

The role of temperature and desiccation stress in limiting the local-scale distribution of the owl limpet, *Lottia gigantea*

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Summary

1. Small-scale distributions of rocky intertidal organisms may be determined in part by temperature and desiccation stress during low tide and the ability of organisms to resist such stresses. Biophysical modelling techniques, coupled with data on the physiological tolerance of organisms can be used to predict the frequency and severity of abiotic stress events.
2. The limpet *Lottia gigantea* is a major competitor for primary space on rocky shores along the west coast of North America. The goal of this study was to quantify the thermal and desiccation tolerance of this species and to predict the frequency of stressful events at Hopkins Marine Station (HMS) in Pacific Grove, CA, USA.
3. *Lottia gigantea* were exposed to thermal stress regimes in the laboratory using protocols designed to mimic prolonged exposures in the field. The production of heat shock protein 70 (Hsp70) was measured as an indicator of sub-lethal stress, and mortality rates were measured.
4. Peak expression of Hsp70 occurred during exposures at 32 °C, while the lethal limits of the limpets ranged between 30 and 42 °C, depending on the exposure regime. Lethal temperatures were approximately 5 °C lower in 50–60% relative humidity conditions compared to 100% relative humidity trials.
5. Results from the physiology experiments were compared to field surveys of the upper limits of *L. gigantea* at HMS and with heat-budget model predictions of limpet body temperatures on a variety of substratum orientations.
6. The highest frequency of sub-lethal and lethal stress events was predicted to occur on high-shore westerly and southerly slopes and horizontal surfaces, microhabitats where *L. gigantea* is comparatively rare at HMS. Predicted stress and mortality events were rare at low-shore sites, vertical surfaces, and sloping rock faces oriented towards the northeast and northwest.
7. The combination of direct measurements of sub-lethal and lethal temperature thresholds using realistic stress exposures and predictions from a heat-budget model give insight into the role of thermal disturbance in affecting the local-scale distributions of intertidal organisms. These methods hold great promise for enhancing our understanding of the role of abiotic factors in limiting species distributions and making predictions about responses to future climatic conditions.

Key-words: heat budget model, rocky intertidal zone, heat shock protein 70, body temperature, shore height, solar irradiance, lethal temperature, wave exposure

Introduction

In both the terrestrial and marine realms, species distributions can be influenced by abiotic factors, including extremes in

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environmental conditions such as temperature, precipitation, desiccation, wind and wave forces. On mountain tops, small excursions along the vertical and horizontal axes can produce marked changes in wind and sun exposure, snow accumulation, precipitation and temperature, changes that are often reflected in the biological community (Poore & McVean 1957; Johnson & Billings 1962; Ferreyra *et al.* 1998). In the intertidal

zone, abiotic conditions may shift even more dramatically over scales of just a few meters. For organisms that live at this interface of the marine and terrestrial realms, survival requires withstanding a broad array of abiotic and biotic stresses.

The distribution of a species on the shore is set by numerous factors, such as interactions with other species, that occur within a constantly shifting set of environmental parameters as the tide rises and falls (Stephenson & Stephenson 1949; Southward 1958; Connell 1961; Newell 1964). The potential for high temperatures and desiccation to limit the range of an organism can be intimately tied to the small-scale variation in conditions that exists between fully submerged and fully terrestrial habitats, along with the physiological tolerance of a species to conditions in each microhabitat (Helmuth & Hofmann 2001; Somero 2002; Davenport & Davenport 2005; Bertness *et al.* 2006). The role of thermal and desiccation stress in setting the distributional limits of organisms, over both large and small scales, has been an integral part of studies of this system (Broekhuysen 1940; Lewis 1954; Foster 1971; Wolcott 1973; Branch 1981; Williams & Morritt 1995; Stillman & Somero 2000; Harley 2003; Harley & Helmuth 2003; Harley 2008).

On a rocky shore, there are three principal factors that determine the suitability of a habitat for occupation with regard to temperature and desiccation stress. First, height on the shore determines the total amount of time the organism is exposed to the air, a time during which temperature may fluctuate substantially (Helmuth 1999; Denny *et al.* 2006; Finke *et al.* 2007). Second, the orientation of the substratum and neighbouring structures (including other species) affects the amount of solar radiation received by an organism, and thereby its heat influx (Gates 1980; Campbell & Norman 1998). Finally, the orientation of the microhabitat relative to incoming waves, coupled with local topographic features which can increase or decrease wave swash, influences the degree of temperature buffering provided by seawater (Harley & Helmuth 2003; Helmuth & Denny 2003; O'Donnell & Denny 2008).

These environmental characteristics of a microhabitat can be used to predict the potential severity of temperature and desiccation stress of that site. A biophysical model of body temperature, generally referred to as a heat-budget model, allows researchers to make hindcasts or forecasts of an organism's body temperature, using a variety of environmental data and selected physical characteristics of the organism (Porter & Gates 1969; Bell 1995; Helmuth 1999; Denny & Harley 2006). With a heat-budget model in hand, one can begin to speak to the suitability of a particular microhabitat, with regard to temperature and desiccation stress, for the organism (Denny *et al.* 2006). To make quantitative predictions about the effect of stress on the distributions of the organism, however, this process requires knowledge of the physiological tolerance of the organism (Newell 1976; Branch 1981; Davenport & Davenport 2005; Helmuth *et al.* 2006).

The focus of this study is the owl limpet *Lottia gigantea* Sowerby, 1834 (Fig. 1), a common competitor for primary space in the mid-intertidal zone on rocky shores from central California to northern Baja California (Stimson 1973). These



Fig. 1. A *Lottia gigantea* on its territory in the field. The limpet is approximately 60 mm in length. Smaller *L. digitalis* and *L. scabra* 'riders' are commonly found on the shell of adult *L. gigantea*.

limpets graze territories on the rock surface, feeding on algae and actively removing other organisms that intrude on the territory (Lindberg *et al.* 1998). Individual adult *L. gigantea* typically occupy a territory for multiple years and may be restricted to that area by the actions of neighbouring limpets or other species occupying primary space around the territory (Galbraith 1965; Stimson 1970). *Lottia gigantea* is typically found on wave-exposed shores on vertical or sloped substratum, occasionally occurring on horizontal platforms (Denny & Blanchette 2000). We detail a combination of physiological and biophysical techniques, along with field surveys of limpet upper limits, aimed at testing the hypothesis that the upper limits and along-shore distribution of *L. gigantea* could be driven, in part, by physiological limitations in the response to extreme temperatures and desiccation stress during aerial emersion. Can the upper vertical limits of *L. gigantea* populations be set by occasional high temperature events during low tide periods, or are they physiologically robust enough that rare, stressful low tides are inconsequential? By measuring the temperature and desiccation tolerance of *L. gigantea* in conditions that approximate stressful events from the field, and comparing these data with hindcasts of limpet body temperatures produced by a heat-budget model in various microhabitats, we predict which microhabitats should be unsuitable for long-term occupation by *L. gigantea*. These predictions are then compared to observed distributional patterns in the field.

Materials and methods

COLLECTIONS

All *L. gigantea* were collected from north and northeast facing vertical or near-vertical walls in the intertidal zone at Hopkins Marine

Station (HMS), Pacific Grove, California (36°37'3" N, 121°54'25" W). Collections were made between December 2006 and March 2007. Prior to all experiments, limpets were held in an outdoor seawater table for a minimum of 5 days, where they were splashed with seawater and were able to graze freely. The water temperature in holding table was as same as the ambient seawater temperature off HMS, typically between 11 and 14 °C. The outdoor aquaria were shaded from the sun, and air temperatures ranged between approximately 8 and 20 °C.

LABORATORY HEAT STRESS EXPERIMENTS

To produce heat stress profiles similar to those experienced in the field by limpets, a temperature-controlled recirculating wind tunnel allowed the body temperature of limpets to be raised gradually by manipulating the temperature of the substratum and air. Using output from the heat-budget model developed by Denny and Harley (2006) for *L. gigantea*, a heating rate of 8 °C h⁻¹ was chosen to represent typical conditions during an extreme high temperature event in the field. The relative humidity in the chamber was maintained between 50% and 60%, based on relative humidity measurements taken next to *L. gigantea* in the field during prolonged heat stress events (L. Miller, personal observation). In the field, limpet body temperature is largely dependent on substratum temperature due to the large contact area of the foot (Denny & Harley 2006). In the wind tunnel, limpets were placed on a heated aluminium plate that was used to control limpet body temperature while air was circulated through the tunnel at 0.5 m s⁻¹. Each heat stress trial ran for a total of 7 h, with the temperature starting and ending at 14 °C, and changing at the fixed 8 °C h⁻¹ rate. When limpets reached the designated maximum body temperature in the middle of a trial, that temperature was held steady until it was time to begin reducing the temperature to 14 °C (again at 8 °C h⁻¹) all within the 7 h time limit (Fig. 2). Air temperature in the wind tunnel was raised and lowered at 8 °C h⁻¹ from the same

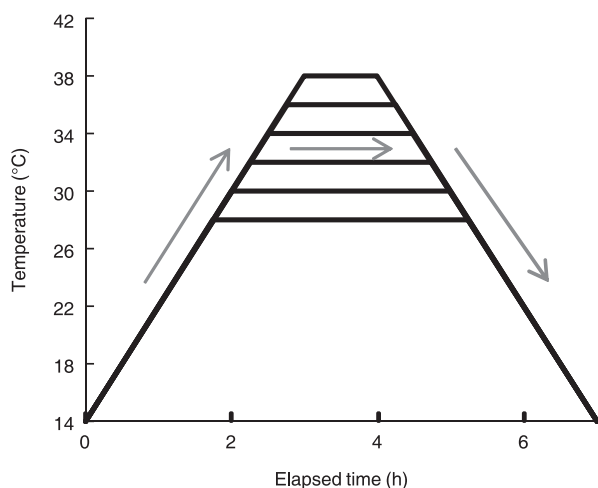


Fig. 2. Illustration of the temperature stress profiles used. Limpet body temperature was raised and lowered at a rate of 8 °C h⁻¹. All limpets began the experiment at 14 °C, close to seawater temperature, before being heated to the target maximum temperature. The maximum temperature was held steady until it was time to begin cooling to return to the 14 °C final temperature at the fixed cooling rate. Total elapsed time of all trials was 7 h.

14 °C starting point. Air temperature in the field at HMS rarely exceeds 30 °C, even on the warmest days, so all wind tunnel experiments used a maximum air temperature 30 °C.

The recirculating wind tunnel was used to quantify survival of *L. gigantea* at high temperatures. Each lethal temperature trial used five *L. gigantea*, and each temperature trial was replicated three times. Maximum body temperatures in these trials were 30, 32, 34, 36 or 38 °C. Following a heat stress exposure, experimental limpets were returned to running seawater for 24 h. If the limpet could not maintain a grip on the substratum after 24 h, it was scored as 'ecologically dead' (*sensu* Wolcott 1973).

Intertidal organisms' survival at high temperatures has often been assayed while the organisms were either submerged or emersed at high humidity (Sanders *et al.* 1991; Clarke *et al.* 2000; McMahon 2001; Somero 2002; Davenport & Davenport 2005). Unlike the experiments described above, these procedures precluded desiccation of the test limpets during heat stress. To facilitate comparison between these methodologies and the more realistic wind-tunnel experiments described above, the thermal tolerance of *L. gigantea* was also determined while emersed at 95–100% relative humidity. A simple closed chamber was designed with a platform to hold the limpets in air above a reserve of seawater in the lower section. The temperature inside the chamber was raised and lowered using the same temperature profiles used in the wind tunnel, and survival was assayed as described above. Trials were run using maximum temperatures of 32, 34, 36, 38, 40 and 42 °C. Groups of five limpets were used in each temperature trial, and each trial was replicated three times.

The number of surviving limpets in each trial was expressed as a fraction *S* of the total number of limpets in the trial. *S* was averaged for the three replicates at each temperature. The lethal temperature, *T*, at which 50% of the limpets in a trial died (LT50) was estimated by a fitting a sigmoidal curve to the survival data

$$S = 1 - \frac{1}{1 + \exp(-(T - a)/b)} \quad (1)$$

where *a* and *b* are best-fit coefficients determined by a curve-fitting routine implemented in Matlab software (The Mathworks, Natick, MA). The value of *a* is equal to the LT50 temperature.

SUB-LETHAL STRESS

In addition to measuring lethal limits for *L. gigantea*, the recirculating wind tunnel was used to expose limpets to sub-lethal temperature and desiccation stress. The intensity of sub-lethal stress was measured using a common physiological stress marker, heat shock protein 70 (Hsp70). Groups of five *L. gigantea* were stressed in the recirculating wind tunnel using maximum temperatures ranging from 24 to 36 °C for 7 h using the same exposure profiles as the lethal temperature determinations. To control for the effects of handling and exposure in the environmental chamber, a control group of five limpets was placed in the environmental chamber and held at 14 °C for 7 h. These control limpets remained wetted for the entire duration of the exposure, so that desiccation was likely a minor stress. The limpets targeted for protein expression work recovered in flowing seawater for 1 h, and were then frozen in liquid nitrogen. An additional field control sample of five *L. gigantea* was collected from the field at HMS while still submerged by a high tide. These limpets were immediately frozen following collection. The frozen limpets were stored at -70 °C until they could be processed.

Hsp70 assays were carried out using a protocol similar to that of Hofmann and Somero (1995). Each limpet was partially thawed and

a small portion of the pallial gills and mantle tissue (approximately 1–2 mg) was dissected from the edge of the mantle (Fisher 1904). Tissues were homogenized in buffer by boiling for 5 min followed by 2 min in an automatic homogenizer (Tissuelyser, Qiagen, Valencia, CA). The samples were boiled and homogenized twice more before centrifugation at 14 000 g for 15 min. The supernatant was stored at –20 °C.

Quantification of the total protein content of each sample was carried out using the BCA Pierce protocol (BCA Protein Assay Kit, Pierce Inc.) in 200- μ l well plates. Each sample was loaded in triplicate, and each plate contained a set of pre-diluted bovine serum albumin (BSA) standards, also loaded in triplicate. Absorbances were measured in a SpectraMax 340pc spectrophotometer (Molecular Devices, Sunnyvale, CA). A standard curve was calculated from the absorbance of the standards, and the average of the three replicates of each sample was taken as the protein concentration for that sample. The protein quantification was carried out two times to determine if concentrations changed appreciably due to freeze and thaw cycles, but no change was detected.

SDS-PAGE gel electrophoresis was used to separate proteins for Western blotting. After boiling for 3 min at 100 °C and centrifuging at 14 000 g for 1 min, 7 μ g of total protein from each limpet, along with Laemmli sample buffer and distilled water, were loaded into a lane of a 10% Tris–HCl pre-cast gel (Ready-Gel Polyacrylamide gel, Bio-Rad Laboratories, Hercules, CA). In addition to the heat-shocked samples, a molecular weight standard was loaded in one lane (Precision Plus Protein Standard, Bio-Rad Laboratories), along with 30 ng of human Hsp70 in another lane (NSP-555, StressGen–Assay Designs, Ann Arbor, MI). Gels were submerged in Tris–glycine running buffer with 0.1% SDS. The gels were placed in a 4 °C cold room and electrophoresed at 200 V for 50 min.

The proteins were electrophoretically transferred from the gel to the solid phase on nitrocellulose membranes. Each gel was sandwiched with the nitrocellulose membrane between four pieces of filter paper and submerged in transfer buffer (Tris–glycine and 20% methanol). The transfer was carried out in a 4 °C cold room at 80 V for 75 min.

Membranes were stored dry before the start of the immunoassay. Each membrane was incubated with gentle shaking in blocking buffer (phosphate-buffered saline (PBS) with 5% non-fat dried milk and 0.1% Tween-20) for 1 h. After three 5-min washes in PBS, membranes were incubated for 1 h with gentle shaking in primary antibody mixed 1 : 5000 in PBS with 5% BSA (antibody MA3-008, clone 2A4, mouse monoclonal, Affinity BioReagents). The membranes were washed six more times for 5 min each before incubation in the secondary antibody (SAB-100, 1 : 5000 dilution in PBS with 5% BSA, StressGen–Assay Designs). The incubation in secondary antibody (1.5 h), was followed by three, 5-min washes in PBS with 0.1% Tween-20. Proteins were visualized by exposing the membranes to enhanced chemiluminescence reagents (GE Healthcare Bio-Sciences Corp) and exposing to x-ray film. Multiple exposure times between 30 s and 20 min were used for each membrane to ensure proper exposure.

The exposed x-ray film was digitally scanned on a transparency scanner. The image files were transferred to the image analysis program Image-J (Rasband 1997–2009) for densitometry. The density of each sample band at 70 kDa was measured relative to the density of the 30 ng human Hsp70 standard on each gel. The presence of the human standard on all gels allowed for comparison of relative density values across multiple western blots. Data were log transformed to better conform to assumptions of normality and tested for homogeneity of variances using Cochran's test. The results for

the limpets collected at high tide in the field were not included in the statistical analysis due to the difference in protocol, since they were not acclimated in the laboratory or handled during experiments.

LIMPET UPPER LIMITS

We surveyed the upper limits of *L. gigantea* at HMS during October 2008. Between five and seven sites facing northwest, northeast, southwest or southeast, were surveyed. Much of the intertidal substratum at HMS faces in one of these four directions. Each site was comprised of a smooth rock face sloped between 30° and 70° above horizontal. The height of the highest *L. gigantea* on each face was measured using a theodolite and stadia rod with reference to a US National Geodetic Survey marker located at HMS. These heights were then converted to height above Mean Lower Low Water (MLLW, National Tidal Datum Epoch 1983–2001, Monterey, CA).

HEAT-BUDGET MODELLING

The heat-budget model of Denny and Harley (2006) was used to produce hindcasts of *L. gigantea* body temperatures on a number of hypothetical shore positions at HMS. The model, written and run using Matlab software, used the same parameters as the previously published model. An expanded 7-year set of historical weather data collected at HMS, from 1 August 1999 through 31 July 2006, were used to produce a time series of body temperatures at 10-min intervals for the 7-year period. The modelled limpet was 42 mm long, and was positioned in one of several orientations: on a horizontal surface, or on sloped surfaces elevated above horizontal in 10° increments, facing different directions (north, east, south etc.) around the compass in 22.5° steps. The limpets were modelled at elevations of 0.5–2.0 m above MLLW in 0.5 m increments on a simulated wave-protected shore (i.e. no wave splash). These conditions were meant to produce a 'worst-case scenario' for *L. gigantea* on the shore at HMS.

The predictions from the heat-budget model were used to calculate the frequency and severity of high temperature stress events over a 7-year period at HMS. Additionally, the conditions on days leading up to stress events were examined to address the potential for short-term physiological acclimation to high temperatures. We identified 130 days in the 7-year temperature record where the body temperature of a limpet on a southwest-facing sloped rock at 2.0 m above MLLW was predicted to exceed 28 °C. For each day predicted to raise body temperatures above this threshold, the temperature profiles from the previous 2 weeks were extracted from the data. The maximum temperature on each of the 14 days prior to each high temperature event was sampled, and the mean difference between the daily maximum body temperature and the 28 °C threshold was calculated for each day.

Results

LETHAL TEMPERATURE TRIALS

When *L. gigantea* were subjected to a 7-h aerial exposure regime at 100% relative humidity, the median lethal temperature (LT50) was 37.4 °C (Fig. 3). Limpets died at maximum temperatures as low as 34 °C, and all limpets died when the maximum temperature was 42 °C.

Survival rates in the recirculating wind tunnel at a more realistic 50–60% relative humidity were lower than the

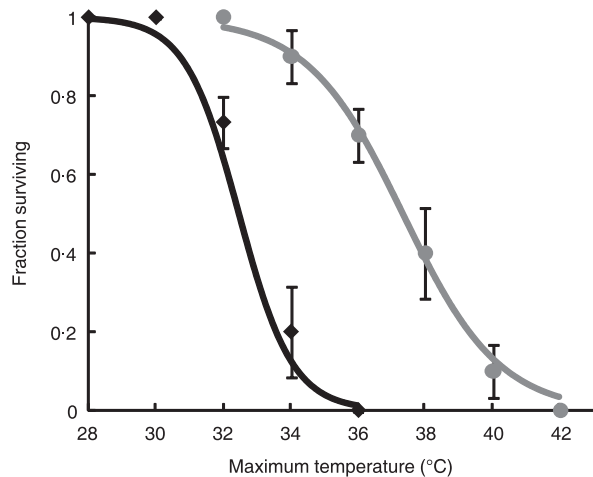


Fig. 3. Survival of *Lottia gigantea* after exposure to thermal stress and 24 h recovery. Circles (●) represent limpets exposed to thermal stress in 100% relative humidity, diamonds (◆) represent limpets exposed to thermal stress at 50–60% relative humidity in the recirculating wind tunnel. The thermal stress profiles used in these trials are explained in the text. Five limpets were used in each trial, and values are mean fraction surviving \pm 1 SEM for three replicates. The curves are drawn according to eqn 1.

high-humidity treatments (Fig. 3). The LT50 for limpets in the low-humidity trials was 32.5 °C, with mortality beginning to occur at 30 °C.

SUBLETHAL TEMPERATURE TRIALS

The primary antibody used in these experiments resolved one band of 70 kDa that showed low levels of constitutive expression and was strongly induced by temperature stress (Fig. 4a). There was a significant effect of temperature treatment on Hsp70 expression (ANOVA, $F_{4,20} = 4.66$, $P = 0.008$) for limpets stressed in the recirculating wind tunnel. The mean density of the Hsp70 band increased in treatments with increasing temperature up to 32 °C, beyond which expression declined (Fig. 4b). Field control limpets exhibited the lowest levels of inducible Hsp70 expression.

LIMPET UPPER LIMITS

The upper limit of *L. gigantea* differed significantly among the four shore directions surveyed (Kruskal–Wallis test, $\chi^2_{0.05,3} = 17.94$, $P < 0.001$). Sites facing northwest and northeast had significantly higher upper limits than southwest facing sites (*post hoc* comparison, Zar 1999), with southeast sites falling in between the two groups (Fig. 5).

HEAT-BUDGET MODEL

Using the results from the sub-lethal and lethal stress experiments, two threshold conditions were chosen to define ‘stressful’ conditions in the heat-budget model. The threshold for the onset of Hsp70 expression was defined as any day where the

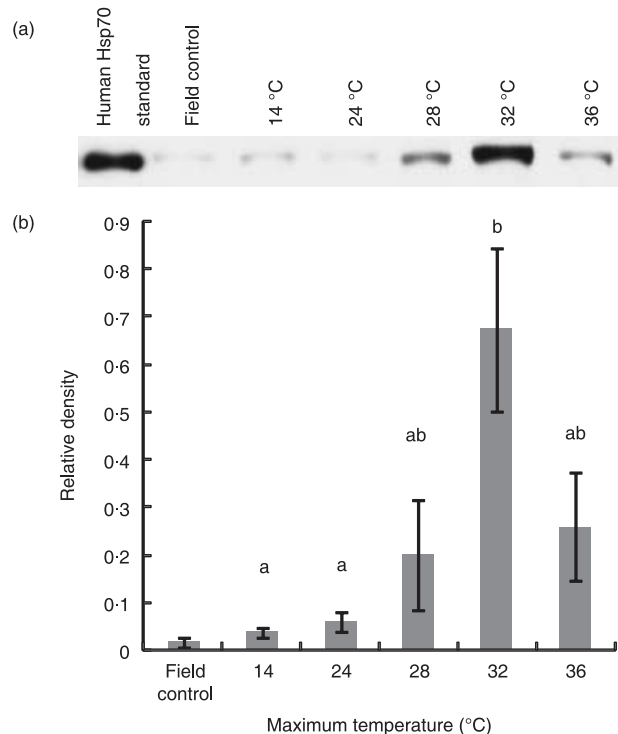


Fig. 4. (a) Representative expression of Hsp70 from limpets emersed in the recirculating wind tunnel. Temperature labels represent the maximum temperature reached in the trial. The standard was 30 ng of human Hsp70. (b) Induced expression of Hsp70 in *L. gigantea* relative to a standard of human Hsp70 after aerial exposure to high temperatures and desiccation for 7 h. Values are average density for five individuals \pm 1 SEM. Letters above each temperature treatment denote groups that are not significantly different. The field control samples were not included in the statistical analysis due to differences in treatment protocol.

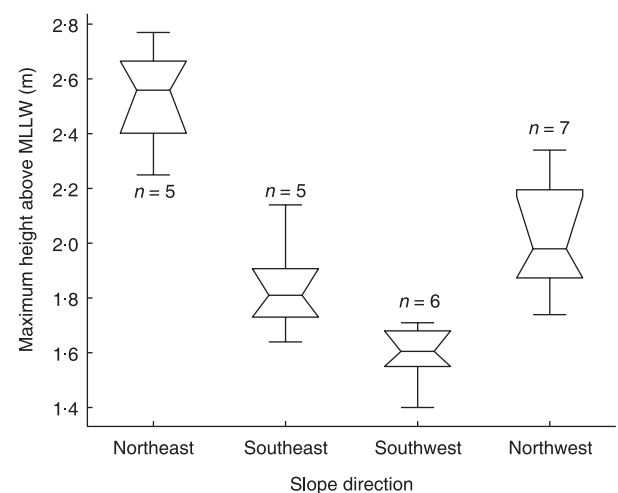


Fig. 5. Surveyed heights of highest *L. gigantea* living on sloped walls facing one of four compass directions at HMS.

limpet body temperature in the model was predicted to exceed 28 °C for more than 3.5 h. This corresponded to the minimal exposure regime in the recirculating wind tunnel that produced a significant increase in Hsp70 relative to control conditions.

The mortality threshold used in the heat-budget model was defined as any day where limpet body temperature was predicted to exceed 32 °C for more than 2.5 h, based on the lowest temperature conditions conservatively expected to produce mortality in the recirculating wind tunnel trials. The cumulative number of stress events varied with the altitude, azimuth, and shore height of the modelled substratum (Fig. 6), with southwest-facing sites angled between 40° and

50° above horizontal producing the largest number of high temperature days, and higher shore heights producing higher peak temperatures. The greatest number of mortality events, six events in 6 years, occurred at a south–southwest facing slope elevated 50° above horizontal at 2.0 m above MLLW. Results are shown for 1.0, 1.5 and 2.0 m above MLLW (Fig. 6), whereas the number of sub-lethal stress and mortality events at 0.5 m above MLLW approached zero.

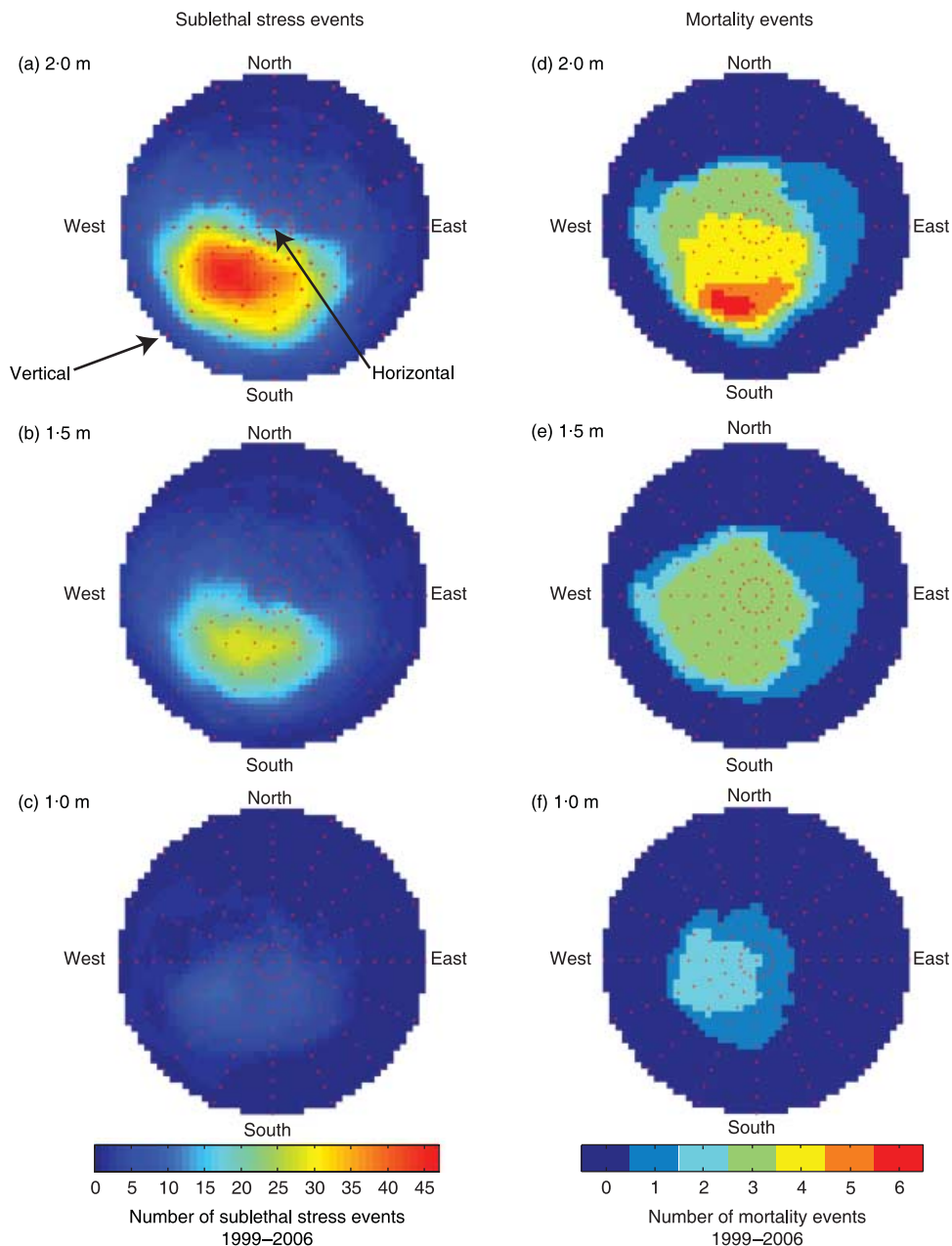


Fig. 6. Predicted cumulative occurrence of stress and mortality events for *L. gigantea* modelled on the shore at HMS during the time period 1999–2006. Cumulative days where limpet body temperature exceeded 28 °C for 3.5 h are shown for shore heights (a) 2.0 m, (b) 1.5 m and (c) 1.0 m above Mean Lower Low Water. Cumulative days where limpet body temperature exceeded 32 °C for 2.5 h are shown for shore heights at (d) 2.0 m, (e) 1.5 m, and (f) 1.0 m. Compass direction of modelled rock is indicated around the perimeter. Radial axes indicate slope of substratum above horizontal, with vertical walls at the perimeter. Modelled orientations are indicated by circles (●), data for other orientations were produced by cubic interpolation. Rocks oriented between west and southeast, and angled between 0° and 70° above horizontal were the most stressful microhabitats, and higher shore heights produced more stress and mortality events.

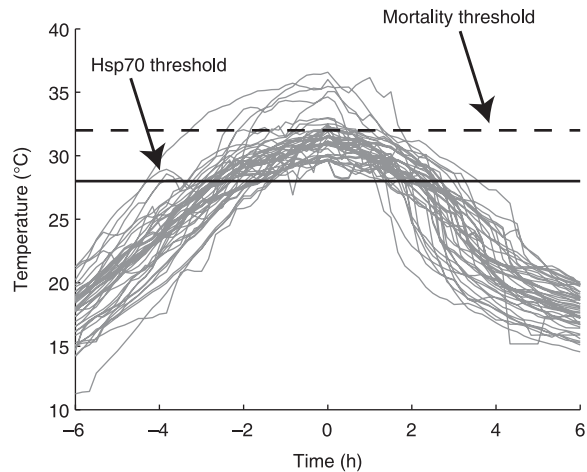


Fig. 7. Predicted body temperatures for a *L. gigantea* modelled at a site 2 m above MLLW on a southwest-facing slope, on a wave-protected shore at HMS for the time period 1999–2006. Each grey line represents predicted body temperatures from a day in the data set where the body temperature exceeded 28 °C for more than 3.5 h ($n = 44$). Times are centred around the peak temperature of each day. The solid line represents the threshold above which Hsp70 expression should be induced. The dashed line represents the temperatures that would result in more than 10% mortality in the limpet population at this modelled site.

A representative set of temperature traces for one of the most stressful modelled orientations, a southwest-facing slope oriented 40° above horizontal at 2.0 m above MLLW, are shown in Fig. 7. The threshold for onset of Hsp70 production was exceeded 46 times at this model site, with many of these exposures lasting more than the 3.5 h time threshold of our wind-tunnel experiments. These prolonged exposures should further increase the thermal and desiccation stress on the limpet above that measured here, resulting in more thermally-induced protein damage and subsequent Hsp70 production. Over the 7 years of weather data used in the model, only 6 days were predicted to exceed the mortality threshold of *L. gigantea* on the southwest-facing slope, but those included 2 days with exposures sufficient to kill 70–90% of the limpets at the modelled sites (i.e. body temperatures in excess of 34 °C for more than 2 h).

An analysis of the conditions during the 2 weeks preceding a day where body temperature was predicted to exceed 28 °C for a limpet on a southwest slope at 2.0 m above MLLW is shown in Fig. 8a. The mean difference between the maximum temperature on each day leading up to the stress event and a 28 °C threshold ($\pm 95\%$ confidence limits) was calculated. Average daily maximum temperatures were on average 2.6 °C cooler than the 28 °C threshold during the 2 weeks before a high temperature event, but this difference decreased as the target date drew closer. One to two days preceding a day where limpet body temperature exceeded 28 °C often consisted of maximum body temperatures that equalled or exceeded the temperature on the target day, indicating that many stress events could be multi-day occurrences rather than isolated

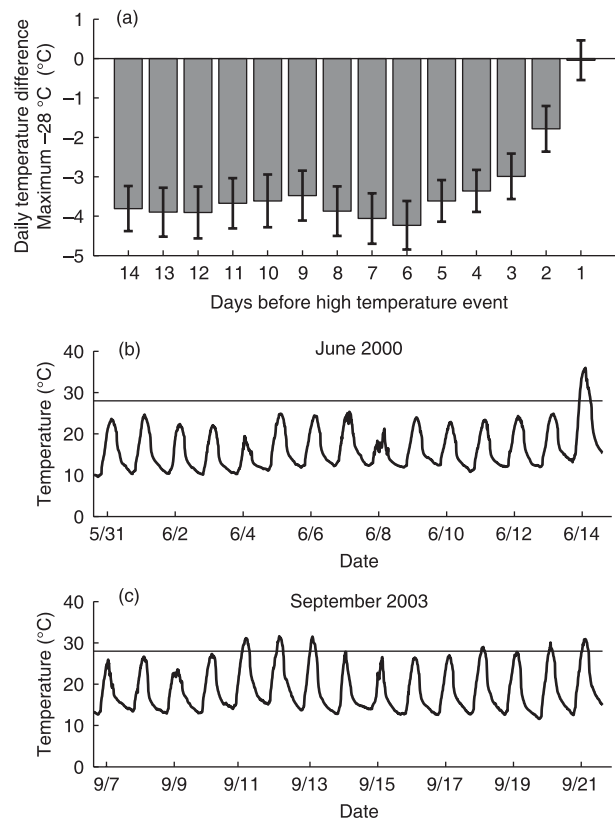


Fig. 8. Predicted limpet body temperatures on days prior to high temperature events. (a) Average difference ($\pm 95\%$ confidence limits) between the maximum temperature on a given day prior to a high temperature event and the 28 °C threshold temperature ($n = 130$). The limpet was modelled on a southwest-facing sloped surface 2.0 m above MLLW. (b) Representative body temperatures for the modelled limpet on the 14 days before a high temperature event on 14 June 2000, when a maximum temperature of 36 °C was reached. Temperatures were mild on the days leading up to the high temperature event. (c) A high temperature event at the same modelled site on 21 September 2003 was preceded by several days where temperatures exceeded the 28 °C Hsp70 induction temperature threshold.

events. We must include the caveat that the mean differences and confidence limits were calculated as if each day leading up to a high temperature event was an independent event, but there may be some autocorrelation among conditions on adjacent days. Two representative periods leading up to hot days are shown in Fig. 8b–c. Conditions preceding a 28 °C day may be consistently mild (Fig. 8b) or may consist of repeated days where body temperature reaches or exceeds the 28 °C threshold (Fig. 8c).

Discussion

THERMAL STRESS AND LIMPET DISTRIBUTIONS

The rocky intertidal zone is commonly acknowledged as one of the most stressful habitats on earth. The steep gradients in conditions along both horizontal and vertical axes can create

greatly different conditions within microhabitats separated by only 10's of centimeters. For mobile organisms, or during the initial settlement period for sessile organisms, the choice of microhabitat can mitigate or intensify the severity of abiotic stresses, influencing survival and fitness (Williams & Morrill 1995; Davison & Pearson 1996; Bertness *et al.* 1999; Gray & Hodgson 2004).

For *L. gigantea*, upper distributional limits on vertical walls or sloped northeast-facing microhabitats at HMS should not be limited by thermal stress anywhere in the 0.5–2.0-m vertical zone modelled here. The *L. gigantea* used in the wind tunnel experiments survived temperature and desiccation stress levels well-beyond the most stressful conditions predicted by the heat-budget model for these microhabitats during the study period. Northwest-facing slopes were also generally benign, with steeply sloped habitats producing 0–10 sub-lethal-stress events and no mortality events at upper shore levels during the 7-year time series.

In contrast, high-shore conditions at HMS at horizontal or southwest- and southeast-facing sloped microhabitats could be severe enough to limit the vertical distribution of *L. gigantea*. These sites receive more solar irradiance, particularly during the middle of the day when the sun is high overhead and air temperatures are highest (Campbell & Norman 1998). The resulting predicted limpet body temperatures exceeded the mortality threshold determined in the laboratory between two and six times during the 7 years of model data, depending on site orientation and height. In earlier work (Denny *et al.* 2006), we concluded that acute thermal mortality was not sufficient to set an upper limit to the vertical range of *L. gigantea*. The longer stress exposure used here, 7 vs. 3.5 h, lowered the mortality temperature threshold sufficiently to predict occasional acute temperature-induced mortality at higher shore levels on these sun-exposed surfaces. From our field surveys, the general pattern of height of the upper limit of *L. gigantea* progresses NE > NW > SE > SW, which corresponds to the predicted incidence of stressful conditions at these microhabitat types produced by the heat-budget model.

Occasional acute temperature and desiccation stresses have been reported to cause mortality and potentially alter the upper limits of intertidal species (Sutherland 1970; Foster 1971; Tsuchiya 1983; Wethey 1983; Bertness *et al.* 1999; Harley 2008). The study of these events is often complicated by their rarity, driven by the need for a confluence of ocean and weather conditions sufficient to raise temperatures to extreme levels (Denny *et al.* 2009). Calm seas, a mid-day low tide, and warm, sunny conditions must occur simultaneously. At HMS, these conditions occur only a few times per year, and the frequency of potentially lethal high temperatures is therefore very low within a given year. However, *L. gigantea* is a long-lived species, with individuals living for more than 20 years (Fenberg 2008), which increases the chances of experiencing these rare stress events. Our observations of *L. gigantea* distributions at HMS agree with the predictions of the heat-budget model and physiology experiments. *Lottia gigantea* that live on open rock faces tend to cover a larger vertical range on northwest- and northeast-facing slopes compared to southwest

and southeast-facing slopes. However, these patterns in the upper limit of *L. gigantea* cannot be solely attributed to the influence of acute thermal stress events. The intertidal zone at HMS is primarily oriented northward and eastward, with ocean swells approaching from the north, so that the total area available for habitation should be greater on rocks of these aspects. The high relief of the granite topography also provides many microhabitats that could serve as refuges from thermal stress by shading organisms and concentrating wave splash (O'Donnell & Denny 2008), allowing *L. gigantea* to extend their territories further up the shore.

The upper limits of limpet distributions can also be potentially set by other abiotic and biotic factors. Both competitive and facilitative interactions with neighbouring species can influence the suitability of a patch of rock. Other grazers may reduce the standing crop of algae (Castenholz 1961), whereas sessile organisms such as macroalgae, barnacles and mussels may occupy potential limpet territories (Stimson 1970, 1973). Alternatively, neighbouring algae and mussels could provide beneficial shade and retain moisture, ameliorating temperature and desiccation stresses at small scales (Bertness & Leonard 1997; Bertness *et al.* 1999). At higher shore heights, the time of emersion and higher solar irradiation may limit the growth of the microalgal food supply of *L. gigantea*, making the habitat unsuitable for occupation (Castenholz 1961).

Beyond rare mortality events, the frequency and severity of sub-lethal stress events could limit the distribution of *L. gigantea* as well. The expression of Hsp70, a common marker for general physiological stress (Feder & Hofmann 1999), was induced between 24 and 28 °C in *L. gigantea*. It should be noted that the recovery time used in these experiments (1 h) was relatively short, and longer recovery times may have revealed enhanced Hsp expression at lower temperatures (Hofmann & Somero 1996; Tomanek & Somero 2000). Based on these data, sub-lethal stress events could occur multiple times per year in many of the microhabitats at HMS. Expression of Hsp's carries great metabolic costs as well as indicating that the organism has sustained some measure of cellular damage (Hofmann & Somero 1996; Feder & Hofmann 1999; Tomanek 2002). We are only beginning to understand the long-term effects of repeated sub-lethal stress events on the survival, growth, and reproduction of intertidal organisms (Helmuth & Hofmann 2001; Somero 2002). The range of onset temperatures for Hsp70 production in *L. gigantea* resembles that of other mid-intertidal species from the west coast of North America including other limpets, snails, and mussels (Roberts *et al.* 1997; Tomanek & Sanford 2003; Dong *et al.* 2008). The environmental conditions on horizontal platforms or on southwest- and southeast-facing sloped rocks could be severe enough that repeated sub-lethal temperature and desiccation stresses inhibit the fitness of *L. gigantea* living there, but this remains unstudied.

Care must be taken in any generalization of our predictions from the heat-budget model. The frequency, intensity and duration of thermal and desiccation stress events in the intertidal zone are intimately associated with the particular microhabitat of an organism (Grainger 1969; Denny *et al.* 2006). Organisms that seek refuge in crevices, under an algal

canopy, or in the interstices of biogenic structures such as mussel beds will experience fewer stressful days due to shading and retention of moisture (Kensler 1967; Raffaelli & Hughes 1978; Halpin *et al.* 2002; Gray & Hodgson 2004; Bazterrica *et al.* 2007). Organisms that live out on open surfaces should be in much greater danger of reaching extreme temperatures (Breen 1972; Roberts *et al.* 1997; Gilman 2006; Berger & Emllet 2007; Jost & Helmuth 2007). *Lottia gigantea* represents a good model organism for this type of modelling, as the territorial nature of this species often restricts it to a defined area on the substratum that has little or no canopy cover. For limpets thus constrained, the model does a good job of predicting body temperature, especially on warm days (Denny & Harley 2006).

APPLYING LABORATORY DATA TO THE FIELD

Accurately predicting how intertidal organisms will cope with prolonged aerial exposure during low tide requires experimental methods that reasonably recreate conditions in the field (Newell 1976; Branch 1981; Helmuth *et al.* 2006; Jost & Helmuth 2007). Simplified laboratory stress protocols have traditionally been used for comparative physiological studies, creating difficulties in the transition from idealized conditions in the laboratory to the extremely variable conditions of the real world. These difficulties raise a number of concerns that must be addressed before we can produce meaningful measures of thermal and desiccation tolerance for intertidal species. Much of the existing literature on thermal stress in intertidal organisms relies on lethal or sub-lethal experimental data derived from high humidity trials in closed chambers or tests in which organisms were submerged in seawater (Broekhuysen 1940; Orr 1955; Fraenkel 1968; Stirling 1982; McMahon & Britton 1985; Cleland & McMahon 1986; McMahon 1991; Sanders *et al.* 1991; Tomanek & Somero 1999; Clarke, Mill & Grahame 2000; Tomanek & Somero 2000; McMahon 2001; Sorte & Hofmann 2005; Davenport & Davenport 2005; Berger & Emllet 2007). The prevalence of these protocols is understandable from a logistical standpoint, as the high-humidity or submerged trials can be carried out with simple water baths. Because much of the work on thermal tolerance in intertidal organisms has been focused on comparisons across species, these standardized methods allow researchers to remove much of the variability of the natural system. However, these methods have the potential to be misleading when the goal is to predict performance in the field. In the current study, *L. gigantea* were able to survive much higher temperatures when kept in saturated air (Fig. 3), a condition that is extremely unlikely to occur in the field at HMS. The median lethal temperatures for the 100% and 50% relative humidity trials decreased by nearly 5 °C in the lower humidity trial. Clearly the method by which lethal temperature determinations are made will have substantial effects on the application of the data to species performance in the field. The practice of exposing intertidal organisms to thermal stress in seawater could complicate comparisons with both 100% RH and low-humidity aerial exposures due a number of

factors. Submerged respiration rates and aerial respiration rates may differ markedly (Carefoot 1987). In air, the inability to dispose of metabolic waste products can create changes in the osmolality of both internal and external water stores, whereas desiccation will further concentrate solutes in the remaining water at low humidity, enhancing physiological stress (Segal & Dehnel 1962; Wolcott 1973; Newell 1976; Lowell 1984). For these reasons, caution is warranted when trying to apply results from submerged stress trials to emersion in the field, as they may overestimate the tolerance of the organism to stressful emersed conditions. Although the lethal temperatures differed greatly between the treatments used here, the onset temperature for the heat shock response of *L. gigantea* (24–28 °C) was similar to previously published values for related limpets stressed in water (23–29 °C, Sanders *et al.* 1991) and for *Chlorostoma (Tegula)* snails stressed in water (24–27 °C, Tomanek & Somero 1999) or at low tide in the field (24–27 °C, Tomanek & Sanford 2003).

The time and temperature profile of stress exposures used here represents only a small fraction of the large variety of potential field conditions that a limpet might experience. The maximum temperatures used in this study encompass the likely range of high temperatures that occur in the field at HMS. However, based on the results from the heat-budget model, there is wide latitude in the length of aerial emersion during low tide (Fig. 7). During calm ocean conditions, *L. gigantea* living in the upper portion of their range at HMS might reasonably remain emersed for 12 h or more, potentially causing even more severe physiological stress than the conditions used in this study. In addition to the time of exposure, the rate of heating may impact the extent of the heat shock response, potentially by damaging cellular processes before the onset of heat shock protein production. The rate of heating has been demonstrated to affect the thermal limits of insects (Chown *et al.* 2009), but unfortunately studies on the effects of heating rate in intertidal organisms are rare. Tomanek & Somero (2000) observed a pronounced increase in the heat shock response of low-shore *Chlorostoma brunnea* when they were heated relatively slowly in air compared to being immersed directly in hot water, but the related mid-shore snail *C. funebralis* showed no difference in Hsp expression under the same heating conditions. These results again demonstrate that comparisons between submerged and emersed stress protocols should be approached with caution, as body temperature can respond much more quickly to changes in water temperature than air temperature due to the thermal conductivities of these fluids. Measuring the temperature of a water bath used to heat a container full of air may give an inaccurate estimation of body temperature of the animal in the container, so heating rate in air should be tied directly to the organism body temperature (as in the current study). Model results from this study indicate that the rate of heating for *L. gigantea* in the field may vary approximately between 2 and 10 °C h⁻¹ on warm days (Fig. 7). Slower heating rates must necessarily be accompanied by prolonged exposure times in order to reach stressful temperatures, and the effects of this combination of conditions on *L. gigantea* are currently unknown.

ACCLIMATIZATION

Single, acute physiological stress events have been the focus of this study, but conditions in the field may occasionally lead to repeated stress events over multiple days. The physiological capacity of limpets to withstand repeated stressful days has not been explored in great detail. Studies on other intertidal species have demonstrated that physiological acclimatization can occur, altering the heat shock response and potentially shifting the lethal limits of an organism (Markel 1974; Roberts *et al.* 1997; Tomanek & Somero 1999; Buckley *et al.* 2001; Tomanek 2005; but see Berger & Emlet 2007). Physiological acclimatization may occur over just a few days in response to weather conditions, and can happen on a seasonal time-scale as well (Newell 1976). Just as the lethal temperature for *L. gigantea* varies with the conditions (time and humidity) in a single exposure, prior acclimatization to warmer seasonal temperatures or repeated acute stress events will likely influence the survival of the limpet during a particular stressful low tide.

Further study is required to determine the response of *L. gigantea* to these repeated multi-day high temperature exposures. The results, in terms of sub-lethal stress or mortality, from repeated events are difficult to predict. On the one hand, short-term physiological acclimatization might occur and allow *L. gigantea* a better chance of surviving each subsequent exposure. On the other hand, the cumulative effects of multi-day stress events could cause *L. gigantea* to succumb to lower maximum temperatures.

Conclusions

Studies of the type described here represent progress towards the integration of biomechanical and physiological techniques with the goal of testing ecological hypotheses (Huey 1991; Chown & Terblanche 2007). We can combine physiological performance data, gathered under realistic conditions, with computer simulations of organismal temperatures in the field to make predictions about where and when the distribution of an ecologically dominant species may be limited by extreme temperature events. This study and others like it provide ecologists with a robust set of tools to examine questions on the importance of thermal stress and microhabitat selection in determining the identity of species that comprise the community at a site. As with terrestrial species (Kearney & Porter 2004; Buckley & Roughgarden 2006; Pincebourde *et al.* 2007; Mitchell *et al.* 2008; Kearney *et al.* 2008), a growing number of biophysical models for important intertidal species such as the limpets, mussels, and algae (Bell 1995; Helmuth 1999; Denny & Harley 2006; Gilman *et al.* 2006), coupled with increasingly detailed studies of the physiology of these organisms (Gracey *et al.* 2008) will form the basis for mechanistic models to study species interactions and make predictions about future community development, especially in the face of an uncertain climatic future (Helmuth *et al.* 2006).

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References

- Bazterrica, M.C., Silliman, B.R., Hidalgo, F.J., Crain, C.M. & Bertness, M.D. (2007) Limpet grazing on a physically stressful Patagonian rocky shore. *Journal of Experimental Marine Biology and Ecology*, **353**, 22–34.
- Bell, E.C. (1995) Environmental and morphological influences on thallus temperature and desiccation of the intertidal alga *Mastocarpus papillatus* Kutzing. *Journal of Experimental Marine Biology and Ecology*, **191**, 29–55.
- Berger, M.S. & Emlet, R.B. (2007) Heat-shock response of the upper intertidal barnacle *Balanus glandula*: thermal stress and acclimation. *Biological Bulletin*, **212**, 232–241.
- Bertness, M.D. & Leonard, G.H. (1997) The role of positive interactions in communities: lessons from intertidal habitats. *Ecology*, **78**, 1976–1989.
- Bertness, M.D., Leonard, G.H., Levine, J.M. & Bruno, J.F. (1999) Climate-driven interactions among rocky intertidal organisms caught between a rock and a hot place. *Oecologia*, **120**, 446–450.
- Bertness, M.D., Crain, C.M., Silliman, B.R., Bazterrica, M.C., Reyna, M.V., Hidalgo, F. & Farina, J.K. (2006) The community structure of western Atlantic Patagonian rocky shores. *Ecological Monographs*, **76**, 439–460.
- Branch, G.M. (1981) The biology of limpets: physical factors, energy flow, and ecological interactions. *Oceanography and Marine Biology: an Annual Review*, **19**, 235–380.
- Breen, P.A. (1972) Seasonal migration and population regulation in the limpet *Acmaea (Collisella) digitalis*. *The Veliger*, **15**, 133–141.
- Broekhuysen, G.J. (1940) A preliminary investigation of the importance of desiccation, temperature and salinity as factors controlling the vertical distribution of certain intertidal marine gastropods in False Bay, South Africa. *Transactions of the Royal Society of South Africa*, **28**, 255–291.
- Buckley, B.A., Owen, M.-E. & Hofmann, G.E. (2001) Adjusting the thermostat: the threshold induction temperature for the heat-shock response in intertidal mussels (genus *Mytilus*) changes as a function of thermal history. *Journal of Experimental Biology*, **204**, 3571–3579.
- Buckley, L.B. & Roughgarden, J. (2006) Climate, competition, and the coexistence of island lizards. *Functional Ecology*, **20**: 315–322.
- Campbell, G.S. & Norman, J.M. (1998) *An Introduction to Environmental Biophysics*. Springer-Verlag, New York.
- Carefoot, T.H. (1987) Gastropoda. *Animal Energetics* (Vol. 2, eds T.J. Pandian and F.J. Vernberg), pp. 89–172. Academic Press Inc, New York.
- Castenholz, R.W. (1961) Effect of grazing on marine littoral diatom populations. *Ecology*, **42**, 783–794.
- Chown, S.L. & Terblanche, J.S. (2007) Physiological diversity in insects: ecological and evolutionary contexts. *Advances in Insect Physiology* (ed. S.J. Simpson), Amsterdam, Elsevier, **33**: 50–152.
- Chown, S.L., Jumbam, K.R., Sørensen, J.G. & Terblanche, J.S. (2009) Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. *Functional Ecology*, **23**: 133–140.
- Clarke, A.P., Mill, P.J. & Grahame, J. (2000) Biodiversity in *Littorina* species (Mollusca: Gastropoda): A physiological approach using heat-coma. *Marine Biology*, **137**, 559–565.
- Cleland, J.D. & McMahon, R.F. (1986) Upper thermal limit of nine intertidal gastropod species from a Hong Kong rocky shore in relation to vertical distribution and desiccation associated with evaporative cooling. *Proceedings of the Second International Marine Biological Workshop: The Marine Flora and Fauna of Hong Kong and Southern China, Hong Kong, 1986* (ed. B. Morton), pp. 1141–1152. Hong Kong University Press, Hong Kong.
- Connell, J.H. (1961) Influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. *Ecology* **42**: 710–723.
- Davenport, J. & Davenport, J.L. (2005) Effects of shore height, wave exposure and geographical distance on thermal niche width of intertidal fauna. *Marine Ecology Progress Series*, **292**, 41–50.
- Davison, I.R. & Pearson, G.A. (1996) Stress tolerance in intertidal seaweeds. *Journal of Phycology*, **32**, 197–211.
- Denny, M.W. & Blanchette, C.A. (2000) Hydrodynamics, shell shape, behavior

- and survivorship in the owl limpet *Lottia gigantea*. *Journal of Experimental Biology*, **203**, 2623–2639.
- Denny, M.W. & Harley, C.D.G. (2006) Hot limpets: predicting body temperature in a conductance-mediated thermal system. *Journal of Experimental Biology*, **209**, 2409–2419.
- Denny, M.W., Miller, L.P. & Harley, C.D.G. (2006) Thermal stress on intertidal limpets: long-term hindcasts and lethal limits. *Journal of Experimental Biology*, **209**, 2420–2431.
- Denny, M.W., Hunt, L.J.H., Miller, L.P. & Harley, C.D.G. (2009) On the prediction of extreme ecological events. *Ecological Monographs* (in press).
- Dong, Y., Miller, L.P., Sanders, J.G. & Somero, G.N. (2008) Heat-shock protein 70 (Hsp70) expression in four limpets of the genus *Lottia*: interspecific variation in constitutive and inducible synthesis correlates with *in situ* exposure to heat stress. *Biological Bulletin*, **215**: 173–181.
- Feder, M.E. & Hofmann, G.E. (1999) Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Annual Review of Physiology*, **61**, 243–282.
- Fenberg, P.B. (2008) *The effects of size-selective harvesting on the population biology and ecology of a sex-changing limpet species, Lottia gigantea*. PhD thesis, Biology, University of California, San Diego. 114.
- Finke, G.R., Navarrete, S.A. & Bozinovic, F. (2007) Tidal regimes of temperate coasts and their influences on aerial exposure for intertidal organisms. *Marine Ecology Progress Series*, **343**, 57–62.
- Fisher, W.K. (1904) The Anatomy of *Lottia gigantea* Gray. *Zoologische Jahrbucher. Abteilung fur Anatomie und Ontogenie der Tiere*, **20**, 1–66.
- Foster, B.A. (1971) On the determinants of the upper limit of intertidal distribution of barnacles (Crustacea: Cirripedia). *Journal of Animal Ecology*, **40**, 33–48.
- Fraenkel, G. (1968) The heat resistance of intertidal snails at Bimini, Bahamas; Ocean Springs, Mississippi; and Woods Hole, Massachusetts. *Physiological Zoology*, **41**, 1–13.
- Galbraith, R.T. (1965) Homing behavior in the limpets *Acmaea digitalis* and *Lottia gigantea*. *American Midland Naturalist*, **74**, 245–246.
- Gates, D.M. (1980) *Biophysical Ecology*, Springer-Verlag, New York, USA.
- Gilman, S.E. (2006) Life at the edge: an experimental study of a poleward range boundary. *Oecologia*, **148**, 270–279.
- Gilman, S.E., Wetthey, D.S. & Helmuth, B.S.T. (2006) Variation in the sensitivity of organismal body temperature to climate change over local and geographical scales. *Proceedings of National Academy of Sciences USA*, **103**: 9560–9565.
- Gracey, A.Y., Chaney, M.L., Boomhower, J.P., Tyburczy, W.R., Connor, K. & Somero, G.N. (2008) Rhythms of gene expression in a fluctuating intertidal environment. *Current Biology*, **18**: 1501–1507.
- Grainger, J.N.R. (1969) Factors affecting the body temperature of *Patella*. *Verhandlungen der Deutschen Zoologischen Gesellschaft*, **3**, 479–487.
- Gray, D.R. & Hodgson, A.N. (2004) The importance of a crevice environment to the limpet *Helcion pectunculus* (Patellidae). *Journal of Molluscan Studies*, **70**, 67–72.
- Halpin, P.M., Sorte, C.J., Hofmann, G.E. & Menge, B.A. (2002) Patterns in variation in levels of Hsp70 in natural rocky shore populations from microscales to mesoscales. *Integrative and Comparative Biology*, **42**, 815–824.
- Harley, C.D.G. (2003) Abiotic stress and herbivory interact to set range limits across a two-dimensional stress gradient. *Ecology*, **84**: 1477–1488.
- Harley, C.D.G. (2008) Tidal dynamics, topographic orientation, and temperature-mediated mass mortalities on rocky shores. *Marine Ecology Progress Series*, **371**, 37–46.
- Harley, C.D.G. & Helmuth, B.S.T. (2003) Local- and regional-scale effects of wave exposure, thermal stress, and absolute versus effective shore level on patterns of intertidal zonation. *Limnology and Oceanography*, **48**, 1498–1508.
- Helmuth, B. (1999) Thermal biology of rocky intertidal mussels: quantifying body temperatures using climatological data. *Ecology*, **80**, 15–34.
- Helmuth, B.S.T. & Denny, M.W. (2003) Predicting wave exposure in the rocky intertidal zone: Do bigger waves always lead to larger forces? *Limnology and Oceanography*, **48**, 1338–1345.
- Helmuth, B.S.T. & Hofmann, G.E. (2001) Microhabitats, thermal heterogeneity, and patterns of physiological stress in the rocky intertidal zone. *Biological Bulletin*, **201**, 374–384.
- Helmuth, B.S.T., Mieszkowska, N., Moore, P. & Hawkins, S.J. (2006) Living on the edge of two changing worlds: forecasting the responses of rocky intertidal ecosystems to climate change. *Annual Review of Ecology, Evolution, and Systematics*, **37**: 373–404.
- Hofmann, G.E. & Somero, G.N. (1995) Evidence for protein damage at environmental temperatures: Seasonal changes in levels of ubiquitin conjugates and hsp70 in the intertidal mussel *Mytilus trossulus*. *Journal of Experimental Biology*, **198**, 1509–1518.
- Hofmann, G.E. & Somero, G.N. (1996) Protein ubiquitination and stress protein synthesis in *Mytilus trossulus* occurs during recovery from tidal emersion. *Molecular Marine Biology and Biotechnology*, **5**, 175–184.
- Huey, R.B. (1991) Physiological consequences of habitat selection. *The American Naturalist*, **137**: S91–S115.
- Jost, J. & Helmuth, B. (2007) Morphological and ecological determinants of body temperatures of *Geukensia demissa*, the Atlantic ribbed mussel, and their effects on mussel mortality. *Biological Bulletin*, **213**, 141–151.
- Kearney, M. & Porter, W.P. (2004) Mapping the fundamental niche: physiology, climate, and the distribution of a nocturnal lizard. *Ecology*, **85**: 3119–3131.
- Kearney, M., Phillips, B.L., Tracy, C.R., Christian, K.A., Betts, G. & Porter, W.P. (2008) Modelling species distributions without using species distributions: the cane toad in Australia under current and future climates. *Ecography*, **31**: 423–434.
- Kensler, C.B. (1967) Desiccation resistance of intertidal crevice species as a factor in their zonation. *Journal of Animal Ecology*, **36**, 391–406.
- Lewis, J.R. (1954) Observations on a high-level population of limpets. *Journal of Animal Ecology*, **23**: 85–100.
- Lindberg, D.R., Estes, J.A. & Warheit, K.I. (1998) Human influences on trophic cascades along rocky shores. *Ecological Applications*, **8**: 880–890.
- Lowell, R.B. (1984) Desiccation of intertidal limpets: effects of shell size, fit to substratum, and shape. *Journal of Experimental Marine Biology and Ecology*, **77**: 197–207.
- Markel, R.P. (1974) Aspects of the physiology of temperature acclimation in the limpet *Acmaea limatula* Carpenter (1864): an integrated field and laboratory study. *Physiological Zoology*, **47**, 99–109.
- McMahon, R.F. (1991) Upper critical temperature limits, tissue temperatures during emersion and evaporative cooling at elevated temperatures in Western Australian intertidal gastropods: Evidence for a physiological barrier at the high tide mark. *Proceedings of the Third International Marine Biological Workshop: The Marine Flora and Fauna of Albany, Western Australia* (eds F.E. Wells, D.I. Walker, H. Kirkman & R. Lethbridge), pp. 661–673. Western Australia Museum, Perth.
- McMahon, R.F. (2001) Acute thermal tolerance in intertidal gastropods relative to latitude, superfamily, zonation and habitat with special emphasis on the Littorinoidea. *Journal of Shellfish Research*, **20**, 459–467.
- McMahon, R.F. & Britton, J.C. (1985) The relationship between vertical distribution, thermal tolerance, evaporative water loss rate, and behavior on emergence in six species of mangrove gastropods from Hong Kong. *The Malacofauna of Hong Kong and Southern China. II*, Vols. 1 and 2. *Second International Workshop: Hong Kong, 6–24 April 1983* (eds B. Morton & D. Dudgeon), pp. 563–582. Hong Kong University Press, Hong Kong.
- Mitchell, N.J., Kearney, M.R., Nelson, N.J. & Porter, W.P. (2008) Predicting the fate of a living fossil: how will global warming affect sex determination and hatching phenology in tuatara? *Proceedings of the Royal Society B*, **275**: 2185–2193.
- Newell, G.E. (1964) Physiological aspects of the ecology of intertidal molluscs. *Physiology of Mollusca* (eds K.M. Wilbur & C.M. Yonge), pp. 59–81. Academic Press, New York, USA.
- Newell, R.C. (1976) Adaptations to intertidal life. *Adaptation to Environment: Essays on the Physiology of Marine Animals* (ed. R.C. Newell), pp. 1–82. Butterworths, London, UK.
- O'Donnell, M.O. & Denny, M.W. (2008) Hydrodynamic forces and surface topography: Centimeter-scale spatial variation in wave forces. *Limnology and Oceanography*, **53**, 579–588.
- Orr, P.R. (1955) Heat Death. I. Time-temperature relationships in marine animals. *Physiological Zoology*, **28**, 290–294.
- Pincebourde, S., Sinoquet, H., Combes, D. & Casas, J. (2007) Regional climate modulates the canopy mosaic of favourable and risky microclimates for insects. *Journal of Animal Ecology*, **76**: 424–438.
- Porter, W.P. & Gates, D.M. (1969) Thermodynamic equilibria of animals with environment. *Ecological Monographs*, **39**, 228–244.
- Raffaelli, D.G. & Hughes, R.N. (1978) The effects of crevice size and availability on populations of *Littorina rudis* and *Littorina neritoides*. *Journal of Animal Ecology*, **47**, 71–83.
- Rasband, W.S. (1997–2009) *Image J. US National Institutes of Health*, Bethesda, Maryland, USA.
- Roberts, D.A., Hofmann, G.E. & Somero, G.N. (1997) Heat-shock protein expression in *Mytilus californianus*: acclimatization (seasonal and tidal-height comparisons) and acclimation effects. *Biological Bulletin*, **192**, 309–320.
- Sanders, B.M., Hope, C., Pascoe, V.M. & Martin, L.S. (1991) Characterization of the stress protein response in two species of *Collisella* limpets with different temperature tolerances. *Physiological Zoology*, **64**, 1471–1489.
- Segal, E. & P.A. Dehnel (1962) Osmotic behavior in an intertidal limpet, *Acmaea limatula*. *Biological Bulletin*, **122**: 417–430.

- Somero, G.N. (2002) Thermal physiology and vertical zonation of intertidal animals: Optima, limits, and costs of living. *Integrative and Comparative Biology*, **42**, 780–789.
- Sorte, C.J.B. & Hofmann, G.E. (2005) Thermotolerance and heat-shock protein expression in Northeastern Pacific *Nucella* species with different biogeographical range. *Marine Biology*, **146**: 985–993.
- Southward, A.J. (1958) The zonation of plants and animals on rocky sea shores. *Biological Reviews*, **33**, 137–177.
- Stephenson, T.A. & Stephenson, A. (1949) The universal features of zonation between tide-marks on rocky coasts. *Journal of Ecology*, **37**, 289–305.
- Stillman, J.H. & Somero, G.N. (2000) A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus *Petrolithes*: Influences of latitude, vertical zonation, acclimation, and phylogeny. *Physiological and Biochemical Zoology*, **73**: 200–208.
- Stimson, J. (1970) Territorial behavior of the owl limpet *Lottia gigantea*. *Ecology*, **51**, 113–118.
- Stimson, J. (1973) The role of the territory in the ecology of the intertidal limpet *Lottia gigantea*. *Ecology*, **54**, 1020–1030.
- Stirling, H.P. (1982) The upper temperature tolerance of prosobranch gastropods of rocky shores at Hong Kong and Dar ES Salaam, Tanzania. *Journal of Experimental Marine Biology and Ecology*, **63**, 133–144.
- Sutherland, J.P. (1970) Dynamics of high and low populations of the limpet, *Acmaea scabra* (Gould). *Ecological Monographs*, **40**, 169–188.
- Tomanek, L. (2002) The heat-shock response: Its variation, regulation, and ecological importance in intertidal gastropods (genus *Tegula*). *Integrative and Comparative Biology*, **42**, 797–807.
- Tomanek, L. (2005) Two-dimensional gel analysis of the heat-shock response in marine snails (genus *Tegula*): interspecific variation in protein expression and acclimation ability. *Journal of Experimental Biology*, **208**, 3133–3143.
- Tomanek, L. & Sanford, E. (2003) Heat-shock protein 70 (Hsp 70) as a biochemical stress indicator: an experimental field test in two congeneric intertidal gastropods (Genus: *Tegula*). *Biological Bulletin*, **205**, 276–284.
- Tomanek, L. & Somero, G.N. (1999) Evolutionary and acclimation-induced variation in the heat-shock responses of congeneric marine snails (Genus *Tegula*) from different thermal habitats: implications for limits of thermotolerance and biogeography. *Journal of Experimental Biology*, **202**, 2925–2936.
- Tomanek, L. & Somero, G.N. (2000) Time course and magnitude of synthesis of heat-shock proteins in congeneric marine snails (Genus *Tegula*) from different tidal heights. *Physiological and Biochemical Zoology*, **73**: 249–256.
- Tsuchiya, M. (1983) Mass mortality in a population of the mussel *Mytilus edulis* L. caused by high temperature on rocky shores. *Journal of Experimental Marine Biology and Ecology*, **66**, 101–111.
- Wethey, D.S. (1983) Geographic limits and local zonation: the barnacles *Semibalanus (Balanus)* and *Chthamalus* in New England. *Biological Bulletin*, **165**, 330–341.
- Williams, G.A. & Morritt, D. (1995) Habitat partitioning and thermal tolerance in a tropical limpet, *Cellana grata*. *Marine Ecology Progress Series*, **124**, 89–103.
- Wolcott, T.G. (1973) Physiological ecology and intertidal zonation in limpets (*Acmaea*)—critical look at limiting factors. *Biological Bulletin*, **145**, 389–422.
- Zar, J.H. (1999) *Biostatistical Analysis*, 4th edn. Prentice-Hall, New Jersey. 929 pgs.

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