

1 **An intertidal life: Combined effects of acidification and winter heatwaves on a**  
2 **coralline alga (*Ellisolandia elongata*) and its associated invertebrate community.**

3

4 Running Title

5 Coralline algae buffer climate change effects

6

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## 32 **Abstract**

33 In coastal marine ecosystems coralline algae often create biogenic reefs. These calcareous algal reefs  
34 affect their associated invertebrate communities *via* diurnal oscillations in photosynthesis, respiration  
35 and calcification processes. Little is known about how these biogenic reefs function and how they  
36 will be affected by climate change. We investigated the winter response of a Mediterranean intertidal  
37 biogenic reef, *Ellisolandia elongate* exposed in the laboratory to reduced pH conditions (i.e. ambient  
38 pH – 0.3, RCP 8.5) together with an extreme heatwave event (+1.4°C for 15 days). Response  
39 variables considered both the algal physiology (calcification and photosynthetic rates) and  
40 community structure of the associated invertebrates (at taxonomic and functional level). The  
41 combination of a reduced pH with a heatwave event caused *Ellisolandia elongata* to significantly  
42 increase photosynthetic activity. The high variability of calcification that occurred during simulated  
43 night time conditions, indicates that there is not a simple, linear relationship between these two and  
44 may indicate that it will resilient to future conditions of climate change.

45 In contrast, the associated fauna **were** particularly negatively affected by the heatwave event, which  
46 impoverished the communities as opportunistic taxa became dominant. Local increases in oxygen  
47 and pH driven by the algae can buffer the microhabitat in the algal fronds, thus favouring the survival  
48 of small invertebrates.

49

50 **Keywords (6-10)** climate change, coralline algae, Mediterranean ecosystem, heatwaves,  
51 acidification, peracarida, polychaeta

52

### 53 **Introduction**

54 Anthropogenic impacts and environmental changes threaten rocky shore ecosystem biodiversity,  
55 structure and functioning (Agostini et al., 2018; Milazzo et al., 2019). Intertidal habitats are  
56 characterized by organisms adapted to a world of extremes, but their resilience to future changes in  
57 the extent of those extremes is unknown (Wolfe et al., 2020). While the patterns and projections of  
58 global warming (GW) and ocean acidification (OA) are well recognized and understood in the open  
59 ocean (Gattuso et al., 2015), the effects of these changes on coastal ecosystems are less well known.  
60 Coastal ecosystems are already facing dramatic changes in carbonate chemistry (at variable time-  
61 scales) and thereby already experiencing saturation levels predicted globally for the end of the  
62 century. Due to the continuous anthropogenic production of greenhouse gases, especially of carbon  
63 dioxide (CO<sub>2</sub>), the average global temperature on Earth's surface increased by 0.7 °C during the last  
64 century (global warming) with 93% of the excess heat transferred in the ocean. Furthermore, increases  
65 in the frequency of discrete extreme warming events have been observed (marine heatwaves –  
66 MHWs, Wernberg et al., 2016; Darmaraki et al., 2019; Oliver et al., 2018). About 25% of the  
67 increased atmospheric *p*CO<sub>2</sub> (risen by over 100 ppm from the pre-industrial era) has been absorbed  
68 by the ocean, leading to a decrease in its pH and a modification in its carbonate system (Bindoff et  
69 al., 2019). Predictions for the end of the century suggest a worst case scenario (RCP 8.5) in which pH

70 decreases to 7.78 and temperature increases by 4.8° C compared to the pre-industrial era. Due to their  
71 small water volumes, it is difficult to predict the magnitude of climate change-induced fluctuations  
72 affecting coastal features such as tidal ranges,  $p\text{CO}_2$ , temperature, salinity, oxygen (Duarte et al.,  
73 2013; Melzner et al., 2019).

74 Among benthic intertidal habitats, biogenic reefs are of particular interest, since these three-  
75 dimensional carbonate structures create spaces and niches promoting marine biodiversity (Hiscock,  
76 2014). Acting as ecosystem engineers, calcifying organisms such as coralline algae, corals,  
77 polychaetes, molluscs, bryozoans and sponges, are able to vary, directly or indirectly, the availability  
78 of resources for other species by modifying, maintaining and creating habitats (Jones et al., 1994).  
79 They directly influence habitats by providing shelter, feeding areas, nursery, and substrate for growth.  
80 Indirectly, they influence habitats by mitigating the effects of physical pressures and modifying  
81 interactions between species (Menge, 1995). Thus, these habitats affect the structure of the associated  
82 communities by influencing biological and environmental processes. Despite their importance, the  
83 functioning of these biogenic reefs, with exception of corals, is poorly understood.

84 In the Mediterranean Sea, the most important biogenic reefs found in intertidal rocky habitats are  
85 built by coralline algae (Phylum Rhodophyta, Order Corallinales), which, besides building submerged  
86 structures, dominate the inorganic carbon budget (Foster, 2001; Mackenzie et al., 2004; Martin &  
87 Gattuso, 2009). Extant in the Mediterranean area for ~140 My (Rindi et al., 2019), the Corallinales  
88 play a major role in the ecological dynamics by providing food and micro-habitat for many other  
89 species (Asnaghi et al., 2015). They produce chemical stimuli that induce invertebrate larvae to settle  
90 and metamorphose (Pearce & Scheibling, 1990), and provide refugia from predators (Yiu & Feehan,  
91 2017). They can also represent a food source for juvenile sea urchins (Himmelman & Steele, 1971).  
92 Different factors influence the quality of macroalgae as a substratum (*e.g.* cell walls component,  
93 chemical defences and palatability, Martínez-Laiz et al., 2018), but the most important feature is their

94 three-dimensional complexity (Grabowski & Powers, 2004; Gestoso et al., 2013), for example the  
95 fractal (Walfe et al., 2008) and interstitial architecture (Dibble et al., 1996; Dibble et al., 2006).

96 Coralline algae are the major producers of calcite with a high magnesium content (HMC: high-Mg  
97 calcite). Being related to the photosynthetic process, the deposition of CaCO<sub>3</sub> is regulated by  
98 irradiance, hence varying according to depth and season (Blake & Maggs, 2003; Kamenos et al.,  
99 2008; El Haïkali et al., 2004; Egilsdottir et al., 2015). Hydrodynamic and sedimentological  
100 conditions, climate change and anthropogenic pollution influence the presence and distribution of  
101 coralline algae (Bressan et al., 2001; Martin et al., 2013; Ragazzola et al., 2016). A change of these  
102 factors can lead to an ecological shift from a dominance of calcifying organisms to that of fleshy  
103 algae (Kroeker et al., 2012) and a consequently less diverse and structurally simpler ecosystem  
104 (Thrush et al., 2009).

105 It has been shown that, under climate change scenarios that include both warming and  
106 acidification, the growth and structure of Mediterranean algae reefs are impaired. Owing to their high  
107 Mg-content (HMC), their thalli are vulnerable to ocean acidification (Marchini et al., 2019). The  
108 community associated with these reefs may become simplified and depleted by this impaired growth.  
109 However, it is still unknown if the physiological processes of these calcifying algae are able to  
110 mitigate the effect of climate change on its associated community, for instance by modulating the  
111 carbonate chemistry *via* diurnal oscillations in photosynthesis, respiration and calcification processes,

112 We investigated the response of the intertidal biogenic fringing reef constructed by *Ellisolandia*  
113 *elongata* to a simulation of reduced pH conditions as predicted for 2100 (RCP 8.5) coupled with a  
114 winter heatwave event. Through this experimental study, we aimed to elucidate the physiological  
115 variations occurring in the foundation species and within the community associated with the reef. We  
116 studied (1) the calcification and photosynthetic rates of *E. elongata*, (2) the mitigation effect on the  
117 associated fauna, and (3) the associated fauna community structure.

118

119

120 **Material and methods**

121 *Target species*

122 *Ellisolandia elongata* (J.Ellis & Solander) K.R. Hind & G.W. Saunders (Rhodophyta, Corallinales)  
123 is an articulated coralline alga with a crustose base and flexible pinnate fronds (5-7 mm), composed  
124 of flexible uncalcified joints (genicula) connecting calcified segments (intergenicula) (Babbini and  
125 Bressan, 1997) (Fig 1). Being one of the most abundant calcareous macroalgae of the Mediterranean  
126 Sea (El Haïkali et al., 2004), *E. elongata* is distributed from southern Spain to Greece and from  
127 Lebanon to Algeria (Bressan and Babbini, 2003), on hard rocky substrata from 0 - 5 m depth. It forms  
128 conspicuous intertidal habitats ('corniche') on natural vertical walls exposed to strong current and  
129 waves, but the species is found also in transitional and more sheltered environments (e.g. tidal pools,  
130 breakwaters and pontoons in marinas). Its adaptability derives from the high tolerance to fluctuations  
131 in salinity, temperature and  $p\text{CO}_2$  (Morris and Taylor, 1983; Huggett and Griffiths, 1986) supported  
132 by the morphology and plasticity of its fronds, making *E. elongata* flexible and resistant to  
133 hydrodynamism (Vogel, 1994; Harder et al., 2004; Ragazzola et al., 2017). Morphological and  
134 structural properties of the corniche favour the settlement of an a highly diverse associated  
135 community, dominated by molluscs, crustaceans and polychaetes (Izquierdo & Guerra-García, 2011;  
136 Guerra-García et al., 2012, 2014; Marchini et al., 2019), thus making *E. elongata* habitats important  
137 Mediterranean biodiversity host-spots.



138  
139 Figure 1. Dried branch of *Ellisolandia elongata*. Natural colour is lost after death.

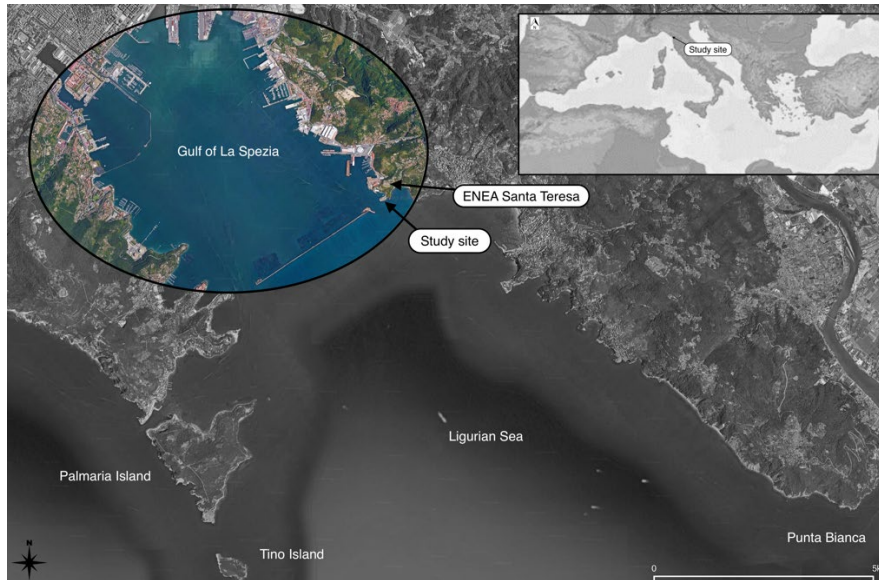
140

141 *Study site and sample collection*

142 The study site (44° 04' 51.79" N, 9° 52' 53.98" E; Fig. 2) is located in the Gulf of La Spezia (Ligurian  
143 Sea, North-West Mediterranean Sea, Figure 2), extending from Tino Island (West end) to Punta  
144 Bianca (East end), 5 km in width and 10 km in length. A breakwater divides the external and deep  
145 part of the Gulf (maximum depth: 25 m, close to Palmaria Island) from the inner and shallow part  
146 (maximum depth: 10 m, in the City Harbour) (Gasparini et al., 2009).

147 On 4<sup>th</sup> October 2017, 36 turfs (low-lying layer of algae - several mm to cm tall) of *E. elongata* were  
148 collected from the corniche. Twelve samples (5 cm × 5 cm each) were randomly sampled by  
149 snorkelling (50 cm below the average sea level), using hammer and chisel. While removing each  
150 sample from the vertical wall, each turf was wrapped with plastic bags pre-filled with ambient  
151 seawater, to avoid losing the associated vagile fauna. Once in the bag, turfs were transferred to an  
152 insulated cooler (one bag per turf) and immediately transported to ENEA laboratory (30 minutes).

153



154

155 Figure 2. The study site. The Gulf of La Spezia (North Western Mediterranean Sea, coloured map)  
156 with the location of the study site (QGIS modified, big picture). ENEA Santa Teresa (44°05'01"N,  
157 9°52'55"E) is located at 50 m above sea-level and 300 m away from the study site.

158

### 159 *Experimental design*

160 We wished to test the following null hypotheses: H<sub>01</sub>) *Ellisolandia elongata* physiology (i.e.  
161 calcification and photosynthesis) is not affected by climate change; H<sub>02</sub>) *E. elongata* does not affect  
162 the general community structure, especially polychaetes and peracarid crustaceans, associated with  
163 the ecosystem under climate change.

164 In the lab, twelve experimental aquaria were allocated four turfs per tank. The experiment started  
165 after one month of acclimatization (5<sup>th</sup> October – 5<sup>th</sup> November 2017) under ambient environmental  
166 conditions (temperature, salinity, pH and dissolved oxygen): *p*CO<sub>2</sub> was gradually increased, over a  
167 10-day period, in order to decrease the pH to 7.78 (RCP 8.5: IPCC, 2014, 2019). The multi-proxy  
168 manipulative system used for this study was a modified version of the semi closed flow-through  
169 systems used by Lucey et al. (2016) (See S1, Fig. S1). During the first experimental phase (*t*<sub>0</sub>) (10  
170 November- to 15<sup>th</sup> December 2017) (Tab. S1.2, S1.3) Three tanks (with 8 turfs per tank) were kept in  
171 conditions of ambient temperature and pH (CC*t*<sub>0</sub>), i.e. at values measured in the field (pH ~ 8.10).



172 The remaining three tanks (with 8 turfs per tank) were maintained at ambient temperature and reduced  
173 pH (= ambient pH – 0.3) ( $CA_{t_0}$ ), following IPCC (scenario RCP 8.5 expected for 2100 (pH ~ 7.78).  
174 Prior to the experimental phase, the pseudo-replicates (n=8) in each aquarium were split in half and  
175 introduced into further six replicate aquaria (three for pCO<sub>2</sub> /T and three for Control/T, n=4 in each).  
176 From 17<sup>th</sup> December - 9<sup>th</sup> January 2018, a Marine HeatWave (MHW) (temperature increase respect  
177 to the mean: approx. +1.4 °C, duration: 15 days) (Fig. S2) was induced in half of the treatments,  
178 changing the design from the first to the second experimental phase ( $t_1$ ) as follows:

- 179 - three tanks were kept in control condition (ambient pH + ambient temperature) ( $CC_{t_1}$ ),
- 180 - three tanks were exposed to ambient temperature and reduced pH ( $CA_{t_1}$ ),
- 181 - three tanks were exposed to increased temperature and ambient pH ( $TC_{t_1}$ ),
- 182 - three tanks were exposed to increased temperature and reduced pH ( $TA_{t_1}$ ).

183 Temperature (t), pH (Seven-go, Mettler Toledo, Swiss; Resolution: 0.01 pH / 1 mV / 0.1 °C. Error  
184 limits: ± 0.01 pH / ± 1 mV / ± 0.5 °C; calibrated with National Bureau of Standard (NBS) solutions  
185 (4.01, 7.00 and 9.21) salinity and oxygen (Hach HQ30d Flexi + Hach LDO Probe, Hach Company,  
186 USA) were monitored twice a day for the entire duration of the experiment in each tank.

187 Total alkalinity (TA) was measured once a day to monitor the carbonate system in one randomly  
188 selected tank of each treatment as well as in the two tanks containing solely rocks (total n = 6; Tab.  
189 S1.4). TA was measured potentiometrically using an automatic titrator (794 Basic Titrino, Metrohm  
190 Italiana Srl, Italy) calibrated with Natural Bureau of Standard (NBS) buffers and with a standard  
191 reference (Batch #165- Andrew G. Dickson). The full seawater carbonate system of the seawater was  
192 calculated from pH, TA, salinity ( $S_p$ ), and t using the Excel Macro CO2SYS (Version 2.1, Lewis and  
193 Wallace, 1998) with the equilibrium K1 and K2 for carbonic acid taken from the refit according to  
194 Mehrbach et al. (1973) and refit by Dickson and Millero (1987). Once a week, water samples were  
195 collected for nutrient analyses (N-NO<sub>3</sub> ( $\mu\text{mol}\cdot\text{L}^{-1}$ ), P-PO<sub>4</sub> [ $\mu\text{mol}\cdot\text{L}^{-1}$ ], Si-Si(OH)<sub>4</sub> ( $\mu\text{mol}\cdot\text{L}^{-1}$ ) and N-  
196 NO<sub>2</sub> ( $\mu\text{mol}\cdot\text{L}^{-1}$ )) (Autoanalyzer 3, Bran and Luebbe, UK). In order to guarantee food supply for the

197 associated fauna, a mix of green micro algae (~ 160 mL, of  $2.3 \cdot 10^5$  algae $\cdot$ L<sup>-1</sup>, *Chlorella* sp.) was  
198 added to each tank once a week and the protein skimmers were turned off for the following 24 hr to  
199 ensure food retention in the system.

200

#### 201 *Incubations and physiological measurement of Ellisolandia elongata*

202 For physiological studies, turf replicates (n=3) were incubated bubble-free in a 500 ml clear closed  
203 cell chamber under saturating light irradiance of  $(660.00 \pm 226.72)$   $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup> (mean  $\pm$   
204 standard deviation (S.D.)) (HQI Mal-Halide Lamp - HITLITE 150W - 14000 K; Germany) for 1.5  
205 hours. During the incubation, closed cell chambers were kept in a water bath at constant temperature  
206 during the incubation ( $(17.76 \pm 0.63)$  °C). To measure respiration, a second chamber was covered in  
207 aluminium foil to mimic night conditions and incubated alongside the light incubation. To account  
208 and correct for metabolic effects by other organisms, reference incubations without algae were  
209 performed at the same time.

210 Oxygen fluxes were estimated by measuring the oxygen concentrations at the beginning and end  
211 of each incubation using an oxygen probe. Measurements of pH were conducted using a pH probe  
212 (Seven-go, Mettler Toledo, Swiss; Resolution: 0.01 pH / 1 mV / 0.1 °C. Error limits:  $\pm 0.01$  pH /  $\pm 1$   
213 mV /  $\pm 0.5$  °C) calibrated with National Bureau of Standard (NBS) solutions and converted into total  
214 scale values using Tris/HCl and 2-aminopyridine/HCl buffer solutions after Dickson *et al.* (2007).  
215 Prior to the seawater chemistry measurements, the water samples were filtered through 0.45  $\mu$ m  
216 HAWP filters (MILLIPORE S.A., France) to remove any material that could interfere with T<sub>A</sub>  
217 titrations.

218 Total Alkalinity was measured as described above.

219

#### 220 *Associated fauna*

221 At the end (t1) of the MHW experiment, turf replicates (n=3 out of 4) were collected from each tank  
222 and preserved in separate containers with 70% ethanol for further analyses. Each sample, containing  
223 alga turf and associated fauna, was washed with tap water while being gently rubbed, and then sieved  
224 by means of 320  $\mu\text{m}$  and 63  $\mu\text{m}$  meshes (ASTM model, Giuliani, Italy). This procedure was repeated  
225 at least three times for each sample to ensure the removal of all the vagile fauna, both meio and macro,  
226 contained within the turf. Due to the differences in turf size and three-dimensional complexity, the  
227 abundances of fauna were standardised by the volume of each turf, which was measured by the  
228 displacement of cleaned turfs immersed in fresh water (Marchini et al., 2019; Sedano et al., 2020).

229 Samples of associated vagile fauna were preserved in 70% ethanol and analysed under a  
230 stereomicroscope (AZ100, Nikon, Japan). Individuals were classified into higher-rank taxonomic  
231 groups (nematodes, nemertean, forams, molluscs, polychaetes, crustaceans) and counted.  
232 Polychaetes and peracarid crustaceans were the most commonly occurring groups, and were  
233 identified at species level whenever possible and counted.

234

235 *Data analyses*

236 *Winter Marine Heatwaves*

237 Prior the calculation of MHW, sea surface temperature data (SST) were downloaded from the  
238 Copernicus Marine environment monitoring service catalogue (CMEMS). The database  
239 corresponded to SST\_MED\_SST\_L4\_REP\_OBSERVATIONS\_010\_021 (Buongiorno Nardelli et al., 2013;  
240 Pisano et al., 2016), which had a resolution of 0.04 degrees and covered the period 25 August 1981 -  
241 31 December 2018. Data were obtained by combining Pathfinder V5.3 (PFV53) AVHRR data with a  
242 bias-corrected version of the CMEMS NRT L4 data up to 2017 in order to provide a full time series  
243 of consistent daily gap-free maps (L4) at the original PFV53 resolution (0.0417° x 0.0417°). The data  
244 were interpolated through an Optimal Interpolation algorithm on the original Pathfinder grid (at  
245 0.0417° x 0.0417° spatial resolution), and they were representative of night SST values (00:00 UTC).

246 For the calculation of heat waves recorded in the area during the climatological winter (from  
247 January to March), both the 90th and 99th percentile of the SST anomaly relative to a 36-year daily  
248 climatology has been computed (Hobday et al., 2016; Darmaraki et al., 2019). A heat wave was  
249 defined as the period for which the anomaly showed values above the threshold for at least five  
250 consecutive days.

251

### 252 *Algal physiology*

253 Primary production was estimated from oxygen fluxes. Net production ( $P_n$ ) and respiration (R) rates  
254 (in  $\mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ ) were calculated following the equation used in Noisette et al. (2013),  
255 Kolzenburg et al. (2019), Egilsdottier et al. (2016), Williamson et al. (2017) and Ju-Hyoung et al.  
256 (2018).

257 Calcification rates (G) ( $\mu\text{mol CaCO}_3 \text{ g}^{-1} \text{ DW h}^{-1}$ ) were estimated using the alkalinity anomaly  
258 technique and were calculated following the equation used in Noisette et al. (2013), Kolzenburg et  
259 al. (2019) and Egilsdottier et al. (2016).

260 Gross production ( $P_g$ ) was calculated as follows:

$$261 \quad P_g = P_n - R$$

262 In order to examine differences among treatments, a one-way ANOVAs were carried out using the  
263 statistical software SPSS (IBM, Italy). Data normality was tested with the Shapiro-Wilk test and  
264 Brown-Forsythe was used as equal variance test.

265

### 266 *Associated fauna*

267 Prior to the analyses, the species (or the macro-groups) that were not present in more than 5% of the  
268 samples, were put altogether in “other” category and treated as a separate group.

269 The effects of experimental conditions on the vagile fauna communities (macro-groups, peracarids  
270 and polychaetes) were tested with PERMANOVA (Anderson, 2001) using 9999 permutations, based

271 on the Bray-Curtis similarity matrix. A fourth root transformation was applied to prevent the most  
272 abundant taxa from dominating the analysis. The PERMANOVA was performed to test differences  
273 among treatments ( $CC_{t0}$ ,  $CA_{t0}$ ,  $CC_{t1}$ ,  $CA_{t1}$ ,  $TC_{t1}$ ,  $TA_{t1}$ ), but since we were not interested in comparing  
274 all pairs of levels of the factor “Treatment”, but rather to compare one level vs another level, a contrast  
275 analysis design was formulated *a priori* (Anderson et al., 2008). After contrast test, a test of  
276 homogeneity of dispersions (PERMDISP) was applied to see the distribution of the data clouds  
277 around their centroids. In case of significant PERMANOVA results, PERMDISP indicates if the  
278 contrasts were due to a different distribution of data around their centroids (in this case indicating that  
279 PERMANOVA results should be taken with caution; Anderson et al., 2008). In order to identify  
280 which taxa were mostly contributing to the dissimilarities among contrasts, a SIMPER test was used.  
281 Also, non-metric multi-dimensional scaling (nMDS) was plotted showing the variation of the  
282 different communities, macro-groups, peracarids and polychaetes, under different conditions. In order  
283 to evaluate the diversity among the peracarid and polychaete communities, under different treatments,  
284 Shannon and Pielou indices were calculated, then differences were analysed *via* PERMANOVA  
285 based on Euclidean distance similarity matrix (Anderson et al., 2008).

286 The effect of algal physiological activities (i.e. respiration, photosynthesis, calcification under dark  
287 and light conditions) on assemblage structures were investigated, both between and within treatments  
288 by using distance-based linear models (DistLM). Prior to DistLM, correlation matrices were produced  
289 in PRIMER 6 to identify collinearities among variables. Where pairs of variables had a Pearson’s  
290 correlation coefficient of  $R \geq 0.9$ , one of the co-correlating variables was excluded in order to remove  
291 redundant variables from the analysis, in accordance with the methodology proposed by Anderson et  
292 al. (2008). The best DistLM model was chosen based on the Akaike Information Criterion (AIC) after  
293 a step-wise selection procedure with 9999 permutations (Anderson et al., 2008). Step-wise selection  
294 was chosen as it allows for both the addition and removal of a term to the model at each step  
295 (Anderson et al., 2008). Distance-based redundancy analysis (dbRDA) plots were used to provide the

296 best possible 2D visualisation of DistLM results for individual algal physiological variables, with  
297 samples identified by vectors proportional to their contribution to the total variation.

298 Additionally, species of polychaetes and peracarids were classified (based on Poore, 2001;  
299 Scipione, 2013; Faulwetter et al., 2014; Guerra-Garcia et al., 2014; Jumars et al., 2015; Larsen et al.,  
300 2015) into five trophic guilds: filter feeders, detritivores, herbivores, predators and omnivores. The  
301 multivariate analyses were then repeated on the dataset of trophic guilds abundance, after fourth root  
302 transformation for polychaetes and square root transformation for peracarids.

303 All analyses were performed *via* PRIMER 6 (version 6.1.13) & PERMANOVA+ (version 1.0.3)  
304 (Anderson et al., 2008).

305

## 306 **Results**

### 307 *Algal physiological responses*

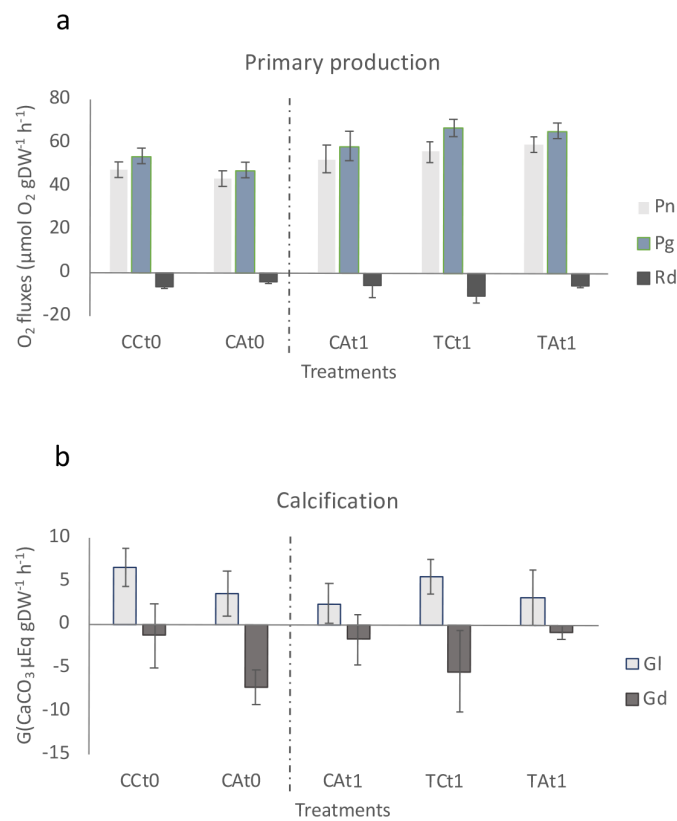
#### 308 *Respiration and photosynthesis.*

309 Both respiration and photosynthesis were overall very stable among treatments (Fig 3a) apart for a  
310 significant increase in photosynthesis between  $TAt_l$  and  $CA_{t_0}$  ( $p= 0.010$ ). The average net  
311 photosynthetic rates at  $t_l$  ranged from  $(47.4 \pm 3.9) \mu\text{mol O}_2 \text{ gDW}^{-1} \text{ h}^{-1}$  for CC to  $(59.7 \pm 3.7) \mu\text{mol}$   
312  $\text{O}_2 \text{ gDW}^{-1} \text{ h}^{-1}$  for  $T_A$ . Overall, the average rate seemed higher where there was an increase in  
313 temperature (TC:  $(56.1 \pm 4.9) \mu\text{mol O}_2 \text{ gDW}^{-1} \text{ h}^{-1}$  and  $T_A$ ) rather than acidification alone (CA:  $52.5 \pm$   
314  $6.5 \mu\text{mol O}_2 \text{ gDW}^{-1} \text{ h}^{-1}$ ). The average respiration rates ranged between  $(-6.3 \pm 4.9) \mu\text{mol O}_2 \text{ gDW}^{-1}$   
315  $\text{h}^{-1}$  for  $CC_{t_l}$  and  $(-10.8 \pm 4.9) \mu\text{mol O}_2 \text{ gDW}^{-1} \text{ h}^{-1}$  for  $TAt_l$  treatments. Both acidified treatments had  
316 very similar respiration rates ( $CA_{t_l}$ :  $(-6.1 \pm 5.3) \mu\text{mol O}_2 \text{ gDW}^{-1} \text{ h}^{-1}$ ;  $TAt_l$ :  $(-6.1 \pm 0.1) \mu\text{mol O}_2 \text{ gDW}^{-1}$   
317  $\text{h}^{-1}$ ).

#### 318 *calcification during light and dark conditions*

319 For both light and dark conditions, calcification there were not significant difference among  
320 treatments (Fig 3b). However under light, the average calcification was slightly lower in the acidified

321 treatments (CA:  $(2.5 \pm 2.3)$   $\text{CaCO}_3 \mu\text{Eq gDW}^{-1} \text{h}^{-1}$ ; TA:  $(3.2 \pm 3.2)$   $\text{CaCO}_3 \mu\text{Eq gDW}^{-1} \text{h}^{-1}$ ) compared  
 322 to CC ( $6.6 \pm 2.2$   $\text{CaCO}_3 \mu\text{Eq gDW}^{-1} \text{h}^{-1}$ ) and TC ( $(5.54 \pm 2.01)$   $\text{CaCO}_3 \mu\text{Eq gDW}^{-1} \text{h}^{-1}$ ). The average  
 323 calcification in the dark across treatments were very similar (CC:  $(-1.3 \pm 3.7)$   $\text{CaCO}_3 \mu\text{Eq gDW}^{-1} \text{h}^{-1}$   
 324  $^{-1}$ ; CA:  $(-1.7 \pm 2.8)$   $\text{CaCO}_3 \mu\text{Eq gDW}^{-1} \text{h}^{-1}$ ; TA:  $(-0.8 \pm 0.8)$   $\text{CaCO}_3 \mu\text{Eq gDW}^{-1} \text{h}^{-1}$ ), except from TC  
 325 that displayed lower values ( $(-5.3 \pm 4.6)$   $\text{CaCO}_3 \mu\text{Eq gDW}^{-1} \text{h}^{-1}$ ). In all the light and dark treatments,  
 326 calcification measurements were high variability among replicates.



327  
 328 Figure 3: a) Net and gross photosynthesis (Pn and P<sub>g</sub>) rates at the experimental 275 irradiance, and  
 329 respiration (R) rates. b) Calcification rates in the dark (Calc\_D) and at the experimental irradiance  
 330 (Calc\_L) in the 4 treatments. Negative values for algal calcification correspond to the decalcification  
 331 activity quantified as increase in total alkalinity. Error bars are  $\pm$  SD and are expressed in terms of O<sub>2</sub>  
 332 release (negative values for respiration correspond to O<sub>2</sub> consumption); n = 3 for each treatment.  
 333 Dotted line separates t0 from t1

334

335 *Associated fauna*

336 *Macrogroups*

337 In all treatments, for both experimental phases ( $t_0$  and  $t_1$ ) Foraminifera represented over 73% of all  
338 individuals.

339 Before the experimental phase ( $t_0$ ), the most abundant taxon (mean  $\pm$  S.D.) in both treatments  
340 ( $CCt_0$  and  $CAt_0$ ) were molluscs ( $CCt_0$  ( $10086 \pm 7803$ ) ind  $L^{-1}$ ,  $CAt_0$  ( $9566 \pm 9172$ ) ind  $L^{-1}$ ), followed  
341 by polychaetes ( $CCt_0$  ( $2750 \pm 2903$ ) ind  $L^{-1}$ ,  $CAt_0$  ( $3205 \pm 2469$ ) ind  $L^{-1}$ ) and crustaceans ( $CCt_0$  ( $1242$   
342  $\pm 641$ ) ind  $L^{-1}$ ,  $CAt_0$  ( $1992 \pm 1514$ ) ind  $L^{-1}$ ). The most represented species in all treatments at  $t_1$  was  
343 the Mediterranean mussel *Mytilus galloprovincialis* Lamarck, 1819. Under control condition,  $CCt_1$ ,  
344 molluscs dominated the community ( $CCt_1$  ( $5387 \pm 2190$ ) ind  $L^{-1}$ ), followed by polychaetes (( $4499 \pm$   
345  $1651$ ) ind  $L^{-1}$ ) and crustaceans, including also Ostracoda and Copepoda (( $4499 \pm 1651$ ) ind  $L^{-1}$ ), while  
346 under the simulated heatwaves ( $TCt_1$ ) polychaetes dominated (( $5440 \pm 1912$ ) ind  $L^{-1}$ ), over molluscs  
347 (( $5114 \pm 985$ ) ind  $L^{-1}$ ) and crustaceans (( $2674 \pm 843$ ) ind  $L^{-1}$ ). The community structure changed  
348 again under reduced pH conditions ( $CAt_1$ ), where the dominant taxa were crustaceans (( $5228 \pm 4076$ )  
349 ind  $L^{-1}$ ), followed by polychaetes (( $4745 \pm 2980$ ) ind  $L^{-1}$ ) and molluscs (( $3686 \pm 1513$ ) ind  $L^{-1}$ ).  
350 Finally, the community cultured under reduced pH and a simulated heatwave ( $ATt_1$ ) exhibited a  
351 structure similar to  $TCt_1$ , with a dominance of polychaetes (( $3704 \pm 2439$ ) ind  $L^{-1}$ ), but followed first  
352 by crustaceans (( $3306 \pm 1687$ ) ind  $L^{-1}$ ) and then molluscs ( $2223 \pm 1216$ ) ind  $L^{-1}$ .

353 The PERMANOVA main test, performed to test differences among treatments (CC, TC, CA, TA)  
354 and time ( $t_0$  and  $t_1$ ), detected significant differences among treatments (Tab. 1). The contrast analysis  
355 revealed several significant differences (Tab. 1), mainly associated to the high temperature simulation  
356 ( $CCt_0$  vs  $TCt_1$ ;  $CCt_1$  vs  $TCt_1$ ). One unexpected result was represented by the control of high  
357 temperature ( $TCt_1$ ) and the combined effect of acid and temperature ( $TAt_1$ ). Despite the high  
358 significance level observed, the deviations from the centroids among the samples were strong as well  
359 (potentially due to the high variability among samples observed earlier), indicating that caution



360 should be applied when considering the effects of the acidification associated to the heatwave. In fact,  
361 when the acid control ( $CA_{t1}$ ) was compared to the combined effects of acid and temperature ( $TAt_1$ ) a  
362 marginal significant difference in the community was observed (Tab. 1). In this case the dispersion  
363 among the centroids was not significant, suggesting that an effective difference due to the high  
364 temperature was plausible.

365 Non-metric multidimensional scaling (nMDS) showing the distribution of the community  
366 according to the different treatments and experimental phases (Fig. 4a), confirmed a high variability  
367 of the community structure within each experimental condition and phase, yet showing some degree  
368 of segregation for samples where altered temperatures have been applied.

369 According to SIMPER, the taxa mostly contributing to their dissimilarity were foraminifers  
370 (17.6%), crustaceans (15.6%) and polychaetes (15.40%) (Tab. S3.1, supplementary material).

371 Table 1 - Results of PERMANOVA and PERMDISP based on fourth root data transformations and  
 372 Bray-Curtis similarity matrix for different taxonomic assemblages, tested for: treatment (CC=  
 373 ambient temperature and pH; CA= ambient temperature, reduced pH; TC= increased temperature,  
 374 ambient pH; TA= increased temperature, reduced pH) and time ( $t_0, t_1$ ) (see material and methods for  
 375 details). F = pseudo – F from permutation procedure. Significant  $p$ -values in bold.  
 376

		PERMANOVA				PERMDISP (deviation from centroids)		
Source		df	F	$p$	Effective permutations	F	t	$p$
Macro-groups	Treatment (main test)	5	3.5	<b>&lt;0.001</b>	9926	7.17	-	<b>&lt;0.001</b>
	TC $t_1$ vs CC $t_0$	1	14.4	<b>&lt;0.001</b>	8121	-	4.5	<b>&lt;0.001</b>
	TC $t_1$ vs CC $t_1$	1	7.9	<b>&lt;0.001</b>	8149	-	3.5	<b>0.001</b>
	TC $t_1$ vs TAt $t_1$	1	12.0	<b>&lt;0.001</b>	8166	-	3.1	<b>0.002</b>
	CC $t_0$ vs CC $t_1$	1	2.0	0.119	8174	-	0.3	0.752
	CA $t_0$ vs CA $t_1$	1	3.5	<b>0.032</b>	8145	-	2.7	<b>0.024</b>
	CC $t_0$ vs CA $t_1$	1	5.2	<b>0.006</b>	8178	-	0.5	0.621
	CA $t_1$ vs TAt $t_1$	1	3.2	<b>0.049</b>	8197	-	0.1	0.928
	CC $t_1$ vs CA $t_1$	1	2.1	0.115	8182	-	0.2	0.862
	CA $t_0$ vs CC $t_0$	1	0.8	0.533	8163	-	2.5	0.051
	Residuals	48						
Polychaetes	Treatment (main test)	5	9.3	<b>&lt;0.001</b>	9915	4.3	-	<b>0.008</b>
	TC $t_1$ vs CC $t_0$	1	17.1	<b>&lt;0.001</b>	8149	-	3.8	<b>0.001</b>
	TC $t_1$ vs CC $t_1$	1	1.1	0.402	8182	-	0.5	0.652
	TC $t_1$ vs TAt $t_1$	1	2.1	0.065	8151	-	1.8	0.136
	CC $t_0$ vs CC $t_1$	1	13.6	<b>&lt;0.001</b>	8161	-	3.1	<b>0.003</b>
	CA $t_0$ vs CA $t_1$	1	15.1	<b>&lt;0.001</b>	8167	-	2.3	<b>0.045</b>

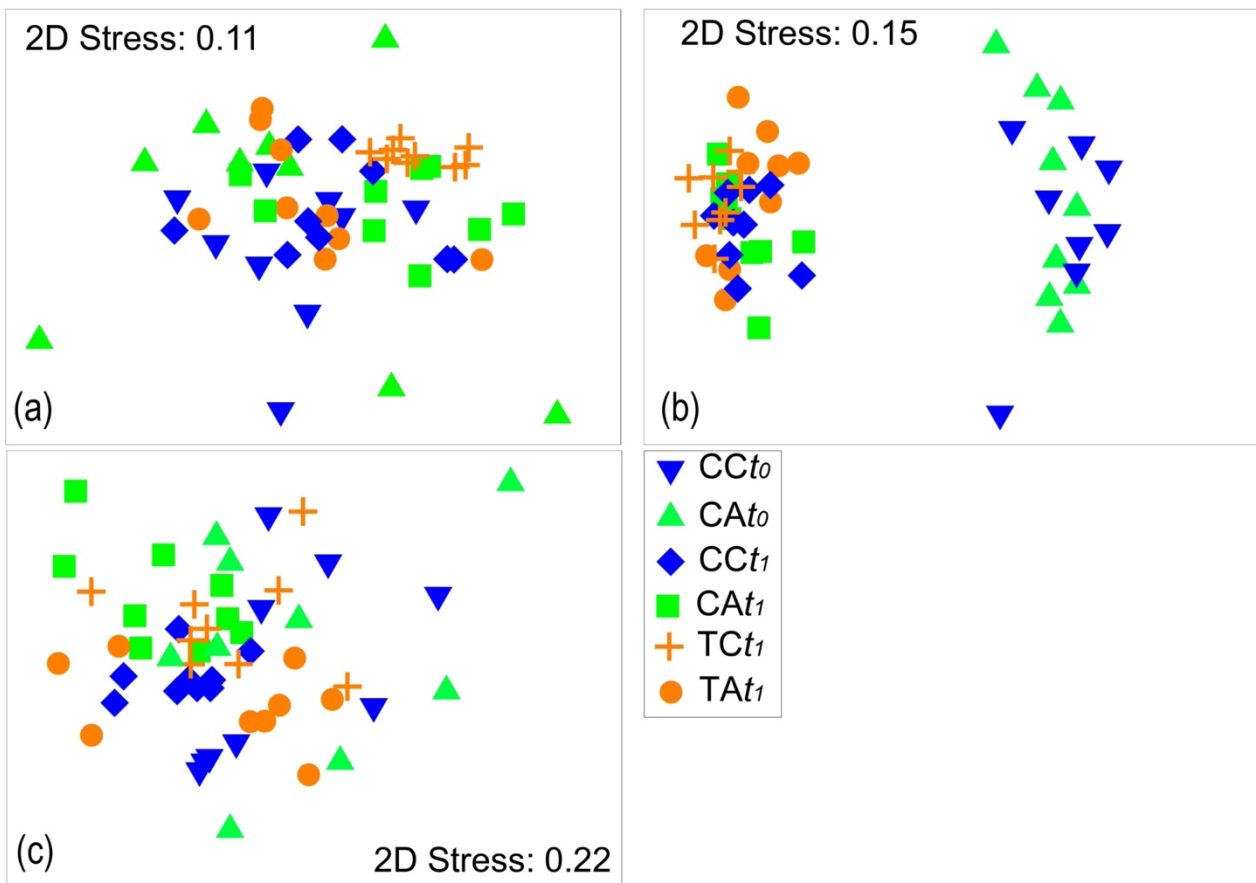
	<i>CCt<sub>0</sub> vs CA<sub>t1</sub></i>	1	14.7	<b>&lt;0.001</b>	8133	-	2.7	<b>0.013</b>
	<i>CA<sub>t1</sub> vs TA<sub>t1</sub></i>	1	1.1	0.369	8135	-	0.9	0.431
	<i>CC<sub>t1</sub> vs CA<sub>t1</sub></i>	1	1.0	0.426	8101	-	0.2	0.859
	<i>CA<sub>t0</sub> vs CC<sub>t0</sub></i>	1	0.7	0.707	8164	-	0.6	0.632
	Residuals	47						
Peracarids	Treatment (main test)	5	2.9	<b>&lt;0.001</b>	9901	4.4	-	<b>0.010</b>
	<i>TC<sub>t1</sub> vs CC<sub>t0</sub></i>	1	2.4	0.056	8151	-	1.4	0.219
	<i>TC<sub>t1</sub> vs CC<sub>t1</sub></i>	1	2.9	<b>0.019</b>	8175	-	1.9	0.088
	<i>TC<sub>t1</sub> vs TA<sub>t1</sub></i>	1	2.6	<b>0.038</b>	8159	-	0.8	0.464
	<i>CC<sub>t0</sub> vs CC<sub>t1</sub></i>	1	4.5	<b>0.002</b>	8157	-	5.2	<b>&lt;0.001</b>
	<i>CA<sub>t0</sub> vs CA<sub>t1</sub></i>	1	3.8	<b>&lt;0.001</b>	8126	-	1.7	0.189
	<i>CC<sub>t0</sub> vs CA<sub>t1</sub></i>	1	5.0	<b>&lt;0.001</b>	8075	-	1.0	0.433
	<i>CA<sub>t1</sub> vs TA<sub>t1</sub></i>	1	2.8	<b>0.025</b>	8119	-	0.3	0.788
	<i>CC<sub>t1</sub> vs CA<sub>t1</sub></i>	1	2.6	<b>0.002</b>	8137	-	3.0	<b>0.013</b>
	<i>CA<sub>t0</sub> vs CC<sub>t0</sub></i>	1	0.8	0.580	8154	-	1.1	0.341
	Residuals	48						

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382 Figure 4. Non-metric multi-dimensional scaling (nMDS) plot of (a) macro groups, (b) polychaetes,  
 383 and (c) peracarids associated to *Ellisolandia elongata* in experimental tanks. Treatments: CC=  
 384 ambient temperature and pH; CA= ambient temperature, reduced pH; TC= increased temperature,  
 385 ambient pH; TA= increased temperature, reduced pH; experimental phases:  $t_0$ ,  $t_1$  (see material and  
 386 methods for details).

387

### 388 *Polychaetes*

389 In general, polychaete communities associated with *Ellisolandia elongata* were characterised by a  
 390 relatively high number of species (see Supplementary Material – Faunistic Remarks).

391 In all experimental phases the most represented polychaete species was the Exogoninae syllid  
 392 *Sphaerosyllis hystrix*; in the first experimental phase, *Exogone verugera* showed a distinctively higher  
 393 abundance under reduced pH conditions (CA $t_0$ ) compared to control conditions (CC $t_0$ ). All other  
 394 species showed a strong variation between replicates within the same experimental condition. The

395 community resulting after the second experimental phase ( $t_1$ ) showed a marked reduction in  
396 polychaete abundance and a shift between species. Some polychaete species (e.g. *Amphiglena*  
397 *mediterranea*, *Dodecaceria concharum*, *Exogone naidina*, *Platynereis dumerilii* and *Syllis gracilis*  
398 complex) showed a strong reduction in the second experimental phase, while some species reporting  
399 high abundances in  $t_1$  were scarcely represented in  $t_0$  (e.g. *Perinereis cultrifera*, *Eurysyllis*  
400 *tuberculata*) or even absent (e.g. *Aphelochaeta* sp., *Exogone dispar*, *Nereis perivisceralis*, *Salvatoria*  
401 *limbata*). The high casual variation retrieved for the majority of polychaete taxa made the  
402 identification of species either favoured or disadvantaged by specific experimental treatments. For  
403 some species, as *Amphiglena mediterranea* and *Eurysyllis tuberculata*, significant contributions to  
404 the dissimilarity between experimental conditions were not related to a consistent pattern.  
405 Nonetheless, in all pairwise comparisons *Haplosyllis granulosa* was more abundant in acidified  
406 conditions if compared to ambient pH, while *Perinereis cultrifera* showed higher abundances under  
407 control conditions, and *Salvatoria limbata* showed higher abundances in samples subjected to the  
408 heatwave (Tab. S3.2, supplementary material).

409 Shannon diversity and Pielou evenness did not vary significantly across treatments and  
410 experimental phases, with the exception of evenness values between  $CC_{t_0}$  and  $CC_{t_1}$  (pseudo -  $F_{1,16} =$   
411 5.98,  $p = 0.007$ ).

412 Contrast tests highlighted significant differences in the communities of the natural control ( $CC_{t_0}$ )  
413 and the heatwave control at  $t_1$  ( $TC_{t_1}$ ; Tab. 1), although, also in this case the PERMDISP showed a  
414 high dispersion of samples among centroids. More interesting is the fact that most of the differences  
415 observed were attributable to the time samples remained in the tanks, as also evident in the graphical  
416 representation of the nMDS (Fig. 4b).

417

#### 418 *Crustaceans*

419 As well as for the analyses on macro groups, different turfs of *Ellisolandia elongata* within each  
420 treatment tank showed a great variability, as shown by the high S.D.. The analyses of crustacean

421 fauna revealed that, in all treatments, and for both experimental phases, the dominant sub class was  
422 Copepoda, whose individuals were isolated with a 63  $\mu\text{m}$  size mesh. This micro fauna represented an  
423 important part of the crustacean community associated to *E. elongata*. Copepoda, commonly  
424 abundant in control conditions in both phases ( $CCt_0$  ( $1837 \pm 683$ ) ind  $L^{-1}$ ;  $CCt_1$  ( $2839 \pm 1831$ ) ind  $L^{-1}$ )  
425 were higher under reduced pH conditions ( $CAt_0$  ( $2129 \pm 2490$ ) ind  $L^{-1}$ ;  $CAt_1$  ( $4130 \pm 2985$ ) ind  
426  $L^{-1}$ ), and lower under the heatwave condition ( $TCt_1$  ( $1717 \pm 561$ ) ind  $L^{-1}$ ), with a positive effect of  
427 reduced pH in the case of combination of both factors ( $TAt_1$  ( $2433 \pm 1186$ ) ind  $L^{-1}$ ).

428 *Superorder Peracarida*. Regarding macro fauna (320  $\mu\text{m}$  size mesh), the crustacean species identified  
429 belonged to the superorder Peracarida (see Supplementary Material – Faunistic Remarks). The  
430 species with highest density ( $> 100$  individuals per 1 ml of substrate) in the first experimental phase  
431 under control conditions,  $CCt_0$ , were *Paranthura costana* ( $(297 \pm 126)$  ind  $L^{-1}$ ), *Carpias*  
432 *galloprovincialis* ( $(204 \pm 292)$  ind  $L^{-1}$ ), *Quadrimeaera inaequipes s.s.* ( $(180 \pm 188)$  ind  $L^{-1}$ ), *Munna*  
433 *cfr. wolffi* ( $(170 \pm 275)$ ), and *Cymodoce* sp. ( $(143 \pm 261)$  ind  $L^{-1}$ ). Under reduced pH conditions,  $CAt_0$ ,  
434 species relative abundances were reduced, but the most represented species ( $> 100$  individuals) were  
435 still *Paranthura costana* ( $(151 \pm 151)$  ind  $L^{-1}$ ) and *Carpias galloprovincialis* ( $(146 \pm 188)$  ind  $L^{-1}$ ),  
436 whereas *Quadrimeaera inaequipes* was halved ( $(63 \pm 62)$  ind  $L^{-1}$ ). Less represented species ( $(5 \pm 15)$   
437 ind  $L^{-1}$ ) were *Monocorophium* sp. and *Ampithoe ramondi*.

438 Shannon diversity and Pielou evenness indexes did not show significant differences when  
439 comparing community structure across treatments, while only time resulted significant for Shannon  
440 index, by comparing  $CCt_0$  and  $CCt_1$  (pseudo- $F_{1, 16} = 10.79$ ,  $p = 0.006$ ).

441 Contrast analyses resulted in significant differences, unrelated to dispersion among centroids,  
442 among all contrasts with the exclusion of  $TCt_1$  vs  $CCt_0$  and  $CAt_0$  vs  $CCt_0$  (Tab. 1). Contrasting  
443 significant effects were observed between  $TCt_1$  vs  $TAt_1$  and  $CAt_1$  vs  $TAt_1$ .

444 The nMDS plot showed the separation of the data cloud of  $TAt_1$  from the  $TCt_1$  and  $CAt_1$  points  
445 (Fig. 4c). The SIMPER test revealed that the species that mainly contributed to the dissimilarities  
446 among the significant ones were isopods, which had different responses to the treatment (Tab. S3.3,

447 supplementary material). When comparing  $CCt_I$  vs  $TCt_I$ , the species that mostly contributed to their  
448 dissimilarity was the sphaeromatid *Cymodoce emarginata* (11.6%). It occurred in lower average  
449 abundance when exposed the heatwaves compared to current temperatures, followed by the anthuroid  
450 *Mesanthura romulea* (10.1%), which displayed the opposite pattern. In the acidified conditions ( $CAt_I$   
451 vs  $TAt_I$ ), other species mostly contributed to the dissimilarity between samples raised in current vs  
452 increased temperature conditions: the janirid *Carpias galloprovincalis* (12.9%), which dominated the  
453 community under current temperatures but dropped to low average abundances under the heatwaves,  
454 and one amphipod, *Quadrinemaera inaequipes* (10.62%), which took the lead under high-temperature  
455 conditions (Tab. S3.3, supplementary material).

456

#### 457 *Trophic guilds*

458 When species were grouped into trophic guilds, PERMANOVA and PERMDISP results were similar  
459 to those obtained with species abundance data. Namely, polychaetes showed significant responses to  
460 factor time, while peracarids responded significantly to combined acidification and winter heatwave  
461 conditions (Tab. 2; see also Supplementary Material – Faunistic Remarks).

462 According to SIMPER (Tab. S3.4, supplementary material), differences between the beginning  
463 ( $t_0$ ) and the end ( $t_1$ ) of the experiment for polychaetes were due to a dramatic decrease of predator  
464 species, which contributed 24.24% to the dissimilarity of  $CCt_0$  vs  $CCt_1$ , and 32.03% for  $CAt_0$  vs  $CAt_1$ .  
465 On the other hand, omnivorous polychaete species increased with time, and contributed 24.79% to  
466 the dissimilarity of  $CCt_0$  vs  $CCt_1$ , and 21.7% for  $CAt_0$  vs  $CAt_1$ .

467 For peracarids, significant response to altered pH and temperature were partially due to an increase  
468 of opportunistic, omnivorous species, which mostly contributed to dissimilarity for both  $CAt_I$  vs  $TAt_I$   
469 (30.94%) and  $TCt_I$  vs  $TAt_I$  (30.73%) (Tab. S3.5, supplementary material). Instead, abundance of  
470 predators and herbivorous guilds decreased when experimental conditions were harsher ( $TAt_I$ ).  
471 Predator peracarids contributed 25.75% to the dissimilarity of  $CAt_I$  vs  $TAt_I$ , and 18.79% for  $TCt_I$  vs

472  $TAt_1$ , while herbivorous species contributed 25.75% to the dissimilarity of  $CA_t_1$  vs  $TAt_1$ , and 28.91%  
 473 for  $TCt_1$  vs  $TAt_1$ .

474

475 Table 2 - Results of PERMANOVA and PERMDISP based on Bray-Curtis similarity matrix based  
 476 on abundance data of different trophic guilds, tested for: treatment (CC= ambient temperature and  
 477 pH; CA= ambient temperature, reduced pH; TC= increased temperature, ambient pH; TA= increased  
 478 temperature, reduced pH) and time ( $t_0$ ,  $t_1$ ) (see material and methods for details). F = pseudo – F from  
 479 permutation procedure. Significant  $p$ -values in bold.

480

		PERMANOVA				PERMDISP (deviation from centroids)		
Source		df	F	$p$	Effective permutations	F	t	$p$
Polychaetes	Treatment (main test)	5	4.8	<b>&lt;0.001</b>	9904	4.6	-	<b>0.002</b>
	$TCt_1$ vs $CCt_0$	1	3.2	<b>&lt;0.001</b>	8123	-	2.3	<b>0.010</b>
	$TCt_1$ vs $CCt_1$	1	0.1	0.886	8167	-	1.9	0.082
	$TCt_1$ vs $TAt_1$	1	0.8	0.522	8139	-	1.1	0.323
	$CCt_0$ vs $CCt_1$	1	3.9	<b>&lt;0.001</b>	8098	-	2.8	<b>&lt;0.001</b>
	$CA_{t_0}$ vs $CA_{t_1}$	1	12.5	<b>&lt;0.001</b>	8168	-	1.4	0.196
	$CCt_0$ vs $CA_{t_1}$	1	3.3	<b>0.002</b>	8170	-	1.8	0.115
	$CA_{t_1}$ vs $TAt_1$	1	0.8	0.517	8183	-	0.5	0.674
	$CCt_1$ vs $CA_{t_1}$	1	0.2	0.853	8187	-	3.0	<b>0.031</b>
	$CA_{t_0}$ vs $CCt_0$	1	0.8	0.720	8152	-	1.3	0.490
	Residuals	48						
Peracarids	Treatment (main test)	5	2.1	<b>0.009</b>	9924	2.7	-	0.060
	$TCt_1$ vs $CCt_0$	1	1.0	0.440	8200	-	0.9	0.371
	$TCt_1$ vs $CCt_1$	1	1.5	0.210	8209	-	0.7	0.504



$TCt_l$ vs $TAt_l$	1	3.1	<b>0.033</b>	8126	-	2.1	0.051
$CCt_0$ vs $CCt_l$	1	1.7	<b>0.170</b>	8160	-	1.9	0.118
$CAt_0$ vs $CAt_l$	1	0.4	0.831	8199	-	1.1	0.330
$CCt_0$ vs $CAt_l$	1	1.8	0.148	8153	-	0.5	0.718
$CAt_l$ vs $TAt_l$	1	3.1	<b>0.023</b>	8235	-	0.5	0.669
$CCt_l$ vs $CAt_l$	1	1.5	0.199	8153	-	1.7	0.216
$CAt_0$ vs $CCt_0$	1	1.7	0.133	8130	-	1.7	0.207
Residuals	48						

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484 *Micro habitat changes and responses of the associated fauna*

485 Data from the algal physiology were used as indicators of micro habitat changes within the fronds  
486 (i.e. photosynthesis as an increase in O<sub>2</sub> and pH; respiration as a decrease in O<sub>2</sub> and pH reduction).  
487 Concerning macro groups, the best model (based on AIC) from the DistLM analysis was the one  
488 based on three algal physiologic variables (Tab. 3): Respiration (Resp), Net Photosynthesis (Photo),  
489 and calcification in dark conditions (Calc\_D) (Fig. 5a). Although the last two variables were not  
490 significant, their presence in the model was considered in some way useful to improve it.

491

492 Table 3: Best models from DistLM analysis based on AIC for Macro-groups, Polychaetes, Peracarids,  
493 Polychaetes feeding guilds and Peracarids feeding guilds. Resp (Respiration); Photo (Net  
494 Photosynthesis); Calc\_D (calcification in dark conditions); Calc\_L (calcification in light conditions).  
495 Significant *p*-values in bold.

	Variable	AIC	df	F	p
Macro-groups	Resp	208.5	52	3.9	<b>0.018</b>
	Photo	208.0	51	2.4	0.069
	Calc_D	207.9	50	2.0	0.128

Polychaetes	Resp	380.1	51	11.7	<b>&lt;0.001</b>
	Calc_D	376.9	49	2.3	<b>0.035</b>
	Calc_L	376.2	48	2.5	<b>0.024</b>
Peracarids	Calc_L	407.1	52	2.6	<b>0.014</b>
	Photo	406.4	51	2.6	<b>0.013</b>
	Resp	406.0	50	2.3	<b>0.034</b>
	Calc_D	405.6	49	2.2	<b>0.039</b>
Polychaetes feeding guilds	Resp	340.6	52	6.9	<b>&lt;0.001</b>
	Calc_D	336.6	51	5.9	<b>&lt;0.001</b>
	Calc_L	336.5	50	2.1	0.104
Peracarids feeding guilds	Photo	362.0	52	4.2	<b>0.004</b>
	Calc_L	359.6	51	4.3	<b>0.004</b>
	Resp	359.2	50	2.3	0.079

496

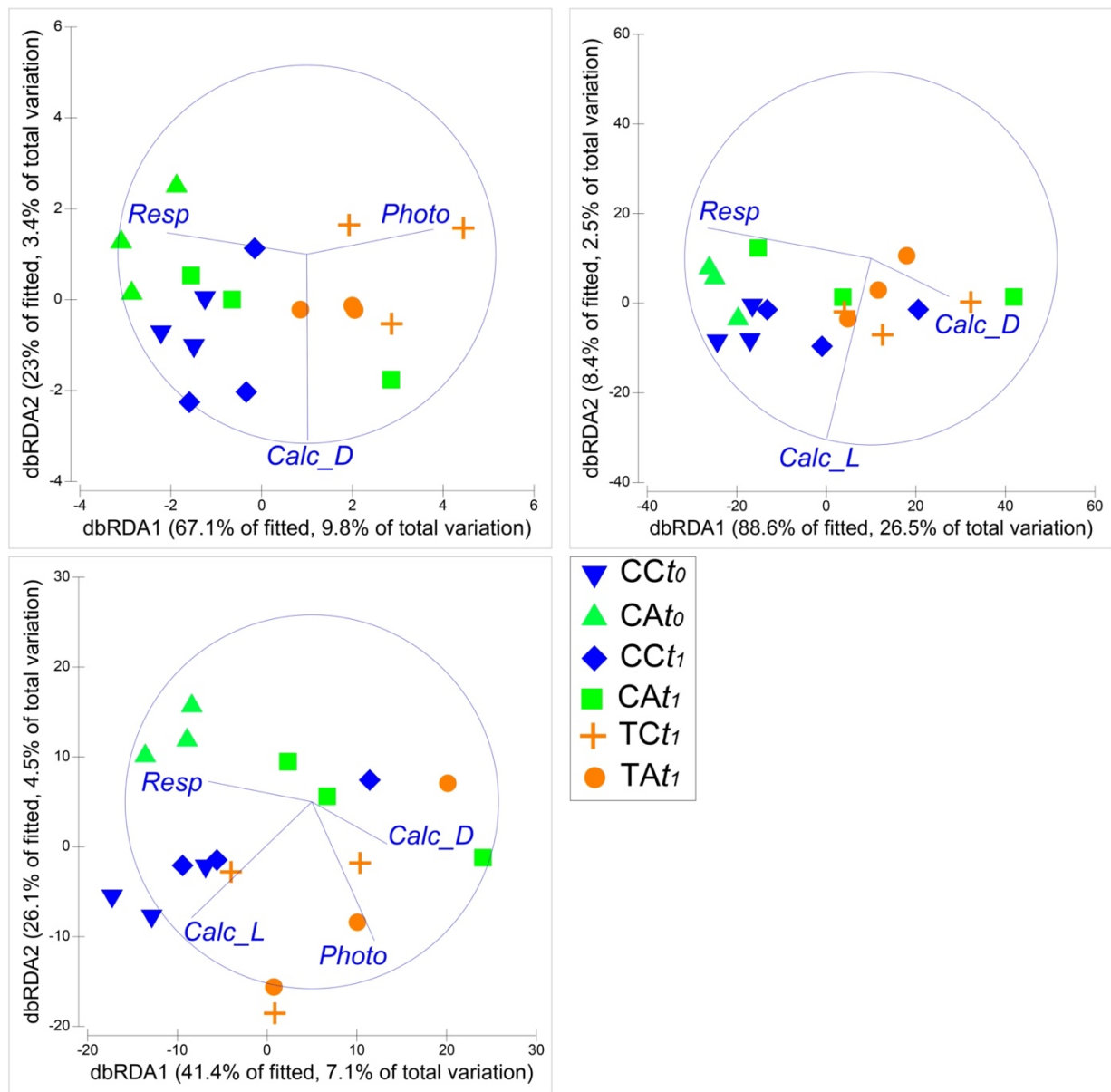
497

498 The importance of individual algal variables to the model were shown in the dbRDA plot  
499 (dbRDA1: 67.1% of fitted, 9.8 of total variation; dbRDA2: 23% of fitted, 3.4% of total variation)  
500 where vectors were proportional to their contribution to the total variation, with respiration being the  
501 most significant one. Treatments with reduced pH and control temperature ( $CA_{t0}$ ,  $CA_{t1}$ ) were grouped  
502 close to respiration, where the oxygen consumption by the algae exacerbated the lowering of pH  
503 decrease within the micro-environment. Under winter heatwave conditions, a reduction of oxygen  
504 availability occurred (the solubility of  $O_2$  decrease with increasing temperature), thus  $TA_{t1}$ ,  $TC_{t1}$

505 assemblages were associated to high photosynthesis, corresponding to conditions of oxygen release  
506 by the algae.

507 For vagile polychaete assemblages, the best model was based on: Resp, Calc\_D and calcification  
508 in light conditions (Calc\_L) (Tab. 3; Fig. 5b). Polychaete assemblages exposed to altered pH and  
509 temperature conditions were far from conditions of high respiration and calcification under light  
510 conditions, which increase the CO<sub>2</sub> content and probably exacerbate the already harsh environment.

511 Regarding peracarids, the best model was based on all four variables: Resp, Photo, Calc\_L and  
512 Calc\_D (Tab. 3; Fig. 5d). Similarly, to the previous cases, high rates of respiration and calcification  
513 under light conditions were only associated to control assemblages (CC<sub>t0</sub>, CC<sub>t1</sub>), while acidic  
514 assemblages were associated to higher rates of calcification under dark conditions (CA<sub>t1</sub>), which  
515 contrasts environmental acidification by locally increasing T<sub>A</sub> (skeletal dissolution). Notably,  
516 assemblages grown under heatwaves (TC<sub>t1</sub>, TA<sub>t1</sub>), where O<sub>2</sub> solubility has obviously decreased, are  
517 associated to conditions of high photosynthesis rates, which ensure local provision of biogenic O<sub>2</sub>.



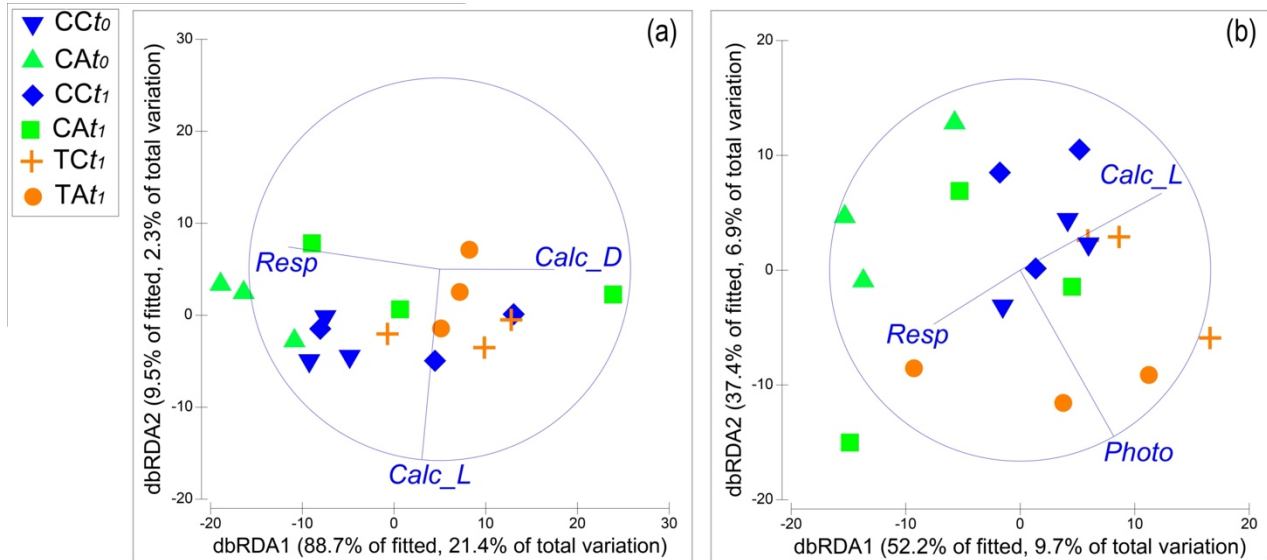
518

519 Figure 5. Distance-based redundancy analysis (dbRDA) plots to visualize distance-based linear  
 520 models results in a 2-dimensional space for algal physiological responses (photosynthesis-Photo,  
 521 respiration-Resp, calcification in dark conditions-Calc\_D and calcification under light-Calc\_L) for:  
 522 a) macrogroups, b) polychaetes, c) peracarids. Treatments: CC= current temperature and pH; CA=  
 523 current temperature, reduced pH; TC= increased temperature, current pH; TA= increased  
 524 temperature, reduced pH; experimental phases:  $t_0$ ,  $t_1$ .

525

526 When data of species abundance were re-organised into abundance of trophic guilds (Fig. 6), the  
 527 patterns of correlation between assemblage structure and algal physiology were confirmed (Tab. 3).

528 Opportunistic (omnivores or detritivores) polychaete species (Fig. 6a) dominating samples at phase  
 529  $t_1$ , were dissociated from respiration and light calcification. Similarly, peracarids that survived the  
 530 most intensive experimental treatment ( $TA_{t_1}$ ), were more associated to photosynthesis and  
 531 calcification under light conditions (Tab. 3; Fig. 6c).



532  
 533 Figure 6. Distance-based redundancy analysis (dbRDA) plots to visualize distance-based linear  
 534 models results in a 2-dimensional space for algal physiological responses (photosynthesis-Photo,  
 535 respiration-Resp, calcification in dark conditions-Calc\_D and calcification in the light-Calc\_L) for:  
 536 a) polychaetes, b) peracarids. Treatments: CC= current temperature and pH; CA= current  
 537 temperature, reduced pH; TC= increased temperature, current pH; TA= increased temperature,  
 538 reduced pH; experimental phases:  $t_0$ ,  $t_1$ .

539

## 540 Discussion

541 Temperature and pH have fundamental effects on algal physiology, from chemical to population  
 542 levels. Calcifying algae have shown variable responses to warming experiments (Martin & Gattuso,  
 543 2009; Cornwall et al., 2019; Nannini et al., 2015) which highlight the high plasticity of this algal  
 544 group across different marine environments (McCoy et al., 2019). In our study, overall there were no  
 545 significant changes in the physiology of *Ellisolandia elongata* turfs apart for the combined treatments  
 546 of low pH and winter heatwave, which resulted in an increase in photosynthetic activity ( $CA_{t_0}$  and

547 TAt<sub>l</sub>). This can be interpreted as an influence of temperature to the photosynthetic activity combined  
548 with a downregulation of the carbon concentrating mechanisms due to the increased availability of  
549 CO<sub>2</sub> (Murru & Sandgren, 2004). While winter heatwaves did not have any negative effect in this  
550 study, several studies showed how summer heatwaves have, on the other hand, detrimental effect on  
551 calcifying algae (Graba-Landry et al., 2018; Rendina et al., 2019). The different effect of summer and  
552 winter heatwaves was most certainly due to the temperature of the ambient condition. For example,  
553 Rendina et al. (2019) showed how a summer heatwave had a significant effect (on both  
554 photosynthesis and calcification) on the intertidal *Corallina officinalis* Linnaeus, 1758 only if the  
555 background water temperature was increased by 3 °C (in accordance with the IPCC prediction for  
556 2100). A meta-analysis study showed that 5.2°C above ambient conditions could be the tipping point  
557 for the coralline algae to be negatively impacted by heatwaves (Cornwall et al., 2019). This is related  
558 to the upper temperature tolerance limits of the algae, which varies depending on the species and the  
559 environment, they live in. Global warming is a stronger threat to algal performance than OA,  
560 particularly in highly illuminated habitats (Martin & Gattuso, 2009; Vásquez-Elizondo & Enriquez,  
561 2016) such as the shallow water of the Mediterranean Sea. The decrease in pH did not have a  
562 detrimental effect on the physiology of *Ellissolandia elongata*, moreover, as mentioned above, there  
563 was an increase in photosynthetic activity when combined with the winter heatwave. Recent literature  
564 has shown how coralline algae might be more resilient to OA than previously thought (Ragazzola et  
565 al., 2013; Kamenos et al., 2016; Cornwall et al., 2019), especially over multiple generations.  
566 However, among algal physiological processes, calcification seems to be most affected (Ragazzola  
567 et al., 2012; Johnson et al., 2019; Kolzenburg et al., 2019; Ordoñez et al., 2019). During this study,  
568 we had a very high variability in calcification under light and dark conditions but without significant  
569 effect among treatments. This high variability was already shown for other physiological processes  
570 (McCoy et al. 2019) and may indicate the potential for resilience to future conditions. Moreover, most  
571 of the studies so far have been carried out on single species and the influence of the entire turfs (each  
572 containing several different specimens) in counteracting climate change is still unknown.

573 High proportion of transient marine species in benthos (Costello & Myers, 1996; Snell-Taylor et al.,  
574 2018), and high variability of species associated to *Ellisolandia elongata* turfs (Marchini et al., 2019;  
575 Sedano et al., 2020), produce abundant, variegated and complex epifaunal communities, resulting in  
576 notable dissimilarity among samples, even those that have been grown in laboratory tanks under the  
577 same experimental treatments. This high variability probably has a role in masking the effects of the  
578 artificially-induced experimental conditions, when indices of community structure are adopted as a  
579 metric. Despite a remarkable variability, the general structure of the community (at macro-group  
580 level) responded to the experimental conditions, namely, to simulated heatwaves under acidified  
581 conditions (CA<sub>T1</sub> vs TA<sub>T1</sub>) in terms of altered density of specific taxa.

582 The polychaete community showed a strong separation between the first and the second experimental  
583 phase, with an overall decrease in polychaete abundance, the disappearance of some species, an  
584 increase in the abundance of some other taxa, and a shift between congeneric species with allegedly  
585 similar ecological features. It is noteworthy that several species showing a strong reduction between  
586 the first and the second phase of the experiment are those characterised by reproduction through  
587 pelagic larvae, and their absence might be due to the incapacity to carry out their reproductive cycle  
588 within the experimental tank. However, at least the sabellid *Amphigelaena mediterranea* undergoes  
589 development within the semi-permanent tube (Rouse & Gambi, 1998) and therefore the strong  
590 decrease in the abundance of this species in the second phase of the experiment is likely due to other  
591 processes. The increase in some species might be due to a higher adaptability to experimental tank  
592 conditions, possibly mediated by direct development. Lastly, the shift between congeneric species of  
593 Exogoninae syllids (the couples *Exogone verugera*-*E. dispar* and *Salvatoria neapolitana*-*S. limbata*,  
594 all characterised by direct development) can be due to casual differences in the sampled algal turfs  
595 and subsequent magnification of these differences through the months of the experimental treatment.  
596 On the other hand, as observed by Marchini et al. (2019), the identification of polychaete species  
597 responding to lowered pH and/or heatwave pulse is hindered by the high, likely casual, variability  
598 displayed by the majority of species. The most abundant species (i.e. *Sphaerosyllis hystrix*,

599 *Sphaerosyllis pirifera*, *Exogone verugera* and *Exogone dispar*) were not affected by experimental  
600 manipulation of seawater conditions, while three species (*Haplosyllis granulosa*, *Perinereis cultrifera*  
601 and *Salvatoria limbata*) seemed to show a decrease under acidified conditions, and an increase under  
602 heatwave, respectively. However, it is not clear which biological traits are likely to have driven this  
603 response.

604 The community composition of peracarid crustaceans, although not being significantly altered in  
605 terms of diversity and evenness, showed significant changes in species composition following the  
606 experimental treatments, in particular the prolonged exposition to acidic conditions ( $CA_{t_0}$  vs  $CA_{t_1}$ ),  
607 as well as to simulated heatwaves ( $CC_{t_1}$  vs  $CT_{t_1}$  and  $CA_{t_1}$  vs  $TA_{t_1}$ ). Crustaceans, which harden their  
608 cuticle not only by sclerotization, like all other arthropods, but also by calcification (Luquet, 2012),  
609 are especially vulnerable to elevated  $pCO_2$  levels in the seawater, although sensitivity depends on  
610 structure and composition of the cuticle. In particular, adverse effects due to acidic conditions are  
611 expected upon growth rate and molting frequency (Whiteley, 2011). The calcification process, in fact,  
612 is linked to molting cycles: calcium carbonate does not deposit before the molt, and is likely taken up  
613 from the surrounding medium through the gills (Wood & Cameron, 1985; Neues et al., 2007).

614 Among peracarids, isopods display a cuticle containing the highly soluble magnesium calcite  
615 (Andersson et al., 2008), making them potentially more vulnerable to OA effects (Marchini et al.,  
616 2019). But even within the order isopoda, cuticle thickness and composition vary among taxa. For  
617 example, the marine rolling species, sphaeromatids, display a thicker cuticle (Neues et al., 2007),  
618 hence they are expected to be more sensitive to lowered pH. Our data supports this statement: the  
619 sphaeromatid isopod *Cymodoce emarginata*, in fact, appears to be especially sensitive to any  
620 alteration of current conditions, thriving well only in the control samples, while other isopods, as well  
621 as amphipods, are not so strongly affected. *Cymodoce emarginata* also declines under elevated  
622 temperatures, as well as other isopod species (*Munna wolffi*, *Carpas galloprovincialis*), while  
623 members of the isopod superfamily Anthuroidea (*Mesanthura romulea*, *Paranthura costana* and  
624 *Paranthura japonica*) responded contrarily. Instead when elevated  $pCO_2$  levels are accompanied by



625 heatwaves, most isopods showed decline, being outnumbered by amphipods such as *Quadrimaera*  
626 *quadrimana*, confirming the patterns observed by Marchini et al. (2019).

627 Even so, bearing all the preceding remarks in mind, it is possible to observe a clear influence of  
628 the treatments in the community structure at the lower taxonomic levels of identification. Of particular  
629 interest is their response to winter heatwave and acidification conditions (TA), which generally differs  
630 from that of CC and CA conditions. When trophic guilds are considered, a shift of communities  
631 towards dominance of opportunistic, omnivorous species emerges.

632 Comparative analyses exploring the relations between physiological responses of *Ellisolandia*  
633 *elongata* and the community structure of associated invertebrates revealed interesting patterns. *E.*  
634 *elongata* contribute to a local increase in oxygen and pH, hence, creating a ‘buffered’ and more  
635 suitable microhabitat for small invertebrates exposed to the extreme experimental conditions  
636 expected under the IPCC scenario RCP 8.5.

637 Furthermore, since temperature within this experiment has been shown to be a major driver of  
638 change in the community whereas ocean acidification effect is much smaller, it may suggest that  
639 coralline algae have a mitigation role regarding ocean acidification, confirming the hypothesis of  
640 Marchini et al. (2019) and others (Guy-Haim et al. 2020). *Ellisolandia elongata* corniche, therefore,  
641 represents an important algal biogenic reef with a potential for the goods and services it can provide  
642 which is still underestimated. More scientific efforts should be made in order to elucidate how these  
643 ecosystems contribute to mitigate the microenvironment, how they maintain the biodiversity, how  
644 their communities respond to threats such as marine bioinvasions and habitat degradation, and how  
645 these reefs can be used in strategies of resilience (i.e. habitat restorations, nature based solution).

646

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650

651

652 **Author contributions**

653 F.R., C.P. and C.L. conceived the study, designed the experiment and drafted the MS. A.M.  
654 coordinated the taxonomy part and contributed in drafting the MS. C.V. did all the statistical analyses.  
655 M.N. did the field work and coordinated (with C.L.) the experimental part ( $t_0$  and  $t_1$ ). M.A. calculated  
656 the MHW. A.B, G.R. did the environmental monitoring and provided support for in field and  
657 experimental phases. G.C. did chemical analyses of seawater from both natural and experimental  
658 conditions. A.C. and J.L. did the polychaete taxonomical work. R.K. performed the algal  
659 physiological tests and analysis (with FR). E.R. did chemical analyses (with G.C. and M.N.) and the  
660 culturing work ( $t_0$ ). C.dM. and M.S. did the sorting of the fauna and taxonomical work (under the  
661 supervision of A.C, J.L., and A.M). All authors contributed in revising the MS.

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