

## Similar metabolic responses of co-occurring post-settlement mussels to temperature change despite distinct geographical distributions

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1 **Similar metabolic responses of co-occurring post-settlement mussels to**  
2 **temperature change despite distinct geographical distributions**

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13

14 **ABSTRACT:**

15 For marine animals with biphasic life stages, different environmental conditions are  
16 experienced during ontogeny so that physiological constraints on early stages could explain  
17 adult distributions and life history traits. The invasive and cool-temperate adapted *Mytilus*  
18 *galloprovincialis* intertidal mussel approaches the eastern limit of its biogeographic  
19 distribution on the south coast of South Africa, where it shares a habitat with the warm-  
20 temperate adapted and indigenous *Perna perna* mussel. As adults, the two species exhibit  
21 different metabolic regulation capacities in response to temperature. We compared the acute  
22 metabolic response to temperature between species during the post-settlement recruit stage.  
23 Aerobic respiration rates of recently settled recruits were measured monthly for five months  
24 for temperatures 5 °C above or below the ambient field seawater temperature at the time of  
25 collection. Unlike adults, the capacity for aerobic metabolic regulation in response to  
26 temperature differed little between species under the conditions tested, indicating a similar  
27 degree of phenotypic or developmental plasticity in response to the thermal environment. In  
28 addition, monthly variations in metabolic patterns indicate unexpectedly high plasticity in  
29 response to recent seasonal thermal history for both species.

30 **KEY WORDS:** Phenotypic plasticity, Ontogeny, Thermal acclimation, Bivalve, Intertidal,  
31 Marginal habitats, Mytilidae, Respirometry

32

### 33 INTRODUCTION

34 Since species fitness, their life history characteristics and ultimately their biogeographic  
35 distribution are driven by environmental tolerance and metabolic capacity (Brown 1984;  
36 Brown et al. 2004; Verberk et al. 2016), establishing metabolic sensitivity to abiotic factors is  
37 important for understanding the dynamics among co-occurring species. Living within an  
38 optimal temperature range is particularly important for ectotherms since temperature directly  
39 affects metabolism and other physiological functions and rates (Pörtner 2002; Angilletta et al.  
40 2010).

41 Population distribution is influenced by animal fitness through different life stages  
42 (Byrne and Przeslawski 2013), especially for species with biphasic life cycles. Both  
43 acclimation potential and environmental tolerance limits can vary with ontogeny and age in  
44 marine ectotherms (Byrne 2011; Freda et al. 2019). For example, post-settlement juvenile  
45 mussels, referred to as “recruits”, often have a higher capacity for rapid phenotypically  
46 plastic responses to temperature (Lou et al. 1982; Gleason et al. 2018) as compared to adults.  
47 This could be explained by developmental plasticity and adjustment to a new environment  
48 (Peyer et al. 2010). Through developmental plasticity, the thermal regime experienced during  
49 early life stages influences fitness and thermal tolerance ranges in advanced stages (Stillwell  
50 et al. 2005; Cavieres et al. 2019). Adult mussels display thermal tolerances based on local  
51 adaptation (Zardi et al. 2011; Tagliarolo and McQuaid 2015), however, understanding the  
52 limits of distribution requires establishing the thermal sensitivities and phenotypic plasticity  
53 for their earlier ontogenetic stages (Gleason et al. 2018; Truebano et al. 2018).

54 Interspecific differences in thermal sensitivity influence the fitness of competing co-  
55 occurring species. In South Africa, the invasive *Mytilus galloprovincialis* dominates mussel  
56 populations on the cool-temperate west coast (Zardi et al. 2007a; McQuaid et al. 2015) and  
57 shows partial habitat segregation with the indigenous *Perna perna* on the warm-temperate

58 south coast, where *M. galloprovincialis* dominates the upper mussel zone and *P. perna* the  
59 lower shore, with overlap in the middle (Bownes and McQuaid 2006, 2009). This simple  
60 pattern emerges from complex effects concerning dispersal, recruitment and adult  
61 interactions (McQuaid et al. 2015), including differences in physiological responses to  
62 temperature change and thermal stress in adults (Tagliarolo and McQuaid 2015). Adult *M.*  
63 *galloprovincialis* display a lower heating activation energy in metabolism (heart rate)  
64 compared to the eastern lineage of *P. perna*, indicating a lack of acclimation, and a lower  
65 cooling activation energy compared to the co-occurring western *P. perna* lineage, indicating  
66 metabolic efficiency at cool temperatures (Tagliarolo and McQuaid 2015). There is currently  
67 no information available on the physiological competitive strategies of the early stages of  
68 post-settlement co-occurring *P. perna* and *M. galloprovincialis* recruits where both primarily  
69 settle in the low-intertidal zone (Porri et al. 2007; Bownes and McQuaid 2009) and display  
70 similar survival there as opposed to the high intertidal zone (Bownes and McQuaid 2009).  
71 We hence expected post-settlement *M. galloprovincialis* to be more sensitive to fluctuation to  
72 high temperature due to its cool-temperate biogeographical adaptation and to therefore  
73 display a lower degree of plasticity or an alternative regulating response, as seen during  
74 emersion in adults (Tagliarolo and McQuaid 2015). Here, we used respirometry to compare  
75 the acute metabolic response to temperature change and metabolic plasticity of co-occurring  
76 recruits of *P. perna* and *M. galloprovincialis* as a possible contributor to the biogeographic  
77 limit of *M. galloprovincialis* distribution on the south coast.

## 78 **MATERIALS AND METHODS**

### 79 **Collection of mussel recruits**

80 Newly recruited *Perna perna* and *Mytilus galloprovincialis* were collected monthly between  
81 May 2018 (year henceforth denoted as '18') and February 2019 (henceforth '19'), in Algoa  
82 Bay, South Africa (33°98' S, 25°67' E), using 8–10 filamentous plastic scouring pads

83 (Menge 1992; Bownes et al. 2008; Figure 1) at fixed positions 50–100 cm apart. These were  
84 fastened to a flat rock (3 x 4 m) within the low intertidal zone. Small recruits (<765 µm and  
85 <686 µm for *Mytilus* and *Perna*; Bownes et al. 2008) were collected for all sampling months,  
86 except for Oct 18 and Jan 19, when the average size comprised ‘large recruits’ (> 765 µm;  
87 Bownes et al. 2008).

88 A sealed Thermochron iButton temperature logger was attached adjacent to one  
89 scouring pad to record temperature every thirty minutes for the month preceding the  
90 collection of pads. Intertidal site air temperatures for sampled months were: 14.1–21.1 °C  
91 (May 18, minimum–maximum), 9.6–21.1 °C (Jul 18), 10.6–22.6 °C (Aug 18), 12.6–32.6 °C  
92 (Oct 18), 15.6–31.1 °C (Dec 18) and 13.6–32.6 °C (Jan 19). Estimated periods of immersion  
93 and associated temperatures (Table 1) were identified as those occurring between sharp  
94 spikes in temperature increase or decrease (Monaco et al. 2019). The scouring pads were  
95 transported in a plastic container filled with a thin layer of seawater to maintain humidity, and  
96 kept moderately cool with a single ice brick during the 90 minutes of transportation from the  
97 field site to the laboratory. The seawater temperature at the time and point of collection was  
98 recorded through three consecutive instantaneous measurements using a thermocouple to set  
99 the (‘at collection’) temperature employed at the controlled-temperature laboratory at the  
100 Aquatic Ecophysiology Research Platform (AERP) of the South African Institute for Aquatic  
101 Biodiversity (Grahamstown, South Africa).

102 In the temperature-controlled room, scouring pads were submerged in unfiltered  
103 seawater collected at the sampling site in two 10 L plastic containers and aerated using air  
104 pumps. Depending on the number of scouring pads in the containers, the whole volume of  
105 seawater was exchanged for fresh seawater kept at the same temperature once or twice a day.  
106 One to three scouring pads were processed per day to collect individual recruits, which were

107 then placed in 0.5  $\mu\text{m}$  filtered seawater at collection temperature for trials on the following  
108 day. This allowed for a fasting period of 18–24 hours prior to respirometry measurements.

### 109 **Respirometry**

110 Daytime oxygen consumption was measured during immersion to record aerobic metabolism  
111 during valve opening (McMahon 1988; Tagliarolo and McQuaid 2015), using a closed  
112 respirometry system. Prior to the experiments, recruits were maintained in aerated freshly  
113 filtered seawater in a water bath set at collection temperature.

114 The oxygen consumption of different sets of animals was measured at the field  
115 seawater ‘collection’ temperature and two others respectively at 5 °C above or below this  
116 (hereafter ‘+5 °C’ and ‘-5 °C’ treatment, respectively). The ‘+5 °C’ and ‘-5 °C’ temperature  
117 change covered what we anticipated to be the thermal ranges experienced by the recruits  
118 during immersion, while aiming to stimulate a metabolic response (Paschke et al. 2018).  
119 Measurements were conducted for a maximum of seven days following collection from the  
120 field. To measure oxygen consumption at the +5 °C and -5 °C temperatures, recruits were  
121 gradually exposed to the new temperature, at a ramping rate of 0.17 °C per minute for 30  
122 minutes (Tagliarolo and McQuaid 2015). This ramping was followed by a 90-minute  
123 acclimation period at the +5 °C and -5 °C temperatures before transfer to respirometry  
124 chambers.

125 The recruits were viewed under a stereo microscope to select healthy-looking  
126 individuals, which responded to gentle stimuli through valve closure, and transferred into 21  
127 individual respirometry chamber wells (80 or 200  $\mu\text{L}$ ) in a Loligo® Systems (Denmark) 24-  
128 well multiplate with optical fluorescence-based oxygen sensors (SensorDish® Reader SDR2,  
129 PreSens, Germany). Three remaining wells, which contained no recruits, were used as  
130 controls for background bacterial respiration rates and were subtracted from experimental

131 recruit respiration rates. Freshly filtered seawater maintained at the measurement temperature  
132 was used to fill up the wells to a convex meniscus, and the microplate was sealed by a sheet  
133 of parafilm, then a silicon seal, finally followed by a compression block. The sealed  
134 multiplate was transferred to a temperature sensor equipped experimental water chamber  
135 (Figure 1) which recirculated externally through a programmed water bath. The multiplate  
136 was kept under darkness for measurements of standard oxygen consumption rates (Nelson  
137 and Chabot 2011; Vorsatz et al. 2021). Oxygen concentration in the wells was recorded for  
138 60–90 minutes at three-minute intervals until a linear decrease in oxygen levels reached 60 %  
139 of the initial levels in the wells to maintain a linear relationship between oxygen level and  
140 time (Jupe et al. 2020).

141       Following respirometry, the recruits were placed in 100 % ethanol and subsequently  
142 measured using an Olympus SZX16 stereo microscope with a built-in camera and Stream  
143 Essentials image capturing and analysis software. Shell length (the longest distance from the  
144 umbo to the furthest posterior tip), shell height (the longest distance between the dorsal and  
145 ventral shell margins) and width (the longest width of the dorsal view) were measured  
146 (Bownes et al. 2008). The volume (L) of each animal was assumed to be that of an ellipsoid  
147 (Filgueira et al. 2006) and calculated as:  $(\pi/6) \times \text{shell height (dm, decimeter)} \times \text{length (dm)} \times$   
148  $\text{shell width (dm)}$ . Respiration rates ( $MO_2$ ) in nanomoles  $O_2 \text{ min}^{-1}$  were calculated from the  
149 linear slope of the change in  $O_2$  over time, during the incubation period, multiplied by the  
150 remaining chamber volume (L) (chamber volume minus the volume of the animal). Total dry  
151 mass ( $\mu\text{g}$ ) values were determined from dry mass ( $\mu\text{g}$ ) vs. length (mm) regression  
152 relationships calculated for bivalve veliger larvae by James (1987) as follows: dry mass ( $\mu\text{g}$ )  
153  $= 47.386 \times (\text{shell length in mm})^{3.663}$ . Recruit sizes varied within and between months and was  
154 an essential covariate. Using length and allometrically calculated dry masses produced the  
155 same model output.



156 **Statistical analysis**

157 Preliminary analyses comparing size-corrected metabolic rates (see below) between replicate  
158 days within the same month, using Kruskal-Wallis and Wilcoxon ranks sum tests, confirmed  
159 that replicates could be pooled ( $p > 0.05$ ).

160 To establish the most important determinants of  $MO_2$ , best-fit mixed generalised least  
161 squares (GLS) regression models were fitted using maximum likelihood estimates (R<sup>®</sup> 4.0.2  
162 statistical software; R Development Core Team 2020) and the ‘nlme’ package (Pinheiro et al.  
163 2012). This analysis is robust to variance differences among the different months that  
164 comprised the present study. Linear regressions of  $MO_2$  ( $\text{nmol O}_2 \text{ min}^{-1}$ ) vs dry mass ( $\mu\text{g}$ )  
165 were fitted after  $\log_{10}$ - $\log_{10}$  transformations using GraphPad Prism 9.0 (GraphPad Software,  
166 San Diego California, USA). All  $MO_2$  and dry mass data were logarithm transformed prior to  
167 analyses due to the linear relationships between  $\log_{10}$ - $\log_{10}$   $MO_2$  and dry mass (Chang &  
168 Hou 2005). Mass-specific metabolic rates ( $\text{nmol O}_2 \text{ min}^{-1} \mu\text{g}^{-1}$ ) were calculated for  
169 visualisation. Due to variability in size ranges among months, the  $MO_2$  response variable was  
170 adjusted for the size covariate by fitting a GLS model with the covariate, and by generating a  
171 new corrected  $MO_{2c}$  response variable from the residuals (step 2). This was done separately  
172 for each species, prior to selection of model variables (Zuur et al. 2009). Separately for the -5  
173 °C and the +5 °C treatment, the selection of significant model variables among species,  
174 temperature (‘collection’ vs. +5 °C or ‘collection’ vs. -5 °C), month and the interactions  
175 among them, was conducted based on the best-fit Akaike’s information criterion (AIC) for  
176 variables that best explained  $MO_2$  (Table S1). The AIC was used for backward model  
177 selection (Zuur et al. 2009). Significant p-values (ANOVA) for interactions further informed  
178 model selection when AIC values were similar. Post-hoc Tukey HSD test-95 % family-wise  
179 confidence levels for interaction ANOVA models corresponding to the full GLS model

180 (Table S1) informed on pairwise differences between temperature treatments and species  
181 within each month.

182 The two species did not always settle in each month, and it was therefore not possible  
183 to do a full factorial model comparing species responses to both +5 °C and -5 °C temperatures  
184 for all months. The effects of species and temperature (+5 °C or -5 °C) were thus evaluated in  
185 six separate comparisons. The first two comparisons tested for the combined effects of  
186 species and temperature, separately for the +5 °C and -5 °C temperatures ('Species and +5  
187 °C' and 'Species and -5 °C' models). Subsequently, four tests determined responses to  
188 temperature treatment for each species (size-corrected  $MO_2$  in response to +5 °C or -5 °C and  
189 month): '*Mytilus* and -5 °C', '*Mytilus* and +5 °C', '*Perna* and -5 °C' and '*Perna* and +5 °C'.

190 Arrhenius plots, describing the relationship between the natural logarithm of mass-  
191 specific respiration rate ( $\ln R$ ) and temperature (T), were calculated as  $\ln R = \ln a - E/k * 1/T$   
192 (Arrhenius 1889). The slope ( $-E/k$ ) defines the Arrhenius activation energy ( $E$ ) and was  
193 compared between species and among months using GraphPad Prism 9.0. The slopes and  
194 intercepts ( $\beta_0$  and  $\beta_1$ ) of all linear regression lines were compared using a t-test, where the  
195 difference between two regression coefficients is divided by the difference of their respective  
196 standard errors (Zar 1984).

## 197 **RESULTS**

198 Respirometry measurements were performed for months during which enough recruits had  
199 settled (Table 1). Lower numbers of animals were tested at the highest temperature

200 (collection + 5 °C) of 26 °C as linear relationships between oxygen decline and time was  
201 difficult to obtain due to lower oxygen tension.

## 202 **Effects of species and temperature on $MO_2$**

203 *Perna* and *Mytilus* recruits reacted similarly to both -5 °C and +5 °C temperatures (Table S1)  
204 as species was not a significant variable for metabolic rate. For both the ‘Species and -5 °C’  
205 and ‘Species and +5 °C’ comparisons, temperature was a significant determinant of metabolic  
206 rate ( $p < 0.001$ ). There were interactions between species and temperature as species differed  
207 within Dec 18 for the -5 °C and +5 °C temperatures (Figure 2 and 3), when *Perna* recruits  
208 displayed lower metabolic rates for the -5 °C temperature and higher rates for the +5 °C  
209 temperature. There was an interaction between species and month in Oct 18 where species  
210 differed for the collection temperature within Oct 18 (Figure 2 and 3), probably owing to  
211 relatively larger sizes of *Perna* recruits collected in that month (Table S2). The best-fit  
212 (selected) models displayed similar fits (AIC) compared to the full models which  
213 incorporated three-way interactions between species, month and temperature (Table S1).  
214 Within May 18 and Dec 18, *Mytilus* metabolic rates were significantly higher for the +5 °C  
215 compared to the collection temperature which was not the case for *Perna* (Figure 3).  
216 Additionally, there were no differences between species for Arrhenius slopes for either the -5  
217 °C (Oct 18 and Dec 18;  $F = 0.5$  and  $2.3$ ,  $p = 0.5$  and  $0.1$  respectively) or the +5 °C  
218 temperatures (May 18, Jul 18, Oct 18 and Dec 18;  $F = 0.3$ – $1.2$ ,  $p = 0.3$ – $0.6$ ).

219 The effects of temperature and temperature-month interactions on *Mytilus* metabolic  
220 rates were significant for both the -5 and +5 °C temperatures (ANOVA,  $p < 0.001$ ). *Mytilus*  
221 metabolic rates at -5 °C temperatures were lower than at collection temperatures within May  
222 18 and Jul 18 (Figure 4A), whereas metabolic rates at +5 °C were higher than those at  
223 collection temperatures within May 18 and Dec 18 (Figure 3 and 4B). Arrhenius slopes for

224 *Mytilus* differed only among months for the +5 °C temperatures where metabolic activation  
225 energies were higher in May 18 than all other months except Dec 18 (Table 2).  
226 For *Perna*, temperature and month interactions on metabolic rate were significant for the +5  
227 °C temperature ( $p < 0.001$ ). Lower metabolism at the -5 °C temperature than at the collection  
228 temperature was observed within Jan 19 (Figure 4C), while higher metabolic rates at the +5  
229 °C temperature than the collection temperature were recorded within May 18 and Jan 19  
230 (Figure 4D). The Arrhenius slopes were higher in Jan 19 than all other months (Table 2) for  
231 both -5 and +5 °C temperatures.

## 232 **DISCUSSION**

233 The aerobic metabolic response of mussel recruits to temperature change was similar between  
234 species and varied across months for both species. We expected inter-specific differences in  
235 physiological response to increased temperature due to *M. galloprovincialis*' dominance in  
236 the cool-temperate west coast and the study site's proximity to the warm edge of *M.*  
237 *galloprovincialis*' distribution on the southeast coast. Here, the transition between the cooler  
238 warm-temperate biogeographic region and the warmer subtropical region limits the northern  
239 spread of *M. galloprovincialis* (Harrison 2002; Assis et al. 2015). Instead, we found similar  
240 activation energies and therefore physiological sensitivities for warming for both species.  
241 Despite their recent spread along the southeast coast and low genetic heterogeneity among  
242 populations (Zardi et al. 2007a), acclimation to warmer waters at the biogeographic edge  
243 could modulate metabolic response. Towards the warm edge of their distribution range in  
244 Chile, *Scurria zebrina* limpets displayed higher thermal optima temperatures compared to  
245 populations in the centre of their biogeographic range (Broitman et al. 2018). Similar to  
246 observations in the present study, adult *M. galloprovincialis* individuals sampled at St Francis  
247 Bay (approximately 80 km west of Algoa Bay), South Africa, displayed similar metabolic  
248 activation energies in response to warming compared to co-occurring *P. perna* (Tagliarolo

249 and McQuaid 2015). Acclimation and the development of tolerance to a warmer and more  
250 variable environment may occur during early ontogeny through differential expression of  
251 various physiological and morphological traits (Peyer et al. 2010; Ravaux et al. 2016; Lardies  
252 et al. 2021), shaping thermal tolerance limits in adults (Ravaux et al. 2016).

253 Higher metabolism in response to increased temperature in the present study occurred  
254 within warmer months (Dec 18 and Jan 19 for *M. galloprovincialis* and *P. perna*,  
255 respectively), when the +5 °C was 1–2 °C higher than the maximum submerged temperature,  
256 and for May 18, when the +5 °C was 3 °C higher than the maximum temperature  
257 experienced. Both early post-settlement *Mytilus* and *Perna* could therefore increase their  
258 metabolism effectively at warmer temperatures, suggesting metabolic plasticity for both  
259 species during the post-settlement phase. In contrast to the similar inter-specific cooling  
260 activation energies for recruits in the present study, adult *M. galloprovincialis* individuals  
261 displayed lower activation energies in response to cooling compared to co-occurring *P. perna*  
262 in St Francis Bay (Tagliarolo and McQuaid 2015), in agreement with biogeographic  
263 distribution. Both *Perna perna* and *Mytilus galloprovincialis* recruits were similarly  
264 insensitive to decreased temperature in early summer (Dec 18), again suggesting similar  
265 metabolic plasticity for both species. Unfortunately, no species comparisons were made for  
266 decreased temperature response within cooler months or within Jan 19, due to uneven  
267 numbers for species as *Perna* display seasonal dependence of reproductive output whereas  
268 *Mytilus* does not (Zardi et al. 2007b). Low metabolic rates at cool temperatures for *Perna*  
269 recruits during the warmest month of Jan 19 reflected a lack of cool temperature acclimation.  
270 In contrast, cool temperature-adapted mussels can maintain normal standard metabolic rates  
271 and low activation energies during temperature decreases, as did *M. galloprovincialis* adults  
272 (Tagliarolo & McQuaid 2015). For *Mytilus*, there was an interaction between decreased  
273 temperature and month for metabolic rates, and lower metabolic rate in response to decreased

274 temperature occurred within the months that comprised lower temperature ranges (May 16  
275 and Jul 18), indicating seasonal patterns.

276 A transplant experiment for *Mytilus californianus* has shown that juvenile mussels  
277 (shell length 5–14.5 mm) can adjust their thermal tolerance range within one month where  
278 adults cannot (Gleason et al. 2018). Similarly, developmental plasticity likely contributed to  
279 seasonal patterns of thermal acclimation observed in the present study. A high degree of  
280 phenotypic plasticity during development can be adaptive when plastic phenotypes directly  
281 result from spatial environmental heterogeneity like temporary pools for tadpoles (Lind and  
282 Johansson 2007; Beldade et al. 2011) or the intertidal environment. The degree of  
283 developmental phenotypic plasticity varies with egg size and species in echinoid  
284 *Strongylocentrotus* larvae as *S. franciscanus* display higher plasticity in feeding organ  
285 morphology compared to congeneric *S. purpuratus* larvae (McAlister 2007). In molluscs, a  
286 higher degree of developmental plasticity in the freshwater quagga mussel (*Dreissena*  
287 *bugensis*), compared to the congeneric *D. polymorpha*, facilitates its wider habitat use in the  
288 Great Lakes of North America (Peyer et al. 2010).

289 In the present study, it is interesting that both *Mytilus* and *Perna* recruits displayed a  
290 similar degree of metabolic plasticity in response to temperature, as shown by their inter-  
291 specific Arrhenius activation energies, despite their different biogeographical distributions.  
292 Future studies should combine comparisons of the metabolic response to warming  
293 temperature between the two species with oxidative stress markers to understand how these  
294 species regulate metabolic efficiency (Salin et al. 2015).

295 At recruitment stage, *Mytilus* and *Perna* within the same lower intertidal environment  
296 can display similar thermal sensitivities in aerobic metabolism, the basis for aerobic scope,  
297 regardless of seasonal variation in expected thermal exposure, and despite different  
298 biogeographic distribution ranges. To fully understand inter-species competition on the south

299 coast for early life stages, future studies should establish the thermal performance curves for  
300 both species for controlled acclimation regimes simulating both summer and winter, using  
301 narrow size ranges. Additionally, sensitivities to other environmental factors, such as the  
302 interaction between temperature and aerial exposure, should be established.

### 303 **CONCLUSION**

304 Early post-settlement *Mytilus* and *Perna* mussels were both able to increase aerobic  
305 respiration when exposed to increased temperature during warmer months, despite *Mytilus*  
306 being close to the warm limit of its distribution range. Both species adjusted their thermal  
307 responses to the temperature range they were recently exposed to during the preceding  
308 month, displaying similar activation energies and plasticity. Metabolic plasticity in post-  
309 settlement mussels are most likely driven by developmental plasticity, although complex  
310 metabolic interactions between factors such as cohort, size, and population may also be at  
311 play.

### 312 *Conflict of Interest*

313 We have no conflicts of interest to disclose.

### 314 *Compliance with ethical standards*

315 All applicable international, national, and/or institutional guidelines for the care and use of  
316 animals were followed. Collection permit from the department of environmental affairs of the  
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325 Research Platform (AERP) at Rhodes University.

326 *Data Availability*

327 Data will be made available on reasonable request.

328 *Authors' Contribution*

329 Data collection and establishment of experimental protocols were performed by A Nel and O  
330 Duna, while study conceptualization and manuscript writing was performed by A Nel, CD  
331 McQuaid, L Giménez and F Porri. The experimental equipment, methods and resources were  
332 governed by CD McQuaid and F Porri.



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456 Table 1: The monthly mean ( $T_{\text{Mean}}$ ) and minimum to maximum ( $T_{\text{Min}}-T_{\text{Max}}$ ) seawater  
 457 temperatures are displayed for thirty-minute interval temperature recordings prior to animal  
 458 sampling ( $N = 904-1187$ ). The coefficient of variation (% C.V.) for monthly submerged  
 459 temperatures was low at 3.0–8.7 %. The  $T_{\text{Mean}}$  and  $T_{\text{Min}}-T_{\text{Max}}$  for water temperatures during  
 460 the previous seven days (7) are displayed in parentheses. The temperatures ( $T_{\text{Exp}}$ ) used for  
 461 recruit respirometry for each sampling are displayed as temperature at the time of  
 462 ‘collection’, and +5 and -5 °C, respectively.

<b>Month</b>	<b><math>T_{\text{Mean}}</math></b>	<b><math>T_{\text{Min}}-T_{\text{Max}}</math></b>	<b><math>(T_{\text{Mean}}, T_{\text{Min}}-T_{\text{Max}})_7</math></b>	<b><math>T_{\text{Exp}}</math></b>
May 18	18.0 °C	16.1–20.1 °C	(17.8, 16.1–18.6 °C)	18, 23 and 13 °C
Jul 18	16.8 °C	15.1–18.6 °C	(16.8, 16.1–17.6 °C)	17, 22 and 12 °C
Aug 18	16.1 °C	14.6–17.6 °C	(16.1, 15.1–17.1 °C)	16 and 21 °C
Oct 18	18.5 °C	14.6–22.6 °C	(19.0, 14.6–22.6 °C)	17, 22 and 12 °C
Dec18	20.9 °C	17.1–24.1 °C	(21.2, 18.1–24.1 °C)	21, 26 and 16 °C
Jan 19	21.8 °C	18.1–24.6 °C	(21.7, 18.1–24.6 °C)	21, 26 and 16 °C

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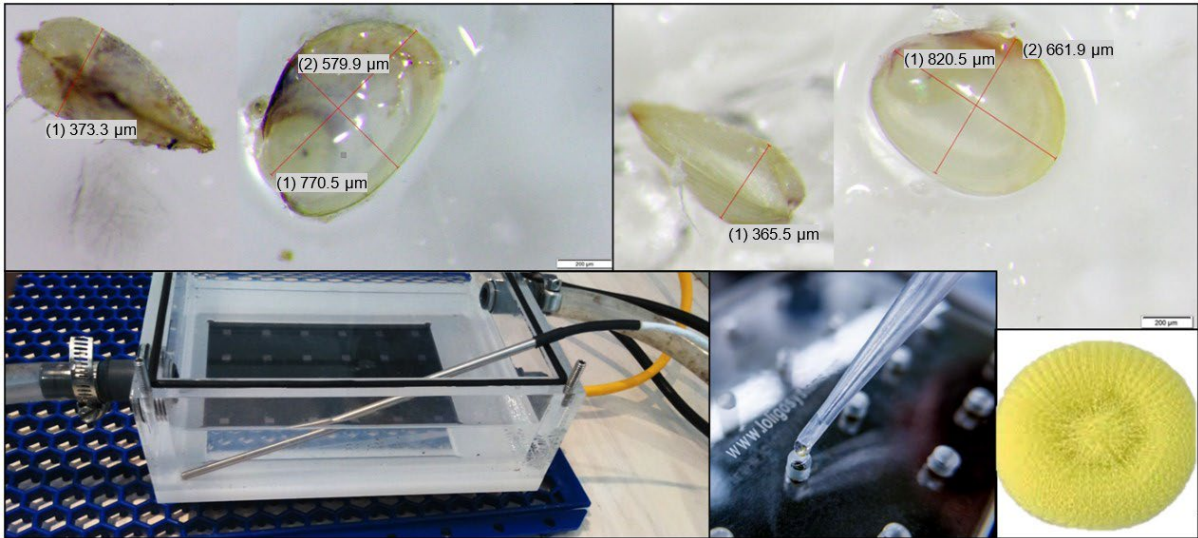
465 Table 2: The slope  $\beta_1$  ( $\pm$  S.E.) and intercept  $\beta_0$  ( $\pm$  S.E.) parameters are displayed for  
466 Arrhenius plots between the natural logarithm of mass-specific respiration rate ( $\ln R$ ) and  
467 inverse temperature ( $1/T$ ). For each test temperature and species, significant differences in  
468  $\beta_1$  and  $\beta_0$  over different months are denoted by different letter superscripts read vertically.

	<i>Mytilus</i>		<i>Perna</i>	
	$\beta_1$	$\beta_0$	$\beta_1$	$\beta_0$
<b><u>T<sub>Low</sub></u></b>				
<b>May 18</b>	-8.1 (4.3) <sup>a</sup>	19.3 (15.0) <sup>a</sup>		
<b>Jul 18</b>	-15.9 (3.6) <sup>a</sup>	47.6 (12.4) <sup>b</sup>		
<b>Oct 18</b>	-4.02 (4.4) <sup>a</sup>	5.5 (15.2) <sup>a</sup>	4.6 (3.6) <sup>a</sup>	-25.1 (12.6) <sup>a</sup>
<b>Dec 18</b>	-5.81 (2.7) <sup>a</sup>	12.6 (9.2) <sup>c</sup>	-2.4 (3.9) <sup>a</sup>	1.0 (13.6) <sup>b</sup>
<b>Jan 19</b>			-15.1 (3.9) <sup>b</sup>	43.3 (13.5)
<b><u>T<sub>High</sub></u></b>				
<b>May 18</b>	-17.9 (3.9) <sup>a</sup>	53.18 (13.2)	-13.5 (5.5) <sup>a</sup>	38.2 (18.8) <sup>a</sup>
<b>Jul 18</b>	-6.8 (3.7) <sup>b</sup>	16.3 (12.5) <sup>a</sup>	-3.4 (5.4) <sup>a</sup>	4.5 (18.6) <sup>b</sup>
<b>Aug 18</b>	-4.8 (5.3) <sup>b</sup>	8.3 (18.2) <sup>b</sup>		
<b>Oct 18</b>	0.4 (4.8) <sup>b</sup>	-9.6 (16.4) <sup>b</sup>	-7.4 (5.3) <sup>a</sup>	16.3 (18.2) <sup>c</sup>
<b>Dec 18</b>	-12.5 (3.7) <sup>ab</sup>	35.2 (12.5) <sup>c</sup>	-20.9 (7.7) <sup>ab</sup>	63.9 (26.0) <sup>bd</sup>
<b>Jan 19</b>			-40.0 (5.9) <sup>b</sup>	127.9 (20.0) <sup>c</sup>

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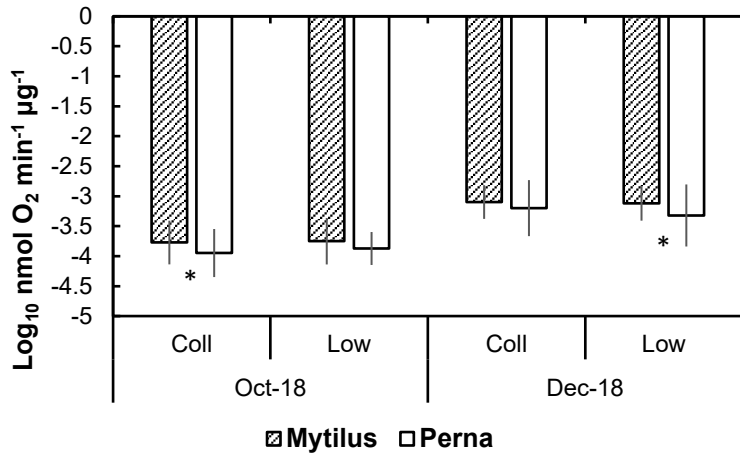


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473 Figure 1: *Mytilus* (top left) and *Perna* (top right) recruits are displayed. Bottom (from left to  
474 right); the circulating water bath, respirometry wells (80 μL), and collection scouring pad for  
475 mussel recruits.

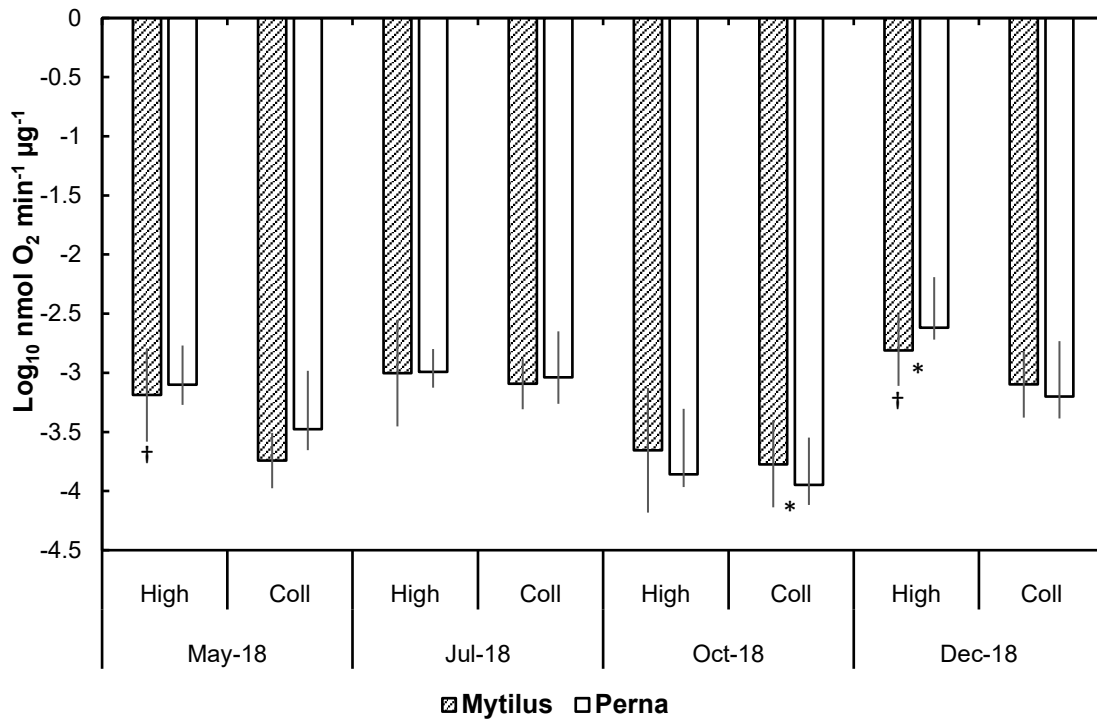
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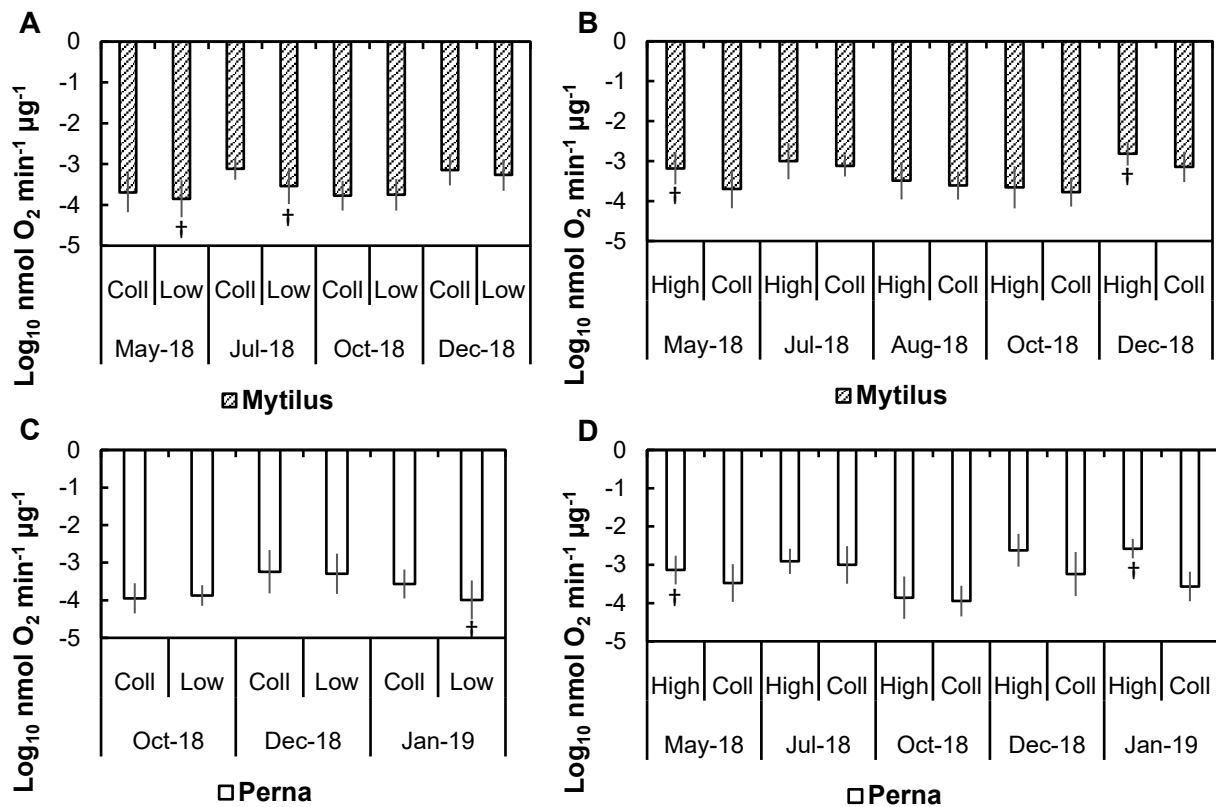
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479 Figure 2: Mean ( $\pm$  S.D.) logarithm transformed mass-specific oxygen consumption values for  
 480 *Mytilus* (N = 16–53) and *Perna* (N = 23–43) recruits in response to the -5 °C (“Low”) and  
 481 collection (“Coll”) temperatures (“Species and -5 °C”) are displayed. The “\*” denotes  
 482 differences between species for the collection temperature within Oct 18 and for the low  
 483 temperature within Dec 18 (Tukey HSD tests for pairwise comparisons for ANOVA testing  
 484 interactions for -5 °C, month and species).



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487 Figure 3: Mean ( $\pm$  S.D.) logarithm transformed mass-specific oxygen consumption values for  
 488 *Mytilus* (N = 16–53) and *Perna* (N = 6–43) recruits in response to +5 °C (“High”) and  
 489 collection (“Coll”) temperatures (“Species and +5 °C”) are displayed. The “\*” denotes  
 490 differences between species for the collection treatment within Oct 18 and for the high  
 491 treatment within Dec 18 (Tukey HSD tests for pairwise comparisons for ANOVA testing  
 492 interactions for +5 °C, month and species). The “†” denotes differences between high and  
 493 collection temperatures for *Mytilus* species within May 18 and Dec 18.



495 Figure 4: Mean ( $\pm$  S.D.) logarithm transformed mass-specific oxygen consumption values are  
 496 displayed. Tukey HSD tests for pairwise comparisons of ANOVA interactions for  
 497 temperature and month revealed differences (denoted by “†”) for *Mytilus* recruits between -5  
 498 °C (“Low”) and collection (“Coll”) temperature (“*Mytilus* and -5 °C”, N = 14–49) within May  
 499 18 and Jul 18 (A). Differences between “Coll” temperatures and the +5 °C (“High”)  
 500 temperature for *Mytilus* recruits (“*Mytilus* and +5 °C”, N = 14–49) occurred within May 18  
 501 and Dec 18 (B). For *Perna* recruits, differences between “Coll” temperatures and the -5 °C  
 502 (“Low”) temperature (“*Perna* and -5 °C”, N = 22–48) occurred within Jan 19 (C), while  
 503 differences between “Coll” temperatures and the +5 °C (“High”) temperature (“*Perna* and +5  
 504 °C”, N = 6–41) occurred within May 18 and Jan 19 (D).

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