



**HAL**  
open science

# Assessing the physiological responses of the gastropod *Crepidula fornicata* to predicted ocean acidification and warming

Fanny Noisette, François Bordeyne, Dominique Davoult, Sophie Martin

► **To cite this version:**

Fanny Noisette, François Bordeyne, Dominique Davoult, Sophie Martin. Assessing the physiological responses of the gastropod *Crepidula fornicata* to predicted ocean acidification and warming. *Limnology and Oceanography*, 2015, 61 (2), pp.430-444 10.1002/lno.10225 . hal-01233060

**HAL Id: hal-01233060**

**<https://hal.sorbonne-universite.fr/hal-01233060>**

Submitted on 24 Nov 2015

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **TITLE**

2 **Assessing the physiological responses of the gastropod *Crepidula fornicata***  
3 **to predicted ocean acidification and warming**

4

5 **Authors:** Fanny Noisette<sup>1,2</sup>, François Bordeyne<sup>1,2</sup>, Dominique Davoult<sup>1,2</sup>, Sophie Martin<sup>1,2</sup>

6

7 **Affiliations**

8 1 Sorbonne Universités, UPMC Univ. Paris 6, UMR 7144, Station Biologique de Roscoff,

9 Place Georges Teissier, 29688 Roscoff Cedex, France

10 2 CNRS, UMR 7144, Station Biologique de Roscoff, Place Georges Teissier, 29688 Roscoff

11 Cedex, France

12

13 **Corresponding author**

14 Fanny Noisette

15 Email: fanny.noisette@live.fr

16 Phone: +33 298292333

17 Fax number: +33 298292324

18

19 **Running title:** Responses of *C. fornicata* to OA and warming

20

21 **Type of paper:** Primary Research Article

22

23

24 **Abstract**

25 Organisms inhabiting coastal waters naturally experience diel and seasonal physico-  
26 chemical variations. According to various assumptions, coastal species are either considered  
27 to be highly tolerant to environmental changes or, conversely, living at the thresholds of their  
28 physiological performance. Therefore, these species are either more resistant or more  
29 sensitive, respectively, to ocean acidification and warming. Here, we focused on *Crepidula*  
30 *fornicata*, an invasive gastropod that colonized bays and estuaries on northwestern European  
31 coasts during the 20<sup>th</sup> century. Small (< 3 cm in length) and large (> 4.5 cm in length),  
32 sexually mature individuals of *C. fornicata* were raised for 6 months in three different  $p\text{CO}_2$   
33 conditions (390, 750 and 1400  $\mu\text{atm}$ ) at four successive temperature levels (10, 13, 16 and  
34 19°C). At each temperature level and in each  $p\text{CO}_2$  condition, we assessed the physiological  
35 rates of respiration, ammonia excretion, filtration and calcification on small and large  
36 individuals. Results show that, in general, temperature positively influenced respiration,  
37 excretion and filtration rates in both small and large individuals. Conversely, increasing  $p\text{CO}_2$   
38 negatively affected calcification rates, leading to net dissolution in the most drastic  $p\text{CO}_2$   
39 condition (1400  $\mu\text{atm}$ ) but did not affect the other physiological rates. Overall, our results  
40 indicate that *C. fornicata* can tolerate ocean acidification, particularly in the intermediate  
41  $p\text{CO}_2$  scenario. Moreover, in this eurythermal species, moderate warming may play a  
42 buffering role in the future responses of organisms to ocean acidification.

43

44 **Keywords:** calcification, coastal system, invasive species, metabolism, mollusk,  $p\text{CO}_2$ ,  
45 temperature

46

47

48

## 49 **Introduction**

50 Predictions indicate that coastal ecosystems will be strongly affected by ocean  
51 acidification and warming, currently two of the most prominent anthropogenic processes  
52 influencing marine life (Harley et al. 2006). Due to the increase in atmospheric CO<sub>2</sub> partial  
53 pressure ( $p\text{CO}_2$ ), pH in surface waters is predicted to decline by 0.06 to 0.32 units and sea  
54 surface temperatures to increase by 1.0 to 3.7°C by the end of the century, depending on the  
55 Intergovernmental Panel on Climate Change (IPCC) representative concentration pathway  
56 considered (Stocker et al. 2013). Modifications in seawater carbonate chemistry due to ocean  
57 acidification lead to a decrease in carbonate ion concentrations ( $\text{CO}_3^{2-}$ ) (Orr et al. 2005) and a  
58 reduction in the calcium carbonate saturation state ( $\Omega$ ), which regulates the thermodynamics  
59 of calcium carbonate ( $\text{CaCO}_3$ ) precipitation (Feely et al. 2009). In estuarine and coastal  
60 waters, pH is more variable than in the open ocean due to intense biological and  
61 biogeochemical processes (Andersson and Mackenzie 2011). In these habitats, ocean  
62 acidification and warming will shift the baselines, exacerbate natural variations in pH and  
63 temperature, and probably threaten the communities living there (Waldbusser and Salisbury  
64 2013).

65 Mollusks constitute a major taxonomic group in estuarine and coastal waters in terms  
66 of community structure and ecosystem functioning (Gutiérrez et al. 2003). Because most  
67 marine mollusk taxa accumulate significant amounts of  $\text{CaCO}_3$  to form protective external  
68 shells, they may be sensitive to the changes in pH and carbonate chemistry induced by ocean  
69 acidification (for review, see Gazeau et al. 2013), although recent studies have shown that  
70 some species could be resistant to elevated  $p\text{CO}_2$  (Range et al. 2011; Ries et al. 2009). Along  
71 with direct impacts on calcification, high CO<sub>2</sub> concentrations may also have indirect effects  
72 on metabolism by disturbing the extracellular acid-base equilibrium, leading to general

73 internal acidosis (Melzner et al. 2009). These potential shifts in acid-base homeostasis have  
74 the potential to change organisms' energy balance (Pörtner et al. 2005).

75 In mollusks, the effects of elevated  $p\text{CO}_2$  and/or decreased pH alone are highly  
76 species-specific (see review in Gazeau et al. 2013), and depend on species sensitivity and any  
77 existing compensation mechanisms (Michaelidis et al. 2005). To better estimate future ocean  
78 acidification effects on mollusk species, various physiological processes have been studied in  
79 bivalves and gastropods such as respiration (Beniash et al. 2010; Bibby et al. 2007), excretion  
80 (Fernandez-Reiriz et al. 2011; Liu and He 2012), feeding (Fernandez-Reiriz et al. 2012;  
81 Marchant et al. 2010), immune response (Bibby et al. 2008; Matozzo et al. 2012) and protein  
82 or enzyme production (Matozzo et al. 2013; Tomanek et al. 2011). However, few studies have  
83 simultaneously assessed the responses of more than three physiological processes to ocean  
84 acidification and warming. The concomitant increase in seawater temperature and  $p\text{CO}_2$  are  
85 likely to affect mollusk metabolism because, in addition to changes in gas solubility and the  
86 proportion of carbon species (Zeebe 2011), temperature also strongly affects physiological  
87 and biochemical reactions (Cossins and Bowler 1987). Because warming can modulate the  
88 metabolism responses to ocean acidification (Ivanina et al. 2013; Melatunan et al. 2013),  
89 investigations of both pH and temperature effects are valuable for understanding the  
90 responses of mollusks in the future ocean.

91 One of the most abundant and widespread shelled mollusks on the French  
92 northwestern Atlantic and Channel coasts is the slipper limpet *Crepidula fornicata*, Linnaeus  
93 1758 (Blanchard 1997). This gastropod native to the northeastern American coast was  
94 introduced in Europe at the end of the 19<sup>th</sup> century, mainly via oysters imported for farming  
95 (Blanchard, 1995). It then colonized European coasts from southern Sweden to southern  
96 France, becoming invasive in some places (Blanchard 1997). *C. fornicata* lives in shallow  
97 sites, especially in bays and estuaries where it can reach very high densities of more than 1000

98 individuals per m<sup>2</sup> (Blanchard 1995). This species is known to be highly robust to  
99 environmental stress, in particular temperature and salinity (Diederich and Pechenik 2013;  
100 Noisette et al. 2015), parameters that have diel and seasonal variations in these coastal  
101 habitats. Established *C. fornicata* populations have largely affected biodiversity and  
102 ecosystem functioning in terms of sediment modifications (Ehrhold et al. 1998), changes in  
103 faunal assemblages (De Montaudouin et al. 1999) and trophic structure (Chauvaud et al.  
104 2000). This species also affects benthic biogeochemical cycles by enhancing filtration,  
105 metabolic activities, CaCO<sub>3</sub> production, and the recycling of nutrients and dissolved carbon  
106 back into the pelagic ecosystem (Martin et al. 2006; Martin et al. 2007; Ragueneau et al.  
107 2002)

108         Although *C. fornicata* is likely highly tolerant to environmental fluctuations, the  
109 combined effects of decreased pH and increased temperature may push this species away  
110 from its physiological optimum. Thus the objective of this work was to quantify the  
111 respiration, ammonia excretion, filtration and calcification responses of small and large  
112 specimens of *C. fornicata* in different temperature and *p*CO<sub>2</sub> conditions. Investigating the  
113 physiology of this key engineer in some coastal ecosystems in a context of climate change is  
114 one way to better understand the sensitivity of this species and its potential future ecological  
115 impact.

116

## 117 **Methods**

118

### 119 *Sampling site and in situ conditions*

120         *C. fornicata* stacks were collected by SCUBA divers on 30 November 2011, in  
121 Morlaix Bay (northwestern Brittany, France), at the “Barre des Flots” site (3°53.015'W;  
122 48°40.015'N) at approximately 11 m depth. No temporal series of abiotic parameters were

123 available for this exact location. However, variations in the physico-chemical parameters  
124 (surface measurements) at a station (called Estacade), located approximately 10 km from the  
125 Barre des Flots site, were obtained from the *Service d'Observation des Milieux Littoraux*  
126 (SOMLIT) between 2010 and 2013, with a sampling step of 15 days. Between October 2010  
127 and March 2013, temperature varied between 8.1°C (January 2011) and 16.5°C (August  
128 2011) with mean values ( $\pm$  SE) of  $10.1 \pm 0.2^\circ\text{C}$  in winter,  $12.7 \pm 0.4^\circ\text{C}$  in spring and  $15.8 \pm$   
129  $0.02^\circ\text{C}$  in summer.

130 In Morlaix Bay (2009 to 2011), phytoplankton groups ( $> 5\mu\text{m}$ ), the most important  
131 food resource of *C. fornicata* (Decottignies et al. 2007), were mainly dominated by planktonic  
132 diatoms in concentrations varying between 10 to 300 cells  $\text{mL}^{-1}$  (depending on the season)  
133 and dinoflagellate species that were found at lower abundances (ca. 25 cells  $\text{mL}^{-1}$ ; Leroy  
134 2011).

135

### 136 *Biological material*

137 *C. fornicata* forms stacks of several individuals in which each individual adheres to the  
138 dorsal surface of the shell of the subjacent partner in the stack. It is a protandrous  
139 hermaphrodite, meaning that the small individuals at the top of the stacks are generally males  
140 and the large ones at the bottom, females (Coe 1936). After sampling, stacks were brought  
141 directly to the *Station Biologique de Roscoff* where they were kept in natural, unfiltered  
142 seawater for 6 weeks at a temperature gradually lowered to 10°C, reflecting the seasonal drop  
143 in temperature between autumn and winter. Sexually mature individuals (more than 1 cm in  
144 length) were selected and separated into two class sizes: small individuals ( $29.5 \pm 0.9$  mm  
145 length) from the top of the stack and larger ones ( $45.4 \pm 0.6$  mm length) from the bottom.  
146 They were separated from the stack and individually labeled with tags glued on their shell.  
147 Empty subjacent shells, whose soft tissue was removed, served as substratum for the sampled

148 live individuals. Other empty shells whose size was similar to that of the substratum shell of  
149 live individuals were also selected for flux corrections (see part “Metabolic rates and O:N  
150 ratios” below). All the shells were gently brushed to remove epibionts without altering  
151 periostracum layer.

152 Length (in mm), volume (in mL) and tissue dry weight (DW in g) of the live  
153 individuals were determined for each incubated specimen at the end of the whole experiment.  
154 Length was measured with calipers, volume was estimated as the volume of seawater moved  
155 when individual was immersed and DW was determined after drying fresh samples at 60°C  
156 for 48 h.

157

#### 158 *Experimental conditions*

159 Single small and large individuals, along with their substratum shell, were randomly  
160 distributed into nine 10 L aquaria with 10 individuals of each class size per each aquarium.  
161 Empty shells were also distributed into nine other 10 L aquaria (4 shells per aquarium). At the  
162 beginning of the experiment, pH was gradually decreased over 2 weeks by 0.02 pH unit per  
163 day from 8.1 until the different pH treatments were reached. *C. fornicata* individuals and  
164 empty shells were then subsequently held for 24 weeks (12 January to 27 June 2012) in three  
165  $p\text{CO}_2$  treatments selected according to the recommendations in Barry et al. (2010): (1) 390  
166  $\mu\text{atm}$  ( $\text{pH}_T = 8.07$ ) represented current  $p\text{CO}_2$ , (2) 750  $\mu\text{atm}$  ( $\text{pH}_T = 7.82$ ) corresponded to the  
167 elevated  $p\text{CO}_2$  level predicted by the IPCC for the end of the century (Solomon et al. 2007)  
168 and (3) 1400  $\mu\text{atm}$  ( $\text{pH}_T = 7.56$ ) represented a  $p\text{CO}_2$  five-fold higher than preindustrial  $p\text{CO}_2$   
169 (280  $\mu\text{atm}$ ) also predicted for 2100 (Stocker et al. 2013).  $p\text{CO}_2$  was adjusted by bubbling  
170  $\text{CO}_2$ -free air (current  $p\text{CO}_2$ ) or pure  $\text{CO}_2$  (elevated  $p\text{CO}_2$ ) in three 100 L header tanks supplied  
171 with unfiltered seawater pumped directly from the foot of the *Station Biologique de Roscoff*.  
172 Each of the three  $p\text{CO}_2$  treatments had six replicate 10 L aquaria, three for live organisms and



173 three for empty shells. They continuously received CO<sub>2</sub>-treated seawater at a rate of 9 L h<sup>-1</sup>  
174 (i.e. a renewal rate of 90% h<sup>-1</sup>) from the header tanks. pCO<sub>2</sub> was monitored and controlled by  
175 an offline feedback system (IKS Aquastar, Karlsbad, Germany) that regulated the addition of  
176 gas in the header tanks. The pH values of the IKS system were adjusted from daily  
177 measurements of pH<sub>T</sub> in the 18 aquaria using a pH meter (826 pH mobile, Metrohm AG,  
178 Herisau, Switzerland) calibrated with Tris HCl and 2-aminopyridine HCl buffers (Dickson et  
179 al. 2007).

180 In each pCO<sub>2</sub> treatment, temperature was raised from 10 to 19°C with an incremental  
181 step of 3°C. The first three temperature levels (10 to 16°C) simulated the natural change in  
182 temperature from winter to summer in Morlaix Bay whereas the last level (19°C)  
183 corresponded to a temperature increase of 3°C predicted for the end of the century (Solomon  
184 et al. 2007). *C. fornicata* individuals were held for three weeks at each temperature before  
185 carrying out the metabolic measurements (see below). This acclimation time was long enough  
186 to overcome the immediate stress response (Meistertzheim et al. 2007). Temperature was  
187 maintained at (1) 10°C (1<sup>st</sup> trial period) from 16 January to 12 February 2012; (2) 13°C (2<sup>nd</sup>  
188 trial period) from 27 February to 25 March 2012; (3) 16°C (3<sup>rd</sup> trial period) from 9 April to 6  
189 May 2012, and (4) 19°C (4<sup>th</sup> trial period) from 21 May to 27 June 2012. Between two  
190 temperature levels, temperature was gradually increased by 0.2°C day<sup>-1</sup> over two weeks. The  
191 18 aquaria were placed in thermostatic baths in which temperature was regulated to within ±  
192 0.2°C using submersible 150 to 250 W heaters controlled by the IKS system.

193 Three independent 10 L aquaria named “control” were maintained at 10°C under  
194 ambient pH (with no pCO<sub>2</sub> control) until the end of the experiment in order to estimate a  
195 potential bias on metabolism induced by the mesocosm experiment over time. Each aquarium  
196 contained 10 small and 10 large slipper limpets on their substratum shell and was supplied

197 with the same seawater sourced from the header tanks. They were kept in a thermostatic bath  
198 regulated at 10°C by an aquarium chiller (TC5, TECO®, Ravenna, Italy).

199 In addition to the natural phytoplankton found in the unfiltered seawater, all slipper  
200 limpets were fed twice a week with a stock solution composed of the diatom *Chaetoceros*  
201 *gracilis* ( $\sim 15 \times 10^6$  cells mL<sup>-1</sup>) and the dinoflagellate *Isochrysis affinis galbana* ( $\sim 26 \times 10^6$   
202 cells mL<sup>-1</sup>); 400 mL of this microalgal mix was added to each aquarium at each feeding.  
203 Seawater flow was stopped for 2 h when organisms were fed and filtering actively. During  
204 this feeding time, pH variation did not exceed 0.05 units.

205 Individuals that did not adhere to their substratum shell and that showed no reaction  
206 when their foot was stimulated were counted as dead and removed from the tanks. Mortality  
207 reached only 8% at the end of the experiment among all  $p\text{CO}_2$  conditions.

208

#### 209 *Seawater parameter monitoring*

210 Seawater parameters were monitored throughout the experiment.  $\text{pH}_T$  and temperature  
211 were recorded daily in each of the 21 aquaria (18 + 3 controls) using a pH meter (826 pH  
212 mobile, Metrohm AG, Herisau, Switzerland) as described above. Total alkalinity ( $A_T$ ) was  
213 measured at each trial period by 0.01 N HCl potentiometric titration on an automatic titrator  
214 (Titroline alpha, Schott SI Analytics, Mainz, Germany). Salinity was also measured at each  
215 trial period with a conductimeter (LF 330/ SET, WTW, Weilheim, Germany). Seawater  
216 carbonate chemistry, i.e. dissolved inorganic carbon (DIC),  $p\text{CO}_2$  and the saturation state of  
217 aragonite ( $\Omega_{Ar}$ ) were calculated for each  $p\text{CO}_2$  level and temperature with CO<sub>2</sub>SYS software  
218 (Lewis and Wallace 1998) using constants from Mehrbach et al. (1973) refitted by Dickson &  
219 Millero (1987).

220

#### 221 *Metabolic rates and O:N ratios*

222 Metabolic rates were assessed at each temperature level after a four-day starvation  
223 period and after the shells were gently cleaned to remove biofilm-forming organisms. Two  
224 small and two large individuals were selected per aquarium. They were incubated individually  
225 in 185 mL (small) and 316 mL (large) acrylic chambers (Engineering & Design Plastics Ltd,  
226 Cambridge, UK) filled with seawater from their respective aquaria. They were put on a plastic  
227 grid above a stirring bar (speed 100 rpm.), which ensured water homogeneity. Chambers were  
228 placed in their original aquaria for incubation to keep the temperature constant. Incubations  
229 were carried out in dark for 2 to 10 h, depending on temperature and limpet size, to maintain  
230 oxygen saturation above 80% until the end of the incubation. At each temperature period,  
231 empty shell incubations were carried out to correct individual rates for fluxes related to the  
232 substratum shell. Blank incubations containing only seawater from the aquarium also helped  
233 to correct fluxes for any microbiological activity in seawater.

234 Oxygen concentrations were measured at the beginning and the end of the incubation  
235 period with a non-invasive fiber-optics system and reactive oxygen spots attached to the inner  
236 wall of the chambers (FIBOX 3, PreSens, Regensburg, Germany). Spots were calibrated at the  
237 beginning of each trial period with 0% and 100% oxygen buffers. Seawater was sampled for  
238 ammonium ( $\text{NH}_4^+$ ) concentration and  $A_T$  measurements with 100 mL syringes at the  
239 beginning of the incubation, directly in the aquaria just after the chambers were closed, and at  
240 the end of the incubation, in the incubation chamber itself. Samples were filtered through 0.7  
241  $\mu\text{m}$  Whatman GF/F filters into 100 mL glass bottles and fixed with reagent solutions for  
242 ammonium or poisoned with mercuric chloride (0.02% vol/vol; Dickson et al. 2007) for  $A_T$   
243 measurements. Vials were stored in the dark pending analysis.  $\text{NH}_4^+$  concentrations were then  
244 determined using the Solorzano method (Solorzano 1969) based on spectrophotometry at a  
245 wavelength of 630 nm (spectrophotometer UV-1201V, Shimadzu Corp, Kyoto, Japan).  $A_T$  (in  
246  $\mu\text{Eq L}^{-1}$ ) values were determined by 0.01 N HCl potentiometric titration on an automatic

247 titrator (Titroline alpha, Schott SI Analytics, Mainz, Germany) and by using the Gran method  
248 (non-linear least-squares fit) applied to pH values from 3.5 to 3.0 (Dickson et al. 2007).

249 Respiration (in  $\mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ ; equation [1]) and excretion (in  $\mu\text{mol NH}_4^+ \text{ g}^{-1}$   
250  $\text{DW h}^{-1}$ ; equation [2]) were directly calculated from oxygen and ammonium concentrations,  
251 respectively. Net calcification (in  $\mu\text{mol CaCO}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ ; equation [3]) was estimated using  
252 the alkalinity anomaly technique (Smith and Key 1975) based on a decrease in  $A_T$  by 2  
253 equivalents for each mole of  $\text{CaCO}_3$  precipitated (Wolf-Gladrow et al. 2007). As ammonium  
254 production increases alkalinity in a mole-per-mole ratio (Wolf-Gladrow et al. 2007), the  
255 alkalinity variation was corrected by the ammonium flux to calculate  $\text{CaCO}_3$  fluxes.

256 [1]  $R = \frac{\Delta\text{O}_2 \times V}{\Delta t \times \text{DW}}$

257 [2]  $E = \frac{\Delta\text{NH}_4^+ \times V}{\Delta t \times \text{DW}}$

258 [3]  $G_n = -\frac{(\Delta A_T - \Delta\text{NH}_4^+) \times V}{2 \times \Delta t \times \text{DW}}$

259 where  $\Delta\text{O}_2$  (in  $\mu\text{mol O}_2 \text{ L}^{-1}$ ) is the difference between initial and final  $\text{O}_2$  concentrations;  $\Delta$   
260  $\text{NH}_4^+$  (in  $\mu\text{mol NH}_4^+ \text{ L}^{-1}$ ) is the difference between initial and final  $\text{NH}_4^+$  concentrations;  $\Delta A_T$   
261 is the difference between initial and final total alkalinity ( $\mu\text{mol Eq L}^{-1}$ );  $V$  (in L) is the volume  
262 of the chamber minus *C. fornicata* volume;  $\Delta t$  (in h) is the incubation time and  $\text{DW}$  (in g) is  
263 the soft tissue dry weight of incubated *C. fornicata*.

264 In addition, oxygen consumption of the individuals maintained at  $10^\circ\text{C}$  during the  
265 experiment were assessed on six small and six large individuals at each trial period, following  
266 the technique described above. These “controls” tested if mesocosm conditioning induced  
267 metabolic stress over time.

268 The O:N ratio, which corresponds to the amount of oxygen consumed for nitrogen  
269 excreted, was calculated from respiration and excretion rates except for the experiments run at  
270  $10^\circ\text{C}$  for which rates were too low to obtain significant data. Generally, the O:N ratio is

271 considered a common indicator of the proportion of the three metabolic substrates  
272 (carbohydrates, lipids and proteins) used in energy metabolism (Mayzaud and Conover 1988).

273 The atomic ratio of oxygen uptake and excreted nitrogen was calculated following the  
274 equation [4] based on Thomsen & Melzner (2010):

275 [4]:  $O:N = R / E$

276 where R is the respiration rate used as a proxy of the quantity of oxygen consumed by the  
277 individual and E, the excretion rate representing the concentration of nitrogen excreted.

278

### 279 *Filtration rates*

280 At each trial period, the filtration rate of three small and three large slipper limpets per  
281  $pCO_2$  condition (i.e. 1 individual per size per aquarium) was determined by calculating  
282 clearance rates (Coughlan 1969). To do so, 10 and 20 mL of a microalgae mix (*C. gracilis*, *T.*  
283 *affinis galbana*, 1:1) were added to the small and large chambers (same as for metabolic  
284 measurements), respectively, using a 10 mL syringe equipped with a thin tube. The mean  
285 initial concentration of the mix was  $1\,200\,000 \pm 310\,000$  cell  $mL^{-1}$ . In parallel, control  
286 incubations containing only microalgae were carried out to check that phytoplankton cells did  
287 not multiply significantly during the incubation. Water from the chambers was sampled with  
288 the syringe every 15 min until the water became totally clear (around 2 h). Samples were  
289 immediately fixed with 25% glutaraldehyde and frozen at  $-80^\circ C$  pending analyses (Marie et  
290 al. 1999). The number of microalgal cells in each sample was then determined on 200  $\mu L$   
291 aliquots using flux cytometry (Cell Lab Quanta<sup>TM</sup>, SC, Beckman Coulter, USA). Filtration  
292 rates (F, in  $mL\ SW\ g^{-1}\ DW\ min^{-1}$ ) were calculated following equation [5]:

293 [5]  $F = V \times \frac{\ln[Ci] - \ln[Cf]}{\Delta t \times DW}$

294 where [Ci] and [Cf] (in cell  $mL^{-1}$ ) were respectively the initial and final cell concentrations in  
295 the chamber water; V (in L) is the volume of the chamber minus individual *C. fornicata*

296 volume;  $\Delta t$  (in h) is the incubation time and DW (in g) is the tissue dry weight of the  
297 individual incubated.

298

### 299 *Statistical analyses*

300 All statistical analyses were performed using the R software, version 2.15.0 (R Core  
301 Team 2013). Normality and homoscedasticity were checked using Kolmogorov-Smirnov's  
302 test and Levene's test, respectively, before each statistical test. Spatial pseudoreplication  
303 effect was first tested by considering "aquarium" as a random factor ( $p$ -value < 0.05). Then,  
304 statistical analyses were simplified to two-way ANOVAs with repeated measurements on the  
305 same individual through the four trial periods (different temperature levels) separately for  
306 small and large individuals. These analyses were performed for the four physiological rates  
307 (respiration, excretion, calcification and filtration) and the O:N ratio, assuming  $p\text{CO}_2$  and  
308 temperature as fixed factors. Student-Newman-Keuls (SNK) post hoc tests were applied to  
309 identify differences among treatments with a confidence level of 95% when ANOVA showed  
310 significant results. In parallel, any changes in the respiration rate of individuals constantly  
311 maintained at 10°C through time were assessed using a non-parametric Friedman test for  
312 repeated measurements, separately for small and large slipper limpets. All results are given as  
313 mean  $\pm$  standard error (SE).

314

## 315 **Results**

316

### 317 *Seawater parameters*

318 The mean temperature and carbonate chemistry parameters among the  $p\text{CO}_2$  and  
319 temperature conditions are presented in Table 1. Temperature was stable at each trial period  
320 with a variability lower than 0.5°C. The different  $p\text{CO}_2$  levels remained close to the selected

321 values of 390, 750 and 1400  $\mu\text{atm}$  except at 19°C where all  $p\text{CO}_2$  increased from the baseline  
322 (+ 100-200  $\mu\text{atm}$ ).  $A_T$  ranged from  $2365 \pm 2$  to  $2422 \pm 2$   $\mu\text{Eq kg}^{-1}$ .  $\Omega_{Ar}$  decreased by less than  
323 1 only in the 1400  $\mu\text{atm}$   $p\text{CO}_2$  condition. Salinity varied between  $34.2 \pm 0.1$  and  $35.1 \pm 0.1$   
324 among the different  $p\text{CO}_2$  and temperature levels with no effect of the temperature increase  
325 on salinity.

326

### 327 *Respiration, excretion and O:N ratio*

328 Respiration and excretion rates changed significantly with temperature, but not with  
329  $p\text{CO}_2$ , in small and large individuals (Figure 1, Table 2). After pooling results for all  $p\text{CO}_2$   
330 conditions, mean respiration rates in small *C. fornicata* increased from 3.78  $\mu\text{mol O}_2 \text{ g}^{-1} \text{ DW}$   
331  $\text{h}^{-1}$  at 10°C to 11.76  $\mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$  at 19°C. In large individuals, the lowest mean  
332 respiration rate was recorded at 10°C (4.82  $\mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ ) whereas rates did not differ  
333 from 13 to 19°C with a mean value of 11.50  $\mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ . Oxygen fluxes measured on  
334 empty shells represented only 4% of the whole organism fluxes measured and decreased only  
335 slightly with temperature.

336 Mean excretion rates calculated among  $p\text{CO}_2$  conditions for small *C. fornicata*  
337 individuals gradually increased from 0.15  $\mu\text{mol NH}_3 \text{ g}^{-1} \text{ DW h}^{-1}$  at 10°C to 1.47  $\mu\text{mol NH}_3 \text{ g}^{-1}$   
338  $\text{DW h}^{-1}$  at 19°C. Excretion rates of large individuals showed a parabolic trend with an  
339 increase from 10°C (0.16  $\mu\text{mol NH}_3 \text{ g}^{-1} \text{ DW h}^{-1}$ ) to 16°C (1.34  $\mu\text{mol NH}_3 \text{ g}^{-1} \text{ DW h}^{-1}$ )  
340 followed by a decrease at 19°C (0.74  $\mu\text{mol NH}_3 \text{ g}^{-1} \text{ DW h}^{-1}$ ). The ammonium fluxes of empty  
341 shells represented less than 1% of the fluxes estimated for whole organisms and were higher  
342 at 10°C than at the other temperature levels (rates practically nil).

343 O:N ratios varied greatly, ranging from 2.86 to 31.68 with a mean value of  $12.91 \pm$   
344 0.56. They varied with  $p\text{CO}_2$  or temperature according to size (Table 2, Figure 2). In small *C.*  
345 *fornicata* individuals, O:N ratios were the highest at 750  $\mu\text{atm}$  and similar between 380 and

346 1400  $\mu\text{atm}$ . In large individuals, the O:N ratios varied with temperature and were significantly  
347 higher at 16°C.

348

#### 349 *Filtration*

350 Temperature significantly affected filtration rates in both small and large individuals  
351 (Figure 1, Table 2). In small *C. fornicata*, mean filtration rates among  $p\text{CO}_2$  were similar  
352 between 10 and 16°C (25.50 mL  $\text{g}^{-1}$  DW  $\text{min}^{-1}$ ), but increased at 19°C (54.30 mL  $\text{g}^{-1}$  DW  $\text{min}^{-1}$ ).  
353  $p\text{CO}_2$  alone did not affect the filtration rate but the interaction of  $p\text{CO}_2$  and temperature was  
354 significant (Table 2, p-value < 0.001). At 19°C, filtration rates increased significantly with the  
355 increase in  $p\text{CO}_2$ . In large individuals, mean filtration rates increased gradually from 10°C  
356 (5.43 mL  $\text{g}^{-1}$  DW  $\text{min}^{-1}$ ) to 19°C (25.78 mL  $\text{g}^{-1}$  DW  $\text{min}^{-1}$ ) without any effect of  $p\text{CO}_2$   
357 conditions.

358

#### 359 *Calcification*

360 Calcification rates were significantly affected by  $p\text{CO}_2$  increase in both small and  
361 large individuals but not by temperature (Figure 1, Table 2). Pooling all temperature levels  
362 together, mean calcification rates were similar at  $p\text{CO}_2$  of 390  $\mu\text{atm}$  (1.88 and 1.63  $\mu\text{mol}$   
363  $\text{CaCO}_3$   $\text{g}^{-1}$  DW  $\text{h}^{-1}$  in small and large individuals, respectively) and 750  $\mu\text{atm}$  (1.02 and 0.60  
364  $\mu\text{mol CaCO}_3$   $\text{g}^{-1}$  DW  $\text{h}^{-1}$  in small and large, respectively), but significantly lower at 1400  
365  $\mu\text{atm } p\text{CO}_2$  (-2.53 and -1.77  $\mu\text{mol CaCO}_3$   $\text{g}^{-1}$  DW  $\text{h}^{-1}$  in small and large individuals,  
366 respectively). In the highest  $p\text{CO}_2$  condition (1400  $\mu\text{atm}$ ), net calcification rates were  
367 negative, corresponding to dissolution. Although the interaction between  $p\text{CO}_2$  and  
368 temperature was not significant for either small or large limpets,  $p\text{CO}_2$  response appeared to  
369 vary as a function of temperature, particularly at 1400  $\mu\text{atm}$ . In this drastic  $p\text{CO}_2$  condition,  
370 organisms globally dissolved at 10, 13 and 16°C and calcified (or dissolved less) at 19°C.



371 Calcification rates decreased with the decrease in the mean aragonite saturation state  
372 ( $\Omega_{Ar}$ ) which correlated with  $pCO_2$  increase (Figure 3). When  $\Omega_{Ar}$  decreased below the  
373 threshold of 1, calcification rates were always negative reflecting a dissolution process. At the  
374 750 and 1400  $\mu atm$   $pCO_2$  conditions,  $\Omega_{Ar}$  was higher at 19°C than at the other temperature  
375 levels because the saturation state increases with temperature.

376

### 377 *Mesocosm controls*

378 In the aquaria maintained at 10°C throughout the entire experiment, temperature was  
379 stable over the first weeks of the experiment and slowly increased from 8 April to the end of  
380 the experiment until reaching a mean of 12.4°C between 21 April and 15 June because we had  
381 technical problems with the chiller (Table 1). Respiration in small individuals showed high  
382 variation over time (Figure 4, white bars) but no time effect was detected (Friedman test,  $df =$   
383 3,  $\chi^2 = 6.6$ ,  $p = 0.086$ ,  $n = 6$ ). Conversely, respiration rates of large individuals increased  
384 throughout the experiment (Figure 4, gray bars) with a significant time effect (Friedman test,  
385  $df = 3$ ,  $\chi^2 = 9.4$ ,  $p = 0.024$ ,  $n = 6$ ).

386

## 387 **Discussion**

388 An increase in temperature affected three of the four physiological processes assessed  
389 on small and large *C. fornicata* individuals. In particular, respiration and ammonia excretion  
390 rates clearly increased along the tested temperature gradient. In contrast, increases in  $pCO_2$   
391 affected only net calcification of the slipper limpets. Interestingly, the coupled effect of  
392 temperature and  $pCO_2$  improved the rate of calcification in the most drastic conditions,  
393 particularly in small individuals.

394

### 395 *Temperature effect*

396           The respiration ( $0.6 - 34.6 \mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ ) and excretion rates ( $-2 - 4.4 \mu\text{mol NH}_3$   
397  $\text{ g}^{-1} \text{ DW h}^{-1}$ ) measured at  $390 \mu\text{atm } p\text{CO}_2$  in small and large *C. fornicata* individuals ranged  
398 metabolic rates recorded *in situ* in the Bay of Brest in northwestern France ( $4$  to  $45 \mu\text{mol O}_2$   
399  $\text{ g}^{-1} \text{ DW h}^{-1}$  and  $0.5$  to  $2.3 \mu\text{mol NH}_3 \text{ g}^{-1} \text{ DW h}^{-1}$ ; Martin et al. 2006). Both rates increased with  
400 temperature in small and large individuals regardless of  $p\text{CO}_2$ . Although respiration rates  
401 gradually increased with temperature in small *C. fornicata* individuals, they only increased  
402 from  $10^\circ\text{C}$  to  $13^\circ\text{C}$ , remaining stable at higher temperatures in large *C. fornicata*. This  
403 increase is a common response due to the rate-enhancing effects of temperature on  
404 physiological and biochemical reactions in ectotherms (Cossins and Bowler 1987). The  
405 intensity of respiratory and excretory processes were also dependent of body size. The  
406 respiration and excretion rates of small individuals were higher than those of large individuals  
407 because the metabolic rate (per unit biomass) decreases with increasing individual size  
408 (Parsons et al. 1984; Von Bertalanffy 1951). Small individuals have higher energy  
409 consumption because they grow faster than the large individuals (Von Bertalanffy 1964).

410           The filtration rates measured in small and large *C. fornicata* fall into the range of  
411 maximum feeding rates calculated by Newell and Kofoed (1977) in *C. fornicata* between  $11$   
412 and  $20^\circ\text{C}$  ( $18$  to  $41 \text{ mL g}^{-1} \text{ min}^{-1}$ ;  $15^\circ\text{C}$  acclimated individuals). Rates were higher in small  
413 than in large individuals because, again, small organisms feed more actively per unit body  
414 mass (Sylvester et al. 2005). Filtration rates increased with temperature as previously  
415 described in other studies (Newell and Kofoed 1977). In small individuals, rates were constant  
416 between  $10$  and  $16^\circ\text{C}$  and increased only at  $19^\circ\text{C}$  while they increased regularly with  
417 temperature in the large individuals. In Calyptraeidae, small individuals — i.e. males with low  
418 mobility — utilize two feeding strategies: grazing with radula and filtration with gills  
419 (Navarro and Chaparro 2002). Therefore, small individuals may have supplemented their diet  
420 between  $10$  and  $16^\circ\text{C}$  by grazing. For the increased energy requirements at  $19^\circ\text{C}$ , small

421 slipper limpets may also increase their filtration rate to meet these supplementary needs. In  
422 large sedentary individuals (usually females), filtration is the only feeding mechanism  
423 (Navarro and Chaparro 2002) and filtration rate increases with temperature to help cover the  
424 higher energy needs.

425 Surprisingly, temperature did not affect calcification rates although an increase was  
426 expected in response to the increase in metabolism and energy requirements (Martin et al.  
427 2006). Because mollusk shell production is an energetically costly process (Gazeau et al.  
428 2013), the absence of any change in calcification rates may be due to food limitation during  
429 the experiment, especially at elevated temperatures (16 and 19°C). At these temperatures,  
430 providing additional food only twice a week may not have been sufficient to support maximal  
431 individual shell growth under pH stressful conditions. If food had been provided more  
432 regularly and/or in higher quantities, *C. fornicata* calcification may not be potentially  
433 restricted and individuals may have better mitigated the effect of high  $p\text{CO}_2$  (Thomsen et al.  
434 2014). Future experiments should include measuring integrated shell growth at each  
435 temperature level to determine the food effect more completely.

436 Mesocosm experiments cannot perfectly reproduce *in situ* conditions such as natural  
437 diet or tidal cycles. This may lead to an increased stress for the organisms grown in these  
438 systems (Bibby et al. 2008). The mesocosm effect on organisms was tested through  $\text{O}_2$   
439 consumption measurements in individuals kept at a constant temperature throughout the  
440 experiment (“controls”). The respiration rates did not change over time in small individuals,  
441 whereas the respiration in large individuals increased slightly in correlation with a +2°C  
442 temperature increase from the beginning to the end of the experiment, because of technical  
443 problems with the chiller. Although food may have constituted a bias, particularly in the one-  
444 off calcification response to temperature, the absence of strong changes in respiration rates in  
445 “controls”, unexceptional metabolic rates ranging from those measured *in situ* and very low

446 mortality during the experiment (only 8%) all suggest the absence of any acute mesocosm  
447 effect on the other physiological traits of *C. fornicata*.

448

#### 449 *pCO<sub>2</sub> effect*

450 In contrast to temperature, *pCO<sub>2</sub>* did not affect *C. fornicata* respiration or excretion  
451 rates regardless of size. Other studies have underlined a lack of any *pCO<sub>2</sub>* effect on bivalve  
452 and limpet respiration (Dickinson et al. 2012; Fernandez-Reiriz et al. 2012; Marchant et al.  
453 2010), although some mollusk species exposed to high *pCO<sub>2</sub>* levels have shown metabolic  
454 depression (i.e. decrease in oxygen uptake) to compensate — albeit often drastic — *pCO<sub>2</sub>*  
455 increases (Michaelidis et al. 2005; Navarro et al. 2013). Responses of ammonia excretion to  
456 high *pCO<sub>2</sub>* in mollusks are also specific: increase in ammonia excretion can occur under  
457 elevated *pCO<sub>2</sub>* (Fernandez-Reiriz et al. 2011; Langenbuch and Pörtner 2002; Range et al.  
458 2014) while some bivalves show opposing trends (Liu and He 2012; Navarro et al. 2013). The  
459 increase in ammonia excretion under increased *pCO<sub>2</sub>* conditions can be interpreted as an  
460 internal pH regulatory mechanism, sometimes based on protein catabolism (Fernandez-Reiriz  
461 et al. 2012; Thomsen and Melzner 2010). In our study, neither change in excretion rates nor in  
462 O:N ratios calculated were detected between the 390 and 1400  $\mu\text{atm}$  conditions. This  
463 similarity indicates that potential intracellular pH regulation of *C. fornicata* was not induced  
464 by enhancing protein metabolism (Fernandez-Reiriz et al. 2012). Thus, the potential for  
465 metabolic resistance of *C. fornicata* to elevated *pCO<sub>2</sub>* is likely due to another effective  
466 acidosis-buffering system, such as the increase in internal  $\text{HCO}_3^-$  concentrations (Gutowska et  
467 al. 2010; Michaelidis et al. 2005) or higher  $\text{H}^+$  excretion (Melzner et al. 2009; Pörtner et al.  
468 2005).

469 Similarly to the respiration and excretion processes, filtration rates did not change as a  
470 function of *pCO<sub>2</sub>* in either small or large *C. fornicata* in our study. Filtration responses with

471 respect to  $p\text{CO}_2$  depend most of the time on the presence of metabolic depression (Fernandez-  
472 Reiriz et al. 2011; Liu and He 2012; Navarro et al. 2013). The absence of variation in  
473 filtration rates at the different  $p\text{CO}_2$  levels indicates that the quantity of food ingested by *C.*  
474 *fornicata* did not vary either. Food is known to interact with other stressors, such as  $p\text{CO}_2$ ,  
475 and significantly influence metabolic responses (Melzner et al. 2011; Pansch et al. 2014).  
476 Quality or quantity changes in the diet can even worsen the condition of invertebrates (Berge  
477 et al. 2006; Vargas et al. 2013). Although our microalgal mix did not perfectly match the  
478 natural diet of *C. fornicata* (Barillé et al. 2006; Decottignies et al. 2007), the diatoms and  
479 dinoflagellate microalgae provided in the experiment correspond to the main taxa present in  
480 Morlaix Bay, assuming a nutritional quality close to the natural diet. However, we cannot  
481 assure that the quantity of food was not a limiting factor in our experiment. To be sure that  
482 microalgae supplied would not represent a bias, the slipper limpets should be fed *ad libitum*  
483 which represented a technical issue on a 6 month experiment.

484 In our study, net calcification was similar between 390 and 750  $\mu\text{atm } p\text{CO}_2$  and  
485 strongly decreased at 1400  $\mu\text{atm } p\text{CO}_2$  regardless of size, which is a common response in  
486 mollusks (Beniash et al. 2010; Melatunan et al. 2013; Range et al. 2011). This pattern  
487 contrasts with that reported in Ries et al. (2009), with a parabolic response in *C. fornicata*  
488 calcification with the highest rates observed at 600  $\mu\text{atm } p\text{CO}_2$ . The stability of calcification  
489 rate at 750  $\mu\text{atm } p\text{CO}_2$  (compared to 390  $\mu\text{atm } p\text{CO}_2$ ) may be due to the biological control of  
490 the calcification process and/or the presence of the periostracum, the organic layer covering  
491 the crystalline layers of the shell. This organic layer has been predicted to play a great role in  
492 maintaining shell integrity of mollusks in elevated  $p\text{CO}_2$  conditions (Ries et al. 2009) and to  
493 protect them from dissolution in  $\text{CaCO}_3$ -undersaturated waters (Huning et al. 2013).  
494 Moreover, mollusks may be able to maintain extrapallial fluid in chemical conditions favoring  
495  $\text{CaCO}_3$  precipitation at the calcification site, even if external seawater  $p\text{CO}_2$  is high

496 (Hiebenthal et al. 2013). Regulation of enzymes involved in the calcification process, such as  
497 chitinase (Cummings et al. 2011) or carbonic anhydrase (Ivanina et al. 2013), may also help  
498 maintain calcification in high  $p\text{CO}_2$  conditions. In our study, at 1400  $\mu\text{atm}$ , calcification rates  
499 dropped, perhaps due to physiological changes in the internal acid-base balance affecting shell  
500 deposition (Waldbusser et al. 2011) or to an eroded and/or damaged periostracum (pers. obs.).  
501 Degradation of this protective layer may lead to higher vulnerability of the shell to external  
502 dissolution processes (Range et al. 2012; Ries et al. 2009), which occurs not only in dead  
503 shells but also in live animals (Harper 2000). Furthermore, chemical dissolution increased  
504 with an increase in  $p\text{CO}_2$  and a correlated decrease in  $\Omega_{\text{Ar}}$ ; the combined effect led to a  
505 decrease in net calcification rates observed in both small and large *C. fornicata* individuals at  
506 high  $p\text{CO}_2$  conditions.

507

#### 508 *Combined effects of temperature and $p\text{CO}_2$*

509 In the range of  $p\text{CO}_2$  and temperatures tested, the interaction of these two variables  
510 had no negative effect on *C. fornicata* respiration and excretion rates. As a eurythermal  
511 species even coping with high temperature in some bays during summer (e.g. Bassin  
512 d’Arcachon in southwestern France; De Montaudouin et al. 1999), *C. fornicata* can have an  
513 optimal temperature of 19°C or higher (Diederich and Pechenik 2013; Noisette et al. 2015).  
514 Thus, 19°C may not constitute a real thermal stress and not transgress the metabolic optimal  
515 threshold for this species. Increase in temperature is predicted to enhance sensitivity to high  
516  $p\text{CO}_2$  levels beyond the optimal temperature of the species and close to its upper limit of  
517 thermal tolerance (Pörtner and Farrell 2008). However, at the cold side of a species optimal  
518 temperature, warming can increase resilience to ocean acidification (Gianguzza et al. 2014).  
519 Therefore, an increase in temperature may actually improve tolerance to  $p\text{CO}_2$  increases in *C.*  
520 *fornicata*.

521           Calcification rates of both small and large *C. fornicata* showed a positive trend with  
522 temperature in the most drastic  $p\text{CO}_2$  conditions (1400  $\mu\text{atm}$ ). Temperature-mediated  
523 increases in metabolism and feeding rates may potentially offset reductions in calcification  
524 rates caused by high  $p\text{CO}_2$  conditions (Melzner et al. 2011; Thomsen et al. 2014). In addition  
525 to this physiological effect, moderate warming can mediate the effects of ocean acidification  
526 by the chemical effect on seawater chemistry (Kroeker et al. 2014). Temperature affects  $\text{CO}_2$   
527 solubility in seawater as well as the equilibrium coefficients governing carbonate chemistry  
528 (Millero 2007). As shown in our study, the saturation state of aragonite was greater in warmer  
529 water than in colder water for a given  $p\text{CO}_2$ , thereby enhancing calcification and reducing the  
530 dissolution processes in the high  $p\text{CO}_2$  conditions. These results highlight the importance of  
531 considering the physiological and geochemical interactions between temperature and  
532 carbonate chemistry when interpreting species' vulnerability to ocean acidification. A better  
533 understanding of how warming influences species' responses to high  $p\text{CO}_2$  levels through  
534 both direct (e.g. increases in metabolic rates) and indirect pathways (e.g. changes in carbonate  
535 chemistry) is thus necessary.

536

### 537 *Conclusion*

538           A trade-off between stressors may affect the physiology of organisms in an  
539 unexpected way (Kroeker et al. 2014). In our case, *C. fornicata* appeared to be able to tolerate  
540 slight increases in  $p\text{CO}_2$  but its calcification was affected by drastic conditions with a positive  
541 effect of temperature, thereby mitigating any ocean acidification effects. This outcome  
542 highlights the need of multistressor studies to understand the future of marine species in a  
543 context of climate change in which different physico-chemical factors vary in different ways.  
544 Furthermore, our results indicate that some species can be highly tolerant to future  $p\text{CO}_2$   
545 increases. *C. fornicata* tolerance likely stems from mechanisms that allow it to acclimate or

546 adapt to environmental fluctuations in its habitat (Clark et al. 2013), because species living in  
547 environments with large abiotic variations tend to have high phenotypic plasticity, allowing  
548 them to survive in stressful conditions (Somero 2010). This capacity to resist decreases in pH  
549 may reinforce the ecological role of *C. fornicata* populations in the ecosystems in which they  
550 are established, even under projected future conditions anticipated due to climate change.

551

552

553

554

555

## 556 **References**

- 557 Andersson, A. J., and F. T. Mackenzie. 2011. Ocean acidification: setting the record straight.  
558 Biogeosciences Discussions **8**: 6161-6190.
- 559 Barillé, L., B. Cognie, P. Beninger, P. Decottignies, and Y. Rince. 2006. Feeding responses of  
560 the gastropod *Crepidula fornicata* to changes in seston concentration. Mar. Ecol.-  
561 Prog. Ser. **322**: 169-178.
- 562 Barry, J. P., T. Tyrrell, L. Hansson, G. K. Plattner, and J. P. Gattuso. 2010. Atmospheric CO<sub>2</sub>  
563 targets for ocean acidification perturbation experiments, p. 260. In F. V. J. Riebesell  
564 U., Hansson L. & Gattuso J.-P. [ed.], Guide to best practices for ocean acidification  
565 research and data reporting.
- 566 Beniash, E., A. Ivanina, N. S. Lieb, I. Kurochkin, and I. M. Sokolova. 2010. Elevated level of  
567 carbon dioxide affects metabolism and shell formation in oysters *Crassostrea*  
568 *virginica*. Mar. Ecol.-Prog. Ser. **419**: 95-108.
- 569 Berge, J. A., B. Bjerkeng, O. Pettersen, M. T. Schaanning, and S. Øxnevad. 2006. Effects of  
570 increased sea water concentrations of CO<sub>2</sub> on growth of the bivalve *Mytilus edulis* L.  
571 Chemosphere **62**: 681-687.
- 572 Bibby, R., P. Cleall-Harding, S. Rundle, S. Widdicombe, and J. Spicer. 2007. Ocean  
573 acidification disrupts induced defences in the intertidal gastropod *Littorina littorea*.  
574 Biol. Lett. **3**: 699-701.
- 575 Bibby, R., S. Widdicombe, H. Parry, J. Spicer, and R. Pipe. 2008. Effects of ocean  
576 acidification on the immune response of the blue mussel *Mytilus edulis*. Aquat. Biol.  
577 **2**: 67-74.
- 578 Blanchard, M. 1995. Origine et état de la population de *Crepidula fornicata* (Gastropoda  
579 Prosobranchia) sur le littoral français. Haliotis **24**: 75-86.
- 580 Blanchard, M. 1997. Spread of the slipper limpet *Crepidula fornicata* (L. 1758) in Europe.  
581 Current state and consequences. Sci. Mar. **61**: 109-118.
- 582 Chauvaud, L., F. Jean, O. Ragueneau, and G. Thouzeau. 2000. Long-term variation of the Bay  
583 of Brest ecosystem: benthic-pelagic coupling revisited. Mar. Ecol.-Prog. Ser. **200**: 35-  
584 48.



- 585 Clark, M. S. and others 2013. Identification of molecular and physiological responses to  
586 chronic environmental challenge in an invasive species: the Pacific oyster,  
587 *Crassostrea gigas*. *Ecology and Evolution* **3**: 3283-3297.
- 588 Coe, W. R. 1936. Sexual phases in *Crepidula*. *Journal of experimental zoology* **72**: 455-477.
- 589 Cossins, A. R., and K. Bowler. 1987. *Temperature biology of animals*. Chapman and Hall
- 590 Coughlan, J. 1969. Estimation of filtering rate from clearance of suspensions. *Mar. Biol.* **2**:  
591 356-&.
- 592 Cummings, V. and others 2011. Ocean acidification at high latitudes: potential effects on  
593 functioning of the antarctic bivalve *Laternula elliptica*. *PLoS One* **6**: e16069.
- 594 De Montaudouin, X., C. Audemard, and P.-J. Labourg. 1999. Does the slipper limpet  
595 (*Crepidula fornicata*, L.) impair oyster growth and zoobenthos biodiversity? A  
596 revisited hypothesis. *Journal of Experimental Marine Biology and Ecology* **235**: 105-  
597 124.
- 598 Decottignies, P., P. G. Beninger, Y. Rince, R. J. Robins, and P. Riera. 2007. Exploitation of  
599 natural food sources by two sympatric, invasive suspension-feeders: *Crassostrea gigas*  
600 and *Crepidula fornicata*. *Mar. Ecol.-Prog. Ser.* **334**: 179-192.
- 601 Dickinson, G. H. and others 2012. Interactive effects of salinity and elevated CO<sub>2</sub> levels on  
602 juvenile eastern oysters, *Crassostrea virginica*. *The Journal of experimental biology*  
603 **215**: 29-43.
- 604 Dickson, A. G., and F. J. Millero. 1987. A comparison of the equilibrium constants for the  
605 dissociation of carbonic acid in seawater media. *Deep Sea Research* **34**: 1733-1743.
- 606 Dickson, A. G., C. L. Sabine, and J. R. Christian [eds.]. 2007. *Guide to best practices for*  
607 *ocean CO<sub>2</sub> measurements*. North Pacific Marine Science Organization.
- 608 Diederich, C. M., and J. A. Pechenik. 2013. Thermal tolerance of *Crepidula fornicata*  
609 (Gastropoda) life history stages from intertidal and subtidal subpopulations. *Mar.*  
610 *Ecol.-Prog. Ser.* **486**: 173-187.
- 611 Ehrhold, A., M. Blanchard, J. P. Auffret, and T. Garlan. 1998. The role of *Crepidula*  
612 proliferation in the modification of the sedimentary tidal environment in Mont-Saint-  
613 Michel Bay (The Channel, France). *Comptes Rendus de l'Academie des Sciences*  
614 *Serie Ii Fascicule a-Sciences de la Terre et des Planetes* **327**: 583-588.
- 615 Feely, R. A., S. C. Doney, and S. R. Cooley. 2009. Ocean acidification: present conditions  
616 and future changes in a high-CO<sub>2</sub> world. *Oceanography* **22**: 36-47.
- 617 Fernandez-Reiriz, M. J., P. Range, X. A. Alvarez-Salgado, J. Espinosa, and U. Labarta. 2012.  
618 Tolerance of juvenile *Mytilus galloprovincialis* to experimental seawater acidification.  
619 *Mar. Ecol.-Prog. Ser.* **454**: 65-74.
- 620 Fernandez-Reiriz, M. J., P. Range, X. A. Alvarez-Salgado, and U. Labarta. 2011.  
621 Physiological energetics of juvenile clams *Ruditapes decussatus* in a high CO<sub>2</sub> coastal  
622 ocean. *Mar. Ecol.-Prog. Ser.* **433**: 97-105.
- 623 Gazeau, F. and others 2013. Impacts of ocean acidification on marine shelled molluscs. *Mar.*  
624 *Biol.* **160**: 2207-2245.
- 625 Gianguzza, P., G. Visconti, F. Gianguzza, S. Vizzini, G. Sara , and S. Dupont. 2014.  
626 Temperature modulates the response of the thermophilous sea urchin *Arbacia lixula*  
627 early life stages to CO<sub>2</sub>-driven acidification. *Mar. Environ. Res.* **93**: 70-77.
- 628 Gutiérrez, J. L., C. G. Jones, D. L. Strayer, and O. O. Iribarne. 2003. Mollusks as ecosystem  
629 engineers: the role of shell production in aquatic habitats. *Oikos* **101**: 79-90.
- 630 Gutowska, M. A., F. Melzner, M. Langenbuch, C. Bock, G. Claireaux, and H.-O. Pörtner.  
631 2010. Acid-base regulatory ability of the cephalopod (*Sepia officinalis*) in response to  
632 environmental hypercapnia. *Journal of Comparative Physiology B* **180**: 323-335.
- 633 Harley, C. D. G. and others 2006. The impacts of climate change in coastal marine systems.  
634 *Ecol. Lett.* **9**: 228-241.

- 635 Harper, E. M. 2000. Are calcitic layers an effective adaptation against shell dissolution in the  
636 Bivalvia? *Journal of Zoology* **251**: 179-186.
- 637 Hiebenthal, C., E. E. Philipp, A. Eisenhauer, and M. Wahl. 2013. Effects of seawater  $p\text{CO}_2$   
638 and temperature on shell growth, shell stability, condition and cellular stress of  
639 Western Baltic Sea *Mytilus edulis* (L.) and *Arctica islandica* (L.). *Mar. Biol.* **160**:  
640 2073-2087.
- 641 Huning, A. K. and others 2013. Impacts of seawater acidification on mantle gene expression  
642 patterns of the Baltic Sea blue mussel: implications for shell formation and energy  
643 metabolism. *Mar. Biol.* **160**: 1845-1861.
- 644 Ivanina, A. V. and others 2013. Interactive effects of elevated temperature and  $\text{CO}_2$  levels on  
645 energy metabolism and biomineralization of marine bivalves *Crassostrea virginica*  
646 and *Mercenaria mercenaria*. *Comparative Biochemistry and Physiology Part A:*  
647 *Molecular & Integrative Physiology* **166**: 101-111.
- 648 Kroeker, K. J., B. Gaylord, T. M. Hill, J. D. Hosfelt, S. H. Miller, and E. Sanford. 2014. The  
649 role of temperature in determining species' vulnerability to ocean acidification: a case  
650 study using *Mytilus galloprovincialis*. *PLoS One* **9**: e100353.
- 651 Langenbuch, M., and H.-O. Pörtner. 2002. Changes in metabolic rate and N excretion in the  
652 marine invertebrate *Sipunculus nudus* under conditions of environmental hypercapnia  
653 identifying effective acid-base variables. *J. Exp. Biol.* **205**: 1153-1160.
- 654 Leroy, F. 2011. Influence des conditions trophiques sur le développement larvaire de l'espèce  
655 invasive *Crepidula fornicata* : conséquences sur ses capacités de dispersion. Paris:  
656 Université Pierre et Marie Curie.
- 657 Lewis, E., and D. W. R. Wallace. 1998. Program developed for  $\text{CO}_2$  system calculations.  
658 Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S.  
659 Department of Energy
- 660 Liu, W., and M. He. 2012. Effects of ocean acidification on the metabolic rates of three  
661 species of bivalve from southern coast of China. *Chinese Journal of Oceanology and*  
662 *Limnology* **30**: 206-211.
- 663 Marchant, H. K., P. Calosi, and J. I. Spicer. 2010. Short-term exposure to hypercapnia does  
664 not compromise feeding, acid-base balance or respiration of *Patella vulgata* but  
665 surprisingly is accompanied by radula damage. *J. Mar. Biol. Assoc. U.K.* **90**: 1379-  
666 1384.
- 667 Marie, D., C. Brussaard, F. Partensky, D. Vaultot, and J. Wiley. 1999. Flow cytometric  
668 analysis of phytoplankton, bacteria and viruses. *Current protocols in cytometry* **11**: 1-  
669 15.
- 670 Martin, S., G. Thouzeau, L. Chauvaud, F. Jean, and L. Guérin. 2006. Respiration,  
671 calcification, and excretion of the invasive slipper limpet, *Crepidula fornicata* L.:  
672 Implications for carbon, carbonate, and nitrogen fluxes in affected areas. *Limnology &*  
673 *Oceanography* **51**: 1996-2007.
- 674 Martin, S., G. Thouzeau, M. Richard, L. Chauvaud, F. Jean, and J. Clavier. 2007. Benthic  
675 community respiration in areas impacted by the invasive mollusk *Crepidula fornicata*.  
676 *Mar. Ecol.-Prog. Ser.* **347**: 51-60.
- 677 Matozzo, V., A. Chinellato, M. Munari, M. Bressan, and M. G. Marin. 2013. Can the  
678 combination of decreased pH and increased temperature values induce oxidative stress  
679 in the clam *Chamelea gallina* and the mussel *Mytilus galloprovincialis*? *Marine*  
680 *Pollution Bulletin* **72**: 34-40.
- 681 Matozzo, V., A. Chinellato, M. Munari, L. Finos, M. Bressan, and M. G. Marin. 2012. First  
682 evidence of immunomodulation in bivalves under seawater acidification and increased  
683 temperature. *PLoS One* **7**: e33820.

684 Mayzaud, P., and R. Conover. 1988. O: N atomic ratio as a tool to describe zooplankton  
685 metabolism. *Marine Ecology Progress Series* **45**: 289-302.

686 Mehrbach, C., Culberso, Ch, J. E. Hawley, and R. M. Pytkowic. 1973. Measurement of  
687 apparent dissociation-constants of carbonic-acid in seawater at atmospheric-pressure.  
688 *Limnology & Oceanography* **18**: 897-907.

689 Meistertzheim, A.-L., A. Tanguy, D. Moraga, and M.-T. Thébault. 2007. Identification of  
690 differentially expressed genes of the Pacific oyster *Crassostrea gigas* exposed to  
691 prolonged thermal stress. *Febs Journal* **274**: 6392-6402.

692 Melatunan, S., P. Calosi, S. D. Rundle, S. Widdicombe, and A. J. Moody. 2013. Effects of  
693 ocean acidification and elevated temperature on shell plasticity and its energetic basis  
694 in an intertidal gastropod. *Mar. Ecol.-Prog. Ser.* **472**: 155-168.

695 Melzner, F. and others 2009. Physiological basis for high CO<sub>2</sub> tolerance in marine  
696 ectothermic animals: pre-adaptation through lifestyle and ontogeny? *Biogeosciences*  
697 **6**: 2313-2331.

698 Melzner, F. and others 2011. Food supply and seawater pCO<sub>2</sub> impact calcification and  
699 internal shell dissolution in the blue mussel *Mytilus edulis*. *PLoS One* **6**: e24223.

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

703 Millero, F. J. 2007. The marine inorganic carbon cycle. *Chem. Rev.* **107**: 308-341.

704 Navarro, J. M., and O. R. Chaparro. 2002. Grazing-filtration as feeding mechanisms in motile  
705 specimens of *Crepidula fecunda* (Gastropoda : Calyptraeidae). *Journal of*  
706 *Experimental Marine Biology and Ecology* **270**: 111-122.

707 Navarro, J. M. and others 2013. Impact of medium-term exposure to elevated pCO<sub>2</sub> levels on  
708 the physiological energetics of the mussel *Mytilus chilensis*. *Chemosphere* **90**: 1242-  
709 1248.

710 Newell, R. C., and L. H. Kofoed. 1977. Adjustment of components of energy-balance in  
711 gastropod *Crepidula fornicata* in response to thermal acclimation. *Mar. Biol.* **44**: 275-  
712 286.

713 Noisette, F., J. Richard, I. Le Fur, L. Peck, D. Davoult, and M. Martin. 2015. Metabolic  
714 responses to temperature stress under elevated pCO<sub>2</sub> in the slipper limpet *Crepidula*  
715 *fornicata*. *J. Molluscan Stud.* **81**: 238-246.

716 Orr, J. C. and others 2005. Anthropogenic ocean acidification over the twenty-first century  
717 and its impact on calcifying organisms. *Nature* **437**: 681-686.

718 Pansch, C., I. Schaub, J. Havenhand, and M. Wahl. 2014. Habitat traits and food availability  
719 determine the response of marine invertebrates to ocean acidification. *Global Change*  
720 *Biology* **20**: 265-277.

721 Parsons, T. R., M. Takahashi, and B. Hargrave [eds.]. 1984. *Biological oceanographic*  
722 *processes* (3<sup>rd</sup> ed).

723 Pörtner, H.-O., and A. P. Farrell. 2008. Physiology and climate change. *Science* **322**: 690-  
724 692.

725 Pörtner, H.-O., M. Langenbuch, and B. Michaelidis. 2005. Synergistic effects of temperature  
726 extremes, hypoxia, and increases in CO<sub>2</sub> on marine animals: from Earth history to  
727 global change. *J. Geophys. Res.-Oceans* **110**: C09S10.

728 R Core Team. 2013. R: a language and environment for statistical computing. R Foundation  
729 for Statistical Computing.

730 Ragueneau, O. and others 2002. Direct evidence of a biologically active coastal silicate pump:  
731 ecological implications. *Limnology & Oceanography* **47**: 1849-1854.

- 732 Range, P. and others 2014. Impacts of CO<sub>2</sub>-induced seawater acidification on coastal  
733 Mediterranean bivalves and interactions with other climatic stressors. *Regional*  
734 *Environmental Change* **14 Suppl (1)**: S19-S30.
- 735 Range, P. and others 2011. Calcification, growth and mortality of juvenile clams *Ruditapes*  
736 *decussatus* under increased *p*CO<sub>2</sub> and reduced pH: Variable responses to ocean  
737 acidification at local scales? *Journal of Experimental Marine Biology and Ecology*  
738 **396**: 177-184.
- 739 Range, P. and others 2012. Seawater acidification by CO<sub>2</sub> in a coastal lagoon environment:  
740 effects on life history traits of juvenile mussels *Mytilus galloprovincialis*. *Journal of*  
741 *Experimental Marine Biology and Ecology* **424**: 89-98.
- 742 Ries, J. B., A. L. Cohen, and D. C. Mccorkle. 2009. Marine calcifiers exhibit mixed responses  
743 to CO<sub>2</sub>-induced ocean acidification. *Geology* **37**: 1131-1134.
- 744 Smith, S. V., and G. S. Key. 1975. Carbon-dioxide and metabolism in marine environments.  
745 *Limnology & Oceanography* **20**: 493-495.
- 746 Solomon, S. and others [eds.]. 2007. Contribution of working group I to the fourth assessment  
747 report of the Intergovernmental Panel on Climate Change.
- 748 Solorzano, L. 1969. Determination of ammonia in natural waters by the phenylhypochlorite  
749 method. *Limnology & Oceanography* **14**: 799-801.
- 750 Somero, G. 2010. The physiology of climate change: how potentials for acclimatization and  
751 genetic adaptation will determine "winners" and "losers". *J. Exp. Biol.* **213**: 912-920.
- 752 Stocker, T. F. and others 2013. Climate Change 2013. The Physical Science Basis. Working  
753 Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel  
754 on Climate Change. *In* C. U. Press [ed.]. Groupe d'experts intergouvernemental sur  
755 l'evolution du climat/Intergovernmental Panel on Climate Change-IPCC, C/O World  
756 Meteorological Organization, 7bis Avenue de la Paix, CP 2300 CH-1211 Geneva 2  
757 (Switzerland).
- 758 Sylvester, F., J. Dorado, D. Boltovskoy, A. Juarez, and D. Cataldo. 2005. Filtration rates of  
759 the invasive pest bivalve *Limnoperna fortunei* as a function of size and temperature.  
760 *Hydrobiologia* **534**: 71-80.
- 761 Thomsen, J., I. Casties, C. Pansch, A. Kortzinger, and F. Melzner. 2014. Food availability  
762 outweighs ocean acidification effects in juvenile *Mytilus edulis*: laboratory and field  
763 experiments. *Global Change Biology* **19**: 1017-1027.
- 764 Thomsen, J., and F. Melzner. 2010. Moderate seawater acidification does not elicit long-term  
765 metabolic depression in the blue mussel *Mytilus edulis*. *Mar. Biol.* **157**: 2667-2676.
- 766 Tomanek, L., M. J. Zuzow, A. V. Ivanina, E. Beniash, and I. M. Sokolova. 2011. Proteomic  
767 response to elevated *p*CO<sub>2</sub> level in eastern oysters, *Crassostrea virginica*: evidence for  
768 oxidative stress. *J. Exp. Biol.* **214**: 1836-1844.
- 769 Vargas, C. A. and others 2013. CO<sub>2</sub>-driven ocean acidification reduces larval feeding  
770 efficiency and change food selectivity in the mollusk *Concholepas concholepas*.  
771 *Journal of Plankton Research* **in press**.
- 772 Von Bertalanffy, L. 1951. Metabolic types and growth types. *The American Naturalist* **85**:  
773 111-117.
- 774 Von Bertalanffy, L. 1964. Basic concepts in quantitative biology of metabolism. *Helgoland*  
775 *Marine Research* **9**: 5-37.
- 776 Waldbusser, G. G., and J. E. Salisbury. 2013. Ocean acidification in the coastal zone from an  
777 organism's perspective: multiple system parameters, frequency domains, and habitats.  
778 *Annual Review of Marine Science* **6**: 221-247.
- 779 Waldbusser, G. G., E. P. Voigt, H. Bergschneider, M. A. Green, and R. I. Newell. 2011.  
780 Biocalcification in the eastern oyster (*Crassostrea virginica*) in relation to long-term  
781 trends in Chesapeake Bay pH. *Estuaries and Coasts* **34**: 221-231.

782 Wolf-Gladrow, D. A., R. E. Zeebe, C. Klaas, A. Kortzinger, and A. G. Dickson. 2007. Total  
783 alkalinity: the explicit conservative expression and its application to biogeochemical  
784 processes. *Marine Chemistry* **106**: 287-300.  
785 Zeebe, R. E. 2011. History of seawater carbonate chemistry, atmospheric CO<sub>2</sub>, and ocean  
786 acidification, p. 141-165. *In* R. Jeanloz [ed.], *Annual Review of Earth and Planetary*  
787 *Sciences*, Vol 40. *Annual Review of Earth and Planetary Sciences*. Annual Reviews.

788

789

790

791

792

793

794

795

796

797

798

799

## 800 **Acknowledgments**

801 The authors thank the “Marine Operations and Services Department” at the *Station*  
802 *Biologique de Roscoff* for the underwater sampling. We also thank the “Multicellular Marine  
803 Models” staff, and especially Ronan Garnier, for providing microalgae and their help for  
804 building the aquarium system. In addition, we are grateful to SOMLIT (Service d’Observation  
805 en Milieu LITtoral, INSU-CNRS) for providing the abiotic parameter datasets. We are also  
806 grateful to Roseline Edern for her help with cytometry flux analysis. We really appreciated  
807 the editor and reviewers’ helpful and constructive comments which greatly improved this  
808 manuscript. This work was supported by the CALCAO project, which received funding from  
809 the Brittany Regional Council, and contributed to the “European Project on Ocean

810 Acidification'' (EPOCA) funded by the European Community's Seventh Framework  
811 Programme (FP7/2007-2013) under grant agreement no. 211384. It was also supported by the  
812 Interreg IV a France (Channel) – England Marinexus project no. 4073 funded by the FEDER  
813 program.

814

## Tables

**Table 1:** Mean seawater temperature and parameters of the carbonate system in each  $p\text{CO}_2$  treatment (3 aquaria per treatment) and at each trial period (i.e. temperature level). The  $\text{pH}_T$  (pH on the total scale) and total alkalinity ( $A_T$ ) were measured whereas the other parameters were calculated. Mean  $A_T$  calculated for each trial period ( $n = 3$  for controls  $10^\circ\text{C}$  and  $19 < n < 30$  for other condition  $p\text{CO}_2$  conditions) and  $p\text{CO}_2$  condition was used for the calculations.  $p\text{CO}_2$ ,  $\text{CO}_2$  partial pressure; DIC, dissolved inorganic carbon and  $\Omega_{\text{Ar}}$ , saturation state of seawater with respect to aragonite.

	n	Temperature ( $^\circ\text{C}$ )		$\text{pH}_T$		$p\text{CO}_2$ ( $\mu\text{atm}$ )		$A_T$ ( $\mu\text{Eq kg}^{-1}$ )		DIC ( $\mu\text{mol C kg}^{-1}$ )		$\Omega_{\text{Ar}}$	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
<b>1<sup>st</sup> trial period (<math>10^\circ\text{C}</math>)</b>													
390 $\mu\text{atm}$	23	9.7	0.2	8.14	0.01	322	7	2365	2	2138	4	2.47	0.04
750 $\mu\text{atm}$	23	9.8	0.2	7.82	0.01	729	19	2368	2	2270	4	1.33	0.03
1400 $\mu\text{atm}$	23	9.5	0.2	7.55	0.03	1486	75	2376	2	2366	11	0.78	0.08
control $10^\circ\text{C}$	40	9.2	0.2	8.19	0.02	288	17	2370	3	2115	8	2.73	0.07
<b>2<sup>nd</sup> trial period (<math>13^\circ\text{C}</math>)</b>													
390 $\mu\text{atm}$	27	12.9	0.2	8.12	0.02	356	25	2418	2	2167	8	2.76	0.07
750 $\mu\text{atm}$	27	13.0	0.1	7.81	0.01	781	20	2416	2	2304	3	1.48	0.03
1400 $\mu\text{atm}$	27	12.8	0.1	7.53	0.01	1557	43	2422	2	2405	4	0.82	0.02
control $10^\circ\text{C}$	41	11.0	0.1	8.18	0.01	297	12	2419	2	2152	5	2.88	0.05
<b>3<sup>rd</sup> trial period (<math>16^\circ\text{C}</math>)</b>													
390 $\mu\text{atm}$	28	15.9	0.1	8.08	0.01	376	10	2379	5	2126	5	2.80	0.05
750 $\mu\text{atm}$	28	16.1	0.1	7.82	0.00	748	8	2369	5	2238	2	1.66	0.01
1400 $\mu\text{atm}$	28	16.0	0.1	7.55	0.01	1492	19	2380	5	2345	2	0.94	0.01
control $10^\circ\text{C}$	42	11.4	0.1	8.23	0.01	253	6	2376	4	2083	5	3.13	0.05

**4<sup>th</sup> trial period (19°C)**

---

390 µatm	23	18.4	0.5	8.02	0.01	450	10	2391	2	2152	5	2.70	0.05
750 µatm	23	18.6	0.5	7.77	0.01	858	19	2395	3	2266	4	1.68	0.04
1400 µatm	23	18.4	0.5	7.51	0.01	1652	41	2394	2	2359	4	0.96	0.03
control 10°C	23	12.4	0.1	8.20	0.01	280	12	2393	1	2107	8	3.07	0.08



1 **Table 2:** Summary of two-way repeated measurements ANOVAs followed by Student-Newman-Keuls post hoc tests testing the effect of  $p\text{CO}_2$ ,  
 2 temperature and their interaction on *Crepidula fornicata* physiology. Numbers in bold indicate significant p-values and values with different  
 3 letters are significantly different at  $p < 0.05$ .

	Two-way repeated measurements ANOVAs						Post hoc SNK tests									
	Factors			Factors			Factors			Factors						
	$p\text{CO}_2$		Temperature	$p\text{CO}_2 \times \text{Temperature}$		$p\text{CO}_2$ ( $\mu\text{atm}$ )	Temperature ( $^\circ\text{C}$ )			10	13	16	19			
	df	F	p	df	F	p	df	F	p	390	750	1400	10	13	16	19
<b>Small individuals</b>																
Respiration	2	1.685	0.219	3	14.530	< <b>0.001</b>	6	1.893	0.103				a	b	b	c
Excretion	2	0.386	0.686	3	5.840	<b>0.002</b>	6	1.257	0.296				a	a,b	b	b
Filtration	2	0.271	0.766	3	15.439	< <b>0.001</b>	6	5.996	< <b>0.001</b>				a	a	a	b
Net calcification	2	6.705	<b>0.008</b>	3	1.849	0.152	6	2.307	<b>0.050</b>	a	a	b				
O:N ratio	2	4.944	<b>0.022</b>	2	2.214	0.127	4	0.382	0.819	a	b	a				
<b>Large individuals</b>																
Respiration	2	0.377	0.692	3	8.398	< <b>0.001</b>	6	0.523	0.788				a	b	b	b
Excretion	2	0.563	0.581	3	17.850	< <b>0.001</b>	6	0.371	0.893				a	b	c	b
Filtration	2	1.593	0.236	3	19.311	< <b>0.001</b>	0	2.012	0.083				a	b	b	c
Net calcification	2	13.615	< <b>0.001</b>	3	0.878	0.459	6	0.911	0.496	a	a	b				
O:N ratio	2	0.739	0.494	2	20.714	< <b>0.001</b>	4	1.728	0.170				-	a	b	a

4

5 **Figures**

6

7 **Figure 1:** Individual respiration, ammonia excretion, filtration and net calcification rates in the three  
8  $p\text{CO}_2$  treatments (shaded in grey) at 10, 13, 16 and 19°C for small (< 3 cm in length) and large (> 4.5 cm  
9 in length) *C. fornicata* individuals. Different letters above bars or before  $p\text{CO}_2$  caption indicate significant  
10 differences between temperature or  $p\text{CO}_2$  conditions, respectively. Results are expressed as mean  $\pm$   
11 standard error, n = 6 individuals.

12

13 **Figure 2:** O:N ratios for the three  $p\text{CO}_2$  treatments (shaded in grey) at 13, 16 and 19°C for small and  
14 large *C. fornicata* individuals. Different letters above bars or before  $p\text{CO}_2$  caption indicate significant  
15 differences between temperature or  $p\text{CO}_2$  conditions, respectively. Results are expressed as mean  $\pm$   
16 standard error, n = 6 individuals.

17

18 **Figure 3:** Mean net calcification rates as function of aragonite saturation state, in the three  $p\text{CO}_2$   
19 treatments (shaded in grey), at 10 (○), 13 (△), 16 (□) and 19°C (◇) for all *C. fornicata* individuals (n =  
20 12 individuals).

21

22 **Figure 4:** Respiration rates in the control treatment (10°C) for the different trial periods (i.e. temperature  
23 levels) for single small (white bars) and large (grey bars) *C. fornicata* individuals. Results are expressed  
24 as mean  $\pm$  standard error, n = 6 individuals.

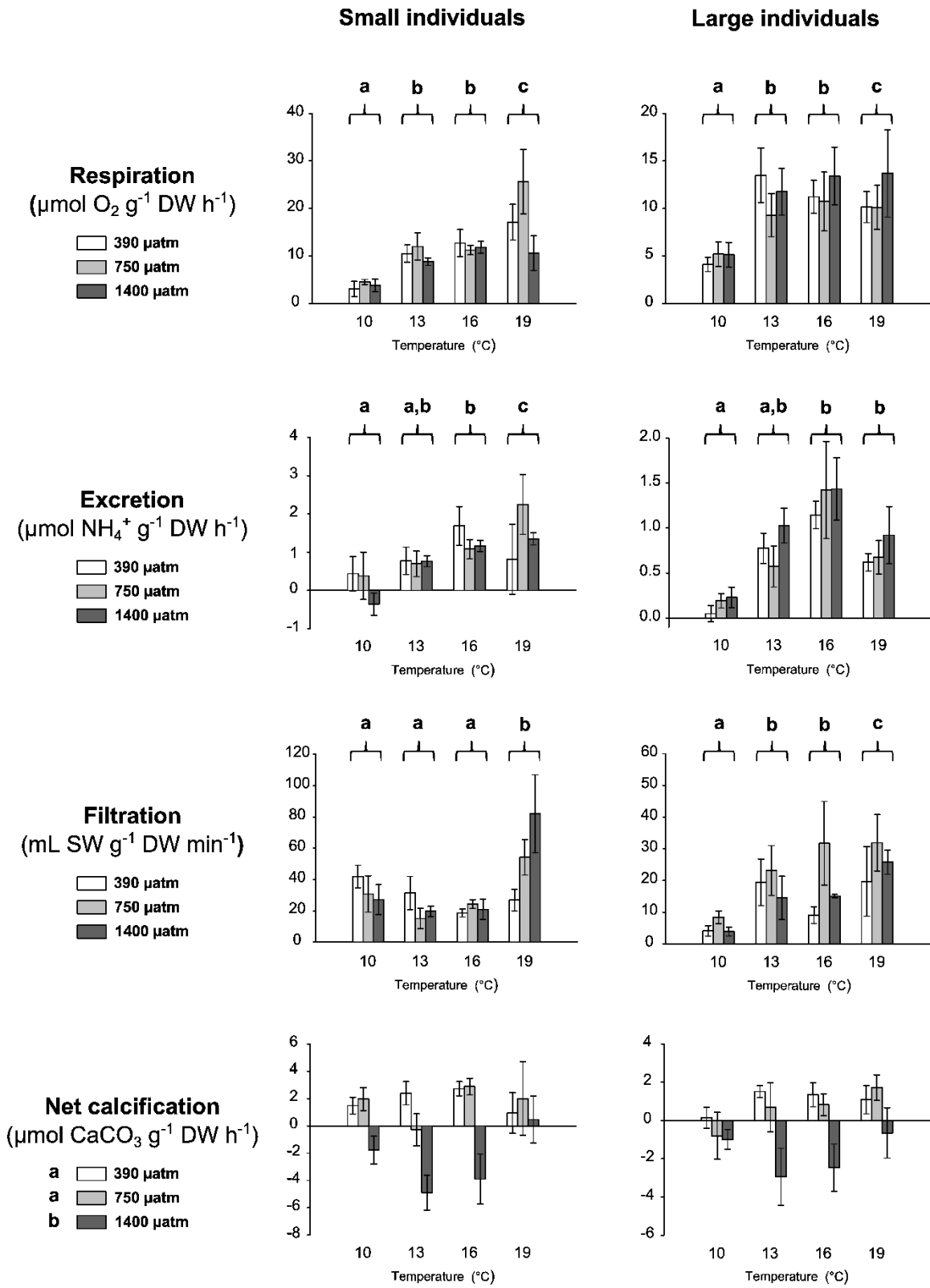
25

26

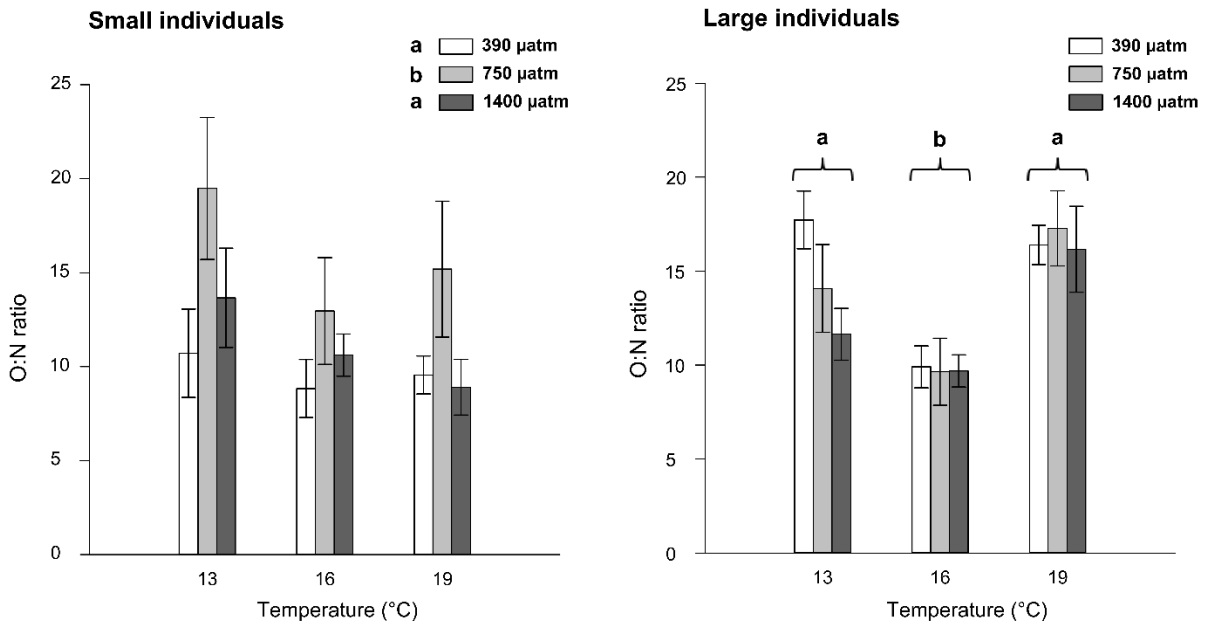
27

28

29



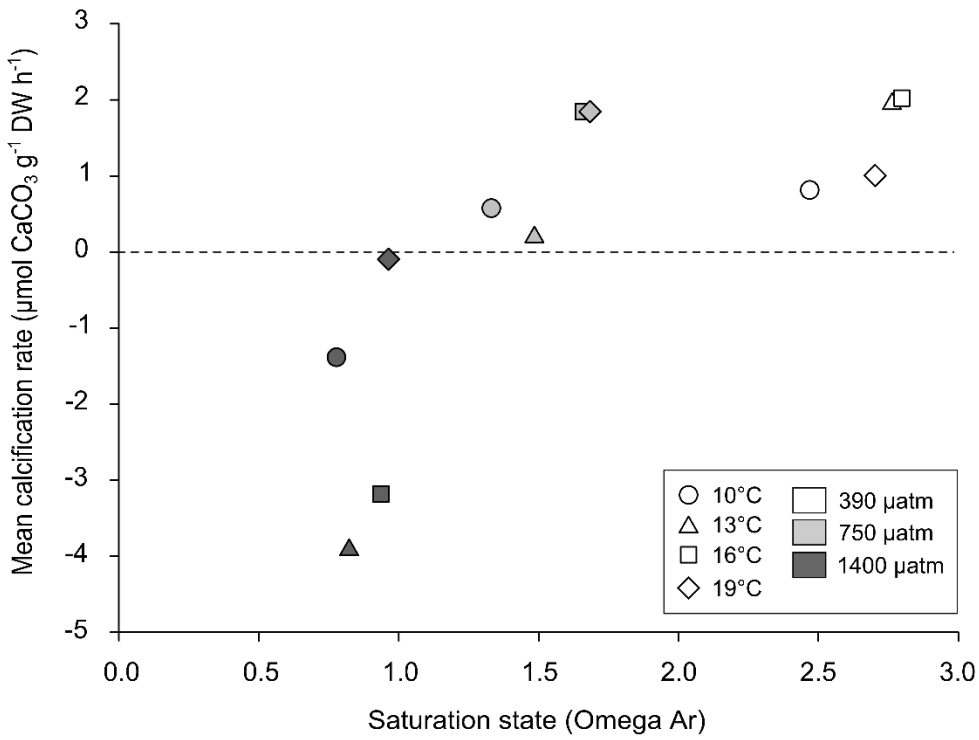
33 Figure 2:



34

35

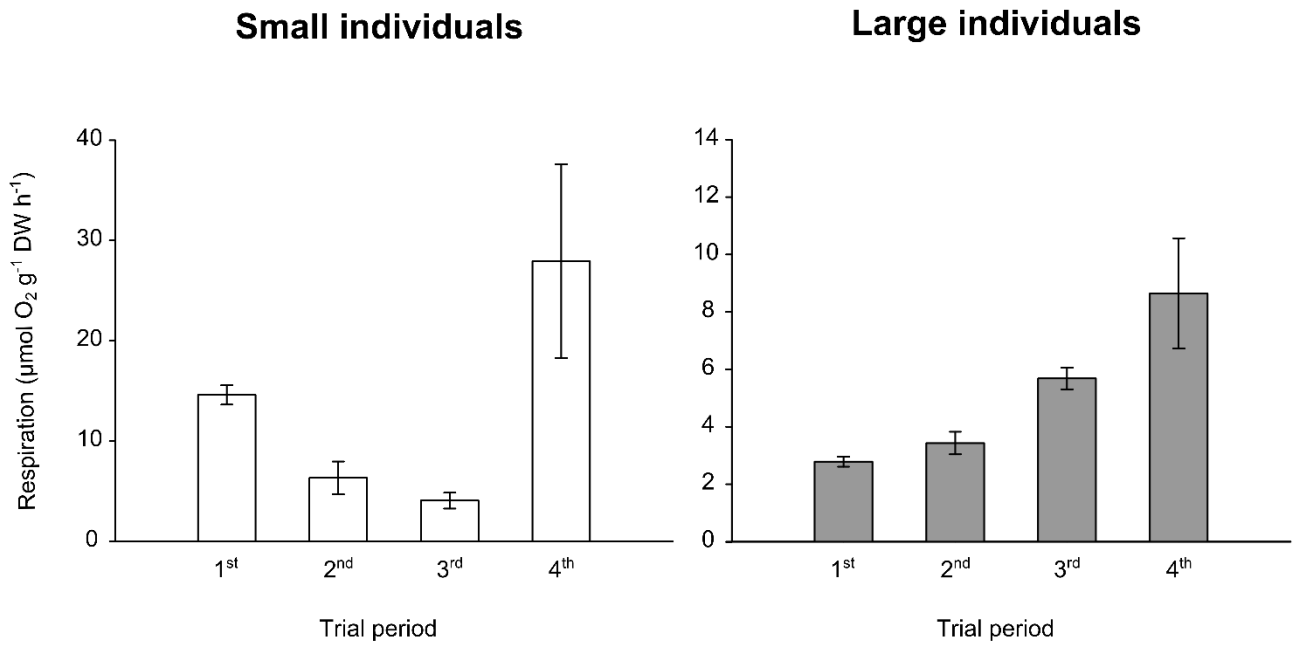
36 Figure 3:



37

38

39 Figure 4:



40