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

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Abstract

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

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

1. Introduction

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

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

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

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

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

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

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

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

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

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

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

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

2.4 Activity Rate

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

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

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

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

2.5 Duration of tenacity

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

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

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

To standardise measurements, limpets were tapped to stimulate shell clamping, three times before the string was attached and again three times before the weight was gently lowered until full 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

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

(**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

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.

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215

216

(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.

Biological Function Analysis: Duration of Tenacity

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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Supplementary Information

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

Glossary

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

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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.

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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