Spreading the risk: Small-scale body temperature variation among intertidal organisms and its implications for species persistence

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ABSTRACT

The intertidal zone of wave-swept rocky shores is a potentially useful system in which to monitor, experimentally manipulate, and possibly forecast the ecological consequences of impending changes in environmental temperature. However, the spatial and temporal complexity of the shoreline's thermal environment challenges ecologists' ability to progress from site-specific measurements to general predictions. In this study, we conducted intensive field experiments to quantify inter-individual variation in body temperature among organisms and surrogate organisms at a typical intertidal site, and we used these measurements to characterize micro-scale variation in potential thermal stress. Within the 336-m extent of our site, local variation was substantial in several metrics of extreme and cumulative thermal exposure. The within-site variation in extreme temperatures rivaled (and in some cases greatly exceeded) variation among sites along 14° of latitude (1660 km of Pacific shoreline). Application of our within-site data to a simple theory of risk suggests that small-scale spatial variation in temperature can reduce the chance of local extirpation that otherwise would accompany an increase in average habitat temperature or an increase in the frequency of extreme thermal events. The magnitude of this "buffering" effect also depends heavily on inter-individual variation in thermal tolerance, a factor that has received relatively little attention from physiologists. We hope that by highlighting the important role of within-site variability (both of temperature and tolerance) in the persistence of intertidal populations, this study will foster further research into the biophysical, physiological, behavioral, and genetic interactions underlying ecological dynamics on wave-washed shores.

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1. Introduction

Rising concentrations of atmospheric carbon dioxide and other greenhouse gases are currently causing a worrisome increase in globally averaged air temperature (IPCC, 2007), and the scientific community has mobilized to predict the ecological effects of consequent climate change. Wave-washed rocky shores provide a potentially important system in which to develop and test these predictive abilities, in large part because the rich history of experimentation on intertidal communities provides a wealth of baseline information on distribution and the roles of competition, predation, facilitation, dispersal, recruitment, disturbance, environmental stress, and patch dynamics (e.g., Bertness et al., 2001; Helmuth and Tomanek, 2002; Helmuth et al., 2006a). In addition, intertidal animals and seaweeds may be particularly sensitive to both direct and indirect effects of environmental temperature change (Sanford, 2002; Helmuth et al., 2006a, 2011-this issue; Somero, 2002, 2010; Lockwood and Somero, 2011-this issue). For example, a variety of intertidal organisms already periodically experience habitat temperatures at or

very near their lethal thermal limits, and the most warm-adapted of these species tend to have the least physiological reserve to cope with warming (e.g., Stillman and Somero, 1996, 2000; Stillman, 2002). Indeed, Harley (2008) has documented recent mass mortalities of mussels and limpets on wave-swept shores. Shifts in geographical range and fluctuations in abundance have already been observed in response to recent warming, particularly in Europe where long-term and broad scale baselines exist (e.g. Lima et al., 2006, 2007; Mieszkowska et al., 2007; Hawkins et al., 2008, 2009). The general interpretation of these findings is that relatively small changes in imposed body temperatures, due to direct or indirect effects of global change, could elicit profound population and ecological shifts, making intertidal communities a bellwether for the consequences of climate change (Southward et al., 1995; Helmuth et al., 2006a).

However, as Pincebourde et al. (2008) note, rocky intertidal shores are "among the most thermally complex" on Earth. Variegated topography and the ever-changing pattern of the tides can cause organisms in close proximity to experience dramatically different thermal environments (e.g., Harley and Helmuth, 2003; Denny et al., 2006; Harley, 2008; Miller et al., 2009). Furthermore, different species respond differently to thermal stress (e.g., Somero, 2010; Marshall and McQuaid, 2010), and the response even within a species can vary from place to place (e.g., Kuo and Sanford, 2009). This small-scale

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environmental and physiological complexity can confound comparison among sites (e.g., Wethey, 2002; Harley and Helmuth, 2003; Harley, 2008), a difficulty that poses a serious challenge for intertidal ecologists as they attempt to predict large-scale effects of rising temperatures (e.g. Poloczanska et al., 2008, barnacle species composition with different future climate scenarios).

How can intertidal ecologists best incorporate local thermal variability into their predictions of the effects of climate change? As a step toward answering this question, we address two fundamental issues. First, how variable is body temperature among individuals at a single site in the intertidal zone? Previous experiments that included measurement of within-site variation generally relied on indirect measurements (e.g., Elvin and Gonor, 1979; Harley, 2008) or a small number (2–6) of direct measurements (e.g. Helmuth and Hofmann, 2001; Helmuth et al., 2006b; Harley, 2008; Petes et al., 2008). In field experiments, we have used a more intense deployment of measuring devices (up to 221), and document extreme inter-individual variation in body temperatures. We then examine the implications of this variability for local species persistence. Specifically, we ask, how does within-site variability affect the fraction of a population killed by heat stress? We conclude that, if sufficiently large, within-site variability in thermal experience can buffer the local effects of either long-term temperature trends or increased frequency of stochastic, extreme events. We suspect that, at least in some cases (e.g., Broitman et al., 2009), local variability will play a more important role in population, species, and community-level responses to global change than mean regional differences among sites. Our results further highlight the need for ecophysiological and evolutionary studies examining the effects of this fine scale of variability on intertidal populations.

2. Materials and methods

We present the results of six independent intertidal experiments that vary in their physical scale (less than 1 m to 100 s of m), duration, time of year, and level of biological similitude. All data were collected at Hopkins Marine Station (HMS) of Stanford University in Pacific Grove, California, USA (36° 37′15″ N, 121° 54′ 15″ W). The granite substratum adjacent to HMS forms a topographically complex shoreline typical of many intertidal sites, and is classed as "moderately exposed" to waves (Ricketts et al., 1985). Measurement locations for this study lie along a 336-m-long horizontal transect at a height of 1.6 to 1.8 m above mean lower low water (MLLW), the same transect used by Denny et al. (2004) to characterize scales of intertidal variation, and the same tidal height as the measurements made by Helmuth and Hofmann (2001), also at HMS.

To avoid semantic confusion when comparing our results to those of others, we use the term "site" to refer to the shoreline at HMS. Within that site, measurements were made for individual objects at specific locations. Thus, the interchangeable terms "individual" and "location" refer to data collected at a single microhabitat within the site.

Relevant terrestrial environmental conditions (solar irradiance and air temperature) during these measurements were recorded by a weather station adjacent to the shore near one end of the transect (Denny et al., 2009). Sea surface temperatures were obtained from daily recordings at HMS.

2.1. Horizontal transect

On April 18, 2002, temperature loggers were installed at each of 221 locations along the transect. Each device comprised a spherical brass ball (2.5 cm diameter), painted black and enclosing a small temperature logger (Thermochron iButton, Maxim Integrated Products, Sunnyvale, CA, USA). Residing in a drilled-and-tapped hole in the ball, the logger was held in firm contact with the brass by a piece of rubber foam, and the ball was attached by a short, threaded, polyvinylchloride rod to a threaded mount

glued to the substratum (Fig. 1). We attempted to install each device with minimal disturbance to the surrounding organisms. Because of its spherical shape, each ball presented the same area for solar heating regardless of the orientation of its mounting; only the properties of the microhabitat–rather than the instrument–vary from location to location. iButtons have a precision of 0.5 °C, and to improve accuracy, each instrument was calibrated prior to deployment. Temperatures were recorded every 10 min for 12 days. These temperature loggers were not intended to mimic any particular organism, but rather to provide a standard measure of temperature that could be compared among locations without complications due to organisms' variable morphology. The "thermal mass" of each logger (the product of its mass [80.65 gm] and specific heat capacity [377 J kg^{-1} °C^{-1}]) is the same as that of a 7.24 gm organism with the specific heat of water (4200 J kg^{-1}°C^{-1}), such as a small mussel. Because the thermal conductivity of the PVC connecting rod is relatively low (0.19 W m^{-1}°C^{-1} compared to 2.3 W m^{-1}°C^{-1} for granite or 0.6 W m^{-1}°C^{-1} for water), the loggers are not as tightly coupled to the temperature of the rock as are organisms such as acorn barnacles and limpets, which maintain effective conductive contact with the substratum (Wethey, 2002; Denny and Harley, 2006).

The distance between adjacent temperature loggers was measured along the shore using large calipers. For the first 98 measurement locations, the spacing between them was 0.46 m, for the next 75 locations, spacing was 1.72 m, and for the remaining 48 locations, spacing was 3.44 m.

A similar set of measurements was conducted at a reduced number (173) of locations (a total transect length of 235 m) beginning on September 11, 2002. Temperatures were recorded every 10 min for 12 days.

2.2. Simulated mussel bed

As a first means to estimate the details of small-scale variation in mussel body temperature, 114 mussel shells (*Mytilus californianus*) were used to construct a small simulated bed approximately 23 cm in diameter. Shells were obtained from the bed at HMS, and were typical of mussels at this site, with lengths varying between 42 and 65 mm. We extracted the resident mussel from each shell and replaced it with silicone sealing compound, which anchored a length of braided Dacron fishing line as a replacement for the mussel's byssus. In 38 of the shells, a calibrated iButton logger was inserted into the center of the "body" to record temperature. Helmuth and Hofmann (2001) have shown that the temperature in a silicone-filled mussel shell accurately approximates to that in a live mussel (Caveats to this conclusion are addressed in the Discussion). The entire set of mussels was tethered to holes drilled in an aluminum plate so that the tightly packed assemblage closely resembled a natural mussel bed (Fig. 2). Granite chips were glued to the exposed areas of the plate to match its
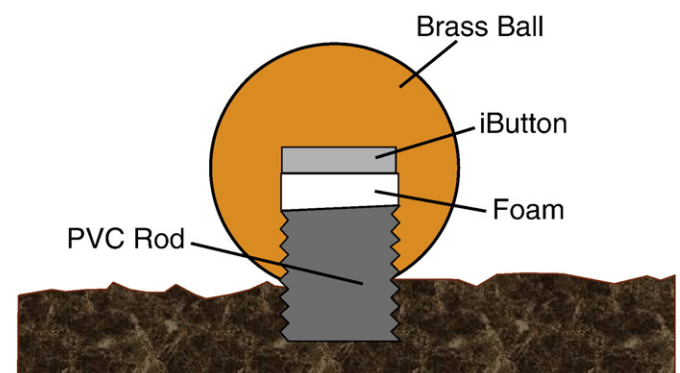

Fig. 1. Brass ball and enclosed data logger used on the Hopkins Marine Station transect.

Fig. 2. The 23-cm diameter, simulated mussel bed deployed at 2.93 m above MLLW at HMS. Colored triangles mark those mussels outfitted with a temperature logger. Blue triangles denote "edge" mussels, green triangles, "near edge" mussels, yellow triangles, "near center" mussels, and red triangles, "center" mussels.

absorptivity to that of the surrounding rock. Instrumented mussels were arranged to form four approximately concentric rings, thereby to characterize the edge, near edge, near center, and center of the clump (Fig. 2). We doubted that the mussels' tethers were sufficiently robust to withstand the impact of waves, so we attached the aluminum plate to the horizontal substratum just above the intertidal zone at HMS, 2.93 m above MLLW. At this height, the bed was occasionally splashed, but never submerged. We filled the space between the plate and the substratum with quick-drying cement to ensure good thermal contact between the plate and the rock. The experiment was initiated on May 21, 2005, and temperatures were recorded every 20 min for 29 days.

2.3. Exposed and protected natural mussel beds

Two representative sites were chosen along the horizontal transect for focused characterization of temperature variation among mussels (M. californianus) in natural beds. Based on force measurements made by Denny et al. (2004) and transect results from April 2002, one bed was chosen at a particularly cool, wave-exposed portion of the transect (the "exposed") bed, and the other at a relatively warm, protected portion of the transect (the "protected") bed. The beds were separated by approximately 24 m along the transect. On July 8, 2010, 21–23 instrumented mussel mimics (silicone-filled shells each containing a calibrated iButton logger as in the simulated bed) were deployed in the existing beds by removing a resident mussel and inserting the logger shell in its place. Each logger shell was held in place with small tabs of epoxy putty (A-788 Splash Zone Compound, Z-Spar Coatings, Pittsburgh, PA). Within each bed, horizontal locations of mussels relative to each other, and vertical location relative to MLLW, were measured using a Topcon GTS-211D total station (Topcon America, Paramus NJ, USA; Fig. 3). Tidal elevations were determined using a USGS benchmark of precisely known elevation near one end of the transect. Temperature was recorded in each shell every 20 min for 24 days. At the end of each experiment, logger mussels were retrieved and temperature loggers

returned to the laboratory for downloading. The experiment was repeated with 15 mussels at the protected site beginning on August 12, 2010, and temperatures were recorded for 28 days.

2.4. Indices of acute and cumulative thermal stress

For all experiments, temperature data were analyzed to quantify for each individual mussel or brass ball several measurements relevant to either acute or cumulative thermal stress. For this purpose we define "stress" as damage to macromolecules (e.g., proteins, and membranes), requiring an energetic investment (i.e., a physiological cost) on the part of the organism to stabilize and repair (Kültz, 2003). As indicators of potential acute stress, we calculated daily minimum and maximum temperatures (T_{min} and T_{max}, respectively; °C), and daily maximum rates of heating and cooling (Q_{max}^+ and Q_{max}^-, respectively; °C h^{-1}), for each individual. The rate of heating and cooling can be an important component of thermal tolerance (Peck et al., 2009). To document potential cumulative stress, we calculated cumulative hours, H, above a temperature threshold (25 °C), cumulative degree hours, $°H$, above the same threshold (for a review, see Newell, 1970, pp. 451–468), and, for the natural beds, the number of discrete days on which body temperature exceeded the temperature threshold. The threshold of 25 °C was chosen because it is typical of the temperature at which a variety of intertidal species begin to produce heat-shock proteins, a biochemical indicator of macromolecular (protein) damage due to sublethal thermal stress (mussels: Mytilus trossulus, M. californianus, Buckley et al., 2001; snails: Chlorostoma (formerly, Tegula) funebralis, C. brunnea, C. montereyi, Tomanek, 2002; limpets: Lottia gigantea, L. digitalis, L. austrodigitalis, L. scutum, L. pelta, Miller et al., 2009; Dong et al., 2008).

2.5. Inter-individual variability in thermal stress

Having quantified these indices of thermal stress for individual objects in the intertidal zone, our primary objective was to analyze their variability among individuals within the local population. To that end, we used three statistics:

(1) R, Range of individual values. For each day in a dataset, we measured $R_{T_{max}}$ and $R_{T_{min}}$, the daily range across individuals of the maximum and minimum temperatures, respectively. That is, we calculated the difference between the maximum recorded value for that day and the minimum recorded value for that day for both T_{max} and T_{min}. Similarly, we calculated $R_{Q_{max}^+}$ and $R_{Q_{max}^-}$, the daily ranges of maximum rates of heating and cooling. We were most interested in quantifying potential cumulative sublethal effects of exceeding the 25 °C threshold, so we did not quantify daily ranges for those metrics.

(2) CV, Coefficient of variation of individual values. The ranges of maximum and minimum temperatures and of the rates of heating and cooling provide a measure of the magnitude of variation in potential stress, but do not place that magnitude in biological context. To provide a measure of context-specific variation, we calculated for each variable its coefficient of variation—the standard deviation of the variable expressed as a fraction of its mean. For example, the coefficient of variation of maximum rate of heating on a given day is:

$$CV_{Q_{max}^+} = \frac{SD_{Q_{max}^+}}{\overline{Q_{max}^+}} \qquad (1)$$

where $SD_{Q_{max}^+}$ is the standard deviation of Q_{max}^+ among individuals that day and $\overline{Q_{max}^+}$ is the mean maximum rate of heating among individuals for the day. These daily CVs are
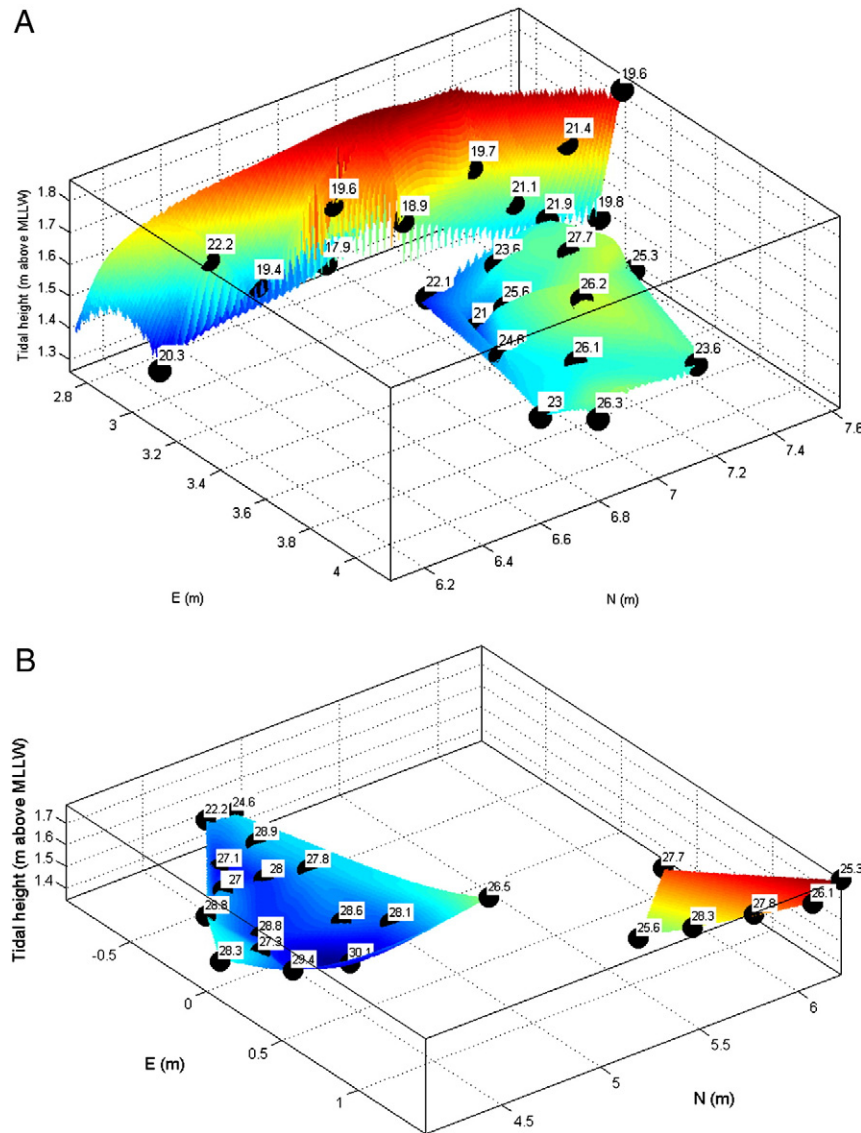
Fig. 3. Spatial representation of the exposed (A) and protected (B) natural mussel beds at HMS. Each black circle represents an individual mussel mimic temperature datalogger from the July 2010 dataset, and corresponding numbers indicate the maximum body temperature measured. Shaded colors indicate height (m) above MLLW. Waves arrive predominantly from the bottom right corner in (A) and the top left corner in (B).

then averaged across all days in the experiment to provide an overall index of the variability in Q_{max}^+ relative to the average Q_{max}^+. Similar calculations were made for Q_{max}^-.

Because the Celsius temperature scale takes the freezing point of water as its zero point–an arbitrary value not particularly relevant to our spring and summertime measurements–it is not meaningful to calculate a coefficient of variation for maximum or minimum temperature per se. Instead, we reset the temperature scale by calculating the differences between each individual's daily value of T_{max} and T_{min} and a more relevant standard—average sea-surface temperature ($\overline{SST}$) during each experiment. Daily and overall average CVs for these adjusted maximum and minimum temperatures ($T_{max}-\overline{SST}$ and $T_{min}-\overline{SST}$, respectively) were then calculated as for Q_{max}^+ and Q_{max}^-. Because the simulated mussel bed was never submerged, $\overline{SST}$ bears little relationship to this experiment, and we consequently do not calculate CV for this experiment.

Because we were interested in inter-individual variation in cumulative sublethal thermal stress rather than variation in mean daily values, we calculated the mean and coefficient of variation of the

individual cumulative totals for hours above threshold and degree hours above threshold. For the counts of discrete days above threshold in the natural beds, we determined only the mode and range.

(3) *W, Index of cumulative physiological consequences of temperature variability.* The metrics described above were designed to quantify aspects of body temperature that are potentially stressful. However, as Sanford (2002) has elegantly pointed out, small changes in "normal" (that is, nonstressful) temperatures can be amplified by metabolism and behavior to have large ecological consequences. To serve as a proxy for these effects, we calculated the hypothetical "work," W, done by each "individual" at each measurement location in our experiments (e.g., prey consumed, biomass added, gametes produced), where the rate of hypothetical work follows a typical nonlinear, temperature-dependent relationship:

$$\frac{dW(T)}{dt} = Q_{10}^{\frac{T-T_{std}}{10}}$$

(2)

Here, T is the instantaneous body temperature, T_{std} is a standard temperature at which $dW/dt = 1$ (we used $\overline{SST}$), and Q_{10} is a rate coefficient that typically varies between 2 and 3 for metabolic work (Cossins and Bowler, 1987; Hochachka and Somero, 2002), but has been reported as high as 4.8 for temperature effects on foraging (Sanford, 2002). We calculated individuals' cumulative work by integrating dW/dt through time for Q_{10}'s of 2, 3, and 4, and we report inter-individual range and coefficient of variation of the accumulated values.

2.6. Mussel thermal tolerance

Tolerance to acute heat stress has been measured for a variety of intertidal species (Table 1). To augment these values, and to provide species-specific data relevant to this study, we measured the thermal tolerance of M. californianus.

Following the procedure of Miller et al. (2009), groups of 20 mussels were collected from the shore at HMS in January and April, 2005, and immediately placed on an aluminum plate in a recirculating wind tunnel. The temperature of both air and plate were raised at a rate sufficient to increase the body temperature of the mussel to a pre-established peak value over the course of 210 min. Peak temperatures ranged from 28 to 41 °C. At the end of the experimental period, mussels were placed in running seawater at 14 °C for 3 days before their status was ascertained. Mussels that gaped and did not respond by closing when prodded were scored as dead; responsive mussels and those with closed shells were scored as alive. The fraction of surviving mussels was determined for 2–3 replicates for each peak temperature, and the median lethal temperature (LT_{50}) was determined using logistic regression (Pincebourde et al., 2008).

2.7. Model of population susceptibility to acute lethal temperatures

Having quantified inter-individual variation in both thermal stress and thermal tolerance, we applied these measurements to a theoretical assessment of the risk of death from acute thermal stress, using theory analogous to that employed to assess the risk of breakage in a structure (Alexander, 1981; Denny, 2006). We modeled the distribution of maximum acute temperature tolerances within a population of hypothetical organisms as a bell-shaped probability density curve, p(T) (Fig. 4A). Given a uniform maximum temperature to which each of these organisms is subjected (T_{max}), the fraction F of individuals killed is the area under this curve and to the left of the T_{max}:

$$F(T_{max}) = \int_{-\infty}^{T_{max}} p(T)dT \tag{3}$$

Table 1
Literature values for acute lethal thermal tolerance limits (°C) in intertidal species. Onset, temperature at which mortality first noted; completion, temperature of 100% mortality; SD, standard deviation calculated as Range/4. Sources: (1) this study; (2) Somero (2002), (3) Tomanek (2002), (4) Miller et al. (2009), (5) Hunt (2006), (6) Pincebourde et al. (2008).

Species	Notes	Onset	Completion	Range R_{tol}	SD	Source
Mytilus californianus[a]		36	41	5	1.25	1
Littorina scutulata		41	48.5	7.5	1.89	2
Littorina keenae		44.5	50.3	5.8	1.45	2
Tegula funebralis		40	42.5	2.5	0.63	3
Lottia gigantea	50–60% RH	30	36	6	1.50	4
	100% RH	32	42	10	2.50	4
Endocladia muricata	4-h exp.	33.8	36.3	2.5	0.63	5
	2-h exp	34.9	37.6	2.7	0.68	5
	2 h exp	34	36.6	2.6	0.65	5
	0.5 h exp	35.5	38.3	2.8	0.70	5
Pisaster ochraceus		35	35	0	0	6

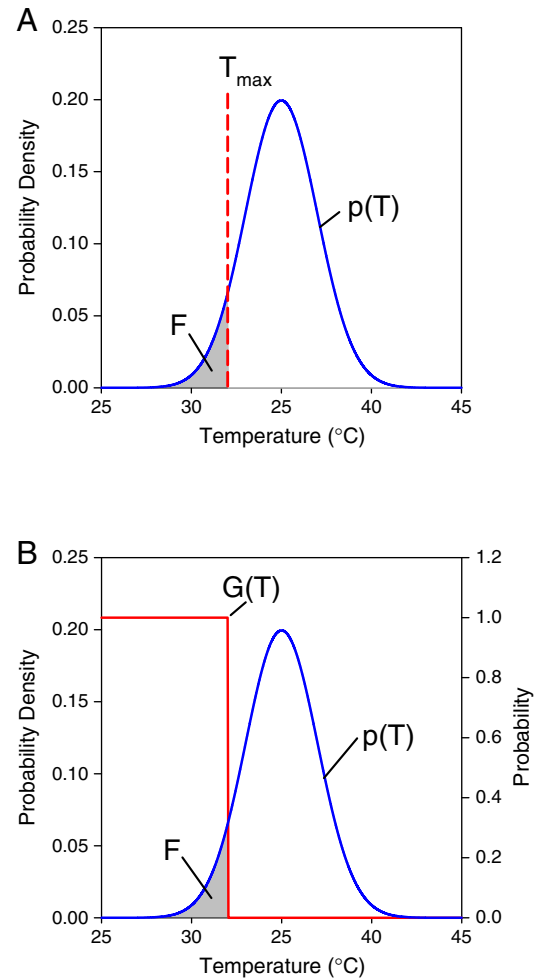[a] The same HMS population in which our field measurements were made.

Fig. 4. (A) Theoretical Gaussian probability density function $p(T)$ of acute thermal tolerance limits for an intertidal population. Assuming a uniform maximum body temperature (T_{max}) is imposed to all individuals, the fraction of the population under the curve of $p(T)$ and to the left of T_{max} (F) will die. (B) The exceedance probability function $G(T)$ describes the probability that individuals will exceed a given temperature. In this example, the break point of $G(T)$, where probability of exceedance shifts from 1 to 0, is the same as T_{max} in (A).

Alternatively, the imposition of lethal temperature stress could be interpreted in terms of the probability of exceeding a given temperature (Fig. 4B). It is certain (that is, probability of exceedance $G, = 1$) that maximum temperature exceeds all temperature tolerance values less than T_{max}, and because T_{max} is indeed the maximum temperature, the probability that maximum temperature exceeds $T_{max} = 0$. The function for the probability of exceedance, $G(T)$, is in this simple case a step function (the scale for G is on the right of Fig. 4B). This formulation for the probability of exceedance allowed us to restate Eq. (3):

$$F = \int_{-\infty}^{\infty} G(T)p(T)dT \tag{4}$$

In essence, each value of probability density p for a given temperature was weighted by the corresponding probability G that T_{max} exceeds that temperature. Note that we no longer needed to define T_{max} in the limits to integration, because the location of T_{max} is implicit in the exceedance function G. Therefore, given the distribution of temperature tolerances in a population and the maximum temperature to which all individuals are subjected, we could use Eq. (4) to calculate the fraction of individuals killed.

However, our empirical results clearly indicated that for a given set of environmental conditions, all individuals at a site on the shore do not experience the same maximum temperature. Instead, there was substantial variation in T_{max} among individuals, such that (barring more information than is currently available), if we picked an individual at random from the population at this site, we could only describe the *probability* that T_{max} for that individual exceeds a given value. In other words, we modeled the distribution of imposed maximum temperatures with a modified exceedance curve; instead of a step function (G equals only to 1 or 0) with a well-defined break at T_{max}, this modified exceedance curve declines from 1 to 0 over a range of temperatures (Fig. 5). Despite this change in how we specified T_{max}, the fraction of individuals killed was still calculated using Eq. (4). Again, each value of probability density p for a given temperature was weighted by the corresponding probability G that T_{max} exceeds that temperature. In summary, provided information about the distributions of both thermal tolerance and maximum imposed body temperature among individuals, we could then predict the fraction of individuals that die from a given episode of acute heat stress. Increased variability in T_{max} changes the shape of G as shown by the dashed lines in Fig. 5, a factor we return to later.

We then applied this model to our central question: How does the magnitude of variation in temperature among close neighbors affect the fraction of the population that might be killed by an extreme event? To answer this question, we modeled the distributions of both

imposed body temperature T, and thermal tolerance of that temperature, as Gaussian functions:

$$G(T) = 1 - \frac{1}{\sigma_{max}\sqrt{2\pi}} \int_{-\infty}^{T} exp - \frac{(x-\mu_{max})^2}{2\sigma_{max}^2} dx \qquad (5)$$

$$p(T) = \frac{1}{\sigma_{tol}\sqrt{2\pi}} exp - \frac{(T-\mu_{tol})^2}{2\sigma_{tol}^2} \qquad (6)$$

Although real-world distributions may not be precisely Gaussian, these idealized expressions allowed us to describe each distribution succinctly by its mean (μ_{max} for maximum temperature, μ_{tol} for temperature tolerance) and its standard deviation (σ_{max} and σ_{tol}, respectively).

Our field data provided guidance as to realistic magnitudes of σ_{max}. Rather than dealing with specific values of μ_{max} and μ_{tol}, we generalized our analysis by dealing with the difference between these two values, $\Delta\mu$, for a range of $\Delta\mu$ from -5 °C to $+5$ °C. Indirect estimates of σ_{tol} were calculated from literature values (Table 1). Assuming that the distribution of thermal tolerances is approximately Gaussian, R_{tol}, the range of temperatures separating 100% survival from 0% survival, is approximately $4\sigma_{tol}$, and we consequently used $R_{tol}/4$ from these studies as rough-and-ready approximations for σ_{tol} (ranging ~0 to 2.5 °C).

We used our field data to assess how well the distribution of measured temperatures matched the assumptions of our theory, and to predict the fraction of individuals killed by the hottest event in our natural bed datasets. We compared this predicted fraction to that observed in the field.

A final note: the approach described above can be used to predict the consequences of extreme low temperatures as well. In this case, the probability, $L(T)$, of encountering a temperature below a given value is substituted for $G(T)$ in Eq. (4). $L(T) = 1 - G(T)$.

3. Results

Environmental conditions for all experiments are summarized in Table 2.

3.1. Inter-individual variation in maximum and minimum temperatures

Our six datasets revealed a notable range of maximum body temperatures within the HMS site, although the details of variation
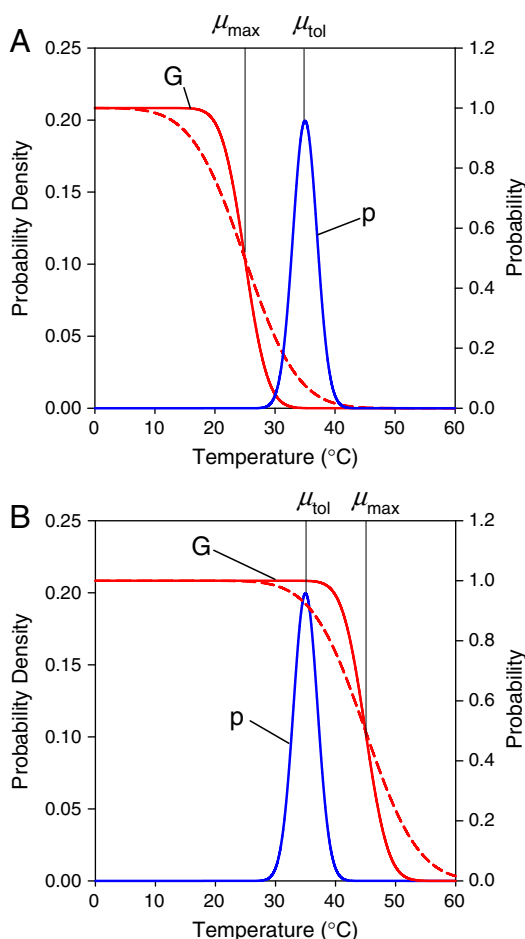

Fig. 5. Theoretical scenarios for population susceptibility to acute thermal stress in which mean thermal tolerance (μ_{tol}) either exceeds mean maximum body temperature (μ_{max}) (A), or $\mu_{tol} < \mu_{max}$ (B). Solid and dashed lines represent different exceedance curves for populations with a smaller or larger standard deviation of imposed maximum body temperatures (σ_{max}), respectively.

Table 2
Environmental conditions recorded at Hopkins Marine Station during each intertidal temperature experiment. SB = simulated mussel bed; NB = natural beds.

	Experiment				
	Transect		Mussel beds		
	April 2002	Sept 2002	SB May 2005	NB July 2010	NB August 2010
Solar irradiance (Wm^{-2})					
Overall maximum	1159	775	1043	1067	924
Average daily max	850	733	962	806	798
SD daily max	203	76	104	214	130
Air temperature (°C)					
Overall maximum	18.1	26.0	18.6	16.8	25.1
Average daily max	13.7	17.9	15.7	14.5	15.9
SD daily max	2.5	3.6	1.3	1.2	3.2
Overall Minimum	6.4	10.6	9.8	10.8	10.5
Average daily min	9.4	12.1	11.2	11.9	11.8
SD daily min	1.2	0.9	0.9	0.7	0.7
Average sea-surface temperature (°C)					
	13.8	15.5	13.6	14.5	14.5

depended on the dataset (Table 3). In April 2002, daily maximum temperatures recorded by the brass balls varied substantially along the 336 m of the transect. The mean range in maximum temperature, $R_{T_{max}}$, was 18.7 °C and the mean CV of T_{max}–$\overline{SST}$ was 0.60. The highest $R_{T_{max}}$ was 25.0 °C, and the overall maximum temperature recorded was 40.4 °C. The temperature patterns were broadly similar along the same transect in September 2002, although the mean CV of T_{max}–$\overline{SST}$ increased to 1.12 (Table 3).

Maximum temperatures also varied dramatically over very small spatial scales within the simulated mussel bed. The average $R_{T_{max}}$ was 12.0 °C, and the highest $R_{T_{max}}$ was 14.5 °C. Neither overall nor average daily maximum temperature was correlated with the volume of the mussel ($P>0.15$).

The metrics of variability of maximum body temperatures of mimics in the natural mussel beds were generally smaller in magnitude than in the other datasets. For example, the mean $R_{T_{max}}$ values at the exposed and protected sites in July were 3.7 °C (max 11.7 °C) and 6.4 °C (max 12.7 °C) respectively. Corresponding mean CVs of T_{max}–$\overline{SST}$ were 0.48 and 0.44. Although the overall maximum temperatures recorded in the exposed and protected natural beds at 1.6 m above MLLW (27.7 °C and 30.2 °C, respectively) were substantially lower than that measured in the simulated bed higher on the shore (50.5 °C at 2.93 m above MLLW), the maximum $R_{T_{max}}$ values were similar (Table 3).

There was considerably less variation among individuals in daily minimum temperatures than daily maximum temperatures. Average daily range of minimum temperature, or mean $R_{T_{min}}$, was less than 25% of the mean $R_{T_{max}}$ in almost every data set. The sole exception was the July exposed natural bed, for which mean $R_{T_{min}}$ was 45% of the mean $R_{T_{max}}$; however, this dataset had the lowest mean $R_{T_{max}}$. The maximum $R_{T_{min}}$ values were also substantially lower than the maximum $R_{T_{max}}$ values (Table 3). Mean $R_{T_{min}}$ and CV values for the exposed (1.6 °C and −0.28, respectively) and protected natural beds in July (1.5 °C, −0.23) were

lower than the values for both the transect and the simulated mussel bed (note: because mean T_{min} is less than $\overline{SST}$, the coefficient of variation of T_{min} is negative). The highest $R_{T_{min}}$ measured in a natural mussel bed was 2.4 °C (protected bed, August). The overall minimum temperature of 4.7 °C (9.1 °C below $\overline{SST}$) was recorded in a brass logger in April; the lowest recorded temperature in a natural mussel bed was 11.0 °C (3.5 °C below $\overline{SST}$). Along the transect, the overall minimum temperature and mean daywise T_{min} were ~5 °C higher in September than in April, reflecting the higher air and sea-surface temperatures (Table 2).

We also noted different patterns between the two natural beds in July maximum, but not minimum, temperatures. The mean $R_{T_{max}}$ in the protected bed in July was larger (Student's t-test, $P \ll 0.001$), with greater variation around the mean, than that in the exposed bed. Individual mean daywise T_{max} in the protected bed was also 5 °C higher than in the exposed bed (Student's t-test, $P \ll 0.001$). That is, individuals in the protected site tended to reach consistently higher temperatures and to exhibit a larger daily inter-individual range of maximum body temperatures than those in the exposed bed (although mean CVs of T_{max}–$\overline{SST}$ were comparable). In contrast, $R_{T_{min}}$ and mean T_{min} values for the exposed and protected beds were not significantly different (Student's t-test, $P>0.05$).

Average daily maximum temperature decreased monotonically from the center to the periphery of the simulated mussel bed (Fig. 6).

3.2. Inter-individual variation in rates of temperature change

Rates of cooling (and variability thereof, indexed using both R and CV) tended to exceed those of heating in each dataset (Table 4), probably due to immersion by the incoming tide, which can rapidly reduce body temperatures. The overall maximum rate of heating of balls on the transect was 7.0 °C h^{-1}, and of cooling was −11.5 °C h^{-1}. Daily maximum rates of heating and cooling (Q_{max}^+ and Q_{max}^-) varied substantially along the transect in April 2002, with an average inter-individual daily range of Q_{max}^+ of 4.9 °C h^{-1} and mean CV of $Q_{max}^+ = 0.45$. The average range (6.7 °C h^{-1}) and mean CV (0.51) of cooling were larger than those for heating (Student's t-test, $P \ll 0.001$). Results for rates of temperature change in September were similar to those in April 2002 (Table 4), although they were more variable with slightly larger mean CVs for Q_{max}^+ and Q_{max}^- of 0.62 and 0.70, respectively.

Rates of heating and cooling also varied substantially across the simulated bed (Table 4). Average $R_{Q_{max}^+}$ was 4.0 °C h^{-1}, and mean CV of Q_{max}^+ was 0.22. Although statistically discernable (Student's t-test, $P<0.05$), the average range (4.6 °C h^{-1}) and mean CV (0.32) of cooling in the simulated bed were not dramatically different than

Table 3
Metrics of inter-individual variation of maximum and minimum daily temperatures in each experiment. SB = simulated bed; NBE = natural bed, exposed; NBP = natural bed, protected. We report the standard deviation of the mean CV as a measure of how variable CV is among days.

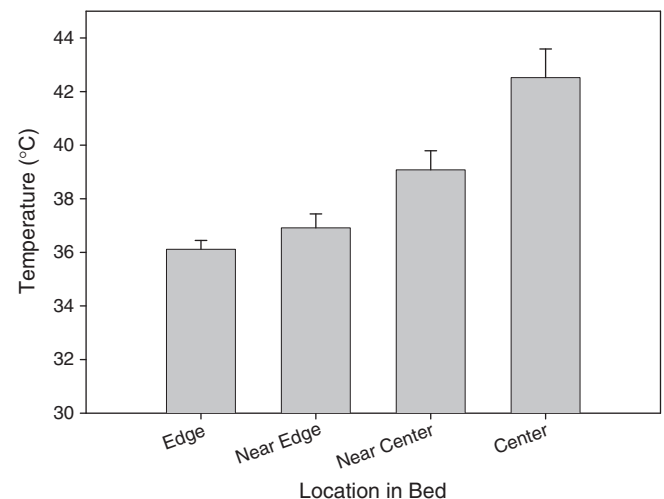| Variable	Experiment					
	Transect		Mussel beds			
	April	Sept	SB	NBE July	NBP July	NBP August
Overall maximum temperature (°C)	40.3	35.7	50.5	27.7	30.2	35.3
Mean range of T_{max} (°C)	18.7	17.6	12.0	3.7	6.4	5.6
SD of range of T_{max} (°C)	5.6	4.0	2.1	2.7	3.5	4.1
Mean site range per latitudinal range	0.55	0.39	0.20	0.26	0.35	0.40
Max. range of T_{max} (°C)	25.0	21.7	14.5	11.7	12.7	15.2
Max. site range per Latitudinal range	2.45	2.13	1.42	1.14	1.25	1.49
Mean individual T_{max} (°C)	29.3	25.3	42.9	22.2	27.4	30.4
Mean SD of T_{max}	3.99	4.57	2.97	0.979	1.818	1.61
Mean CV of T_{max} - $\overline{SST}$	0.60	1.12		0.48	0.44	0.27
SD of CV of T_{max} - $\overline{SST}$	0.49	0.51		0.40	0.19	0.88
Overall Minimum Temperature (°C)	4.7	9.2	8.5	11.8	11.9	11.0
Mean range of T_{min} (°C)	4.1	3.8	2.9	1.6	1.5	1.2
SD of range of T_{min} (°C)	1.3	0.9	0.5	0.3	0.3	0.4
Mean site range per latitudinal range	0.89	0.81	0.62	0.35	0.32	0.25
Max. range of T_{min} (°C)	6.1	5.6	3.8	2.3	2.0	2.4
Max. site range per latitudinal range	1.30	1.20	0.80	0.48	0.43	0.51
Mean individual T_{min} (°C)	6.6	11.3	9.7	12.5	12.6	12.0
Mean SD of T_{min}	0.761	0.705	0.675	0.366	0.387	0.392
Mean CV of T_{min} - $\overline{SST}$	−0.18	−0.23		−0.28	−0.23	−0.30
SD of CV of T_{min} - $\overline{SST}$	0.07	0.05		2.28	1.89	0.11

Fig. 6. Average daily maximum temp (T_{max}) as a function of position within the 23-cm diameter, simulated mussel bed. Error bars are 95% confidence intervals.

Table 4

Metrics of inter-individual variation of maximum and minimum daily rates of temperature change (i.e., maximum rates of heating, Q_{max}^+, and cooling, Q_{max}^-). SB = simulated bed; NBE = natural bed, exposed; NBP = natural bed, protected. We report the standard deviation of the mean CV as a measure of how variable CV is among days.

| | Experiment | | | | | |
| | Transect | | Mussel beds | | | |
Variable	April	Sept	SB	NBE July	NBP July	NBP August
Overall maximum rate (°C h^{-1})	7.0	8.5	11.2	3.2	5.6	8.1
Mean range of max. rate (°C h^{-1})	4.9	5.0	4.0	1.1	2.0	2.2
SD range of max. rate (°C h^{-1})	1.7	1.8	1.7	0.8	1.3	1.5
Max range of max. rate (°C h^{-1})	6.5	7.8	8.2	2.7	5.1	5.2
Mean CV of max. rate	0.45	0.62	0.22	0.36	0.38	0.42
SD of mean CV of max. rate	0.09	0.12	0.05	0.16	0.10	0.18
Overall minimum rate (°C h^{-1})	−11.5	−11.4	−10.7	−7.6	−8.4	−10.9
Mean range of min. rate (°C h^{-1})	6.7	7.0	4.6	1.4	3.0	3.1
SD range of min. rate (°C h^{-1})	2.55	2.75	1.34	1.56	2.01	2.74
Max range of min. rate (°C h^{-1})	11.0	10.9	8.2	7.1	7.6	8.9
Mean CV of min. rate	0.51	0.70	0.32	0.38	0.44	0.45
SD of mean CV of min. rate	0.10	0.12	0.07	0.23	0.12	0.16

Table 5

Metrics of inter-individual variation in cumulative sublethal stress, defined as exceeding a body temperature threshold of 25 °C. For H and °H, measurements have been normalized to a 30-day month (720 h). For days above threshold, the proportions of individuals for that dataset are given in parentheses after each statistic. SB = simulated bed; NBE = natural bed, exposed; NBP = natural bed, protected.

| | | Experiment | | | | | |
| | | Transect | | Mussel beds | | | |
		April	Sept	SB	NBE July	NBP July	NBP August
Hours above threshold (*H*)	Mean	24.3	9.8	221.9	0.3	3.3	6.5
	Max	119.7	67.6	251.4	1.9	8.9	13.5
	Min	0.0	0.0	186.0	0.0	0.0	0
	SD	26.7	16.0	14.5	0.6	2.4	3.5
	CV	1.10	1.64	0.07	2.20	0.71	0.54
Degree hours above threshold (°*H*)	Mean	76.6	26.8	1707.1	0.2	4.8	21.2
	Max	643.6	319.7	2845.4	2.6	13.9	58.4
	Min	0	0	950.5	0	0	0
	SD	117.4	57.1	441.7	0.6	3.8	16.2
	CV	1.53	2.13	0.25	2.85	0.77	0.76
Days above threshold	Mode				0 (0.75)	2 (0.27)	2 (0.40)
	Max				3 (0.04)	5 (0.14)	5 (0.20)
	Min				0 (0.75)	0 (0.08)	0 (0.07)

those of heating, likely because the simulated bed was not immersed by the incoming tide.

As for the metrics of extreme temperatures, metrics of inter-individual variation in the rates of heating and cooling were generally lower within the natural mussel beds relative to the transect or simulated bed. For example, Q_{max}^+ and Q_{max}^- were relatively uniform across the exposed site in July (Table 4). Average $R_{Q_{max}^+}$ for this dataset was 1.1 °C h^{-1} and the mean CV of Q_{max}^+ was 0.36. Average range (1.4 °C) and CV (0.38) of cooling were similar to those for heating. Variability in rates of temperature change was generally higher in the protected site than the exposed site in July (e.g., mean $R_{Q_{max}^+} = 2.0$ °C h^{-1}, mean CV of $Q_{max}^+ = 0.38$; Table 4), but still below the values observed for balls on the transect.

Again, the protected natural bed in July tended to exhibit larger metrics of inter-individual variability in rates of temperature change than the exposed bed. Maximum ranges of both rates of heating and cooling were higher in the protected bed than in the exposed bed (Table 4; Student's *t*-test, $P < 0.005$ and $P < 0.001$, respectively); however, the mean CVs were not significantly different between beds (Student's *t*-test, $P > 0.05$).

3.3. Inter-individual variation in cumulative sublethal stress

Cumulative hours and degree hours above the 25 °C threshold varied widely among locations on the 336-m transect (Table 5). The coefficients of variation in April 2002 were 1.10 and 1.53 for H and °H, respectively. Even greater inter-location variability was observed in September 2002 (CVs of 1.64 for H and 2.13 for °H). Variation in cumulative thermal stress was less across the 23-cm diameter of the simulated bed than along the transect, but still substantial. The coefficient of variation was 0.84 for hours above threshold and 0.56 for degree hours above threshold. When experimental totals were standardized to a 30-day month, mussels in the simulated bed spent on average ~10–20 or ~60–850 times as many hours above threshold as individuals in the transect or natural beds, respectively (Table 5), less than surprising given that the height of the simulated

bed was well above the height at which mussels are found on this shore.

In contrast to the patterns of maximum temperature and maximum rates of temperature change, in which the protected site exhibited greater variation, inter-individual variation in cumulative thermal stress in the exposed natural bed in July exceeded that in the protected bed. The coefficient of variation was 2.20 for H and 2.85 for °H in the exposed bed, but only 0.71 and 0.77 in the protected bed. However, the protected site mussels on average experienced substantially more sublethal stress (Table 5, Fig. 7). Notably, exposed bed inter-individual variation in cumulative thermal stress (measured as its coefficient of variation) over ~2 m was also greater than that along the 336 m transect.

There was also a significant range in both the number and temporal pattern of discrete days in which body temperature exceeded the 25 °C threshold, both within and between the two natural beds (Table 5; Fig. 8). Even in the warm, protected bed in August, some individuals never exceeded the threshold, while others experienced up to 5 episodes of sublethal stress in two multi-day windows.
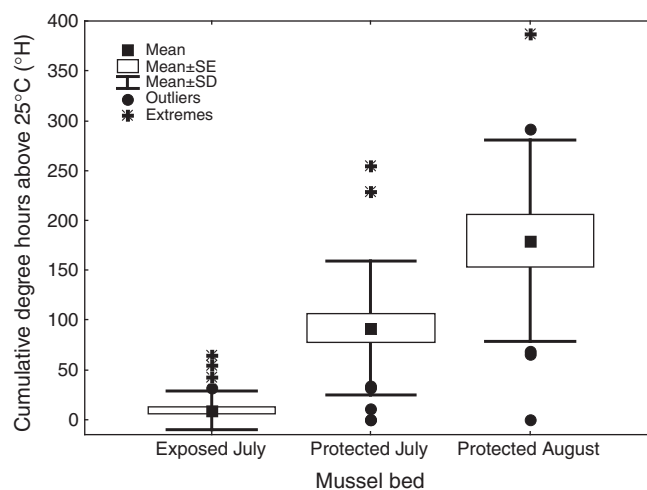

Fig. 7. Inter-individual variation in a common measure of sublethal stress (°*H*, standardized to a 30-day month) in mussels from exposed and protected natural beds at HMS.
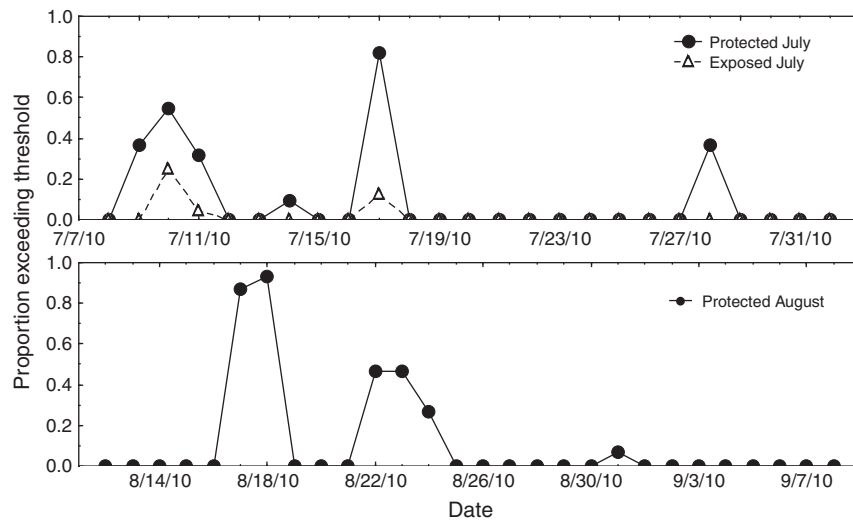
Fig. 8. Variation in the proportion of individual mussels in the natural beds that exceeded the 25 °C sublethal stress threshold on each day in July (A) and August (B).

3.4. Inter-individual variation in cumulative physiological work

In contrast to our metrics of acute and sublethal thermal stress, cumulative physiological work (*W*) exhibited surprisingly little inter-individual variation (Table 6). Among individuals on the transect, the CV of *W* in April ranged from 0.10 ($Q_{10} = 2$) to 0.32 ($Q_{10} = 4$). Regardless of the chosen Q_{10}, the CV of cumulative work in the natural mussel beds remained even lower, never exceeding 6% of the mean.

3.5. Lack of spatial structure

Due to the topographic complexity of individual mussel beds, we observed little spatial structure within each of the natural bed data sets. For example, spatial autocorrelation of both maximum body temperatures and cumulative degree hours above threshold (assessed by Moran's I index, Fortin et al., 2002) was positive but generally low (≤0.2) at inter-individual distances less than 0.5–1 m, decreasing to insignificant values near zero at higher separation (data not shown). We also examined the overall temperature records of mussels within each of the natural-bed datasets using Principal Components Analysis (PCA). When inter-individual distance in physical (*X, Y ,Z*) space was plotted against inter-individual distance in three-dimensional PCA-space (the first 3 principal components explained 70% or more of the variation), there was no correlation between physical separation and temperature regime ($r^2 < 0.01$; data not shown).

3.6. Thermal tolerance of M. californianus

Individual *M. californianus* began to die when body temperature exceeded 36 °C, approximately half were killed by exposure to 38 °C ($LT_{50} = 38.2$ °C), and all died when their body temperature exceeded 41 °C (Fig. 9).

3.7. Effects of local variation on mortality

The distributions of maximum temperatures in our experiments were approximately Gaussian (Fig. 10), meeting the assumption of our model. Data in Table 3 show that the mean σ_{max} can be at least as high as approximately 4.6 °C for objects in the intertidal zone. Our measurements did not coincide with any extreme thermal events, and because σ_{max} increased with increasing mean temperature in our dataset, we suspect that σ_{max} can be larger than we observed. To accommodate this possibility, we used σ_{max} values as high as 10 °C in our model scenarios.

Table 6
Metrics of inter-individual variation in cumulative physiological work (*W*; arbitrary units) at three different temperature sensitivities (Q_{10}s). In each case, measurements have been normalized to a 30-day month (720 h). SB = simulated mussel bed; NBE = natural bed, exposed; NBP = natural bed, protected.

|		Q_{10}	Mean	Maximum	Minimum	SD	CV
Transects	April	2	919	967	610	70.3	0.08
		3	768	1388	556	158.2	0.21
		4	842	1954	522	265.5	0.32
	September	2	1343	1659	1159	99.7	0.07
		3	1323	1976	1027	184.2	0.14
		4	1330	2362	945	269.3	0.20
Mussel beds	SB	2	1470	1893	1239	149.1	0.10
		3	2776	4613	1949	602.4	0.22
		4	4765	9658	2842	1531.4	0.32
	NBE	2	737	768	706	14.5	0.02
		3	750	802	702	23.8	0.03
		4	761	832	701	31.0	0.04
	NBP July	2	756	800	719	18.6	0.03
		3	787	862	725	32.8	0.04
		4	814	915	733	45.4	0.06
	NBP Sept	2	740	758	721	12.4	0.02
		3	763	798	723	24.0	0.03
		4	789	848	726	36.6	0.05
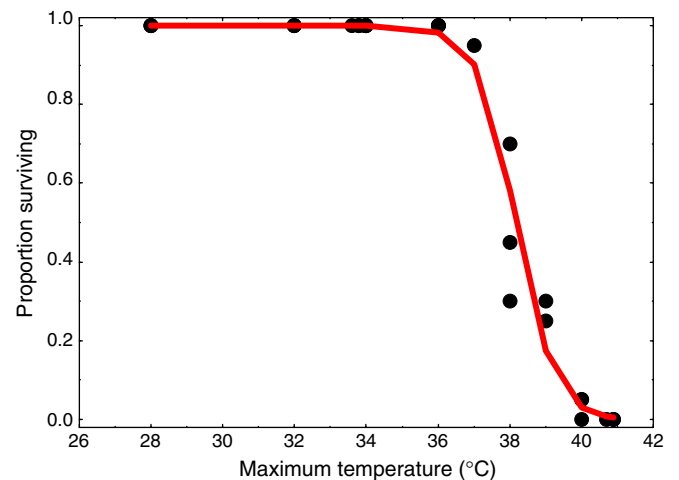
Fig. 9. Proportion of *Mytilus californianus* (out of 20 mussels in each replicate) surviving three days after aerial thermal stress as a function of maximum body temperature reached. Red line is a logistic regression, with an LT_{50} of 38.2 °C.
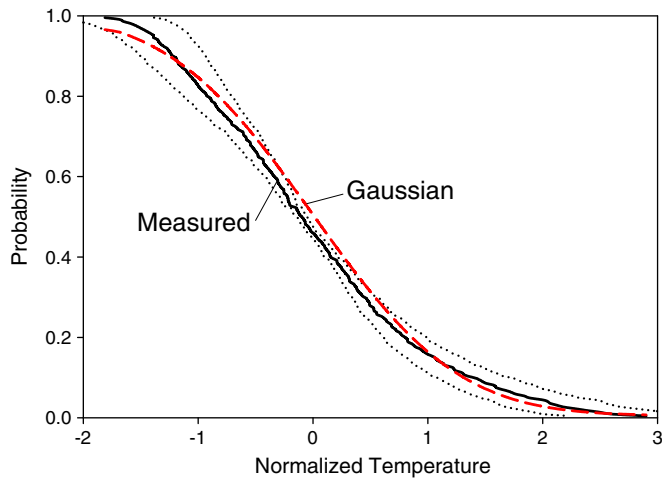
Fig. 10. The distribution of daily maximum temperatures among balls on the 336-m transect was approximately Gaussian. Each individual T_{max} on a given day was converted to a normalized temperature (a z score, Zar, 1999) by subtracting the mean T_{max} for that day and dividing by the standard deviation of T_{max} for that day. All normalized maximum daily temperatures for the entire experiment were then averaged and ranked to create the exceedance curve shown here. Dotted lines are ± 1 SD of the mean exceedance. For comparison, a Gaussian exceedance curve with the same mean and standard deviation is shown.

In our natural mussel beds, the highest mean maximum temperature occurred on August 18, 2010 ($\mu_{max} = 30.1$ °C in the protected bed), with a standard deviation of 3.1 °C among 15

individuals. Estimated median (~mean) thermal tolerance of *M. californianus* is 38.2 °C with a standard deviation of 1.25 °C. Inserting these values into Eqs. (5) and (6), and then into Eq. (4) predicts that only 0.8% of mussels would be killed (a practically undetectable level), and we observed no mortality. Thus, our results at HMS are consistent with risk theory, although they hardly form a stringent test.

The theoretical effect on mortality of variation in maximum temperature is shown in Fig. 11. In each panel, the abscissa is a measure of how variable maximum temperature is among individuals, expressed as its standard deviation σ_{max}, and the ordinate is F the fraction of individuals killed by acute thermal stress (evaluated by inserting Eqs. (5) and (6) into Eq. (4)). Each curve is for a given value of $\Delta\mu$, the difference between mean maximum temperature and mean thermal tolerance, ranging from -5 °C to $+5$ °C. The standard deviation of thermal tolerance σ_{tol} increases from 0.50 °C in A, to 1.25 °C in B, to 2.50 °C in C, spanning much of the range of measured values (Table 1). The implications of these theoretical results are discussed below.

4. Discussion

Our measurements document in detail the dramatic variation in maximum body temperatures and overall thermal experience among individuals within what ecologists would consider a single site (Tables 3–6). To make productive use of these data, we need to place them in perspective, a task we divide into five parts: (1) First, we use the theory of risk to explore the effect of inter-individual variation on the fraction of a local population killed by an extreme thermal event. We find that inter-individual variability, both in body
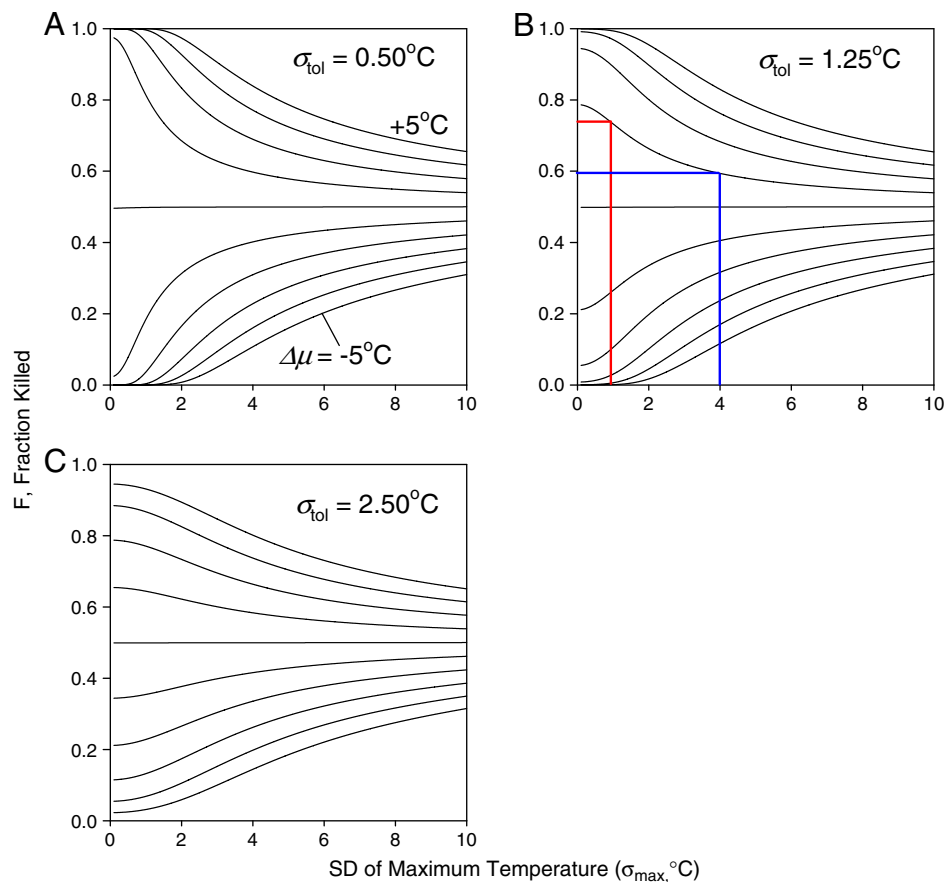

Fig. 11. Theoretical risk of being killed by the acute imposition of elevated temperature depends on both the standard deviation of maximum temperatures among individuals, σ_{max} (shown on the abscissa) and the standard deviation of temperatures tolerance among individuals, σ_{tol}, which increases from A to B to C. Each black line corresponds to a different $\Delta\mu$, from μ_{max} 5 °C below μ_{tol} to 5 °C above μ_{tol}. The red and blues lines are discussed in the text.

temperature and thermal tolerance, can limit the damage done by extreme temperatures. (2) Having explored the effects of temperature variation within a site, we then place this information in the context of variation at larger scales. We show that our within-site variation is comparable to the variation among sites along 14° of latitude, potentially complicating the search for latitudinal trends. (3) We next examine possible sources of the variability we have measured. Numerous factors contribute to variation in body temperature, and understanding their magnitude and interaction is key if we are to move from small-scale observations to large-scale predictions. (4) We then briefly review the organismal consequences (both lethal and sublethal) of variation in temperature, and echo recent calls for more research into the ecological physiology of intertidal organisms. (5) And lastly, we speculate on the evolutionary consequences of within site temperature variation.

4.1. The theory of risk

The theory of risk developed in the Materials and methods can be applied to any environmental variable. For example, if one knows the mean and standard deviation of imposed degree-hours above threshold and the mean and standard deviation of a species' tolerance of this thermal stress, the theory of risk can be used to predict the fraction of the local population killed by imposition of this stressor. However, information is scarce regarding the tolerance of intertidal organisms to degree-hours above threshold, so it is currently not feasible to apply the theory to this factor. The same practical constraint applies to the tolerance of hours above threshold and the rates of heating and cooling. Consequently, we frame our discussion of risk in the context of extreme body temperatures, with an emphasis on maximum temperature, the factor more likely to be exacerbated by climate change.

Three general conclusions emerge from our theoretical analysis of the risk of death from elevated body temperature. First, the effect of variation in mean maximum body temperature depends on whether mean maximum temperature is greater than or less than mean thermal tolerance. If $\mu_{max} < \mu_{tol}$, increasing variation in imposed maximum temperature increases the fraction of individuals killed. The reason why is illustrated by the dashed red line in Fig. 5A. Increasing σ_{max}–that is, increasing the spread of maximum temperatures around μ_{max}–has the effect of reducing G for temperatures below μ_{max} and increasing G for temperatures above μ_{max}. When $\mu_{max} < \mu_{tol}$, the reduction in G for low temperatures has little effect in Eq. (4); at these low temperatures values of p are near 0 anyway. In contrast, for temperatures above μ_{max}, G is increased at temperatures for which p is substantial. The net result is an increase in F, the fraction of individuals killed.

In contrast, if $\mu_{max} > \mu_{tol}$ (due to an acute thermal event, for example a heat wave), increasing variation in T_{max}, as observed in our field data, decreases the fraction killed. In this case (shown by the dashed red line in Fig. 5B), the increase in G for temperatures above μ_{max} has little consequence because, at these high temperatures, values for p are near 0. But the decrease in G for temperatures below μ_{max} now affects p values near μ_{tol} (where they are relatively large), and F is decreased.

In light of these theoretical results, we reach the intuitively reassuring conclusion that an ecologically important effect of inter-individual variation in maximum temperature is to make it increasingly unlikely that *all* of a local population will be killed by an increase in mean maximum temperature (either acute or chronic). Yes, if $\mu_{max} < \mu_{tol}$, increasing variation in T_{max} increases the fraction of individuals killed, as a result of extremely high temperatures experienced by some individuals. But, as long as $\mu_{max} < \mu_{tol}$, no more than 50% of the population will die. Furthermore, if $\mu_{max} > \mu_{tol}$, increasing variation in T_{max} can substantially reduce the risk of extirpation. Consider an example in which $\sigma_{tol} = 1.25$ °C (Fig. 10B). If

μ_{max} exceeds μ_{tol} by 1 °C and the standard deviation of T_{max} is 1 °C (typical of the values we measured for mussels at HMS), approximately 73% of individuals will die (red lines). If, instead, the standard deviation of T_{max} is 4 °C (typical of the values we measured for balls along the transect, and perhaps applicable to some individual intertidal organisms), only 59% of individuals will die (blue lines). This increased 14% "buffer" of surviving individuals might allow the population to persist. These results suggest that variation in maximum body temperature from individual to individual can potentially act analogous to variation from time to time (Benedetti-Cecchi et al., 2006) in reducing the effects of rising mean temperature.

The second general conclusion evident in this hypothetical example concerns the variation in thermal tolerance. The narrower the variation (that is, the smaller σ_{tol} is), the more pronounced the effect of variation in maximum temperature on survival. As a representative example, consider a situation in which μ_{max} differs from μ_{tol} by ± 1 °C (Fig. 12). If σ_{tol} is 0.5 °C–corresponding to a range of about 2 °C between all individuals surviving and all individuals dying–there is a substantial change in the fraction of individuals killed as the variation in maximum temperature increases; an increase in F if $\mu_{max} < \mu_{tol}$, a decrease if $\mu_{max} > \mu_{tol}$. The larger σ_{tol} is, the smaller the effect of variation in T_{max}. This effect is especially evident if σ_{max} is 1–2 °C, characteristic of our dataset for mussels in natural beds.

The importance of σ_{tol} in determining risk highlights a fundamental problem in physiological measurement. Because thermal tolerance is likely to depend on past thermal history, reliable measurement of the distribution of tolerances must be conducted using individuals that have experienced field conditions. However, if these conditions included events stressful enough to kill some individuals, the measured distribution of tolerances is affected by the absence of these susceptible organisms—the mean is increased and the standard deviation decreased. For example, truncating the lower end of a Gaussian tolerance distribution such that 10% of individuals are killed, increases the mean of the remaining individuals by an amount equal to 19.5% of the distribution's original standard deviation, and the standard deviation of the truncated tolerance distribution is only 63% that of the original. In theory, the presence of such truncation effects should be evident from the shape of measured tolerance distributions —they should be skewed such that their mode is less than their mean. However, definitive detection of such skew will require more extensive measurements than have been conducted to date, and a
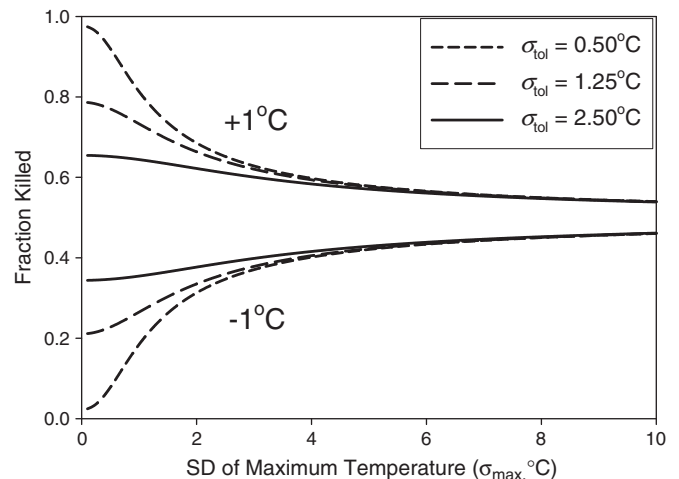

Fig. 12. The predicted fraction of the population killed by imposition of an acute temperature stress depends on the variation among individuals in the temperature applied (σ_{max}, shown on the abscissa) and the standard deviation of temperatures tolerance among individuals, σ_{tol}, which is shown by the various dashed lines. For any σ_{max}, the smaller σ_{tol} is, the greater the fraction killed. In this example, $\Delta\mu$ is either +1 °C or −1 °C.

thorough assessment of the influence of history on σ_{tol} awaits future research.

Lastly, we view these theoretical results from the perspective of climate change. That is, if we know the mean and standard deviation of thermal tolerance, and the standard deviation of imposed body temperature, how does the fraction of surviving organisms change as mean temperature rises? This question is answered by noting in Fig. 10 the intersection of a line of constant σ_{max} with the curves for various $\Delta\mu$. The results are shown in Fig. 13. Keeping tolerance constant, when there is little within-site variation in T_{max} (that is, small σ_{max}), increasing mean maximum temperature results in an abrupt increase in the fraction of individuals killed. Essentially, the local population is barely affected until temperature reaches a critical level, and catastrophe then ensues. In contrast, if within-site T_{max} is more variable (higher σ_{max}, again as seen in our field datasets), the fraction of individuals killed increases gradually as temperature rises (Fig. 13), and at least a small fraction of the population always survives.

Note that the abscissa in Figs. 10, 12 and 13, in which we calculate the fraction of individuals killed, is given in terms of temperature. Shifts in temperature can happen either over time for a given site, or between sites at any given time. Thus, our conclusions regarding the fraction of individuals killed can easily be applied to questions of either climate change or latitudinal gradients in temperature. Variation in tolerance among sites (as seen for whelks, Kuo and Sanford, 2009, and mussels, Logan, 2010) or size classes would complicate calculations slightly, but as long as variation in tolerance is known and quantified, it could be incorporated into the predictions.

It will be valuable to test the predictions of our risk theory. As noted above, our field observations under relatively benign conditions are consistent with theory, but do not constitute a stringent test. A true test will require longer-term field measurements that coincide with a thermal event sufficiently stressful to kill a measurable fraction of the mussel population, such as that observed by Harley (2008).

4.2. Local versus latitudinal variation

The variation we observe at HMS rivals variation documented at larger scales. In a study covering 1660 km of Northeast Pacific coastline, Helmuth et al. (2006b) measured the maximum temperature of the competitive dominant occupier of mid-intertidal space

(the mussel, M. californianus) from 34.4°N (southern California) to 48.4°N (northern Washington State). They do not report data for rates of heating and cooling or for hours and degree-hours above threshold, but we can compare our local ranges in temperature to their measurements spanning this coast.

The hottest daily average maximum temperature measured by Helmuth et al. (2006b) in their coastwide survey was 23.4 °C at Alegria in southern California, and the coldest was 13.2 °C at Tatoosh Island in northern Washington State, a difference of 10.2 °C, and we take this difference as a standard for comparison. The range of daily average maxima within the small confines of one of our natural mussel beds (NBP August) was 3.9 °C, 38% of the difference between Tatoosh Island and Alegria. The largest range of daily maximum body temperatures among individuals within the protected natural mussel bed at HMS is 149% (NBP August) of the range of daily average maxima among sites along 1660 km of coast (Table 3). Although the type of logger was different in the two cases, a comparison of variability among brass balls on our transect to that of mussels along the coast is nonetheless informative. The maximum and average ranges of T_{max} among brass balls on the 336-m transect are 245% and 55%, respectively, of the variation in average daily maxima for mussels across 14° of latitude. Clearly, within-site variation in maximum body temperature is comparable to between-site variation.

The same analysis can be applied to variation in minimum temperature. The range of daily average minimum temperature measured by Helmuth et al. (2006b) was 4.7 °C, varying between 12.7 °C at Coal Oil Point in southern California and 8.0 °C at Tatoosh Island. The range of daily average minimum temperatures within a natural mussel bed at HMS (NBE July) is 2.7 °C, 57% of the variation among sites spanning 14° of latitude, and the average daily range of T_{min} within HMS represents 25% to 35% of the range of average daily minima along this entire latitudinal range (Table 3). Analogous values for brass balls on the transect are 130% and 89%. As with maximum temperature, within-site variation in minimum temperature is clearly comparable to variation among sites.

4.3. Sources of variability

Body temperatures of intertidal organisms are set by the interaction of multiple physical factors (Gates, 1980; Campbell and Norman, 1998; Nobel, 1991; Helmuth, 1999, 2002; Denny and Harley, 2006). Heat energy can be delivered to the plant or animal by: absorption of direct, reflected, or scattered sun and infrared light; by convective transfer from hot air; by conductive transfer from warm substratum or neighbors; and by condensation of water. Heat can be lost by: radiation of infrared light, convective and conductive transfer to cool air, substratum, or neighbors, and by evaporation. The "mix" of these factors varies through time with variation in the environment, through space with variation in topography and height on the shore, and between organisms of different size, shape, coloration and behavior.

Given sufficient information about each of these factors, the body temperature of individual organisms can be accurately predicted. In this sense, the small-scale variability we have measured is fundamentally deterministic. However, at the scale of individual organisms, sufficient information about many important physical aspects of the environment is essentially unobtainable. Today, a random splash may cool one mussel and not its neighbor; tomorrow's splash may cool both or neither. Just as the flip of a coin can be predicted in theory but not in practice, the complexity of the wave-swept environment will always inject an element of randomness into the prediction of individual body temperatures. For example, although Harley (2008) carefully measured the height on shore and substratum orientation for 75 groups of mussels (two primary components of solar heat input), these details predicted only 39% of the variation in mortality during a stressful thermal event. This study was not designed to
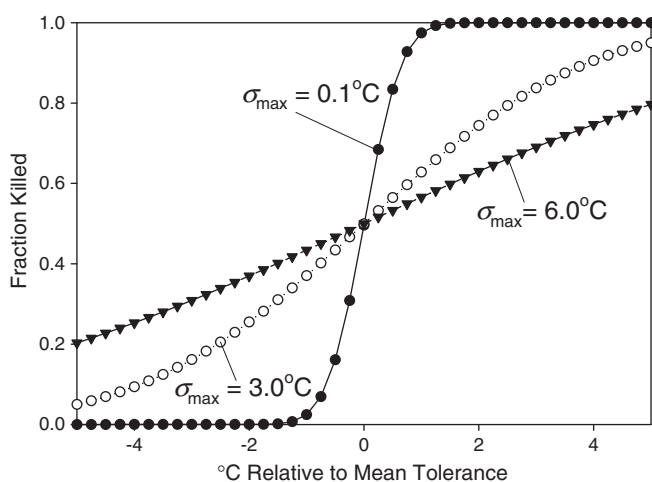

Fig. 13. The effect of increasing mean maximum temperature from 5 °C below mean tolerance temperature to 5 °C above mean tolerance temperature (shown on the abscissa) varies with the variation of maximum temperature among individuals. The larger σ_{max} is, the more gradual the increase in the fraction of individuals killed, and, for $\mu_{max} > \mu_{tol}$, the lower the fraction of individuals killed.

separate the predictable components of inter-individual variation from those that are unpredictable, but three observations are nonetheless pertinent here.

1. Denny et al. (2004) measured variation in a variety of environmental and biological factors along the transect at HMS, and concluded that in all but one case (the abundance of a predatory whelk), the amount of variation observed increased with the spatial scale of observation (specifically, a pattern known as $1/f$ noise). It appears that this general pattern may apply to our measurements of temperature. Arrayed over 336 m, our brass temperature loggers showed larger ranges of daily maximum temperature (21 °C to 25 °C) than did mussel mimics within 2 m of each other. Some of the increased variation in the brass loggers is likely due to their lower thermal mass, which responds more rapidly to shifting environmental conditions than does a mussel in a bed. However, it is also likely that the brass loggers encountered a wider range of topographical and environmental conditions than those available to mussels in the constrained space of our experimental beds, and we know that the transect extends into habitats where no mussel beds are found. It seems possible that we would have observed more variation in both maximum and minimum temperatures had we measured variability among mussels in natural beds over larger scales. If this is indeed true, our measurements of within-site variation in mussel body temperature are conservative, and the implications for our models of population viability even more pronounced.

2. Although we have attempted to make our mussel mimics as realistic as current technology allows, there may still be differences between the temperatures we recorded and those that would have been experienced by live mussels under the same circumstances. For example, our mimics cannot exhibit behavior. The primary behavior of mussels potentially relevant to acute body temperature regulation is valve gaping, which could moderate temperature by increasing evaporative cooling (albeit at the risk of desiccation; Nicastro et al., 2010). Previous studies have failed to demonstrate an effect of gaping on body temperature in *M. californianus* (Fitzhenry et al., 2004) or on survival of elevated temperatures in another mussel (*Modiolus demissus*; Lent, 1968), and gaping behavior during emersion in mussels appears to function more to maintain mantle fluid oxygenation for aerobic respiration than to modulate body temperature (e.g., Coleman, 1973; Bayne et al., 1976). However, it seems premature to conclude that gaping (or other behaviors) has no effect on body temperature. In a similar vein, our mimics measure temperature at the center of a silicone body, which may differ from the core temperature of a mussel in which haemolymph and/or seawater circulate. Further study is required to determine how much, if any, of the small-scale variation we have measured is due to these measurement issues, and we hope that this study will spur renewed interest in exploring ways to better measure body temperature in the field.

It is worth noting, however, that advances in measurement will require both technological and physiological insight. Suppose an ideal technique could be developed that non-invasively and continuously measured temperature at 10 locations within a mussel. Would we then know any more about inter-individual body temperature variation than we do now? We suggest that we would not. Instead, it is likely that the increased ability to measure temperature would only confuse matters by raising issues of *intra*-individual variation. A mussel's heart lies near the middle of its body, whereas its gonads line the inside of the shell. Which organ's temperature should we use as "the" body temperature? Critical thermal maxima of heart rate indicate absolute organismal limits to physiological function, but sublethal temperature stress to the gonads might well affect reproductive output. Only with increased understanding of the physiological details of organisms' response to temperature will we

ever be able to truly account for the effects of small-scale thermal variation.

3. In our simulated mussel bed, temperatures were highest in the center and lowest at the edge. Helmuth (1999) predicted the opposite pattern when comparing temperatures in the interior of a continuous mussel bed to the edges of a patch within the bed. The difference is most likely explained by contrasting air flow in the two situations. In our isolated small bed, mussels at the edge probably experienced more convective cooling than mussels in the center, which were sheltered from the wind by their neighbors. In contrast, wind is likely to "skim" over a patch in a continuous bed (Denny, 1988), so that edge mussels in a patch do not have the same advantage in convective cooling. They do, however, present more area with which to absorb sunlight, resulting in higher body temperatures than the shaded individuals in the bed's interior. Hunt (2006) found that temperatures were highest in the center of clumps of seaweed (*Endocladia muricata*) rather than at the edges.

4.4. Ecological physiology of thermal tolerance and cumulative stress

Our analyses of the consequences of local variability imply an important role for the intrapopulation range of thermal tolerance. However, general application of this model to intertidal ecology is limited by several shortcomings in our understanding of ecological physiology.

First, the thermal tolerance values extracted from the literature must be treated circumspectly. The acute tolerance of an organism to elevated body temperature depends on a variety of factors. For example, working with the limpet *Lottia gigantea*, Miller et al. (2009) found a substantial shift in both σ_{tol} and LT_{50}–the temperature required to kill half the individuals tested–depending on the relative humidity of the air in which the animals were tested (Table 1). The duration of exposure to high temperature can also affect thermal tolerance. In general, the longer the exposure, the lower the temperature required to kill an organism (e.g., Newell, 1970; McQuaid and Scherman, 1982), but in the case of the alga *Endocladia muricata*, the shift in μ_{tol} as exposure time increased was not accompanied by an apparent shift in σ_{tol} (Table 1). The sea star *Pisaster ochraceus* forms another curious case, for which Pincebourde et al. (2008) report an exceedingly narrow range of thermal tolerance: All individuals survived exposure to 35 °C, but none survived exposure to temperatures above 35 °C. This narrow σ_{tol} could lead to the sorts of abrupt shifts in survival predicted by our theory of risk. If the susceptibility to thermal catastrophes differs between *P. ochraceus* (a keystone predator) and its preferred prey (mussels, the dominant competitor for primary space on the substratum), interesting ecological consequences could ensue. However, *P. ochraceus* can behaviorally modulate the T_{max} to which it is exposed by seeking out cooler microhabitats and/or accumulating cool seawater in its coelomic space (Pincebourde et al., 2009), and can thereby potentially modify its susceptibility to thermal stress. Indeed, in some species such behavioral mechanisms could override the small-scale variation in T_{max} we have documented among stationary "individuals," and this possibility requires further exploration. It is also likely that the history of exposure to high temperatures has an effect on thermal tolerance (e.g., Schonbeck and Norton, 1979; Harley and Paine, 2009), although the type of effect may well be complicated, depending on mean acclimation temperature (most likely seasonal seawater temperature), the frequency and intensity of previous thermal stress events, and acclimatory capacity (Stillman, 2002; Stillman and Tagmount, 2009). Unfortunately, the interactions of these potential factors in setting thermal tolerance remain to be investigated in detail for many intertidal taxa.

Second, as alluded to above, it is quite possible that sublethal thermal stress effects, rather than acute lethal temperature events of the kind we have modeled, play a prominent role in intertidal

population and community ecology, as has recently been implicated on a global scale for lizard extinctions (Sinervo et al., 2010). While much of our discussion to this point has focused on maximum body temperatures and acute lethal effects, none of the mussels in our natural beds exceeded temperatures that were found to be lethal in our laboratory experiment or in a previous study (Logan, 2010), and very few approached within even 5 °C of lethal body temperature. Meanwhile, we have demonstrated dramatic inter-individual variation in cumulative time spent above a physiological stress threshold (25 °C) (see also Harley and Helmuth, 2003) as well as the number of discrete events in which individuals' body temperature exceeded that threshold, again among mussels separated by less than 2 m. Notably, these above-threshold events tend to cluster in 2–3 day windows, as modeled for limpets (Miller et al., 2009). Repeated exposure to elevated temperatures could lead either to acclimatization (with an increase in tolerance) or to cumulative stress and accumulated damage (resulting in a decrease in tolerance). Unfortunately, our current grasp of physiology is insufficient to allow us to make reliable general predictions about the ecological consequences of these repeated episodes (Helmuth et al., 2005; Denny and Helmuth, 2009).

Finally, even more subtle effects of variable thermal history, such as our hypothetical measure of inter-individual variation in cumulative work (W), may prove fundamental to intertidal dynamics. For example, Sanford (2002) has shown that "normal" ocean temperature variation modulates the foraging rate of *P. ochraceus*, a keystone predator on the west coast of North America. As noted above, small-scale temperature effects on predator foraging could have important, cascading consequences for intertidal communities. In our analyses, cumulative W varied among individuals by only 6% or less of the mean, while exposure to "stressful" conditions in the same individuals varied by up to 285% of the mean. Ecologists require relevant physiological substitutes for W–including division of this variation in work between maintenance metabolism and metabolic 'surplus' (e.g., work for growth, predation, or reproduction; see Kordas et al., 2011-this issue)–that can be related to organismal fitness and population outcomes. Comparative analyses of these various metrics of cumulative thermal history using such ecophysiological indices are urgently needed.

4.5. Evolutionary implications of extremely local temperature variability

Lastly, we return to the opening observation that a number of intertidal species already appear to experience habitat temperatures at or very near their lethal thermal limits. Our empirical and theoretical results represent an important caveat to this conclusion. We have demonstrated substantial ranges in extreme maximum body temperature among mussels separated by as little as 2 m, and we have also shown that the pattern of thermal variability itself varies dramatically between two mussel beds separated in space by only 24 m. Furthermore, our model demonstrates how, even when μ_{max} exceeds μ_{tol}, local species persistence derives, at least in part, from such small-scale variability.

What are the potential evolutionary implications of this small-scale variability? At the simplest level, population outcomes will depend on the frequency of extreme, lethal events. For example, Denny et al. (2006) and Miller et al. (2009) estimated that intertidal limpets experience lethal body temperature episodes every 1 to 5 years. The population genetic and evolutionary consequences of such events will depend in part on generation time, which is less than 2 years in *Mytilus* and many other intertidal invertebrates. Individuals with lower thermal tolerance, but that reproduce before the return time of lethal events, will likely have comparable reproductive fitness to more thermally tolerant conspecifics in the same bed. In fact, as recent work has shown for intertidal copepods, there may be significant fitness trade-offs associated with the ability to tolerate extreme body temperatures (Willett, 2010), suggesting that, in

between extreme events, less thermally tolerant individuals might have higher fitness than the high temperature "specialists."

When these fitness considerations are applied in a species (such as *M. californianus*) with the homogenizing population genetic influences of broadcast spawning (leading to a general lack of genetic population structure; Levinton and Suchanek, 1978; Sarver and Foltz, 1993; Addison et al., 2008), lottery recruitment to a mosaic of micro niches with wide ranges of imposed body temperatures over very small spatial scales (such that thermally tolerant genotypes might settle in relatively stress-free locations, or vice versa, the latter known as "phenotype-environment mismatch"; Marshall et al., 2010), limited adult mobility, and other stochastic factors, we might predict the net result of this small-scale variability to be a maintenance of substantial physiological diversity within local populations. Such local functional variability has indeed been documented in *M. californianus* (large within-site standard deviations in critical heart rate temperature; Logan, 2010) and intertidal copepods (large within-pool variance in temperature tolerance despite a very high degree of between-pool genetic population structure; Willett, 2010), among others. At the genetic level, habitat heterogeneity appears to maintain genetic polymorphisms in broadcast-spawning barnacles (e.g., Schmidt et al., 2000). Even in cases where the evidence supports genetic divergence in broadcast spawners over wider spatial scales despite habitat variability (e.g., Oregon vs. California in purple sea urchins; Pespeni et al., 2010), there remains substantial local genetic heterogeneity. We suggest that the micro-scale variability we have documented plays a prominent role in maintenance of this local genetic and functional variation.

Whether small-scale variation in imposed body temperatures might buffer populations from the effects of climate change and an increased frequency of lethal and sublethal events enough for physiological diversity, physiological plasticity, and/or physiological evolution (e.g., evolution of μ_{tol}) to keep pace is an open, and vitally important, question. Clearly, integrative studies examining the interplay of variability in individual environmental experience, population genetic diversity and structure, and variation in physiological performance are needed to begin to answer this question.

Acknowledgments

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