

Timing Metabolic Depression: Predicting Thermal Stress in Extreme Intertidal Environments

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Submitted September 5, 2019; Accepted May 15, 2020; Electronically published August 25, 2020

Online enhancements: supplemental PDF.

ABSTRACT: Anticipatory changes in organismal responses, triggered by reliable environmental cues for future conditions, are key to species' persistence in temporally variable environments. Such responses were tested by measuring the physiological performance of a tropical high-shore oyster in tandem with the temporal predictability of environmental temperature. Heart rate of the oyster increased with environmental temperatures until body temperature reached $\sim 37^{\circ}\text{C}$, when a substantial depression occurred ($\sim 60\%$) before recovery between $\sim 42^{\circ}$ and 47°C , after which cardiac function collapsed. The sequential increase, depression, and recovery in cardiac performance aligned with temporal patterns in rock surface temperatures, where the risk of reaching temperatures close to the oysters' lethal limit accelerates if the rock heats up beyond $\sim 37^{\circ}\text{C}$, coinciding closely with the body temperature at which the oysters initiate metabolic depression. The increase in body temperature over a critical threshold serves as an early-warning cue to initiate anticipatory shifts in physiology and energy conservation before severe thermal stress occurs on the shore. Cross-correlating the onset of physiological mechanisms and temporal structures in environmental temperatures, therefore, reveals the potential role of reliable real-time environmental cues for future conditions in driving the evolution of anticipatory responses.

Keywords: predictability, rocky shore, *Isognomon nucleus*, thermal performance, tropical.

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Am. Nat. 2020. Vol. 196, pp. 501–511. © 2020 by The University of Chicago. 0003-0147/2020/19604-5946\$15.00. All rights reserved.

DOI: 10.1086/710339

Introduction

Species respond to environmental changes at multiple temporal scales by detecting and reacting to environmental cues, which can induce phenotypic and/or genotypic changes over ecological and evolutionary timescales (Levins 1968; Via and Lande 1985; Bennett and Huey 1990). While predictable and periodic changes in environments may select for genotypes with rigid endogenous biological rhythms, such as circadian and circatidal rhythms (Aschoff 1960; Zann 1973), acute and stochastic changes will favor the evolution of more flexible and rapid response systems (Stevenson 1985). Stochastic environments are generally unpredictable over long time periods, but the autocorrelative characteristics of a broad range of environmental processes enable the prediction of future conditions using reliable signals over shorter timescales. These include local/short-term processes such as the probability of rain following cumulus cloud formation (Cooper et al. 2013) or the dependency of intertidal rock temperature on previous days' temperatures (Dong et al. 2017) to large-scale/long-term processes such as the frequency of freezing events anticipated by the date of snowmelt (Wheeler et al. 2014) or the prevalence of hot days resulting from preceding precipitation deficits (Mueller and Seneviratne 2012). Temporal structures in these environmental processes thus enable predictions for future conditions and can, if effectively exploited by organisms via physiological/behavioral responses, play a critical role in driving the evolution of adaptive strategies to enhance performance in such stochastic environments.

The high-shore area of intertidal habitats represents one such stochastic environment, with very challenging thermal regimes (Tomanek and Helmuth 2002; Harley 2008). Species inhabiting these environments have reduced opportunities to gain energy from feeding (Little 1989; Williams and Little 2007) and often experience lethal physical conditions during low tides (Marshall et al.

2015). During periods of emersion, thermal and desiccation stressors induce a wide variety of physiological damage that requires adaptive behavioral and physiological responses to survive (Garrity 1984; Somero 2002; Williams et al. 2005). The thermal regimes that individuals experience during low tides are often highly contingent upon local, fine-scale weather conditions, which interact with spatial features such as shore topography, orientation, and the presence of refuges to produce complex spatial and temporal thermal mosaics (Helmuth and Hofmann 2001; Helmuth et al. 2006; Harley 2008; Dowd et al. 2015).

While mobile species can actively select microhabitats to mediate variable thermal conditions, sessile species are generally unable to do so after settlement, and their body temperatures therefore are largely governed by environmental heat fluxes (Helmuth 1998). Sessile species are also constrained in their feeding opportunities, as they are unable to search actively for food and to feed must rely on wave splash/tidal immersion, which are limited for species living higher on the shore. As a result, high-shore sessile species are largely dependent on physiological responses to conserve energy and buffer thermal stress during low tide periods (Stevenson 1985; Williams and Somero 1996; Buckley et al. 2001). Physiological responses are temperature dependent in ectotherms, where metabolic rates generally increase with body temperatures until they break down at high temperatures, causing hypoxia and reduced mitochondrial capacity and hence affecting aerobic metabolism (Pörtner 2001; Somero et al. 2017). Variation in metabolic rate, however, is not only driven by the chemodynamic effects of temperature per se but also can be regulated by molecular pathways that can suppress or elevate physiological responses (Hand and Hardewig 1996). Metabolic depression is such a response, and it is invoked to suppress metabolism during extreme variations of the external environment (Storey and Storey 1990). Metabolic depression has been found in a wide range of taxa that experience extreme desiccation, anoxia, and cold or heat stresses (Hand and Hardewig 1996; Guppy and Withers 1999; Storey and Storey 2004; Richards 2010). By suppressing metabolism, the energy required to survive extreme conditions is effectively conserved (Hand and Hardewig 1996) and can later be allocated to critical functions when normal metabolism resumes.

The speed with which adaptive physiological mechanisms such as metabolic depression can be invoked is, however, constrained by the time required to activate transcriptional and translational processes. Increased expression of heat-shock proteins in snails experiencing thermal stress, for example, may require a few hours (Tomanek and Somero 2000; Tomanek 2002; Zhang et al. 2014). Such a delay renders the timely acquisition of physiological responses challenging in intertidal ectotherms, since mortality-inducing heat

events can occur rapidly during low tides (Williams and Somero 1996; Mislán et al. 2009). To be adaptive, therefore, the induction of physiological responses should be anticipatory and triggered by environmental signals that reliably predict the occurrence of severe thermal stress, such that adjustments across molecular, organellar, cellular, and organismal levels can be achieved at an appropriate timescale to maintain physiological functions (Somero et al. 2017).

Intertidal rock temperatures are temporally autocorrelated within a finite timescale (i.e., lower/higher present temperatures are, in general, associated with lower/higher future temperatures; Helmuth et al. 2006; Dong et al. 2017). Such a temporal structure in environmental dynamics provides a cue for adaptive physiological adjustments, triggered by body temperatures that usually precede, and can be used to predict, forthcoming severe thermal stress. Using the harsh and variable thermal environments experienced on tropical rocky shores, we investigated the coupling between the onset of metabolic depression and temporal structure in environmental temperatures on a high-shore species in Thailand. Two species are abundant on the high shore at the study site—the oyster *Isognomon nucleus* (Lamarck, 1819) and a littorinid snail *Echinolittorina malaccana* (Philippi, 1847). In other locations, the littorinids are known to undergo metabolic depression at high body temperatures (Marshall et al. 2011), but the relevance of metabolic depression to environmental predictability remains unclear. Given the short-term temporal structure in rock temperature, we hypothesized that the oysters may exhibit anticipatory physiological adjustments to future stressful conditions, following reliable cues in rock temperatures, in order to conserve energy in this extreme and dynamic environment. To test this hypothesis, we compared the oysters' physiological performance (heart rate) to onshore environmental temperatures to explore how the observed responses aligned with environmental signals and whether environmental temperature could be an effective cue for induction of metabolic depression.

Material and Methods

Onshore Thermal Environments

The oyster, *Isognomon nucleus* (max. size ~20 mm), forms dense beds in the high shore at 2.5–3 m + mean sea level (Samakraman et al. 2009) along the east coast of the Gulf of Thailand. To record rock surface temperatures, iButton dataloggers (DS1922L, Maxim) were deployed on two shores (sites A and B) within Tam Pang Bay, Sichang Island (13°08'52"N, 100°48'11"E). Sichang Island experiences mixed, semidiurnal tides and, when low tides occur during early-afternoon, rock surface temperatures often exceed 50°C and can reach >60°C during the hot and dry

season (March to June). Rock temperatures are lower from November to February, during which *I. nucleus* settle, when low tides fall at nighttime (Samakraman 2013). In this study, temperatures were logged at either hourly (from March to May 2016) or half-hourly intervals (from May to October 2016, from January to May 2017, and from May to August 2017) along three tidal heights across the oysters' vertical distribution (see the supplemental PDF available online; $\Sigma n = 2 \text{ shores} \times 3 \text{ tidal heights} \times 4 \text{ recording periods} \times 1\text{--}3 \text{ replicates} = 67 \text{ continuous time series}$).

Thermal Sensitivity of Heart Rate

Large *Isognomon nucleus* (15–20 mm in shell length; Samakraman et al. 2009) were collected haphazardly in the hot and dry season (May 2016 and May 2017) from the areas where dataloggers were deployed, and they were maintained in aerated natural seawater at ambient conditions <24 h prior to heart rate measurements.

Heart rate was used as an indicator of metabolism and recorded using noninvasive plethysmography (Burnett et al. 2013). Infrared sensors (CNY 70, Vishay) were attached using cyanoacrylate glue onto individual oysters, the shells of which were gently filed smooth and blotted dry. Individuals in plastic containers were held, in air, in a programmable water bath (GP200, Grant), which heated the oysters at a rate of 0.22°C per minute (to mimic the heating rate of the rock surface at the study sites) from 25° to >55°C (~2.5 h duration). Humidity was not measured inside the plastic containers, but body temperatures of additional oysters were measured using a fine-tipped thermocouple (Type K) and recorded using a digital thermometer (TM-947SD, Lutron, Taiwan), which accounted for the effects of humidity and other heat fluxes (e.g., conduction) in modifying body temperatures inside the plastic containers. Infrared signals were amplified and filtered (AMP03, Newshift, Portugal) before being visualized (PicoScope 2204, Pico Technology). The number of heartbeats was counted manually every minute (expressed as beats per minute [bpm], $\Sigma n = 2 \text{ years} \times 12\text{--}14 \text{ replicates} = 26 \text{ oysters}$ in total).

Statistical Analyses

To investigate variations in heart rate with body temperature, segmented regressions were performed to calculate the Arrhenius breakpoint temperature (ABT; Stillman and Somero 1996). The ABT occurs when the scaling between metabolic rate and body temperature changes in relationship (Cossins and Bowler 1987). Although Arrhenius relationships might be oversimplistic when describing complex

biological processes (Schulte 2015), the ABT is useful to indicate alterations in overall metabolic processes and thermal damage causing denaturation of proteins (Cossins and Bowler 1987), and is thus extensively used to identify changes in metabolic patterns in intertidal species (e.g., Stillman and Somero 1996; Stenseng et al. 2005; Dong and Williams 2011; Marshall et al. 2011). Heart rate (HR) and Temperature (Temp) were first transformed to $\ln(\text{HR})$ and $1,000/(\text{Temp} + 273)$, and segmented regression models with three to six breakpoints were fitted using transformed HR as the response and transformed Temp as the explanatory variable. Three to six breakpoints were considered, because the M-shaped HR trajectories clearly refuted models with less than three breaks (see fig. 1) and because excessive segments will result in spurious breakpoints, which are not indicative of major shifts in the HR-Temp relationship (i.e., overfitting without clear biological meaning). Akaike information criterion (AIC) was used to compare models with different numbers of breakpoints, and the model with the lowest AIC score was selected. From the set of breakpoints fitted in the selected model, critical breakpoints were further identified where the slopes before and after the breaks changed in sign (i.e., from a positive to negative slope, or vice versa). Three such critical breakpoints were identified for each individual (cBP1–cBP3; fig. 1). Flatline temperature (FLT, or temperature when HR falls to zero; Somero 2007) was calculated by regressing back-transformed HR against body temperature (HR in bpm and temp in degrees Celsius) after the final breakpoint, and extrapolating HR to zero to obtain the x -intercept as the FLT. For each individual, depression breadth (difference in temperatures between cBP1 and cBP3) and persistence breadth (difference in temperatures between cBP3 and the final breakpoint; see fig. 1) were calculated. Data were pooled across 2016 and 2017, as critical breakpoint HRs, breakpoint temperatures, and FLT did not differ (t -tests; all $P > .05$). All segmented regressions were performed using the segmented package in R (Muggeo 2008).

Temporal Structure of Temperature Variation during Daytime Emersion

To quantify general temporal patterns in the oyster's thermal environment, percentages of time during the day (including both submersion and emersion from all four recording periods, excluding nighttime temperatures) when temperatures exceeded each critical breakpoint (cBP1–cBP3), final breakpoint, and FLT were calculated. Temperature time series within periods of daytime aerial emersion were further extracted from the dataloggers (time periods immediately after the loggers were emersed and before the tide returned; see fig. S1; figs. S1–S9 are available online). For onshore temperatures that were increasing

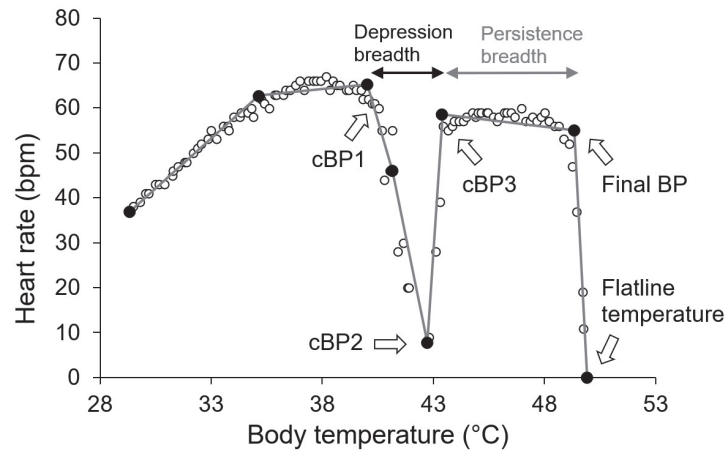


Figure 1: Example of cardiac thermal performance of *Isognomon nucleus* over an acute warming ramp. Open circles are raw data of heart rates, while filled circles are breakpoints fitted from the best segmented regression model, in addition to the first data point and the extrapolated flatline temperature (FLT). Filled circles are joined by gray lines to demonstrate changes in slopes. Initially, heart rate increases as temperature increases but depresses to ~41%, on average, of the peak value at critical breakpoint 1 (cBP1) until critical breakpoint 2 (cBP2), when heart rate increases again. Such an increase peaks at critical breakpoint 3 (cBP3, which delimits depression breadth with cBP1), after which heart rate remains relatively stable with temperature changes (the persistence breadth), until temperature continues to rise above the final breakpoint (final BP), when heart rate drops rapidly until FLT.

(indicating that the rock surface is heating up), subsequent temperature readings until the next submergence or dusk were extracted to form an emersion period with starting temperature T , where T is the first temperature of the extracted segment during that emersion period. Starting temperatures, T , were binned into 1°C intervals from 27° to 46°C, where the remaining emersion period following T represents the time series of rock temperatures after the rock has reached T since emersion. The probability of attaining cBP3 or above (P_{cBP3}) was computed for each T by dividing the number of emersion periods with temperatures above 47.0°C (= mean cBP3 temperature) by the total number of emersion periods. The mean and standard deviation of P_{cBP3} (over the 1–3 iButtons and four recording periods at each shore and tidal height) and their ratios were calculated for each T . Third-order polynomials were fitted to the relationships between T and the mean and mean/SD ratios of P_{cBP3} along each tidal height in each site using the `nlstools` package in R (Baty et al. 2015). Inflection points were bootstrapped 1,000 times from the fitted polynomial parameters, which indicated the switch in curvature from a gradual to rapid increase in P_{cBP3} with temperatures. Estimates of both the mean and SD of P_{cBP3} were not correlated with sample sizes among starting temperatures (sample sizes ranged from six to 12; see the supplemental PDF). To further assess whether the temporal patterns in mean P_{cBP3} will change when the rock is cooling down (e.g., emersion during late afternoon or at night), the computation of P_{cBP3} was repeated as described above but using emersion temperature time

series from both day and night, and temperature segments extracted from the time series after the rock temperature dropped below T . All relevant data have been deposited in Open Science Framework (<https://osf.io/7fjs5/>; Hui 2020).

Results

Cardiac Thermal Performance of Isognomon nucleus

Oyster HR followed a distinctive M-shaped trajectory as body temperature increased (fig. 2), with three critical

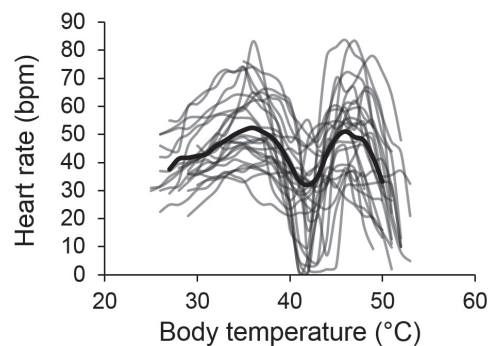


Figure 2: Cardiac thermal performance of individuals of *Isognomon nucleus* (fitted using spline functions) over an acute warming ramp. Each transparent curve represents individual cardiac performance over different temperatures, while the black curve represents the average ($n = 26$ in total from the hot and dry seasons of both 2016 and 2017).

Table 1: Mean, SD, minimum, and maximum of critical breakpoint (cBP1–cBP3) and final breakpoint (final BP) temperatures of heart rate of *Isognomon nucleus* during acute warming ramps

Metrics	cBP1	cBP2	cBP3	Final BP	FLT	LT ₅₀
Mean (°C)	36.8	42.1	47.0	50.6	51.6	54.3
SD	2.3	1.4	2.6	1.4	1.4	.6
Min (°C)	32.1	38.8	42.3	46.4	49.6	53.9
Max (°C)	41.9	44.5	53.2	53.2	56.1	55.0

Note: Critical breakpoints are body temperatures at which the slope between heart rate and body temperature changes in sign (i.e., from positive to negative, or vice versa) on an Arrhenius plot. Flatline temperatures (FLT) were extrapolated from the decreasing heart rate after the final breakpoint, using back-transformed raw data. Lethal temperatures at 50% mortality (LT₅₀) were obtained from independent sets of oysters using logistic regressions (see the supplemental PDF). For cBP2, cBP3, final BP, and FLT, $n = 26$. For cBP1, $n = 25$. For LT₅₀, $n = 3$ (three replicate logistic models; see the supplemental PDF).

breakpoints (table 1) and four to six evident Arrhenius breakpoints (table S1, available online). After increasing with body temperature to the first critical breakpoint (cBP1; $\sim 37^\circ\text{C}$, HR averaged at 54.9 ± 13.3 bpm, mean \pm SD), oyster HR dropped to $41\% \pm 30\%$ of the HR at cBP1, and reached a minimum of 0 bpm (22 ± 16 bpm) at cBP2 ($\sim 42^\circ\text{C}$), before rising to peak again at cBP3 ($\sim 47^\circ\text{C}$; HR averaged at 55.0 ± 14.5 bpm; see complete descriptions of breakpoint temperatures in table 1). The estimated FLT ($51.6^\circ \pm 1.4^\circ\text{C}$) of the oyster occurred rapidly after the final breakpoint (~ 5 min under the same heating rate). Depression breadth (difference between cBP1 and cBP3 temperatures) was negatively correlated with persistence breadth (difference between cBP3 and the final breakpoint temperatures; $r = -0.79$, $P < .001$; fig. S2), suggesting that the HR could persist over a wider temperature range before the final breakpoint if the depression duration was shorter. Depression/persistence breadths were, however, not correlated with heart rates at cBP1 or cBP3 (r ranged from -0.03 to 0.2 ; $P > .05$).

Thermal Environment

Rock surface temperature exceeded the cBP1 of the oyster for $62.7\% \pm 4.3\%$ (mean \pm SD, site A) and $61.3\% \pm 1.7\%$ (site B) of daytime periods over the four recording periods in 2016 and 2017 (figs. 3, S3; see above for descriptions of breakpoints), with the highest recorded temperature being 62.5°C . Rock temperature exceeded the FLT of the oysters for $2.6\% \pm 2.2\%$ (site A) and $2.9\% \pm 0.5\%$ (site B) of the time, with $\sim 0.7\%$ of the time being higher than the oysters' LT₅₀ ($\sim 54^\circ\text{C}$ in air; see fig. S4; G.A.W. and M.G., unpub-

lished data), confirming that the oyster experiences both extreme and lethal thermal stress periods on the shore.

Alignment between Cardiac Function Breakpoints and Environmental Risk

Temperature distributions within emersion periods in each day (i.e., the time period until the next submergence or dusk) were strongly autocorrelated, with the rock surface likely to become hotter if the starting temperature was hot (generally $>35^\circ\text{C}$; see fig. S5). Both the mean probability of the occurrence of temperatures \geq cBP3 (P_{cBP3}) and the mean/SD ratio of P_{cBP3} increased with higher starting temperatures (fig. 4). Such increases were gradual and tapering before $\sim 37^\circ\text{C}$ (inflection points fitted by the polynomials) but accelerated rapidly afterward at higher starting temperatures (figs. 4, S6, S7). The inflection points aligned closely with the cBP1 of the oysters, indicating that the risk for the oysters to exceed cBP3 on the shore accelerated rapidly if their body temperature reached cBP1 during emersion periods. When the rock was cooling during late afternoon or night, however, P_{cBP3} reduced to minimal values (mean \pm SD = 0.04 ± 0.03 , ranging from 0 to 0.13) across different starting temperatures (fig. S8).

Discussion

Extreme Environments Experienced by Oysters on Tropical Rocky Shores

The extreme onshore thermal environment, exceeding the FLT of the oyster *Isognomon nucleus* 1%–4% of the time during daytime periods, would be a strong driver for selection of physiological mechanisms to avoid mortality and maintain cellular functions at high temperatures (Somero 2002; Giomi et al. 2016). Heat tolerance of the oyster, as a result, is substantially higher (heart failure occurring $>52^\circ\text{C}$ during acute heating in air) than lethal temperatures of most intertidal, including tropical, mollusks (Liu and Morton 1994; Williams and Morritt 1995; Luk 2011; Nguyen et al. 2011). The strong desiccation stress associated with high rock temperatures can further affect the oysters (desiccation at 45°C can cause a reduction of $>15\%$ wet weight in 5 h; M.C., unpublished data; see the supplemental PDF) and, in combination, these stressors impose conflicting needs to conserve water but cool evaporatively via “gaping” (opening the valves; Hicks and McMahon 2003; Nicastro et al. 2012). Individuals at different life stages, however, might experience dissimilar thermal/desiccation regimes because of differences in body size, and thus realized body temperatures (Helmuth 1998), or risks of mortality because of enhanced tolerance in adults compared with juveniles (Liu and Morton 1994; Jenewein and Gosselin 2013). Despite the fact

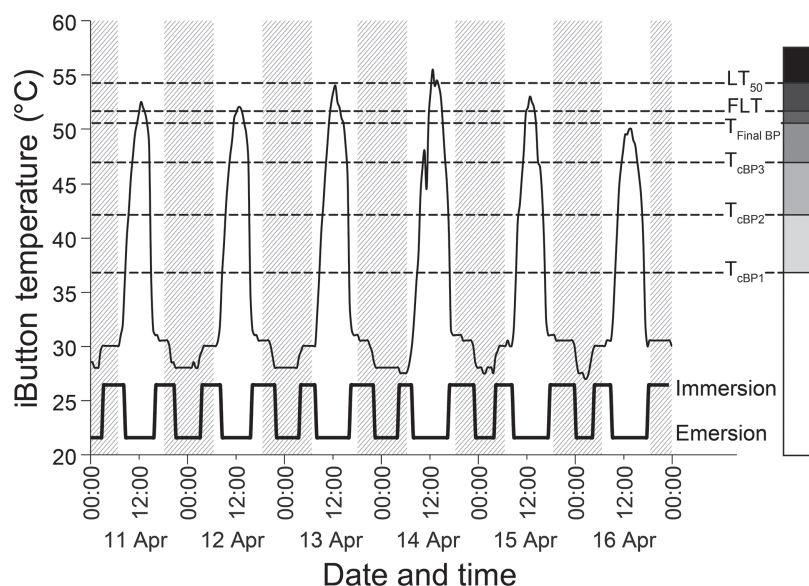


Figure 3: Example of a rock surface temperature time series measured half-hourly by iButtons deployed within the tidal range of *Isognomon nucleus* on Sichang Island, Thailand (data are from April 11 to 16, 2017). Shaded regions represent nighttime, and unshaded regions represent daytime. The thick black line indicates the tidal regime. Horizontal dotted lines represent mean body temperatures at different breakpoints in cardiac function (see fig. 1).

that sessile intertidal species can ameliorate thermal stress via larval habitat choice and/or forming aggregations (Lathlean et al. 2013; Jurgens and Gaylord 2018), and the fact that the oysters are often found in dense aggregations, the shell temperatures of *I. nucleus* are similar to rock surface temperatures (infrared imagery, difference between shell and rock = -0.23 ± 0.69 °C; $n = 26$; G.A.W., unpublished data; see the supplemental PDF), so their limited ability to change microhabitats during tidal emersion makes physiological mechanisms the most appropriate strategies to cope with onshore temperature fluctuation (Stevenson 1985; Harley 2008). Molecular responses inducing stress-related proteins (such as heat-shock proteins, ubiquitin, and sequestosomal proteins; Feder and Hofmann 1999; Gracey et al. 2008; Bjelde and Todgham 2013) would be especially important for these species, which are unable to seek thermal refuges.

Depression as a Convergent Trait to Persist in Harsh Environments

The oyster demonstrated a characteristic depression in heart rate at $\sim 37^{\circ}\text{C}$ and, in some individuals, their hearts stopped for nearly 1 min before beating again at temperatures above 40°C . Such suppression of heart rates could be the result of a reduction in aerobic metabolism as temperature increases,

which disrupts mitochondrial membrane fluidity and can reduce the production of aerobic adenosine triphosphate from mitochondrial respiration (Dahlhoff et al. 1991; Weinstein and Somero 1998; Somero 2002). Sealing of the oysters' valves to avoid desiccation will also limit the oxygen available during emersion. These factors may suppress aerobic metabolism directly and reduce the oysters' heart rates from cBP1, irrespective of the temperature distribution during the remaining emersion period. The recovery of heart rates following depression, conversely, may be accomplished by a replenishment of energy, either through direct influx of oxygen (e.g., via gaping to take in air, although such behavior was not observed during the heating process) to recover aerobic metabolism or through switching energetic pathways to anaerobic metabolism such as opine anaerobiosis, which has been demonstrated in other intertidal invertebrates (Storey and Storey 2004; Han et al. 2017).

While the depression in the oysters' heart rates could be caused by limitations in aerobic respiration, metabolic depression is generally invoked as the primary strategy to actively minimize metabolism and thus avoid escalating energetic demands during adverse conditions (Storey and Storey 1990; Marshall and McQuaid 1991, 2011; Guppy and Withers 1999). This conservation strategy will be particularly important for high-shore species, which are briefly splashed/immersed so time for feeding and energy acquisition is heavily constrained (Marshall and McQuaid 1991; Marshall

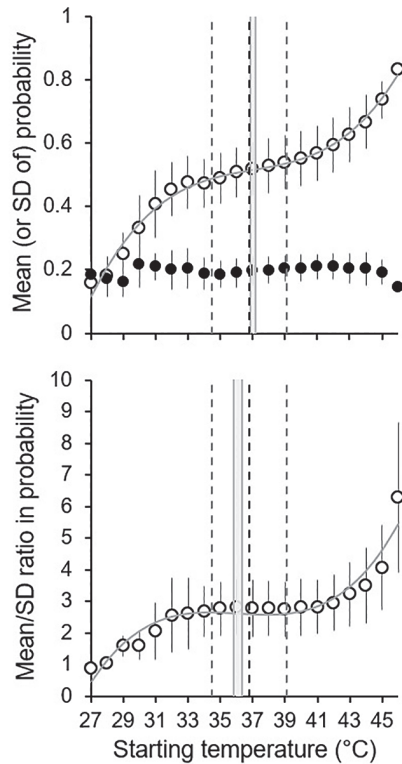


Figure 4: Variation in the mean (open circles, upper panel), standard deviation (filled circles, upper panel), and their ratio (open circles, lower panel) of P_{cBP3} (the probability to exceed critical breakpoint 3 in the remaining emersion period until next tidal submergence/dusk) with increasing starting temperature. Data were pooled over six to 12 iButton data series from two sites and three tidal heights (for details, see the supplemental PDF). Gray curves represent third-order polynomial regressions between the mean (or mean/SD ratio) of P_{cBP3} with increasing starting temperatures, while vertical lines around each circle represent \pm SD in the estimates. Dashed vertical lines represent the mean \pm SD of cBP1 of *Isognomon nucleus*, while gray solid lines with shading represent the inflection points (\pm SD) of the polynomials.

et al. 2011; Sokolova et al. 2012; Bjelde and Todgham 2013). Littorinid snails, which inhabit the same high intertidal environment, also depress metabolism, although to a lesser extent at body temperatures between 35° and 45°C, before the return to rapid, thermally correlated metabolism at higher temperatures (Marshall and McQuaid 2011; Marshall et al. 2011). The selection for metabolic depression among these distantly related species suggests a convergent adaptation to thermally extreme environments. While this strategy allows conservation of energy to survive adverse conditions, it will, however, increase the accumulation of waste metabolites and reduce protein biosynthesis, affecting normal cellular functions (Hand and Hardewig 1996; Williams and McMahon 1998; Uglow and Williams 2001). Metabolic depression is therefore likely to be under strong conflicting se-

lection pressures, owing to the trade-offs between the costs and benefits that will be incurred when body temperatures reach a critical threshold.

When to Depress? Cues from Environmental Temperatures

The benefits of depressing metabolism depend on whether individuals can correctly predict potential adverse conditions. The most obvious cue would be a threshold environmental temperature or pattern, which would trigger the depression response. To achieve this, the temporal structure of temperature distribution could be exploited over evolutionary time to adjust physiological pathways by responding to critical environmental signals (McNamara et al. 2006). In order to be reliable and useful, such a signal must be a consistent predictor of future conditions (Schmidt et al. 2010). In this case, the temporal autocorrelation of rock surface temperature (which correlates strongly with body temperatures of sessile species; Bertness 1989) indicated that lower/higher starting temperatures usually precede lower/higher future temperatures before the next submergence/dusk. Low temperatures, such as those typically associated with the beginning of falling tides ($\sim 25^{\circ}$ – 30° C) lack predictive power as a cue, since the rock surface might become extremely hot or stay cool during the remaining emersion periods, depending on local weather conditions (Helmuth et al. 2014; see also fig. S9). As temperatures increase ($>35^{\circ}$ C), however, the rock is likely to stay hot or become hotter, and the oysters are increasingly likely to experience severe thermal stress.

The risk of encountering severe thermal stress in the remaining emersion period, therefore, changes with the body temperatures experienced by the oysters. The risk to exceed cBP3, for example, increased from more benign conditions (seawater, $\sim 28^{\circ}$ C) and reached a plateau around 37° C before rising again at temperatures $>40^{\circ}$ C. If the oyster experiences a temperature near its first critical breakpoint in HR ($\sim 37^{\circ}$ C), then the future temperature will be much more likely, and more consistently expected, to exceed its third critical breakpoint before the next submergence or dusk. Such temperature ranges (35° – 39° C), therefore, represent a reliable signal, over an ecologically relevant timescale, to actively depress metabolism. Such temperature cues, however, are dependent on their directions (i.e., whether the temperature is increasing or decreasing when attaining these cues). The risk for temperature to exceed cBP3 is only accelerating at $\sim 37^{\circ}$ C when the rock is warming, but not cooling down. This directionality should, therefore, act in combination with the temperature cues to initiate metabolic depression only when the oysters are heating up. Likewise, the ramping rate at which the temperature cues are attained might also modify the depression patterns. If the

oysters are heating up at a much slower rate compared with the onshore conditions, no depression will be expected to occur if the onshore temporal structure in temperature is an important driving force in shaping metabolic depression patterns. These hypotheses could be tested by ramping the oysters' body temperatures up using different ramping rates, as well as reversing the ramping direction to cool the animals to cBP1, and assessing whether depression occurs again.

When oyster individuals settle at different locations, and experience different temporal variations in temperature over different years, individuals will experience variations in risk (see variation in individual HR). Under such a scenario, physiological adaptation involving the temporal signals in risk could be selected for via different genotypes (Levins 1968). Variation in the predictive power of environmental cues, therefore, represents an important selection mechanism shaping the initiation of organismal responses in species experiencing temporally/spatially structured environments (Dall et al. 2005). Temporal structures of environments, if present and preceded by reliable cues, can be exploited by organisms to adjust their physiological/behavioural strategies in preparing for future conditions, as in the initiation of metabolic depression in *Isognomon nucleus* under reliable environmental signals. Such anticipatory responses are not, however, limited to sessile species, but rather are efficient strategies for a broad array of taxa in coping with variable environments. Seasonal migrations in many bird and fish species, for instance, are triggered by changes in day length and/or temperature, which predict seasonal shifts with high probability and consistency (Winkler et al. 2014). Spatial cues such as variations in food abundance are also heavily exploited by species foraging in heterogeneous environments to predict changes in food richness and thus adjust their movement strategies (Morales and Ellner 2002; Morales et al. 2005; Hui and Williams 2017).

Costs and Benefits of Depressing Metabolic Rate

While the exact mechanism of metabolic depression in the oysters remains unknown, by depressing heart rates when body temperatures approach $\sim 37^{\circ}\text{C}$, they are likely to avoid exhausting energetic and/or oxygen reserves and reduce the production of harmful reactive oxygen species (Abele et al. 2002; Storey and Storey 2004). This strategy allows conservation of respiratory substrates/oxygen that would otherwise be used for the elevated metabolic demands during severe thermal stress, which will likely and consistently occur if the rock temperature exceeds the first critical breakpoint. As rock temperature exceeds the first critical breakpoint of the oyster 60% of the time during daytime period, metabolic depression, therefore, provides a substantial energetic saving. If the depression occurs at temperatures not aligned with environmental variations, such an

energy-conservation strategy will not be as effective. If, for example, the oysters start to depress metabolism at benign conditions around 30°C , they will be conserving energy that might not always be of use, since the probability of experiencing severe thermal stress is low and more variable. Conversely, if depression starts only at very high temperatures (e.g., after the inflection of P_{cBP3} against temperatures, where the risk to attain cBP3 increases rapidly), where the oysters are more likely to experience extreme thermal stress, there may be insufficient time to express the necessary suite of molecular responses before mortality-inducing temperatures occur. In intertidal gastropods (*Tegula* spp.), heat-shock proteins require 1–3 h to reach maximal levels following moderate acute thermal stress and up to ~ 30 h to attain peak levels after extreme heat stress (Tomanek and Somero 2000). Upregulation of stress protein production after acute thermal stress also lasts over a period of hours in the mussel, *Mytilus californianus* (Gracey et al. 2008). In contrast to the relatively long time required for stress protein accumulation, the oysters can reach LT_{50} in ~ 35 min after cBP3 if the rock is continuously heating up. While maintaining a high level of heat-shock proteins irrespective of the thermal regime is beneficial as a preparative defense (Dong et al. 2008), such a strategy is energetically costly, particularly for high-shore species where feeding time is constrained and food is limited (Thompson et al. 2004; Williams and Little 2007; Marshall and McQuaid 2011).

Energetic pathways often shift in association with metabolic depression and adverse body temperatures (e.g., shifting aerobic pathways to anaerobiosis) where different substrates are used and waste metabolites are accumulated (Pörtner 2001; Storey and Storey 2004). As a result, while metabolic depression could reduce energetic demands during harsh conditions, substantial debts will be incurred when individuals return to more benign conditions (Somero 2002). The signal to trigger depression is therefore critical to be able to maximize the certainty of energy-conservation benefits at the expense of the negative effects of waste metabolite accumulation. This trade-off might explain the wide individual variation in the oyster cardiac thermal performance in two ways. First, the risk to exceed cBP3 is variable depending on tidal heights, local topography of the shore, and time. Individual oysters are thus effectively acclimatized to different temperature regimes, causing adjustments in physiology (Giomi et al. 2016) and metabolic depression to be initiated over a wide range of temperatures between 32° and 42°C , in order to align with local temporal structures in temperature. Second, the variation in energy reserve among individual oysters, caused by the differences in feeding and emersion durations over tidal heights, could also alter the energy-conservation benefits of metabolic depression. Individuals with sufficient energy reserve, for instance, might opt to depress at a higher

temperature, compared with individuals with less energy reserve. Such variable metabolic depression patterns pertain to variable depression and persistence breadths between individuals. While the negative correlation between depression and persistence breadths could be explained by the effects of waste metabolites accumulated during metabolic depression on persistence at recovery, such a trade-off might also result from the relatively constrained final breakpoint temperatures among oysters (SD ~40% lower than cBP1 and cBP3), causing a more limited persistence breadth when depression breadth is broader. Critical temperatures for metabolic depression, therefore, appear more flexible compared with the final breakpoint, which could be highly constrained because of fundamental limits on biological systems to cope with high temperatures (Araujo et al. 2013).

Conclusion

Temporal structures present in stochastic environments can provide reliable cues for species to invoke active anticipatory responses to enhance performance. On tropical high shores with challenging but autocorrelative temperature regimes, such an anticipatory strategy in physiological adjustments is adaptive, where metabolic depression can be invoked preceding extreme thermal stress to avoid excessive energetic cost. The cross-correlation between the onset of anticipatory responses and environmental cues thus represents a potentially overlooked mechanism for species persistence in extreme, stochastic but temporally structured environments.

Acknowledgments

This project was supported by King Mongkut's Institute of Technology Ladkrabang (KMUTL) Research Fund (grant KREF155801). Many thanks to Priscila Grando, Sujitra Samakraman, and Terence P. T. Ng for help with fieldwork. We thank George Somero (Stanford University), Brian Helmuth (Northeastern University), and Juan Diego Gaitán-Espitia (University of Hong Kong) for stimulating discussions and critical comments toward improving the manuscript. We also thank Jenny Lau, Jay Stachowicz, and two anonymous reviewers for their comments. The authors declare no conflicts of interest.

Statement of Authorship

M.G. and G.A.W. conceived the study. T.Y.H. and G.A.W. prepared the manuscript, while T.Y.H. performed the statistical analyses. T.Y.H. and S.L.Y.L. prepared the graphs and tables. Y-w. D., G-d. H., S.L.Y.L., M.C.F.C., C.M., M.G.,

and G.A.W. contributed to field data collection and physiological experiments.

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