doi.org/10.1371/journal.pone.0210358.

Vizzini, S., Di Leonardo, R., Costa, V., Tramati, C.D.D., Luzzu, F., Mazzola, A., 2013. Trace element bias in the use of CO_2 vents as analogues for low pH environments: implications for contamination levels in acidified oceans. Estuar. Coast. Shelf Sci. 134, 19–30. https://doi.org/10.1016/j.ecss.2013.09.015.

Webster, N.S., Soo, R., Cobb, R., Negri, A.P., 2010. Elevated seawater temperature causes a microbial shift on crustose coralline algae with implications for the recruitment of coral larvae. ISME J 5, 759–770. https://doi.org/10.1038/ismej.2010.152.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A. a, Smith, G.M., Corporation, Ebooks, 2009. Mixed effects models and extensions in ecology with R. Stat. Biol. Heal. https://doi.org/10.1007/978-0-387-87458-6.