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Metabolic responses to temperature stress under elevated pCO₂ in *Crepidula fornicata*

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1 **Title: METABOLIC RESPONSES TO TEMPERATURE STRESS UNDER**
2 **ELEVATED $p\text{CO}_2$ IN THE SLIPPER LIMPET *CREPIDULA FORNICATA***

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27 **Short running head:** *C. fornicata* respiration under high $p\text{CO}_2$

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ABSTRACT

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In the current context of environmental change, ocean acidification is predicted to affect the cellular processes, physiology and behavior of all marine organisms, impacting survival, growth and reproduction. In relation to thermal tolerance limits, the effects of elevated $p\text{CO}_2$ could be expected to be more pronounced at the upper limits of the thermal tolerance window. Our study focused on *Crepidula fornicata*, an invasive gastropod which colonized shallow waters around European coasts during the 20th century. We investigated the effects of 10 weeks' exposure to current (380 μatm) and elevated (550, 750, 1000 μatm) $p\text{CO}_2$ on this engineer species using an acute temperature increase ($1^\circ\text{C } 12\text{h}^{-1}$) as the test. Respiration rates were measured on both males (small individuals) and females (large individuals). Mortality increased suddenly from 34°C , particularly in females. Respiration rate in *C. fornicata* increased linearly with temperature between 18°C and 34°C , but no differences were detected between the different $p\text{CO}_2$ conditions either in the regressions between respiration rate and temperature, or in Q_{10} values. In the same way, condition indices were similar in all the $p\text{CO}_2$ treatments at the end of the experiment but decreased from the beginning of the experiment. This species was highly resistant to acute exposure to high temperature regardless of $p\text{CO}_2$ levels, even though food was limited during the experiment. *C. fornicata* appears to have either developed resistance mechanisms or a strong phenotypic plasticity to deal with fluctuations of physico-chemical parameters in their habitat. This suggests that this invasive species may be more resistant to future environmental changes compared to its native competitors.

Keywords: CO_2 stress, invasive species, ocean acidification, Q_{10} , respiration, temperate waters

INTRODUCTION

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As part of global change, ocean acidification is caused by increasing anthropogenic CO₂ emissions which have increased since the beginning of the industrial revolution (Solomon *et al.*, 2007). Future *p*CO₂ increases are predicted to reduce the pH of surface waters by 0.3 - 0.4 units by the end of the century (Caldeira & Wickett, 2003). Such decreases will produce changes in carbon and carbonate seawater chemistry through decreased carbonate ion concentrations (CO₃²⁻) and a lower calcium carbonate saturation state (Ω). These changes are predicted to have major consequences for marine life (Fabry *et al.*, 2008; Kroeker *et al.*, 2013b) and, especially, could have broad impacts on physiological functions of heterotrophic marine organisms (Pörtner, 2008; Hofmann & Todgham, 2010).

The decrease in pH is likely to have a wide range of effects on marine invertebrates via shifts in acid-base homeostasis, changes in metabolism and energy balance (Pörtner *et al.*, 2005), leading to effects on somatic growth (Berge *et al.*, 2006; Thomsen & Melzner, 2010), respiration (Melatunan *et al.*, 2011; Schalkhausser *et al.*, 2013), excretion (Liu & He, 2012), calcification (Gazeau *et al.*, 2007; Wood *et al.*, 2008; Watson *et al.*, 2012) or feeding rates (Bamber, 1990; Navarro *et al.*, 2013). Many marine invertebrates exposed to elevated *p*CO₂ have exhibited metabolic depression (Willson & Burnett, 2000; Michaelidis *et al.*, 2005; Navarro *et al.*, 2013) as a decrease in respiration rate while others have remained unaffected (Gutowska *et al.*, 2008; Lannig *et al.*, 2010; Clark *et al.*, 2013) or even increased their metabolic rate (Wood *et al.*, 2008; Beniash *et al.*, 2010). These responses are highly species-specific and may vary with organism size (Beniash *et al.*, 2010). The resilience of the species studied, and the capacity to regulate metabolism under stressful conditions are also important (Pörtner, 2008). These physiological impacts are likely to have broad effects on the survival, growth and reproduction of marine species (Shirayama & Thornton, 2005; Byrne, 2011),

83 which would lead to changes in community structure from altered diversity and abundances
84 (Hale *et al.*, 2011; Kroeker *et al.*, 2013a).

85 These physiological impacts are likely modulated by temperature because temperature
86 is a primary driver of physiological function in ectotherms (Hofmann & Todgham, 2010).
87 Increasing temperature affects the rate of all biochemical reactions, and hence cellular
88 processes and physiological functions (Clarke, 1983; Pörtner, 2012), increasing metabolic
89 costs within a limited thermal tolerance window (Peck *et al.*, 2002; Marshall *et al.*, 2003).
90 The interactive effects of increased temperature and elevated CO₂ concentrations are
91 predicted to impair physiological processes (Clarke, 2003; Pörtner, 2008) by narrowing the
92 thermal tolerance window of the organisms (Metzger *et al.*, 2007; Lannig *et al.*, 2010) and
93 elevating vulnerability to extreme temperature (Schalkhausser *et al.*, 2012).

94 In a context of global change, non-indigenous species are expected to be favored in
95 their introduced area (Dukes & Mooney, 1999; Occhipinti-Ambrogi, 2007) mainly because
96 robustness to abiotic variation is often a trait that determines the success of invasive of a
97 species (Hellmann *et al.*, 2008; Lenz *et al.*, 2011). Climatic changes in the physical
98 environment will likely affect the distribution, spread, abundance, impacts and interactions of
99 species, possibly to the advantage of introduced organisms (Occhipinti-Ambrogi, 2007). Thus
100 our study focused on the response of an invasive Calyptraeidae gastropod living on western
101 European coasts, but which originates from North East America. The slipper limpet,
102 *Crepidula fornicata* (Linné 1758) was introduced in Europe at the end of the 19th century,
103 mainly with oysters (*Crassostrea gigas*) which were imported for farming (Blanchard, 1995),
104 and has subsequently colonized European coasts from southern Sweden to southern France
105 (Blanchard, 1997). *C. fornicata* has significant impacts on biodiversity and ecosystem
106 functioning where it has established (De Montaudouin *et al.*, 1999; Decottignies *et al.*, 2007;
107 Martin *et al.*, 2007). It lives in shallow sites, especially in bays and estuaries where very high

108 densities of over one thousand individuals m^{-2} have been reported (Blanchard, 1995). *C.*
109 *fornicata* is known to be strongly resistant to environmental variations, particularly
110 temperature and salinity (Blanchard, 1995; Blanchard, 1997; Diederich & Pechenick, 2013).
111 In light of the different ecological and physiological characteristics of *C. fornicata*, it is
112 important to investigate the impact of future pCO_2 levels, and determine its resistance
113 capacities to high levels of stress to assess the likely future impact of this engineer species in
114 the ecosystems to which it was introduced.

115 The present study was designed to investigate the metabolic responses of *C. fornicata*
116 to high pCO_2 conditions during temperature stress. Short-term experimental approaches using
117 faster temperature elevations than natural changes provide valuable insight into physiological
118 responses of marine invertebrates in term of their ability to resist high levels of stress or their
119 lethal temperature (Sokolova & Pörtner, 2003; Peck *et al.*, 2004; Pörtner *et al.*, 2006; Richard
120 *et al.*, 2012). Following the hypothesis that CO_2 stress will increase sensitivity to temperature
121 change, we evaluated changes in oxygen-consumption of *C. fornicata* individuals previously
122 reared under elevated pCO_2 for 10 weeks during a rapid temperature increase ($1^\circ C\ 12h^{-1}$).
123 Respiration rates were measured as a proxy for metabolism on males (small individuals) and
124 females (large individuals), as in this species there is sexual dimorphism in size.

125

126

FMATERIAL & METHODS

127

128 *Biological material*

129 *Crepidula fornicata* stacks were collected by SCUBA divers on 4 February 2010, in
130 Morlaix Bay (northwest Brittany, France), at the “Barre des Flots” site ($3^\circ 53.015'W$;
131 $48^\circ 40.015'N$) at a depth of 10 meters and at an *in situ* temperature of $11.6^\circ C$ (SOMLIT:
132 *Service d’Observation de la Mer et du Littoral* data). They were transferred directly to

133 aquaria at the Station Biologique de Roscoff where they were held in natural unfiltered
134 seawater at a temperature around 10°C, until they were used in experiments starting on 10
135 March 2010.

136 Males and females at the top and the bottom of stacks respectively, were selected,
137 separated and individually labelled. Small males (23.31 ± 0.16 mm length), which were still
138 slightly mobile, were placed individually on 3 cm Petri dishes one month before the beginning
139 of the trials. Dead individual shells at the base of stacks were kept as the substratum under the
140 largest living immobile females (47.53 ± 0.25 mm length). In *C. fornicata*, size cannot be
141 discriminated from sex because this is a protandrous hermaphroditic organism, changing sex
142 with age and size (Coe 1938). All individuals were gently brushed to remove epibionts and
143 biofilm from their shells before proceeding to the metabolic measurements.

144 Condition indices (CI) were calculated on a pool of 20 specimens in March, before the
145 beginning of the experiment, and on all remaining living and recently dead individuals (male
146 $n = 74$; female $n = 99$) at the end of the temperature increase on 29 May 2010. Shell dry
147 weight (DW_{Shell}), shell length and tissue dry weight (DW_{Tissue}) were determined separately on
148 each individual after drying at 60°C for 48h. Specimens were then ignited in a muffle furnace
149 at 520°C for 6 h, with tissue ash-free dry weight ($AFDW_{\text{Tissue}}$) being obtained by difference.
150 CI were calculated as:

$$151 \quad CI = (AFDW_{\text{Tissue}} / DW_{\text{Shell}}) \times 100.$$

152 Mortality was checked daily during the experiment. Individuals with no reaction when
153 the foot was stimulated were classed as dead and removed from the tanks.

154

155 *Experimental conditions and set-up*

156 After distributing randomly in each of twelve 10-L aquarium tanks comprising the
157 experimental flow-through system (as described in Noisette *et al.*, 2013), 120 males and 120

158 females (i.e. 10 individuals of each sex per aquarium) were held in different $p\text{CO}_2$ conditions
159 between 13 March and 29 May 2010. At the beginning of the experiment, pH was gradually
160 decreased (by bubbling CO_2) over four days at $0.1 \text{ pH units day}^{-1}$ from 8.1 until the required
161 pH was reached. Specimens were subsequently held for ten weeks in four different $p\text{CO}_2$
162 conditions: a current $p\text{CO}_2$ of $380 \mu\text{atm}$ ($\text{pH}_T = 8.07$), and three elevated $p\text{CO}_2$ levels of 550
163 μatm ($\text{pH}_T = 7.94$), $750 \mu\text{atm}$ ($\text{pH}_T = 7.82$) and $1000 \mu\text{atm}$ ($\text{pH}_T = 7.77$). The elevated $p\text{CO}_2$
164 values corresponded to different scenarios predicted by the Intergovernmental Panel on
165 Climate Change (IPCC) for the end of the century (Solomon *et al.*, 2007) and were selected
166 according to the recommendations of Barry *et al.*, (2010). $p\text{CO}_2$ was adjusted by bubbling
167 CO_2 -free air (current $p\text{CO}_2$) or pure CO_2 (elevated $p\text{CO}_2$) in four 100 L header tanks (1 per
168 $p\text{CO}_2$ condition) supplied with natural unfiltered seawater pumped from the sea, directly at the
169 foot of the Station Biologique de Roscoff. Seawater was continually delivered by gravity from
170 each header tank to three aquaria per $p\text{CO}_2$ condition at a constant rate of 9 L h^{-1} (renewal
171 rate: 90% total aquarium volume h^{-1}). $p\text{CO}_2$ was monitored and controlled by a feedback
172 system (IKS Aquastar, Karlsbad, Germany) that regulated the addition of gas in the header
173 tanks. pH values of the pH-stat system were adjusted from daily measurements of pH on the
174 total scale (pH_T) in the aquaria using a pH meter (HQ40D, Hach Lange, Ltd portable LDOTM,
175 Loveland, Colorado, USA) calibrated using Tris/HCl and 2-aminopyridine/HCl buffers
176 (Dickson *et al.*, 2007). The twelve aquaria were placed in four thermostatic baths where
177 temperature was controlled to $\pm 0.2 \text{ }^\circ\text{C}$ using 100 - 150 W submersible heaters.

178 Before the rapid temperature increase experiment, *C. fornicata* individuals were
179 maintained in the different $p\text{CO}_2$ treatments for 10 weeks while temperature was raised
180 successively to mimic the natural rate of temperature change from winter to summer.
181 Temperature was maintained at 10°C from the beginning of the trial to 29 March. It was
182 raised to 13°C from 5 to 19 April and to 16°C from 26 April to 18 May 2010. To reach these

183 set levels the temperature was increased by $0.5^{\circ}\text{C day}^{-1}$ until the new set temperature was
184 achieved. During the experiment, animals were naturally fed by the phytoplankton provided
185 by unfiltered seawater.

186 The rapid temperature increase experiment was conducted between the 18 and 29 May
187 2010. In all four $p\text{CO}_2$ treatments, temperature was increased from 16 to 36°C at $1^{\circ}\text{C }12\text{h}^{-1}$. *C.*
188 *fornicata* oxygen consumption was measured (see below) both in small and large individuals
189 in the different $p\text{CO}_2$ treatments during this rapid temperature increase.

190

191 *Seawater parameters*

192 Seawater parameters were monitored throughout the experiment. pH_T and temperature
193 were recorded daily in each of the 12 aquaria using a pH meter (HQ40D, Hach Lange, Ltd
194 portable LDOTM, Loveland, Colorado, USA). Total alkalinity was determined every 3 weeks
195 by 0.01N HCl potentiometric titration on an automatic titrator (Titroline alpha, Schott SI
196 Analytics, Mainz, Germany). Seawater carbonate chemistry, *i.e.* exact CO_2 partial pressure
197 ($p\text{CO}_2$) and saturation state of aragonite were calculated in each $p\text{CO}_2$ condition using
198 CO_2SYS software (Lewis & Wallace, 1998) using constants from Mehrbach *et al.*, (1973)
199 refitted by Dickson & Millero, (1987). Mean values (\pm standard error, SE) of the parameters
200 in each $p\text{CO}_2$ treatment are presented in Table 1.

201

202 *Oxygen consumption measurements*

203 During the rapid temperature increase trial (18 - 29 May 2010), oxygen consumption
204 of 6 randomly selected labeled individuals of each sex (2 per aquaria) was measured in each
205 of the $p\text{CO}_2$ treatments every two days, at 18, 22, 26, 30 and 34°C . Respiration rates were
206 determined using closed incubations in 75 mL (males) or 180 mL (females) acrylic chambers
207 (Engineering & Design Plastics Ltd, Cambridge, UK) filled with water from the same

208 aquarium (see methods in Morley *et al.*, 2007). Chambers were placed in their respective
209 aquaria during incubations to keep the temperature constant. Incubations varied between 1 h
210 and 3 h depending on temperature and were halted before oxygen saturation fell below 80%
211 saturation. Control incubations without animals (n = 1 control incubation / aquarium /
212 measurement) were carried out to allow correction for microbial activity in seawater.

213 Respiration rates were calculated from the differences in measurements of oxygen
214 concentration during trials and controls using a non-invasive fiber-optical system (FIBOX 3,
215 PreSens, Regensburg, Germany) made up of an optical fiber and reactive oxygen spots
216 attached to the inner wall of the chambers. These spots were calibrated with 0% and 100%
217 oxygen buffers made from the manufacturer instructions. 0% O₂ buffer was prepared by
218 dissolving 10 g of Na₂SO₃ in 1 L of seawater and 100% O₂ buffer was prepared by bubbling
219 air in 1L of seawater for 20 min to achieve oxygen saturation. Previous experiments had
220 demonstrated that oxygen consumption remained linear during all the incubation periods.
221 Chamber contents were mixed gently by inverting chambers several times before each oxygen
222 measurement. Respiration (R) rates (in μmol O₂ g⁻¹ AFDW h⁻¹) were corrected for oxygen
223 consumption in controls and calculated as:

$$224 \quad R = -(\Delta O_2 \times V) / (\Delta t \times AFDW_{\text{Tissue}})$$

225 where ΔO₂ (μmol O₂ L⁻¹) is the difference between initial and final O₂ concentrations during
226 the incubation, V (L) is the chamber volume minus the individual *C. fornicata* volume, Δt (h)
227 is the incubation time and AFDW_{Tissue} (g) is the tissue ash free dry weight of the slipper
228 limpet incubated.

229 Q₁₀ coefficients were calculated by using the standard equation:

$$230 \quad Q_{10} = (R_H / R_L)^{10 / (T_H - T_L)}$$

231 where T_L and T_H were the lowest and highest temperature reached and R_L and R_H the
232 respiration rates in these temperature respectively.

233

234 *Statistical analyses*

235 All statistical analyses were performed using R version 2.15.0 (R Core Team 2013)
236 and STATISTICA software. A logistic regression (general linear model, GLM) was applied
237 to test the differences in mortalities between the different $p\text{CO}_2$ treatments and between sex
238 with temperature as the linear variable. The effects of $p\text{CO}_2$, sex and the interaction of these
239 two factors on condition index (CI) at the end of the experiment and on Q_{10} values were
240 investigated by 2-way analysis of variance (ANOVA). Linear regressions between respiration
241 rates and increasing temperatures were fitted in the four different $p\text{CO}_2$ treatments for males
242 and females separately. Differences between $p\text{CO}_2$ treatments were explored using an
243 ANCOVA with $p\text{CO}_2$ and sex as fixed factors and temperature as co-variable.. Normality was
244 assessed using the Kolmogorov-Smirnov test and Levene's test was used to ensure that
245 variances were homogenous. All the results are presented as mean \pm standard error (SE).

246

247

RESULTS

248

249 Mortality occurred between 34 and 36°C for females and 22 and 36°C for males
250 (Figure 1). There were no significant differences in mortality between the different $p\text{CO}_2$
251 treatments (GLM, $df = 3$, $F = 0.680$, $p = 0.565$) or between males and females (GLM, $df = 1$,
252 $F = 0.580$, $p = 0.449$). Moreover, the interaction between factors $p\text{CO}_2$ and sex of the
253 individuals was not significant (GLM, $df = 3$; $F = 0.21$; $p = 0.888$). At $p\text{CO}_2$ levels of 380,
254 550, 750 and 1000 μatm , the mortality was 29, 19, 19, and 24 for females and 28, 6, 8, and 6
255 for males . At the end of the acute temperature increase nearly twice the number of females
256 had died (91) compared with the males (48) (χ^2 test, $p < 0.05$).

257 The mean condition index before the start of the experiment was 3.00 ± 0.27 (n=10). It
258 varied at the end of the experiment between 1.69 ± 0.13 for males at $p\text{CO}_2$ of 380 μatm and
259 2.41 ± 0.27 for females at $p\text{CO}_2$ of 550 μatm (Table 2). There were no effects of $p\text{CO}_2$, sex
260 or the interaction of these two factors on the condition index at the end of the trial (Table 2).
261 However, the condition index from the beginning of the experiment (3.00 ± 0.27) was
262 different from the mean condition index including all $p\text{CO}_2$ conditions (2.11 ± 0.07) at the end
263 of the trial (t-test, $df = 181$, $t = 3.159$, $p = 0.002$), which means that CI in both males and
264 females decreased significantly from the start to the end of the experiment (Figure 2).

265 Female respiration rates varied between $0.51 \mu\text{mol O}_2 \text{ g}^{-1} \text{ AFDW h}^{-1}$ at 18°C and
266 $p\text{CO}_2$ of 750 μatm and $91.62 \mu\text{mol O}_2 \text{ g}^{-1} \text{ AFDW h}^{-1}$ at 32°C and $p\text{CO}_2$ of 380 μatm . Males
267 had higher rates, which ranged between $5.13 \mu\text{mol O}_2 \text{ g}^{-1} \text{ AFDW h}^{-1}$ at 18°C and $p\text{CO}_2$ of 380
268 μatm and $175.51 \mu\text{mol O}_2 \text{ g}^{-1} \text{ AFDW h}^{-1}$ at 32°C and $p\text{CO}_2$ of 380 (Figure 3).

269 Relationships between respiration rate and temperature were linear at each $p\text{CO}_2$ level
270 (Figure 3). Respiration rose significantly with increasing temperature in all $p\text{CO}_2$ treatments,
271 for both males and females (Table 3, all p-values < 0.02). There were no significant
272 differences between the slopes of the different regressions among the $p\text{CO}_2$ treatments or
273 between sexes (analysis of slopes, $df = 3$, $F = 1.1$, $p = 0.346$). The intercepts of the different
274 regressions also did not significantly vary among $p\text{CO}_2$ (ANCOVA, $df = 3$, $F = 0.350$, $p =$
275 0.789), but there were difference between males and females (ANCOVA, $df = 1$, $F = 62.63$, p
276 < 0.001).

277 Q_{10} values ranged from 1.24 to 2.40 for females and from 1.36 to 2.77 for males
278 among the different $p\text{CO}_2$ treatments (Figure 2). There was no significant $p\text{CO}_2$ effect on Q_{10}
279 values for either males or females (Table 2). Across all $p\text{CO}_2$ treatments, females had
280 significantly lower Q_{10} values than males with means of 1.61 ± 0.11 and 2.00 ± 0.12 for

281 females and males, respectively (Table 2). The interaction between $p\text{CO}_2$ and sex, however,
282 was not significant (Table 2).

283

284

DISCUSSION

285

286 Independently of the impact of $p\text{CO}_2$ we planned to test, one of the major issues of this
287 study was food limitation which was unintentionally imposed on the *C. fornicata* individuals
288 in the experiments. This food limitation was detected because the decrease in condition
289 indices (CI) of both males and females from the beginning to the end of the experiment. Such
290 decreases in CI are usually related to food quantity or quality supplied to organisms (Norkko
291 & Thrush, 2006). Animals were maintained in unfiltered seawater which carried natural
292 phytoplankton at a concentration between 0.2 and 1 $\mu\text{g Chl a L}^{-1}$ (*SOMLIT* data). The water
293 renewal in the aquarium was maintained constant at a rate of 0.9 L h⁻¹ (i.e. 90% of the total
294 volume of each aquarium changed per hour). Water supply in our experimental system was
295 likely too low to provide sufficient food for the experimental animals, which thus relied on
296 internal energy reserves and so decreased their CI. A similar outcome was reported for
297 mussels by Mackenzie *et al.* (2014).

298 The use of stored reserves was similar in the different $p\text{CO}_2$ conditions as CI at the end
299 of the experiment did not differ between the different $p\text{CO}_2$ treatments, and this was the case
300 for both sexes. Previous studies have shown interspecific variability in the responses of
301 condition indices under high $p\text{CO}_2$ levels, ranging from a lack of effect (Cummings *et al.*,
302 2011; Clark *et al.*, 2013; Sanders *et al.*, 2013) to large changes in condition under high $p\text{CO}_2$
303 levels (Hiebenthal *et al.*, 2013; Range *et al.*, 2014). Energy availability is a major component
304 in mitigating the effects of ocean acidification (Pansch *et al.*, 2014). Studies have shown that
305 an abundant food supply might counteract even overcome the negative effects of high $p\text{CO}_2$

306 on adult and juvenile bivalves (Melzner *et al.*, 2011; Thomsen *et al.*, 2013). Thus, it is
307 important to consider that in this study *C. fornicata* were under limited food conditions when
308 interpreting their metabolic responses to elevated $p\text{CO}_2$ conditions during the temperature
309 rise. The data here are representative of conditions where there is temperature stress and food
310 supplies are limited, conditions that can occur in the field.

311 The limitation of food supply was not markedly more important in any of our reduced
312 pH conditions as there were no differences in mortality rates between the different $p\text{CO}_2$
313 treatments in *C. fornicata* males and females. This is a different outcome to that reported for
314 some other mollusk species held in elevated $p\text{CO}_2$ levels (Shirayama & Thornton, 2005;
315 Beniash *et al.*, 2010). However, similarly to our study, Pansh *et al.*, (2014) showed that food
316 availability had no impact on mortalities of the barnacle *Amphibalanus improvises* held in
317 different $p\text{CO}_2$ conditions. In the present study, important mortalities started to occur from
318 32°C and they became larger at and above 34°C for both males and females. These values are
319 consistent with the upper lethal temperature recorded for *C. fornicata* by Diederich &
320 Pechenick, (2013) in a laboratory study investigating a population from Rhode Island, USA,
321 in which only 40% of the adults survived after a 3 h exposure to 34°C, and all died after a 3 h
322 exposure to 36°C. Mortality was higher in females (larger individuals) than in males (small
323 individuals) even if, male started to die at lower temperatures than females. Similarly, Peck *et*
324 *al.*, (2009) demonstrated for 14 species that smaller species survived to higher temperatures
325 than large ones when temperature was raised at 1°C day⁻¹, and Peck *et al.*, (2013) showed that
326 juveniles had higher upper temperature limits than adults in 4 species of marine invertebrates
327 at warming rates of 1°C day⁻¹ and 1°C 3days⁻¹. The mechanisms setting temperature limits at
328 acute rates of warming may not be energy availability (Peck *et al.*, 2014) and females, which
329 had more energetic reserves than males, may thus have not had an advantage.

330 Despite the decreases in CI, mean respiration rates of *C. fornicata* at 18°C and $p\text{CO}_2$
331 of 380 μatm were 31 and 26 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ AFDW h}^{-1}$ for males and females, respectively,
332 which are close to the middle of the range of *in situ* values reported for wild individuals from
333 the Bay of Brest (Brittany, France) (6 to 63 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ AFDW h}^{-1}$: Martin *et al.*, 2006). This
334 indicates that animals in the experiments here had similar oxygen consumption than wild
335 specimens and were not metabolically depressed under insufficient food supply. In both *C.*
336 *fornicata* males and females, respiration rates increased with temperature, as previously
337 demonstrated for this species by Newell & Kofoed, (1977) and most ectotherm metabolic
338 rates are correlated positively with temperature (Cossins & Bowler, 1987). Respiration rates
339 were higher in *C. fornicata* males than in females regardless of the temperature. Generally,
340 mass-specific respiration rates of small individuals are higher than those of larger ones
341 because metabolic rate (normalized to the biomass) decreases with increasing organisms size
342 (von Bertalanffy, 1951; Parsons *et al.*, 1984).

343 The relationship between oxygen consumption and temperature here for *C. fornicata*
344 was similar in all the different $p\text{CO}_2$ treatments. The slopes and intercepts of the regressions
345 were not significantly different across the four $p\text{CO}_2$ conditions which means temperature
346 effect on respiration rate was not affected by the different $p\text{CO}_2$ levels in males or females. In
347 contrast to our results, Lannig *et al.*, (2010) found that an acute temperature rise
348 (1.25°C/12h) caused a more rapid increase in metabolic rate in *Crassostrea gigas* under
349 elevated $p\text{CO}_2$ conditions, and there was a synergistic effect of temperature and $p\text{CO}_2$. The
350 lack of difference in respiration between animals held in different $p\text{CO}_2$ conditions may be
351 related to a stronger ability to up-regulate their metabolism under a temperature stress
352 irrespective of $p\text{CO}_2$. Thus, under warming conditions, *C. fornicata* can generate sufficient
353 energy to cope with any effects of decreased pH (Wood *et al.*, 2010). Q_{10} values were also
354 similar across $p\text{CO}_2$ treatments in both males and females and they were within the expected

355 range of values recorded for marine invertebrates (Branch *et al.*, 1988; Marshall *et al.*, 2003).
356 Even if *C. fornicata* individuals were food limited, their oxygen consumption remained
357 unaffected by elevated $p\text{CO}_2$. A similar lack of $p\text{CO}_2$ effect was reported for growth and shell
358 strength of the barnacle *A. improvisus* (Pansch *et al.*, 2014). In our study, the low food supply
359 did not appear to affect the resistance or resilience of *C. fornicata* to CO_2 stress.

360 Several studies investigating the response of mollusk respiration to elevated $p\text{CO}_2$
361 have demonstrated metabolic depression under high $p\text{CO}_2$ in both bivalves and gastropods
362 (Michaelidis *et al.*, 2005; Bibby *et al.*, 2007; Fernandez-Reiriz *et al.*, 2011; Melatunan *et al.*,
363 2011; Liu & He, 2012; Navarro *et al.*, 2013). Conversely, others observed no $p\text{CO}_2$ effect on
364 mollusk respiration and general metabolism (Gazeau *et al.*, 2007; Marchant *et al.*, 2010;
365 Fernandez-Reiriz *et al.*, 2012; Clark *et al.*, 2013) as reported in our study. In some rare cases,
366 O_2 consumption was reported to increase under high $p\text{CO}_2$ conditions (Wood *et al.*, 2010;
367 Cummings *et al.*, 2011). The effects of high CO_2 concentrations on metabolism appear
368 species-specific and depend on resistance capacities of the organisms (Melzner *et al.*, 2009).
369 It has been widely reported that exposure to environmental high $p\text{CO}_2$ levels leads to changes
370 in homeostasis and extracellular acid-base balance counterbalanced by metabolic depression
371 in many cases (Pörtner *et al.*, 2005; Pörtner, 2008), although it should be noted, as above, that
372 metabolic depression is often not seen in high $p\text{CO}_2$ conditions. Differences in acid-base
373 regulatory capacities by increasing HCO_3^- internal concentrations (Michaelidis *et al.*, 2005;
374 Gutowska *et al.*, 2010) or H^+ excretion (Pörtner *et al.*, 2005) are taxon specific and are more
375 or less effective in mitigating the effects of hypercapnia. It has also been suggested that
376 organisms could maintain low metabolic rates without controlling internal pH by not using
377 pH-sensitive oxygen-binding pigments (Thomsen *et al.*, 2010; Hiebenthal *et al.*, 2013). Such
378 mechanisms may be crucial factors in explaining the observed variation in sensitivities and
379 resistances of marine invertebrates to elevated $p\text{CO}_2$ conditions (Gutowska *et al.*, 2010).

380 It is important to note here that many of the studies to date on the effects of elevated
381 $p\text{CO}_2$ on organisms are short-term and acute (e.g. Tomanek *et al.*, 2011), not reflecting the
382 long-term trade off in energy balance and physiological changes associated with acclimation
383 of new environmental conditions (Clark *et al.*, 2013). For example, metabolic depression acts
384 as a time-limited compensation strategy to survive unfavorable condition such as high CO_2
385 concentrations (Guppy & Withers, 1999; Willson & Burnett, 2000). Because *C. fornicata*
386 were held for 10 weeks in the different $p\text{CO}_2$ treatments in this investigation, it is likely there
387 was enough time for them to acclimate to the new pH, and no difference in oxygen
388 consumption was detected between the different $p\text{CO}_2$ conditions. However, the energetic cost
389 likely produced by the negative effects of elevated $p\text{CO}_2$ may either be relatively small , or
390 difficult to maintain over longer time periods. This could be seen in impacts on other
391 physiological processes than respiration (Catarino *et al.*, 2012). For example, Bibby *et al.*,
392 (2008) demonstrated that exposure to hypercapnic conditions may compromise the ability to
393 express an immune response in mussels. They showed that *Mytilus edulis* phagocytosis
394 declined as function of decreased pH. In the same way, Matozzo *et al.*, (2012) showed that
395 elevated $p\text{CO}_2$ and temperature may strongly affect haemocyte functionality in the bivalves
396 *Chamelea gallina* and *Mytilus galloprovincialis*. Other cellular processes have also been
397 shown to be negatively impacted by high CO_2 concentrations, including protein synthesis in
398 the sipunculid *Sipunculus nudus* (Langenbuch *et al.*, 2006) or enzyme activities in *C. gallina*
399 and *M. galloprovincialis* (Matozzo *et al.*, 2013). However, studies of the impact of reduced
400 pH on immune systems have generally been of short duration and it would be interesting to
401 investigate other physiological parameters than respiration (e.g. calcification, protein
402 production, immunity regulation, fertility) in *C. fornicata* acclimated over several months in
403 the different $p\text{CO}_2$ conditions predicted for the end of the century. As a coastal species
404 adapted to relatively large fluctuations of abiotic parameters, *C. fornicata* in this study were

405 strongly resistant to both elevated $p\text{CO}_2$ and increased temperature. Indeed, resistance to high
406 $p\text{CO}_2$ levels can also come from pre-acclimation or pre-adaptation to fluctuations in the
407 environment where species live (Burnett, 1997). Species living in environments with large
408 abiotic variation have a high phenotypic plasticity which can allow them to survive in
409 stressful conditions (Hofmann & Todgham, 2010). Coastal organisms are more exposed to
410 physico-chemical variations than their open-ocean counterparts that live in more stable
411 thermal and pH environments (Berge *et al.*, 2006; Peck *et al.*, 2006). Species living in shallow
412 waters tolerate not only seasonal and extreme temperature events but also periodic large
413 fluctuations in seawater pH, driven by biological process that sequester and release large
414 amounts of CO_2 (Beniash *et al.*, 2010). This exposure to a wide environmental variation has
415 likely led to the evolution of resistance mechanisms to abiotic factors including variations in
416 $p\text{CO}_2$ and/or pH (Lannig *et al.*, 2010).

417 *C. fornicata* is an invasive species which has successfully colonized European coastal
418 shallow waters. This species is likely to have high phenotypic plasticity and resilience to
419 physico-chemical variations that determined its success. Indeed, successful invasive species
420 generally share characteristics that allow them to establish, colonize and expand their range.
421 Among these characteristics, tolerance to environmental stress is one of the most common
422 (Lenz *et al.*, 2011). In a global change context, the movement of physico-chemical conditions
423 away from the optimum increases the energy required by marine species to fuel the extra
424 processes entrained to resist the stresses involved and to maintain homeostasis. This may
425 result in changes in overall physiological condition (Cummings *et al.*, 2011) that could impact
426 ecological processes and community interactions. The high resilience to altered $p\text{CO}_2$ /low pH
427 levels observed here for *C. fornicata* may confer a competitive advantage to this invasive
428 species over taxonomically or functionally related species (Lenz *et al.*, 2011). For example,
429 the performance of the scallop *Pecten maximus*, which is one of the *C. fornicata* competitors

430 (Thouzeau *et al.*, 2000; Fresard & Boncoeur, 2006), has been shown to be negatively affected
431 by high $p\text{CO}_2$ levels (Schalkhausser *et al.*, 2013). These different sensitivities to
432 environmental factors will likely dictate “winners” and “losers” among marine species that
433 could lead to a restructuring of benthic communities. With other studies, our data suggest this
434 restructuring could favor invasive species as evidence is building that shows they are more
435 resistant to change than their native competitors (Dukes & Mooney, 1999; Occhipinti-
436 Ambrogi, 2007).

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FIGURES CAPTIONS

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Figure 1: Cumulated mortalities during the temperature increase. Males are represented on the graph on the top and females are on the graph in the bottom. The greyscale represent the different $p\text{CO}_2$ levels in which *C. fornicata* individuals where held during the experiment.

Figure 2: Mean (\pm SE) conditions indices at the beginning (black bar), and at the end of the experiment for *C. fornicata* females (white bars) and males (grey bars) in the different $p\text{CO}_2$. $27 > N > 10$

Figure 3: Respiration rates as a function of increasing temperature in each $p\text{CO}_2$ treatment, for *C. fornicata* males (top, triangles) and females (bottom, circles). Detailed statistical analyses relative to the regressions can be found in Table 3.

Figure 4: Mean (\pm SE) Q_{10} values for *C. fornicata* females (white bars) and males (grey bars) in the different $p\text{CO}_2$ treatments. $N = 3$

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TABLES

778

779 **Table 1:** Mean (\pm standard error, SE) carbonate chemistry parameters for each $p\text{CO}_2$
 780 treatment. pH (on the total scale, pH_T) was measured daily and total alkalinity (A_T) was
 781 measured every 3 weeks. Other parameters were calculated with CO2sys software. $p\text{CO}_2$:
 782 CO_2 partial pressure; Ω_{Ar} : saturation state of seawater with respect to aragonite.

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$p\text{CO}_2$ treatment	pH_T n = 69	$p\text{CO}_2$ (μatm) n = 69	Ω_{Ar} n = 69	A_T ($\mu\text{Eq kg}^{-1}$ SW) n = 76
380 μatm	8.13 ± 0.01	324 ± 8	2.72 ± 0.06	2333 ± 1
550 μatm	7.89 ± 0.01	619 ± 16	1.69 ± 0.04	2334 ± 2
750 μatm	7.75 ± 0.01	873 ± 20	1.28 ± 0.03	2335 ± 2
1000 μatm	7.66 ± 0.01	1138 ± 65	1.05 ± 0.02	2334 ± 2

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785

786 **Table 2:** Summary of two-way ANOVAs testing the effects of $p\text{CO}_2$, sex and the interaction
 787 of these two factors on the final condition indices (CI) and the Q_{10} values determined for *C.*
 788 *fornicata* males and females in the different $p\text{CO}_2$ conditions (380, 550, 750 and 1000 μatm).
 789 Bold numbers indicate significant level greater than 95%.

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	df	CI		Q_{10}	
		F-value	p-value	F-value	p-value
$p\text{CO}_2$	3	1.245	0.295	0.657	0.590
sex	1	2.472	0.118	6.124	0.025
$p\text{CO}_2$ x sex	3	1.371	0.254	2.293	0.117

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792 **Table 3:** Relationships between *C. fornicata* male and female respiration rates and
 793 temperature in each $p\text{CO}_2$ treatment

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	$p\text{CO}_2$	Regression equation	n	R	R^2	F	p
males	380	$y = 3.691 x - 34.455$	42	0.60	0.37	22.97	< 0.001
	550	$y = 2.993 x - 18.461$	42	0.46	0.21	10.56	0.002
	750	$y = 2.406 x - 4.543$	41	0.40	0.16	7.55	0.009
	1000	$y = 3.701 x - 41.556$	41	0.56	0.31	17.37	< 0.001
females	380	$y = 1.826 x - 7.635$	42	0.49	0.24	12.72	< 0.001
	550	$y = 1.585 x - 4.218$	42	0.55	0.30	16.89	< 0.001
	750	$y = 2.637 x - 26.240$	42	0.63	0.40	26.66	< 0.001
	1000	$y = 1.442 x + 3.435$	42	0.37	0.14	6.26	0.017

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