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

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

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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*

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).

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Methods

Live specimen collection

Adult (Length = 20 – 40 mm, wet mass approx. 0.5 – 8g) *P.vulgata* and *P.depressa* were collected

during low tide at Plymouth Hoe (50.3635N, -4.13965E) between April and July, 2017. Limpets were

removed from the substrate when away from their home scar, reducing chance of damage and

mitigating against stress experienced by the animals. Limpets that were damaged during removal

were not used in the study and were returned to the shore. Prior to experiments, all limpets were

held in an aerated indoor seawater holding tank at ambient temperature.

Feeding rate

Limpets were transferred to individual 7 L experimental tanks supplied with aerated seawater. Diel

tidal (6 hr) and light (12 hr) cycles were initiated in a closed aquarium system in a laboratory

mesocosm room. In accordance with future predictions of sea surface temperature (SST) around

southwest England obtained from two sources (IPCC, 2007, Tinker et al., 2016), three temperature

treatments (11.0 °C, 13.5 °C and 16.0 °C) were established by heating supply tanks using 55w

submersible water heaters (Aqua Pacific UK Ltd., Nursling). Air temperature was controlled and held

at the appropriate monthly average air temperature from the Western Channel Observatory time-

series. Limpets were held at treatment temperature for 48 hours to acclimate before the start of

feeding experiments. This short acclimatory period reflected the short temporal duration of the

experiment, which was designed to test acute responses to changes in thermal environment. For

longer experiments, acclimation time is increased to days/weeks.

Algal biofilm grown on Perspex sheets (14 x 14 x 0.5 cm) incubated in flow through seawater aquaria

for 1 month was used as a food source in feeding trials. Prior to introducing a Perspex food sheet to

the limpet tanks photographs were taken of the biofilm cover using a Canon D300 dSLR, further

photographs were taken on days 3 and 5 of the trial. Images were analysed using ImageJ 1.51k

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.

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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.

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2.5 Duration of tenacity

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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
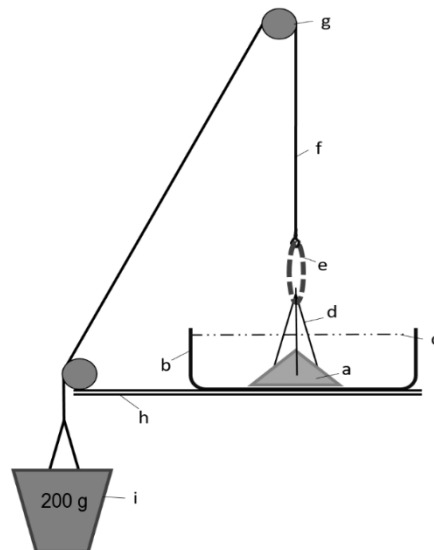
187   squares.

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.

Results

Feeding Rate

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190   Across the species, temperature had a significant impact on the probability of limpets feeding, this

191   was largely attributable to an increase at 13.5ºC in both (temperature χ^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 11ºC (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 χ^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 13.5ºC (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.

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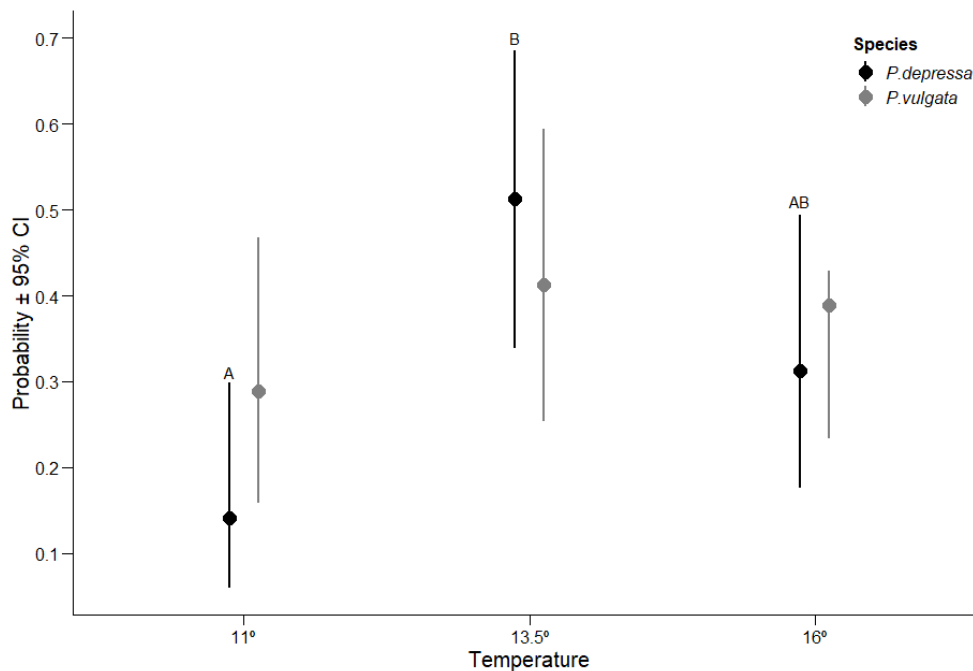
(**Fig. 2.**) Mean (symbols, with 95% confidence intervals (whiskers), back-calculated from log-odds estimates in glm) probability of a limpet [feeding/moving] during the mesocosm trial. Superscripts indicate temperatures differing significantly within species (p < 0.05; Tukey adjusted pairwise estimates).

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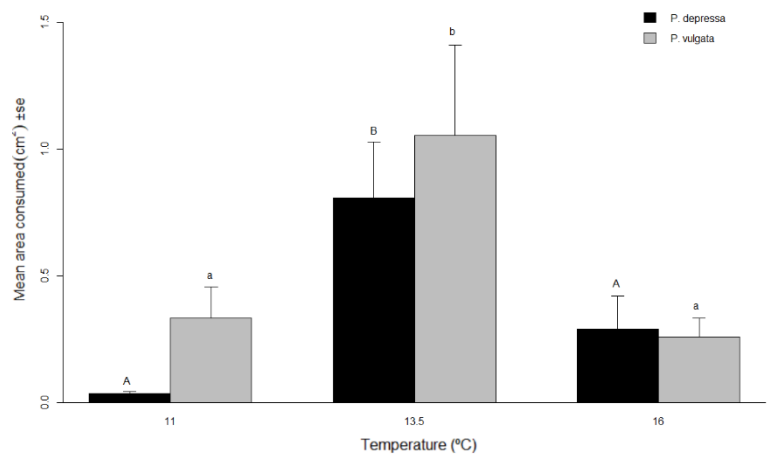
(**Fig. 3.**) Mean (+ s.e) area of algal film consumed by *P. depressa* and *P. vulgata* during trails at each temperature (*P.depressa* n = 11,21,13; *P. vulgata* n = 12, 17, 16). Superscripts indicate treatments differing at P < 0.05 (tests of estimated marginal means using Tukey adjustments) within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

203

Activity

205 Temperature had a significant effect on both the probability of limpets moving (χ^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 (χ^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 11ºC

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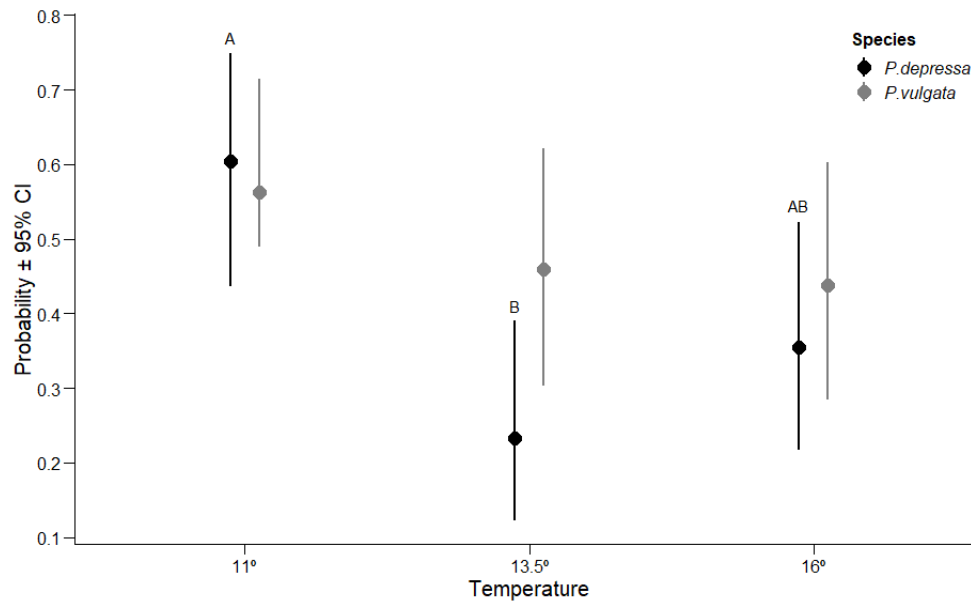
212 individuals nonetheless moved on average 60% further than *P. depressa,* a trend reversed at the

213 higher temperatures.

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(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).

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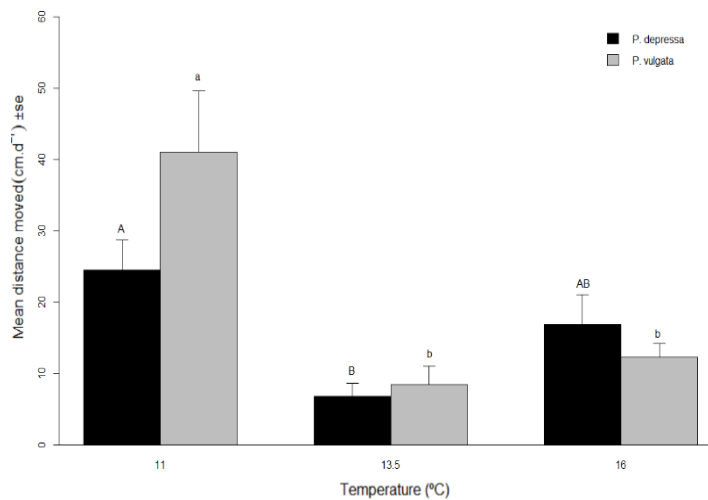
(Fig. 5.) Mean (±se) distance moved by *P. depressa* and *P. vulgata* during trails at each temperature (*P. depressa* n = 18,12,30; *P. vulgata* n = 22, 19, 23). Superscripts indicate treatments differing at P < 0.05 (tests of estimated marginal means using Tukey adjustments) within species .

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*.

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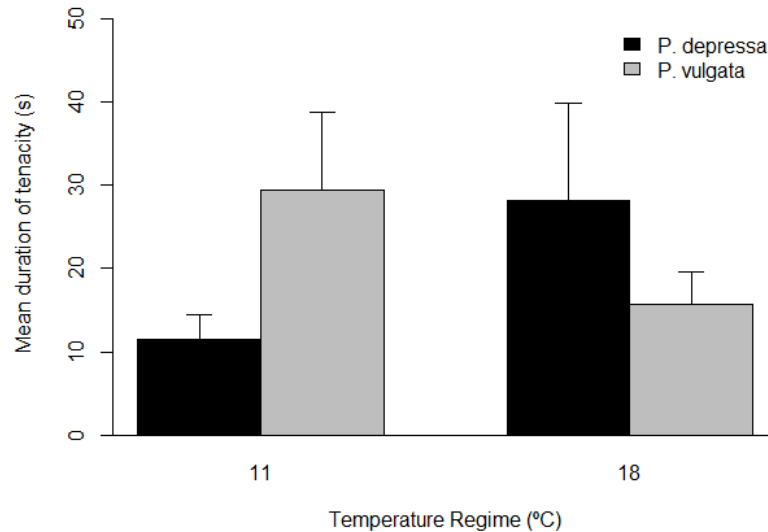
(**Fig. 6.**) Duration of tenacity in *P. depressa* and *P. vulgata* held under under two temperature regimes (all n = 12).

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, whist 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 whist *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 it's 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 spatial 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 Wethey, 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

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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.

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.

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Ethovision Settings

510   Following settings refer to setting within Ethovision and should provide adequate

511   information to recreate tracking conducted during investigation. Arena definition was set using

512   images collected by CCTV cameras, these were calibrated using tank length and width. Trial control

513   settings were set to start acquisition after the subject was detected within the arena for less than 1

514   second. Detection settings were configured to detect limpet movements most accurately. In most

515   cases, Dynamic Subtraction detection was used however separate detection settings were required

516   for light and dark periods. Track smoothing profile was set to smooth across 5 samples and were

517   checked for abnormalities (eg. rogue points, misdetection) which were often replace via

518   interpolation in track editor.

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Supplementary Table. 1 Results of GLMER with binomial errors examining the effects of temperature upon
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

Supplementary Table. 2 LMER examining effects of temperature upon mean area grazed across 6 trails
(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

Supplementary Table. 3 Results of binomial GLMER examining probability of limpet movement in the three
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

Supplementary Table. 4 LMER examining gross distance moved per limpet d in the three different
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

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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		

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

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

Mesocosm experiments analysed effect of temperature on limpet feeding and movement probablity 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 signficantly lower level of feeding.

Duration tenacity measured using pulley and weight system.

P. depressa able to maintain higher biological function at elvated temperature.

Other results suggest *P. vulgata* is more eurythermal.