

2021-03

Handling the heat: Responses of two congeneric limpet species to environmental temperature differences

Redfern, JC

<http://hdl.handle.net/10026.1/16813>

10.1016/j.jembe.2020.151500

Journal of Experimental Marine Biology and Ecology

Elsevier BV

All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.

1 Handling the Heat: Responses of Two Congeneric Limpet Species to 2 Environmental Temperature Differences

3 Journal: **JEMBE**

4 J. C. Redfern ^{ab}, A. Foggo ^a, N. Mieszkowska ^{bc}

5 ^a School of Biological and Marine Sciences (Faculty of Science and Engineering), University of Plymouth, England

6 ^b Marine Biological Association of the U.K., Citadel Hill, PL1 2PB Plymouth, England

7 ^c School of Environmental Science, University of Liverpool, England

8 Abstract

9 Poleward migrations of coastal marine species are occurring due to anthropogenic climate
10 change. Temperature is a known driver of species distributions, however, the specific influence of
11 temperature responsible for ecological disruption are diverse and often species-specific. If we are to
12 predict future impacts of climate change it is imperative we have a comprehensive understanding of
13 the influences of temperature at the scale of individual organisms, especially for ecosystem engineer
14 species. In this study, manipulative mesocosm experiments were conducted to explore how
15 temperature affects limpet feeding and activity rates, and duration of tenacity ability of the foot was
16 used to assess the impact of water temperature on biological function. Mesocosm trials and
17 biological function analysis indicated that *P. vulgata* may be more eurythermal, whilst *P. depressa*
18 has a higher thermal tolerance while performing simple biological functions at higher temperatures.
19 These investigations supplement the established body of research aimed at improving the predictive
20 power of species distribution models (SDM) used to forecast the impact of climate change by
21 pointing to potential importance of sub-lethal effects upon behaviour and performance.

22 **Keywords:** Biogeographic range shifts, Behaviour, sub-lethal effects, *Patella vulgata*, *Patella*
23 *depressa*

24 1. Introduction

25 Temperature is a known determinant of biological processes and patterns across ecological
26 scales and especially in poikilotherms (Markel, 1974; Woods et al., 2003). At a fundamental level,
27 temperature has been demonstrated to influence the speed and efficiency of biochemical reactions
28 (Gillooly et al., 2001; Clarke, 2006) and to covary with many biological traits such as activity, growth,
29 and reproductive output (Brown et al., 2004; Jeffrey and Saenger, 2012; Woodin et al., 2013),
30 affecting function and fitness. These processes occur at the organismal, cellular, and genomic levels,

31 but may have cascading effects upon distribution, abundance, and fitness of organisms and their
32 interactions over a range of spatial and temporal scales (Kordas et al. 2011; Somero, 2005).

33 The impact of temperature on physiology, behaviour, reproductive success, and
34 biogeographical distributions of coastal marine species is well documented (Helmuth et al., 2006;
35 Helmuth, 2009; Lima et al. 2009; Kordas et al., 2011; Woodin et al., 2013). The processes and limits of
36 thermal tolerance and thermal adaptation in marine ectotherms are directly related to the adjustment
37 of aerobic scope, with acclimatisation of thermally sensitive traits leading to adaptive shifts in thermal
38 optima and limits (Pörtner 2002, Somero 2002). Changes in thermal environment also drive
39 phenological shifts, with warming springs and summers driving a change from single to multiple
40 breeding events during the year, or to protracted breeding seasons (Moore et al. 2011). One area
41 where information is lacking, however, is the sub-lethal effects on population dynamics, which directly
42 influence population connectivity and biogeographical distribution as a result.

43 Anthropogenic climate change is driving an increase in the importance of researching
44 temperature and its influence upon individuals and communities (Somero, 2005; Helmuth, 2009).
45 Rocky shore environments are periodically exposed to terrestrial (atmospheric temperature, wind
46 speed, sun exposure, etc.) and marine (water temperature, salinity, diel tidal cycle etc.) abiotic
47 influences, making them valuable sites for studying abiotic stress, biotic interactions, and ecological
48 change (Mieszkowska et al., 2006; Seabra et al., 2011). Global sea surface temperature (SST) has
49 been warming at approximately 0.13°C per decade since the 1980's (IPCC, 2007), this is evident off
50 the coast of Plymouth (England), which experienced a 1 °C increase between 1980 and 2000
51 (Hawkins et al., 2003). Coastal marine species can be effectively used to measure and track the
52 influence of such climate change upon species distributions (Mieszkowska et al., 2006, 2005).
53 However, abiotic factors affecting limpet body temperature and its consequences for physiological
54 and behavioural responses can be multi-faceted, complex, and difficult to distinguish (Gilman et.al.,
55 2006; Helmuth et al., 2006).

56 Patellid limpets are a structurally and functionally important family on rocky shores, having
57 strong influences upon community structure (Branch, 1981). This is mainly due to the impact of
58 grazing on algae which modifies algal abundances, increasing ecosystem stability, biodiversity, and
59 function (Branch, 1981; Coleman et al., 2006; Hawkins and Hartnoll, 1983; Southward, 1964).
60 Changes to the distributions and abundance of limpets may therefore have consequences for other
61 species, with a potential to affect the community structure and ecosystem functioning (Moore et al.,
62 2007). *Patella vulgata* (Linnaeus, 1758) is a boreal species commonly found in the mid-intertidal
63 zone across North West Europe. *Patella depressa* (Pennant, 1777) is a lusitanian species, ranging
64 from Senegal to North Wales, with a distribution that overlaps with *P. vulgata* on rocky shores
65 around southwest England (Southward et al., 1995). These species share similar morphological
66 (feeding apparatus) and behavioural features (diets, habitat) (Hawkins et al., 1989) and there is
67 evidence for strong interspecific competition between them. Boyden et al. (2002) investigated this
68 competition on the northern coast of Portugal, where the species exist in similar densities and found
69 that where they co-occurred, both species experienced higher mortality and lower size and weight
70 compared to locations supporting only one of the pair.

71 Long-term data sets collected around southwest England suggest relative abundances of *P.*
72 *vulgata* and *P. depressa* fluctuate in relation to warmer and cooler periods (Hawkins et al., 2003;
73 Southward et al., 1995; Southward et al., 2004). *P. depressa* has a thermal niche located at higher
74 temperatures than *P. vulgata* which is subject to lesser thermal constraints in UK regional seas.
75 Recent ocean warming trends related to anthropogenic climate change provide suitable climatic
76 conditions for *P. depressa* to increase in abundance near its leading range edge in the UK. Through
77 such processes, climate change is altering local-scale community dynamics, affecting interspecific
78 relationships, and altering large-scale patterns of distribution and abundance (Harley et al., 2006). In
79 north-western Europe, cold adapted boreal species are decreasing in abundance and retreating
80 poleward while lusitanian species are increasing in abundance and advancing as evidenced by *P.*
81 *depressa* and *P. vulgata* (Hawkins et al., 2008; Mieszkowska et al., 2013). Both species appear to

82 experience deleterious effects upon their behaviour at higher temperatures, a sub-lethal
83 physiological impact which is reflected in the shifts in abundance of both species to higher latitudes
84 as the climate continues to warm (Mieszkowska et al. 2006).

85 Despite such documented patterns, it often remains unclear exactly what physiological
86 mechanisms underlie species' responses to increases in temperature which result in change in
87 distributions and abundances (Bjelde and Todgham, 2013). Previous studies observing seasonal
88 variations have pointed to changes in multiple factors causing alterations in limpet feeding rates;
89 these include; state of gonad development, food availability, wave action and rock inclination
90 (Jenkins et al., 2001; Jenkins and Hartnoll, 2001; Santini et al., 2004). In particular, strong
91 correlations between SST and grazing activity have been established (Branch, 1981; Jenkins et al.,
92 2001; Santini et al., 2004; Thompson et al., 2000). In addition to this general trend, temperature has
93 been observed to have a species-specific effect on feeding rate of limpets due to variations in
94 thermal tolerances (Petraitis, 1992).

95 Due to the inherent complexity of biological responses, we often have very poor
96 understanding of how weather and climate patterns are experienced by organisms at their scales. In
97 this study, the impact of temperature on behaviour in two congeneric limpets with differing
98 latitudinal ranges of origin was investigated by analysing activity and feeding rates in a controlled
99 mesocosm environment. In addition, the influence of water temperature upon a simple biological
100 function was studied by examining the effect of water temperature on duration of tenacity in the
101 two species. Water temperature (SST) has been observed to correlate significantly with population
102 abundance of *Siphonaria pectinate* (Rubal et al., 2013).

103

104

105 Methods

106 Live specimen collection

107 Adult (Length = 20 – 40 mm, wet mass approx. 0.5 – 8g) *P.vulgata* and *P.depressa* were collected
108 during low tide at Plymouth Hoe (50.3635N, -4.13965E) between April and July, 2017. Limpets were
109 removed from the substrate when away from their home scar, reducing chance of damage and
110 mitigating against stress experienced by the animals. Limpets that were damaged during removal
111 were not used in the study and were returned to the shore. Prior to experiments, all limpets were
112 held in an aerated indoor seawater holding tank at ambient temperature.

113

114 Feeding rate

115 Limpets were transferred to individual 7 L experimental tanks supplied with aerated seawater. Diel
116 tidal (6 hr) and light (12 hr) cycles were initiated in a closed aquarium system in a laboratory
117 mesocosm room. In accordance with future predictions of sea surface temperature (SST) around
118 southwest England obtained from two sources (IPCC, 2007, Tinker et al., 2016), three temperature
119 treatments (11.0 °C, 13.5 °C and 16.0 °C) were established by heating supply tanks using 55w
120 submersible water heaters (Aqua Pacific UK Ltd., Nursling). Air temperature was controlled and held
121 at the appropriate monthly average air temperature from the Western Channel Observatory time-
122 series. Limpets were held at treatment temperature for 48 hours to acclimate before the start of
123 feeding experiments. This short acclimatory period reflected the short temporal duration of the
124 experiment, which was designed to test acute responses to changes in thermal environment. For
125 longer experiments, acclimation time is increased to days/weeks.

126 Algal biofilm grown on Perspex sheets (14 x 14 x 0.5 cm) incubated in flow through seawater aquaria
127 for 1 month was used as a food source in feeding trials. Prior to introducing a Perspex food sheet to
128 the limpet tanks photographs were taken of the biofilm cover using a Canon D300 dSLR, further
129 photographs were taken on days 3 and 5 of the trial. Images were analysed using ImageJ 1.51k
130 software (National Institute of Health, USA). To accurately threshold the images, each was colour

131 split producing blue, red and green layers. Blue layers best reflected the optical density of the colour
132 spectrum giving clear demarcation of grazing patterns. Therefore, blue layer data was converted to
133 binary by thresholding the image. Image particle analysis was then used to assess the level of biofilm
134 loss over the five days, giving an indicated measure of feeding rate. Six trials were conducted in
135 total, with 48 individuals in each trial.

136 Data describing the feeding response to temperature in the two species were highly dominated by
137 zeroes, therefore analyses were conducted in two stages to avoid statistical issues with zero-inflated
138 mixed models. First a binary response variable was created scoring 1 in animals that fed and 0 for
139 non-feeders. The effects of temperature (fixed factor) and trial (random factor) upon feeding
140 likelihood in the two species were then modelled using binomial family GLMER in package lme4
141 (Bates et al 2015) in R ver. 3.6.1 (R Core Team, 2019). P-values for fixed effects were derived from
142 Wald chi-squared tests with type III sums of squares, pair-wise comparisons between treatment
143 levels were estimated via marginal means with Tukey adjusted p-values. Second, replicates where
144 animals fed were isolated and the effects of the same fixed and random factors upon log
145 transformed area of biofilm removed were tested using a linear mixed model with p-values again
146 derived from type III Wald chi-squared tests. Pair-wise contrasts were obtained using Tukey adjusted
147 tests upon estimated marginal means as above.

148

149 2.4 Activity Rate

150 Movement/activity rate analysis was conducted on 12 individuals per trial over 5 trials using
151 the mesocosm system and water temperature treatments described above. Four animals (two *P.*
152 *vulgata* and two *P. depressa*) for each temperature regime were filmed continuously using HD CCTV
153 cameras (Hikvision DS-2CD2042WD-I) over a five-day period. Infrared video recording allowed
154 filming during dark periods of the diel light cycle. A total of 60 individual limpets were recorded over

155 a 5-day period for each trial. Six hundred hours of footage was recorded which was analysed to
156 calculate total distance moved.

157 Limpet movements were tracked and analysed using Noldus Ethovision XT (13.0) software.
158 Information on methods and settings within the Ethovision software can be found in the
159 Supplementary Information section.

160 The two-stage approach described earlier was taken to the zero-inflated data describing
161 limpet activity. In this case data were aggregated to probability and extent of movement per day
162 (the latter log transformed to normalise residuals), with both trial and identity of the individual
163 limpet included as random factors in the statistical models.

164

165 2.5 Duration of tenacity

166 Effects of temperature upon the capacity of limpets to maintain tonic grip upon the rock was
167 assessed by determining the length of time limpets could remain attached whilst under tension.

168 Twelve limpets of each species were placed in separate temperature controlled tanks at
169 11°C. A further 24 limpets were placed in separate temperature controlled tanks and water
170 temperature increased by 2°C per hour until the required temperature of 18°C was achieved.

171 At the start of the trials, cotton threads attached to the limpets using superglue (Gorilla
172 Super Glue Gel) were hooked onto a piece of 6mm polypropylene string using a 5mm cable tie. The
173 string was then passed through two 19mm Fast Eye pulleys supported by two fixed metal uprights.
174 200g weight consisting of water in a plastic container suspended by polypropylene string was
175 attached at the opposite end (**see Fig. 1**).

176 To standardise measurements, limpets were tapped to stimulate shell clamping, three times
177 before the string was attached and again three times before the weight was gently lowered until full
178 force was applied centrally to the limpet shell.

179 The time taken for the limpet to become detached from the substratum was recorded.
180 During trials limpets were submerged in 7-10 cm of temperature controlled seawater. After the
181 limpet became detached foot size was measured by allowing the limpets foot to reattach to a clear
182 acetate sheet, the foot was then traced allowing area to be quantified using ImageJ.

183 Between-species differences in tenacity were analysed by linear model with log-transformed
184 foot-size as a continuous covariate, species and temperature regime as fixed factors. An initial fully
185 interactive model indicated homogeneity of slopes of all factor by covariate combinations, therefore
186 the final linear model was unsaturated, with p-values derived from F-ratios using type 1 sums of
187 squares.

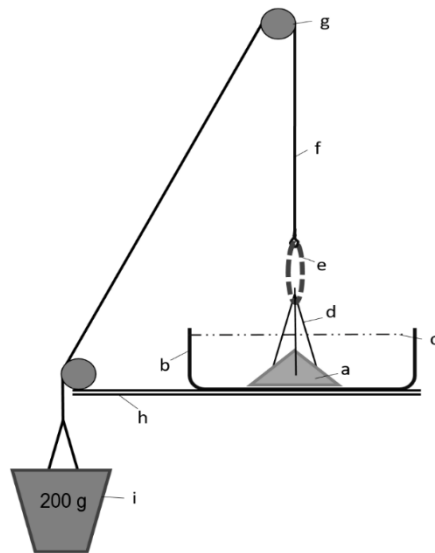
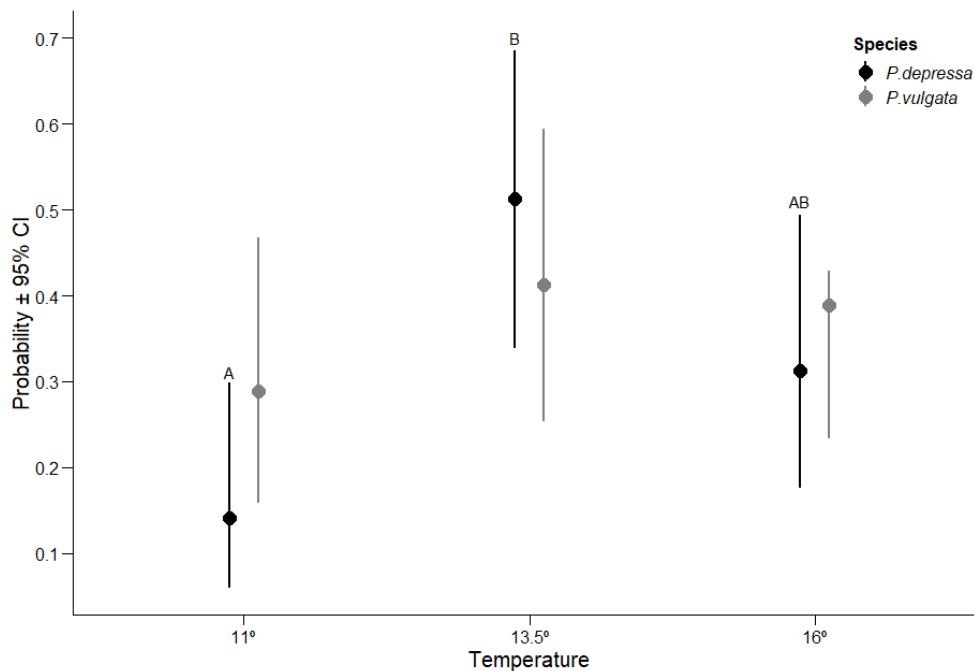


Fig. 1. Tenacity Machine: **a** = limpet, **b** = holding tank, **c** = denotes water level 7-10 cm, **d** = Korbond thread, **e** = 5 mm plastic cable tie, **f** = 6 mm polypropylene string, **g** = 19 mm pulley, **h** = table top, **i** = weight in plastic container. Diagram not to scale.

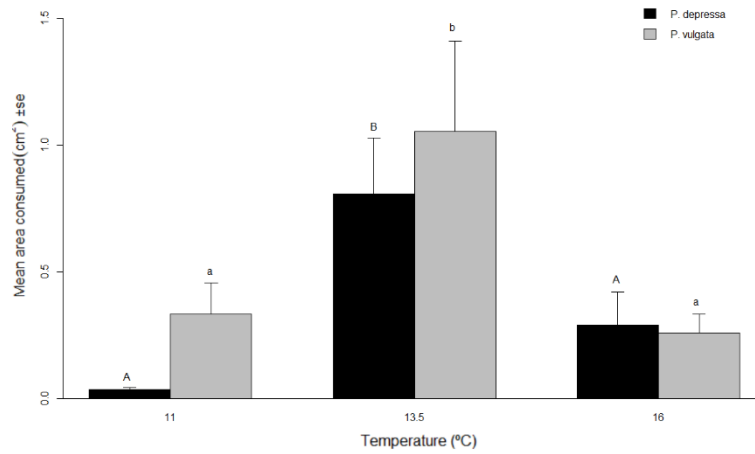
188 **Results**
189 **Feeding Rate**

190 Across the species, temperature had a significant impact on the probability of limpets feeding, this
191 was largely attributable to an increase at 13.5°C in both (temperature $\chi^2 = 11.752$, $df = 2$, $p < 0.01$,
192 see supplementary table S1) (**Fig. 2**). *P. depressa* behaved most variably, being more than twice as
193 likely to feed in temperatures above 11°C (pair-wise tests: $0.01 < p < 0.05$). Probability of *P. vulgata*
194 feeding was less temperature dependent, and whilst it followed the same trends, none of the
195 differences observed was statistically significant. This pattern was reflected in the analysis of area
196 grazed per limpet in those that fed (Temperature effect $\chi^2 = 8.048$, $df = 2$, $p < 0.05$). *P. depressa* not
197 only fed infrequently at the lowest temperature, but also grazed very little when it did, whilst *P.*
198 *vulgata* grazed more extensively, especially at 13.5°C ($p < 0.05$ both pairwise comparisons; **Fig. 3**,
199 table S2). Indeed, both species expressed an increase in feeding rates at the intermediate
200 temperature treatment (13.5°C) compared to both the low (11°C) and high (16°C) temperatures.
201



(**Fig. 2.**) Mean (symbols, with 95% confidence intervals (whiskers), back-calculated from log-odds estimates in glm) probability of a limpet [feeding/moving] during the mesocosm trial. Superscripts indicate temperatures differing significantly within species ($p < 0.05$; Tukey adjusted pairwise estimates).

202



(Fig. 3.) Mean (+ s.e) area of algal film consumed by *P. depressa* and *P. vulgata* during trails at each temperature (*P.depressa* n = 11,21,13; *P. vulgata* n = 12, 17, 16). Superscripts indicate treatments differing at P < 0.05 (tests of estimated marginal means using Tukey adjustments) within species.

203

204 Activity

205 Temperature had a significant effect on both the probability of limpets moving ($\chi^2 = 13.467$, df = 2, p

206 < 0.01; Supplementary table 3; Fig.4) and the amount of movement evident in those that did move

207 ($\chi^2 = 7.438$, df = 2, p < 0.05; Supplementary table 4; Fig.5). Post-hoc tests corrected for multiple

208 comparisons indicate that the activity rate for mobile individuals of both species was higher at 11°C

209 than at warmer temperatures, however this difference was only significant for *P. depressa* between

210 11°C and 13.5°C; mobile *P. vulgata* meanwhile moved less in both warmer temperature treatments.

211 Whilst both species moved more in the coolest treatment, it is notable that mobile *P. vulgata*

212 individuals nonetheless moved on average 60% further than *P. depressa*, a trend reversed at the

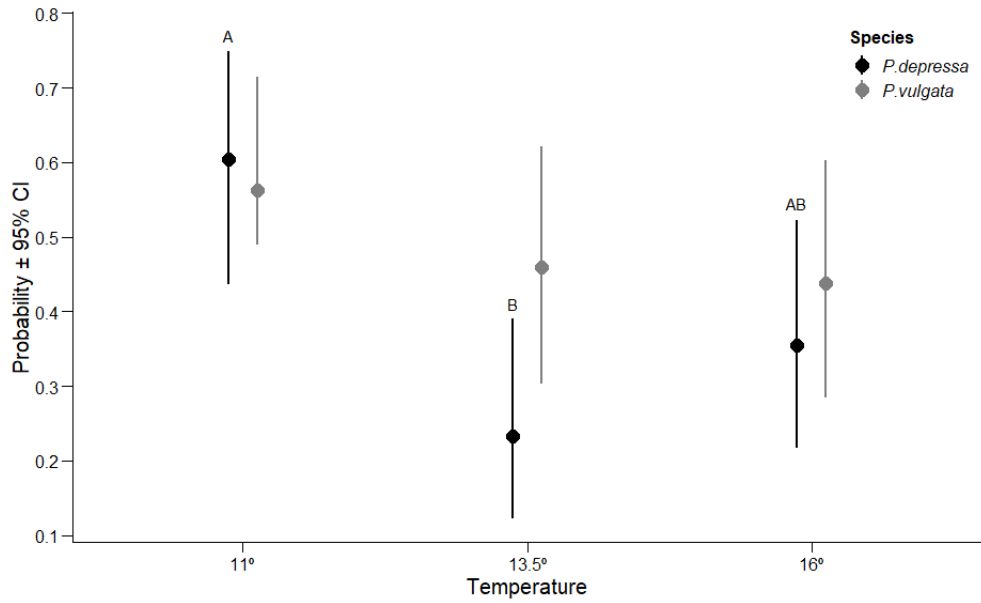
213 higher temperatures.

214

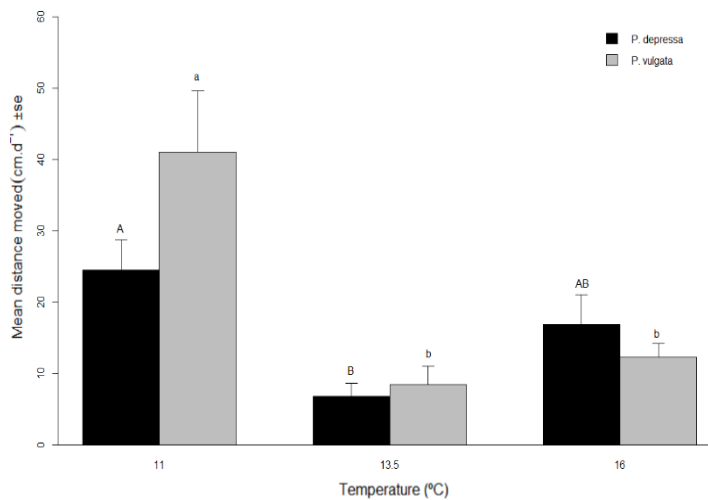
215

216

217
 218
 219
 220
 221
 222
 223
 224
 225
 226
 227



(Fig. 4.) Mean (symbols, with 95% confidence intervals (whiskers), back-calculated from log-odds estimates in glm) probability of a limpet [feeding/moving] during the mesocosm trial. Superscripts indicate temperatures differing significantly within species ($p < 0.05$; Tukey adjusted pairwise estimates).



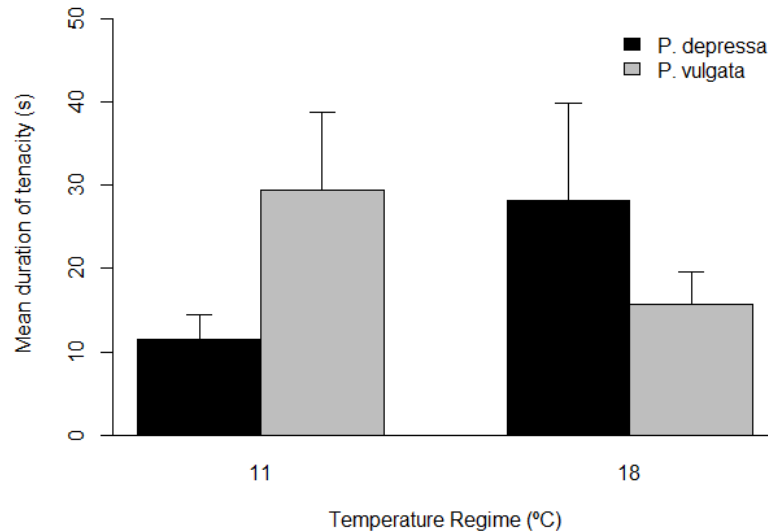
(Fig. 5.) Mean (\pm se) distance moved by *P. depressa* and *P. vulgata* during trails at each temperature (*P. depressa* $n = 18, 12, 30$; *P. vulgata* $n = 22, 19, 23$). Superscripts indicate treatments differing at $P < 0.05$ (tests of estimated marginal means using Tukey adjustments) within species.

228 **Biological Function Analysis: Duration of Tenacity**

229 After accounting for a positive effect of foot size upon tenacity, there were no significant effects of
 230 species or temperature regime in isolation, but a significant heterogeneity of response to
 231 temperature regime across the two species (species x temperature $F_{1,43} = 4.335$, $p < 0.05$; Fig. 6) *P.*

232 *vulgata* was more than twice as tenacious as *P. depressa* at 11°C, with this pattern reversed at 18°C
233 despite the consistently larger size of *P. vulgata*.

234



(Fig. 6.) Duration of tenacity in *P. depressa* and *P. vulgata* held under under two temperature regimes (all n = 12).

235 Discussion

236 In our study, temperature had a significant impact on the probability of limpets feeding as
237 well as the amount of feeding performed. Previously, both laboratory and in situ studies have shown
238 a link between temperature and limpet feeding rates (Branch, 1981; Newell, 1980). Cooler air and
239 water temperatures have previously been shown to reduce feeding rates of both *Patella* spp. and
240 other limpets (*Patella vulgata* (Santini et al., 2004), *Cellana tramoserica* (Petraitis, 1992), *Nacella*
241 *concinna* (Morley et al., 2014), highlighting the importance of seasonal variations in temperature for
242 the intake of food.

243 The results of the present study support the proposition that higher temperatures constrain
244 feeding behaviour in both *P. vulgata* and *P. depressa*. Such sub-lethal thermal responses are likely to
245 have species-specific impacts on metabolism (Gillooly et al., 2001; Thompson et al., 2000),

246 performance and ultimately survival of individuals towards the leading range edges at higher
247 latitudes.

248 Higher temperatures within the thermal range of a species generally increase the average
249 rate of biochemical reactions thus allowing more activity (Halsey et al., 2014). However, contrary to
250 expectation and the observed feeding rates, the intermediate treatment elicited a significantly lower
251 level of non-feeding movement compared to the coolest temperature treatment for both species. In
252 fact, feeding and non-feeding movement were almost inversely related, implying that the animals
253 may have been prioritising either feeding or movement, possibly in the search for amelioration of
254 temperature stress. Activity rates for *P. vulgata* which chose to move were significantly higher at
255 11°C compared to both other temperature treatments. *P. depressa* meanwhile experienced no
256 significant difference in effects upon activity between the lowest and highest temperature
257 treatments, yet although probability of moving was similar in the two higher temperature
258 treatments, both were significantly higher compared to the intermediate trial (**Fig. 5.**). A similar
259 pattern has previously been observed in *Patella caerulea* where activity rates during immersion
260 were 24% higher in winter months compared to summer and 81% higher than in spring months
261 (Santini et al., 2004).

262 Previous studies have cited desiccation and predation risk as being primary factors in
263 determining limpet activity rates (Jenkins and Hartnoll, 2001; Little, 1989; Little and Stirling, 1985).
264 Here, observed patterns of activity rates should be interpreted with caution as abiotic factors
265 experienced by limpets under natural conditions differed in our experiments. Solar gain and wind
266 chill were absent in the mesocosm and whilst we were able to generate tidal emersion / immersion
267 and control air temperature, we could not simulate synchrony of emersion and low night time or
268 daytime highs of air temperature that may augment stresses of emersion at both extremes of the
269 spectrum. Solar radiation is known to have a strong influence on heat flux experienced by limpets
270 and therefore has a large impact on desiccation risk (Denny et al., 2011). Wind speed is also known

271 to influence limpet body temperature and therefore chance of desiccation (Fraser et al., 2016;
272 Williams and Morritt, 1995). These limitations of our study methodology, including the short
273 acclimation periods we used, represent potential sources of confounding in our study; however,
274 these parameters were the same for both species studied.

275 Previous studies have observed a significant effect of temperature on tenacity ability of both
276 limpets (Davenport, 1997; Grenon and Walker, 1981; Morley et al., 2011) and other marine
277 organisms (Flammang et al., 2002; Santos and Flammang, 2007). The results of the present
278 investigation indicate temperature had a significant impact on duration tenacity, affecting each
279 species differently. *P. depressa* exhibited greater tenacity at higher temperatures, whilst the opposite
280 was seen for *P. vulgata*. It has previously been suggested that temperature can affect muscular
281 structures and enzyme activity which may therefore impact muscular reaction speed, muscular
282 force, and endurance (Kordas et al., 2011; Pörtner, 2002). The relationship between temperature
283 and duration of tenacity is likely to reflect an integrated measure of the thermal impact upon several
284 limpet organs. It is likely, therefore that temperature influences the functions of *P. vulgata* and *P.*
285 *depressa* differently and impacts a variety of different life processes; further studies are required to
286 determine the full influence of changing thermal regimes upon the ecology and distribution of the
287 two species.

288 From present results, it can be summarised that *P. depressa* and *P. vulgata* appear to
289 express different thermal responses. Both species appear to perform better at 13.5°C where feeding
290 rates are highest; feeding rates of *P. depressa* are greatly depressed at 11°C whilst *P. vulgata*
291 maintained a similar rate of feeding at the two temperature extremes. This suggests that while *P.*
292 *vulgata* is more eurythermal, *P. depressa* is better able to function at warmer compared to cooler
293 temperatures, mirroring its Lusitanian geographic origins; however, in order to confidently predict
294 future distribution patterns, more research is needed over a wider range of temperatures to identify
295 optimal, upper and lower thermal limits for key functions in each species.

296 An overarching aim of this investigation was to provide information on the mechanistic
297 responses of limpets to sub-lethal temperature stress in the hope that observations made could
298 inform future studies using species distribution modelling (SDM), improving our ability to predict
299 future impacts of climate change. Comprehensive knowledge of species fitness and environmental
300 conditions is vital to improve accuracy of mechanistic SDMs (which factor in biological processes)
301 (Buckley et al., 2010). However, errors in parameterising SDMs can confound accuracy reducing
302 effectiveness. It is vital, therefore, that a more comprehensive knowledge and understanding of
303 thermal impacts upon physiology and behaviour is acquired if outcomes of climate change are to be
304 successfully predicted (Helmuth et al., 2006). Other behaviours performed to regulate body
305 temperature, for example 'mushrooming' in *Cellana grata* which is thought to reduce body
306 temperature by facilitating evaporative cooling (Williams et al., 2005), should be included in future
307 studies investigating impact of behavioural adaptations upon limpet body temperature as they may
308 provide mechanisms allowing species to persist during higher than expected levels of thermal stress.
309 Here, manipulative experimentation results, although not conclusive, indicate that *P. depressa* has a
310 lesser capacity to tolerate cold temperatures than *P. vulgata* which appears to have a wider window
311 of tolerance and functions more effectively at lower temperatures.

312 Intertidal rocky ecosystems are one of the most temporally and spatially complex habitats of
313 all. A broader understanding of context-sensitive (e.g. immersion / emersion) and small-scale
314 environmental conditions experienced by limpets can only improve the predictive power of future
315 SDMs. The development of biomimetic loggers allows realistic data acquisition to be achieved
316 relatively cheaply compared to traditional methods (Lima and Wetthey, 2009). This should be
317 exploited in future studies to provide an array of data allowing ecologists to form a detailed
318 understanding of the complexity of environmental factors affecting organisms on the rocky shore,
319 without the need to displace them. As parameterisation of SDMs improves, the importance of
320 assemblage-level effects upon biological 'error' is likely to increase, with cascading interactions
321 confounding further gains in predictive power. In addition to enhancing the knowledge of the

322 physiological and behavioural responses of the target species to temperature, future studies should
323 investigate how temperature may influence keystone species mediating ecological interactions.
324 Understanding key ecological interactions may be an important step in improving the ability to
325 forecast distribution shifts.

326 Acknowledgements

327 This study was conducted in part at the Mieszkowska Mesocosm, The Marine Biology Association
328 (UK). JR thanks Aaron Barrett at the Brunel Workshop, Plymouth University, Kevin Atkins and
329 Kathryn Pack at the MBA and Tom Pilgrem and Rowan Henthorn for consistent support during field
330 work. The authors are particularly grateful to the editor and referees whose commitment and care
331 helped dramatically improve the manuscript.

332 This research did not receive any specific grant from funding agencies in the public, commercial, or
333 not-for-profit sectors.

334

335

336

337

338

339 References

340

341 Bjelde, B.E., Todgham, A.E., 2013. Thermal physiology of the fingered limpet *Lottia digitalis* under emersion
342 and immersion. *J. Exp. Biol.* 216, 2858–69. doi:10.1242/jeb.084178

343 Boaventura, D., Cancela Da Fonseca, L., Hawkins, S.J., 2002. Analysis of competitive interactions between the
344 limpets *Patella depressa* Pennant and *Patella vulgata* L. on the northern coast of Portugal. *J. Exp. Mar. Bio.*
345 *Ecol.* 271, 171–188. doi:10.1016/S0022-0981(02)00044-8

346 Boyden, C.R., Zeldis, J.R., 1979. Preliminary observations using an attached microphonic sensor to study
347 feeding behaviour of an intertidal limpet. *Estuar. Coast. Mar. Sci.* 9, 759–769. doi:10.1016/S0302-
348 3524(79)80009-2

349 Branch, G.M., 1981. The Biology of Limpets: Physical Factors, Energy Flow, and Ecological Interactions.
350 *Oceanogr. Mar. Biol.* 19, 235–380.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., West, G.B., 2004. *Ecology* 85, 1771-1789.
doi: 10.1890/03-9000

351 Buckley, L.B., Urban, M.C., Angilletta, M.J., Crozier, L.G., Rissler, L.J., Sears, M.W., 2010. Can mechanism inform
352 species' distribution models? *Ecol. Lett.* doi:10.1111/j.1461-0248.2010.01479.x

353 Clarke, A., 2006. Temperature and the metabolic theory of ecology. *Functional Ecology*, 20(2), pp.405-412.

354 Coleman, R.A., Underwood, A.J., Benedetti-Cecchi, L., Åberg, P., Arenas, F., Arrontes, J., Castro, J., Hartnoll,
355 R.G., Jenkins, S.R., Paula, J., Santina, P. Della, Hawkins, S.J., 2006. A continental scale evaluation of the role of
356 limpet grazing on rocky shores. *Oecologia* 147, 556–564. doi:10.1007/s00442-005-0296-9

357 Davenport, J., 1997. Comparisons of the biology of the intertidal subantarctic limpets *Nacella concinna* and
358 *Kerguelenella lateralis*. *J. Molluscan Stud.* 63, 39–48. doi:10.1093/mollus/63.1.39

359 Denny, M.W., Dowd, W.W., Bilir, L., Mach, K.J., 2011. Spreading the risk: Small-scale body temperature
360 variation among intertidal organisms and its implications for species persistence. *J. Exp. Mar. Bio. Ecol.* 400,
361 175–190. doi:10.1016/j.jembe.2011.02.006

362 Flammang, P., Ribesse, J., Jangoux, M., 2002. Biomechanics of adhesion in sea cucumber *Cuvierian tubules*
363 (echinodermata, holothuroidea). *Integr. Comp. Biol.* 42, 1107–1115. doi:10.1093/icb/42.6.1107

364 Fraser, C.M.L., Seebacher, F., Lathlean, J., Coleman, R.A., 2016. Facing the Heat: Does Desiccation and Thermal
365 Stress Explain Patterns of Orientation in an Intertidal Invertebrate? *PLoS One*.
366 doi:10.1371/journal.pone.0150200

367 Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M., Charnov, E.L., 2001. Effects of size and temperature on
368 metabolic rate. *Science* (80-.). 293, 2248–2251. doi:10.1126/science.1061967

369 Gilman, S.E., Wetthey, D.S., Helmuth, B., 2006. Variation in the sensitivity of organismal body temperature to
370 climate change over local and geographic scales. *Proc. Natl. Acad. Sci. U. S. A.* 103, 9560–9565. doi:Doi
371 10.1073/Pnas.0510992103

372 Grenon, J.F., Walker, G., 1981. The tenacity of the limpet, *Patella vulgata* L.: An experimental approach. *J. Exp.*
373 *Mar. Bio. Ecol.* 54, 277–308. doi:10.1016/0022-0981(81)90162-3

374 Halsey, L.G., Matthews, P.G.D., Rezende, E.L., Chauvaud, L., Robson, A.A., 2015. The interactions between
375 temperature and activity levels in driving metabolic rate: theory, with empirical validation from contrasting

376 ectotherms. *Oecologia* 177, 1117–1129. doi:10.1007/s00442-014-3190-5

377 Harley, C.D.G., Randall Hughes, A., Hultgren, K.M., Miner, B.G., Sorte, C.J.B., Thornber, C.S., Rodriguez, L.F.,
378 Tomanek, L., Williams, S.L., 2006. The impacts of climate change in coastal marine systems: Climate change in
379 coastal marine systems. *Ecol. Lett.* 9, 228–241. doi:10.1111/j.1461-0248.2005.00871.x

380 Hawkins, S.J., Hartnoll, R.J., 1983. Algal Grazing By Marine Invertebrates. *Oceanogr. Mar. Biology an Annu. Rev.*
381 21, 195–282.

382 Hawkins, S.J., Moore, P.J., Burrows, M.T., Poloczanska, E., Mieszkowska, N., Herbert, R.J.H., Jenkins, S.R.,
383 Thompson, R.C., Genner, M.J., Southward, A.J., 2008. Complex interactions in a rapidly changing world:
384 Responses of rocky shore communities to recent climate change. *Clim. Res.* 37, 123–133. doi:10.3354/cr00768

385 Hawkins, S.J., Southward, A.J., Genner, M.J., 2003. Detection of environmental change in a marine ecosystem -
386 evidence from the western English Channel. *Sci. Total Environ.* 310, 245–56. doi:10.1016/S0048-
387 9697(02)00645-9

388 Hawkins, S.J., Watson, D.C., Hill, A.S., Harding, S.P., Kyriakides, M.A., Hutchinson, S., Norton, T.A., 1989. A
389 comparison of feeding mechanisms in microphagous, herbivorous, intertidal, prosobranchs in relation to
390 resource partitioning. *J. Molluscan Stud.* 55, 151–165. doi:10.1093/mollus/55.2.151

391 Helmuth, B., 2009. From cells to coastlines: how can we use physiology to forecast the impacts of climate
392 change? *J. Exp. Biol.* 212, 753–760. doi:10.1242/jeb.023861

393 Helmuth, B., Mieszkowska, N., Moore, P., Hawkins, S.J., 2006. Living on the Edge of Two Changing Worlds:
394 Forecasting the Responses of Rocky Intertidal Ecosystems to Climate Change. *Annu. Rev. Ecol. Evol. Syst.* 37,
395 373–404. doi:10.2307/30033837

396 IPCC, 2007. *Climate Change 2007: The Physical Science Basis*. Cambridge University Press.

397 Jeffrey, G.A. and Saenger, W., 2012. *Hydrogen bonding in biological structures*. Springer Sci. Bus. Media.

398 Jenkins, S.R., Arenas, F., Arrontes, J., Bussell, J., Castro, J., Coleman, R.A., Hawkins, S.J., Kay, S., Martinnez, B.,
399 Oliveros, J., Roberts, M.F., Sousa, S., Thompson, R.C., Hartnoll, R.G., 2001. European-scale analysis of seasonal
400 variability in limpet grazing activity and microalgal abundance. *Mar. Ecol. Prog. Ser.* 211, 193–203.
401 doi:10.3354/meps211193

402 Jenkins, S.R., Hartnoll, R.G., 2001. Food supply, grazing activity and growth rate in the limpet *Patella vulgata* L.:
403 A comparison between exposed and sheltered shores. *J. Exp. Mar. Bio. Ecol.* 258, 123–139. doi:10.1016/S0022-
404 0981(01)00211-8

405 Kordas, R.L., Harley, C.D.G., O'Connor, M.I., 2011. Community ecology in a warming world: The influence of
406 temperature on interspecific interactions in marine systems. *J. Exp. Mar. Bio. Ecol.* 400, 218–226.
407 doi:10.1016/j.jembe.2011.02.029

408 Lima, F.P., Burnett, N.P., Helmuth, B., Kish, N., Aveni-Deforge, K., Wetthey, D.S., 2011. Monitoring the intertidal
409 environment with biomimetic devices. In Biomimetic based applications. InTech. doi:10.5772/52807

410 Lima, F.P., Wetthey, D.S., 2009. Robolimpets: measuring intertidal body temperatures using biomimetic loggers.
411 Limnol. Oceanogr. Methods 7, 347–353. doi:10.4319/lom.2009.7.347

412 Little, C., 1989. Factors governing patterns of foraging activity in littoral marine herbivorous molluscs. J.
413 Molluscan Stud. 55, 273–284. doi:10.1093/mollus/55.2.273

414 Little, C., Stirling, P., 1985. Patterns of foraging activity in the limpet *Patella vulgata* L. - A preliminary study. J.
415 Exp. Mar. Bio. Ecol. 89, 283–296. doi:10.1016/0022-0981(85)90133-9

416 Markel, R.P., 1974. Aspects of the Physiology of Temperature Acclimation in the Limpet *Acmaea limatula*
417 Carpenter (1864): An Integrated Field and Laboratory Study. Physiol. Zool. 47, 99–109.
418 doi:10.1086/physzool.47.2.30155627

419 Mieszkowska, N., Firth, L., Bentley, M., 2013. Impacts of climate change on intertidal habitats. MCCIP Sci. Rev.
420 180–192. doi:10.14465/2013.arc19.180-192

421 Mieszkowska, N., Kendall, M.A., Hawkins, S.J., Leaper, R., Williamson, P., Hardman-Mountford, N.J.,
422 Southward, A.J., 2006. Changes in the range of some common rocky shore species in Britain - A response to
423 climate change? Hydrobiologia 555, 241–251. doi:10.1007/s10750-005-1120-6

424 Mieszkowska, N., Leaper, R., Moore, P., Kendall, M.A., Burrows, M.T., Lear, D., Poloczanska, E., 2005. Marine
425 Biodiversity and Climate Change Assessing and Predicting the Influence of Climatic Change Using Intertidal
426 Rocky Shore Biota Final Report for United Kingdom Funders.

427 Moore, P., Hawkins, S.J., Thompson, R.C., 2007. Role of biological habitat amelioration in altering the relative
428 responses of congeneric species to climate change. Mar. Ecol. Prog. Ser. 334, 11–19. doi:10.3354/meps334011

429 Moore, P.J., Thompson, R.C., Hawkins, S.J., 2011. Phenological changes in intertidal con-specific gastropods in
430 response to climate warming. Global Change Biology, 17(2), 709-719.

431
432 Morley, S.A., Lai, C.H., Clarke, A., Tan, K.S., Thorne, M.A.S., Peck, L.S., 2014. Limpet feeding rate and the
433 consistency of physiological response to temperature. J. Comp. Physiol. B 184, 563–570. doi:10.1007/s00360-
434 014-0814-3

435 Morley, S.A., Lemmon, V., Obermüller, B.E., Spicer, J.I., Clark, M.S., Peck, L.S., 2011. Duration of tenacity: A
436 method for assessing acclimatory capacity of the Antarctic limpet, *Nacella concinna*. J. Exp. Mar. Bio. Ecol. 399,
437 39–42. doi:10.1016/j.jembe.2011.01.013

438 Newell G.M., R.C. & B., 1980. The influence of temperature on the maintenance of metabolic energy balance in
439 marine invertebrates. Adv. Mar. Biol., 17 329-396. 17, 329–396.

440 Petraitis, P.S., 1992. Effects of body size and water temperature on grazing rates of four intertidal gastropods.

441 Aust. J. Ecol. 17, 409–414. doi:10.1111/j.1442-9993.1992.tb00823.

442 Pörtner, H.O., 2002. Physiological basis of temperature-dependent biogeography: trade-offs in muscle design
443 and performance in polar ectotherms. J. Exp. Biol. 205, 2217–2230. doi:10.1016/S1095-6433(02)00045-4

444 Pörtner, H.O., 2002. Climate variations and the physiological basis of temperature dependent biogeography:
445 systemic to molecular hierarchy of thermal tolerance in animals. *Comparative Biochemistry and Physiology Part*
446 *A: Molecular & Integrative Physiology*, 132(4), pp.739-761.

447 Rubal, M., Veiga, P., Cacabelos, E., Moreira, J. and Sousa-Pinto, I., 2013. Increasing sea surface temperature and
448 range shifts of intertidal gastropods along the Iberian Peninsula. *Journal of Sea Research*, 77, pp.1-10.

449 Santini, G., Thompson, R.C., Tendi, C., Hawkins, S.J., Hartnoll, M.G., Chelazzi, G., 2004. Intra-specific variability
450 in the temporal organisation of foraging activity in the limpet *Patella vulgata*. *Mar. Biol.* 144, 1165–1172.
451 doi:10.1007/s00227-003-1283-5

452 Santos, R., Flammang, P., 2007. Intra- And interspecific variation of attachment strength in sea urchins. *Mar.*
453 *Ecol. Prog. Ser.* 332, 129–142. doi:10.3354/meps332129

454 Seabra, R., Wetthey, D.S., Santos, A.M., Lima, F.P., 2011. Side matters: Microhabitat influence on intertidal heat
455 stress over a large geographical scale. *J. Exp. Mar. Bio. Ecol.* 400, 200–208. doi:10.1016/j.jembe.2011.02.010

456 Somero, G.N., 2002. Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of
457 living. *Integrative and Comparative Biology*, 42(4), 780-789.

458 Somero, G.N., 2005. Linking biogeography to physiology: Evolutionary and acclimatory adjustments of thermal
459 limits. *Front. Zool.* 2, 1. doi:10.1186/1742-9994-2-1

460 Southward, A.J., 1964. Limpet grazing and the control of vegetation on rocky shores. *Grazing Terr. Mar.*
461 *Environ.* Blackwell, 265–273.

462 Southward, A.J., Hawkins, S.J., Burrows, M.T., 1995. Seventy years' observations of changes in distribution and
463 abundance of zooplankton and intertidal organisms in the western English Channel in relation to rising sea
464 temperature. *J. Therm. Biol.* 20, 127–155. doi:10.1016/0306-4565(94)00043-1

465 Southward, A.J., Langmead, O., Hardman-Mountford, N.J., Aiken, J., Boalch, G.T., Dando, P.R., Genner, M.J.,
466 Joint, I., Kendall, M.A., Halliday, N.C., Harris, R.P., Leaper, R., Mieszkowska, N., Pingree, R.D., Richardson, A.J.,
467 Sims, D.W., Smith, T., Walne, A.W., Hawkins, S.J., 2004. Long-Term Oceanographic and Ecological Research in
468 the Western English Channel. pp. 1–105. doi:10.1016/S0065-2881(04)47001-1

469 Thompson, R.C., Roberts, M.F., Norton, T.A., Hawkins, S.J., 2000. Feast or famine for intertidal grazing
470 molluscs: a mis-match between seasonal variations in grazing intensity and the abundance of microbial
471 resources, in: Jones, M.B., Azevedo, J.M.N., Neto, A.I., Costa, A.C., Martins, A.M.F. (Eds.), *Island, Ocean and*
472 *Deep-Sea Biology: Proceedings of the 34th European Marine Biology Symposium, Held in Ponta Delgada*
473 *(Azores), Portugal, 13--17 September 1999.* Springer Netherlands, Dordrecht, pp. 357–367. doi:10.1007/978-
474 94-017-1982-7_33

- 475 Tinker, J., Lowe, J., Pardaens, A., Holt, J., Barciela, R., 2016. Uncertainty in climate projections for the 21st
476 century northwest European shelf seas. *Prog. Oceanogr.* 148, 56–73. doi:10.1016/j.pocean.2016.09.003
- 477 Western Channel Observatory, Smyth., T website reference: 08/201
478 <http://www.westernchannelobservatory.org.uk/buoys.php>
- 479 Williams, G.A., De Pirro, M., Leung, K.M.Y., Morritt, D., 2005. Physiological responses to heat stress on a
480 tropical shore: The benefits of mushrooming behaviour in the limpet *Cellana grata*. *Mar. Ecol. Prog. Ser.* 292,
481 213–224. doi:10.3354/meps292213
- 482 Williams, G.A., Morritt, D., 1995. Habitat partitioning and thermal tolerance in a tropical limpet, *Cellana grata*.
483 *Mar. Ecol. Prog. Ser.* 124, 89–103. doi:10.3354/meps124089
- 484 Woodin, S.A., Hilbish, T.J., Helmuth, B., Jones, S.J., Wethey, D.S., 2013. Climate change, species distribution
485 models, and physiological performance metrics: Predicting when biogeographic models are likely to fail. *Ecol.*
486 *Evol.* 3, 3334–3346. doi:10.1002/ece3.680
- Woods, H.A., Makino, W., Cotner, J.B., Hobbie, S.E., Harrison, J.F., Acharya, K., Elser, J.J., 2003 *Funct. Ecol.* 17,
237-245. doi: 10.1046/j.1365-2435.2003.00724.x

487

488

489 Supplementary Information

490 This section provides further details and information to supplement the main text. Raw data files for
491 tenacity, feeding and activity trials can be found as excel files via Mendeley Data online submission.

492 Glossary

- 493 • **Noldus Ethovision XT:** widely applied video tracking software that tracks and analyses the
494 behaviour, movement, and activity of animals.
- 495 • **Trial Control Settings:** settings within Noldus Ethovision XT which when set control the start
496 of the tracking trial.
- 497 • **Detection Settings:** settings within Noldus Ethovision XT which can be manipulated to allow
498 the software to identify and track subject animal.
- 499 • **Track Smoothing Profile:** settings within Noldus Ethovision XT which alter the identify
500 tracked points and ‘smooth’ them to give a more accurate tracking measure. This process
501 takes 5 tracked points and averages them to give a more realistic tracking profile.
- 502 • **Dynamic Subtraction:** a method of detection in Noldus Ethovision XT which uses differences
503 between the most recent recorded image and a continually updated reference image. The
504 most recent image is subtracted from the reference image and differences calculated.
- 505 • **Thresholding:** a technique for dividing an image into two (or more) classes of pixels.

506

507
508
509
510
511
512
513
514
515
516
517
518

519
520
521
522
523
524
525
526
527
528

Ethovision Settings

Following settings refer to setting within Ethovision and should provide adequate information to recreate tracking conducted during investigation. Arena definition was set using images collected by CCTV cameras, these were calibrated using tank length and width. Trial control settings were set to start acquisition after the subject was detected within the arena for less than 1 second. Detection settings were configured to detect limpet movements most accurately. In most cases, Dynamic Subtraction detection was used however separate detection settings were required for light and dark periods. Track smoothing profile was set to smooth across 5 samples and were checked for abnormalities (eg. rogue points, misdetection) which were often replace via interpolation in track editor.

529

530 **Supplementary Table. 1** Results of GLMER with binomial errors examining the effects of temperature upon
531 **probability of feeding taking place across 6 trails (random effect) in the two limpet species**

	Chisq	Df	p-value
Species	2.5502	1	0.110
Temperature	11.752	2	0.002 **
Species:Temperature	3.4089	2	0.182

535

536 **Supplementary Table. 2** LMER examining effects of temperature upon mean area grazed across 6 trails
537 **(random effect) in the two limpet species.**

	Chisq	Df	p-value
Species	0.904	1	0.342
Temperature	8.048	2	0.017
Species:Temperature	0.662	2	0.718

541

542 **Supplementary Table. 3** Results of binomial GLMER examining probability of limpet movement in the three
543 **different temperature regimes.**

	Chisq	Df	p-value
Species	5.35	1	0.952
Temperature	13.467	2	0.027 *
Species:Temperature	3.901	2	0.547

546

547 **Supplementary Table. 4** LMER examining gross distance moved per limpet d in the three different
548 **temperature regimes.**

	Chisq	Df	p-value
Species	1.451	1	0.228
Temperature	7.438	2	0.024
Species:Temperature	1.248	2	0.535

552

553

554

555

556

557

558

559

560

561 **Supplementary Table. 5** Results of LM examining effect of temperature upon duration of tenacity in
562 the two species, controlled for effect of foot size (continuous covariate).

	Df	MS	F	p-value
Log(foot size)	1	8476895	3.711	0.061
Temperature	1	19221	0.008	0.927
Species	1	85094	0.037	0.848
Temperature:Species	1	9901365	4.335	0.044
Residual	43	2284052		

563

564

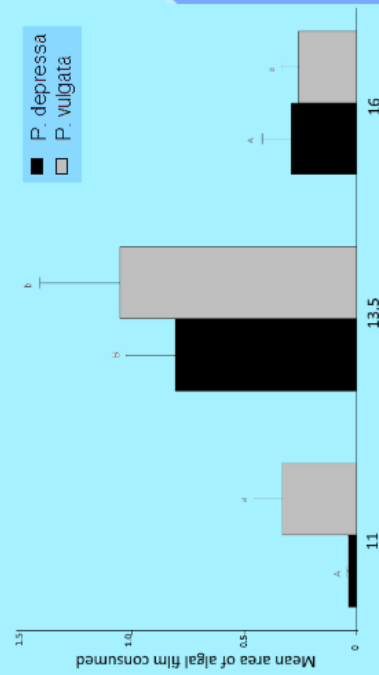
565

Handling the Heat: Responses of Two congeneric Limpet Species to Environmental Temperature Differences

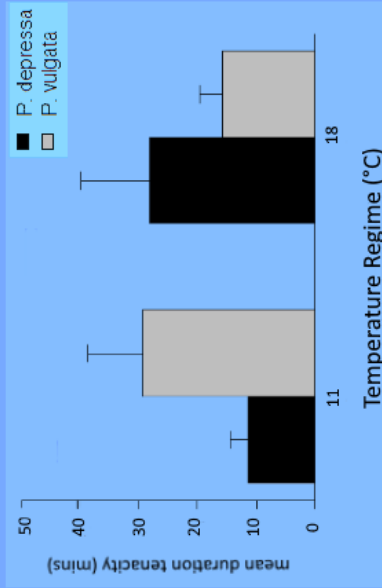
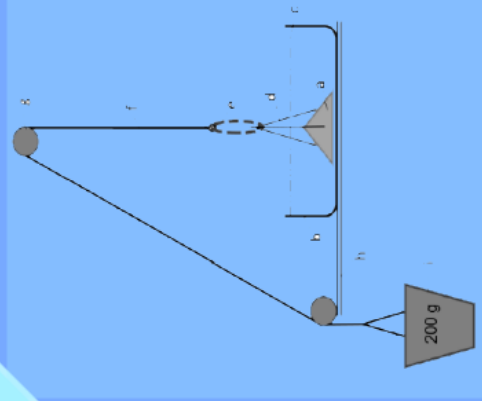
Unpicking the mechanisms behind climate induced distribution shifts observed in Limpet species on the UK coastline

Mesocosm experiments analysed effect of temperature on limpet feeding and movement probability and amount.

The medium temperature elicited a higher level of feeding from both species while the cold temperature treatment (11 °C) caused *P. depressa* to express a significantly lower level of feeding.



Duration tenacity measured using pulley and weight system.



P. depressa able to maintain higher biological function at elevated temperature. Other results suggest *P. vulgata* is more eurythermal.