

August 2009

## The Role of Temperature and Desiccation Stress in Limiting the Local-scale Distribution of the Owl Limpet, *Lottia gigantea*

Luke P. Miller  
*Stanford University*, [luke.miller@sjsu.edu](mailto:luke.miller@sjsu.edu)

Christopher D. G. Harley  
*Stanford University*

Mark W. Denny  
*Stanford University*

Follow this and additional works at: [https://scholarworks.sjsu.edu/biol\\_pub](https://scholarworks.sjsu.edu/biol_pub)



Part of the [Biology Commons](#)

---

### Recommended Citation

Luke P. Miller, Christopher D. G. Harley, and Mark W. Denny. "The Role of Temperature and Desiccation Stress in Limiting the Local-scale Distribution of the Owl Limpet, *Lottia gigantea*" *Functional Ecology* (2009): 756-767.

This Article is brought to you for free and open access by the Biological Sciences at SJSU ScholarWorks. It has been accepted for inclusion in Faculty Publications, Biological Sciences by an authorized administrator of SJSU ScholarWorks. For more information, please contact [scholarworks@sjsu.edu](mailto:scholarworks@sjsu.edu).

1

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

4

5 Luke P. Miller<sup>1\*</sup>, Christopher D. G. Harley<sup>1,2</sup>, Mark W. Denny<sup>1</sup>

6

7 <sup>1</sup>Hopkins Marine Station, Stanford University, Pacific Grove, CA, 93950, USA

8 <sup>2</sup>Department of Zoology, University of British Columbia, Vancouver, BC, V6T1Z4, Canada

9 \*corresponding author's contact information:

10 Marine Science Center, Northeastern University, 430 Nahant Rd, Nahant, MA, 01908, USA

11 Phone: 1-781-581-7370 x329

12 Fax: 1-781-581-6076

13 Email: [contact@lukemiller.org](mailto:contact@lukemiller.org)

14

15 Running head: Stress and distributions of *Lottia gigantea*

16 Summary

- 17 1. Small scale distributions of rocky intertidal organisms may be determined in part by  
18 temperature and desiccation stress during low tide and the ability of organisms to resist  
19 such stresses. Biophysical modeling techniques, coupled with data on the physiological  
20 tolerance of organisms can be used to predict the frequency and severity of abiotic stress  
21 events.
- 22 2. The limpet *Lottia gigantea* is a major competitor for primary space on rocky shores  
23 along the west coast of North America. The goal of this study was to quantify the thermal  
24 and desiccation tolerance of this species and to predict the frequency of stressful events at  
25 Hopkins Marine Station (HMS) in Pacific Grove, California, USA.
- 26 3. *L. gigantea* were exposed to thermal stress regimes in the laboratory using protocols  
27 designed to mimic prolonged exposures in the field. The production of heat shock protein  
28 70 (Hsp70) was measured as an indicator of sublethal stress, and mortality rates were  
29 measured.
- 30 4. Peak expression of Hsp70 occurred during exposures at 32°C, while the lethal limits of  
31 the limpets ranged between 30°C and 42°C, depending on the exposure regime. Lethal  
32 temperatures were approximately 5°C lower in 50-60% relative humidity conditions  
33 compared to 100% relative humidity trials.
- 34 5. Results from the physiology experiments were compared to field surveys of the upper  
35 limits of *L. gigantea* at HMS and with heat-budget model predictions of limpet body  
36 temperatures on a variety of substratum orientations.
- 37 6. The highest frequency of sublethal and lethal stress events was predicted to occur on  
38 high-shore westerly and southerly slopes and horizontal surfaces, microhabitats where *L.*

39 *gigantea* is comparatively rare at HMS. Predicted stress and mortality events were more  
40 rare at low-shore sites, vertical surfaces, and sloping rock faces oriented towards the  
41 northeast and northwest.

42 7. The combination of direct measurements of sublethal and lethal temperature thresholds  
43 using realistic stress exposures and predictions from a heat-budget model give insight  
44 into the role of thermal disturbance in affecting the local-scale distributions of intertidal  
45 organisms. These methods hold great promise for enhancing our understanding of the  
46 role of abiotic factors in limiting species distributions and making predictions about  
47 responses to future climatic conditions.

48

49 Keywords: heat budget model, rocky intertidal zone, heat shock protein 70, body temperature,  
50 shore height, solar irradiance, lethal temperature, wave exposure

## 51 INTRODUCTION

52           In both the terrestrial and marine realms, species distributions can be influenced by  
53 abiotic factors, including extremes in environmental conditions such as temperature,  
54 precipitation, desiccation, wind and wave forces. On mountain tops, small excursions along the  
55 vertical and horizontal axes can produce marked changes in wind and sun exposure, snow  
56 accumulation, precipitation and temperature, changes that are often reflected in the biological  
57 community (Poore & McVean 1957, Johnson & Billings 1962, Ferreyra et al. 1998). In the  
58 intertidal zone, abiotic conditions may shift even more dramatically over scales of just a few  
59 meters. For organisms that live at this interface of the marine and terrestrial realms, survival  
60 requires withstanding a broad array of abiotic and biotic stresses.

61           The distribution of a species on the shore is set by numerous factors, such as interactions  
62 with other species, that occur within a constantly shifting set of environmental parameters as the  
63 tide rises and falls (Stephenson & Stephenson 1949, Southward 1958, Connell 1961, Newell  
64 1964). The potential for high temperatures and desiccation to limit the range of an organism can  
65 be intimately tied to the small-scale variation in conditions that exists between fully submerged  
66 and fully terrestrial habitats, along with the physiological tolerance of a species to conditions in  
67 each microhabitat (Helmuth & Hofmann 2001, Somero 2002, Davenport & Davenport 2005,  
68 Bertness et al. 2006). The role of thermal and desiccation stress in setting the distributional limits  
69 of organisms, over both large and small scales, has been an integral part of studies of this system  
70 (Broekhuysen 1940, Lewis 1954, Foster 1971, Wolcott 1973, Branch 1981, Williams & Morritt  
71 1995, Stillman & Somero 2000, Harley 2003, Harley & Helmuth 2003, Harley 2008).

72           On a rocky shore, there are three principal factors that determine the suitability of a  
73 habitat for occupation with regard to temperature and desiccation stress. First, height on the

74 shore determines the total amount of time the organism is exposed to the air, a time during which  
75 temperature may fluctuate substantially (Helmuth 1999, Denny, Miller & Harley 2006, Finke,  
76 Navarrete & Bozinovic 2007). Second, the orientation of the substratum and neighboring  
77 structures (including other species) affects the amount of solar radiation received by an  
78 organism, and thereby its heat influx (Gates 1980, Campbell & Norman 1998). Finally, the  
79 orientation of the microhabitat relative to incoming waves, coupled with local topographic  
80 features which can increase or decrease wave swash, influences the degree of temperature  
81 buffering provided by seawater (Harley & Helmuth 2003, Helmuth & Denny 2003, O'Donnell &  
82 Denny 2008).

83         These environmental characteristics of a microhabitat can be used to predict the potential  
84 severity of temperature and desiccation stress of that site. A biophysical model of body  
85 temperature, generally referred to as a heat-budget model, allows researchers to make hindcasts  
86 or forecasts of an organism's body temperature, using a variety of environmental data and  
87 selected physical characteristics of the organism (Porter & Gates 1969, Bell 1995, Helmuth  
88 1999, Denny & Harley 2006). With a heat-budget model in hand, one can begin to speak to the  
89 suitability of a particular microhabitat, with regard to temperature and desiccation stress, for the  
90 organism (Denny et al. 2006). To make quantitative predictions about the effect of stress on the  
91 distributions of the organism, however, this process requires knowledge of the physiological  
92 tolerance of the organism (Newell 1976, Branch 1981, Davenport & Davenport 2005, Helmuth et  
93 al. 2006).

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

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

## 114 MATERIALS AND METHODS

### 115 *Collections*

116 All *Lottia gigantea* were collected from north and northeast facing vertical or near-  
117 vertical walls in the intertidal zone at Hopkins Marine Station (HMS), Pacific Grove, California  
118 (36° 37.3' N, 121° 54.25' W). Collections were made between December 2006 and March 2007.

119 Prior to all experiments, limpets were held in an outdoor seawater table for a minimum of five  
120 days, where they were splashed with seawater and were able to graze freely. The water  
121 temperature in the holding table was the same as the ambient seawater temperature off HMS,  
122 typically between 11 and 14°C. The outdoor aquaria were shaded from the sun, and air  
123 temperatures ranged between approximately 8 and 20°C.

#### 124 *Laboratory heat stress experiments*

125 To produce heat stress profiles similar to those experienced in the field by limpets, a  
126 temperature-controlled recirculating wind tunnel allowed the body temperature of limpets to be  
127 raised gradually by manipulating the temperature of the substratum and air. Using output from  
128 the heat-budget model developed by Denny and Harley (2006) for *L. gigantea*, a heating rate of  
129 8°C hr<sup>-1</sup> was chosen to represent typical conditions during an extreme high temperature event in  
130 the field. The relative humidity in the chamber was maintained between 50 and 60%, based on  
131 relative humidity measurements taken next to *L. gigantea* in the field during prolonged heat  
132 stress events (L. Miller, pers. obs.). In the field, limpet body temperature is largely dependent on  
133 substratum temperature due to the large contact area of the foot (Denny & Harley 2006). In the  
134 wind tunnel, limpets sat on a heated aluminum plate that was used to control limpet body  
135 temperature while air was circulated through the tunnel at 0.5 m s<sup>-1</sup>. Each heat stress trial ran for  
136 a total of 7 h, with the temperature starting and ending at 14°C, and changing at the fixed 8°C h<sup>-1</sup>  
137 rate. When limpets reached the designated maximum body temperature in the middle of a trial,  
138 that temperature was held steady until it was time to begin reducing the temperature to 14°C  
139 (again at 8 °C h<sup>-1</sup>) all within the 7 h time limit (Fig. 1). Air temperature in the wind tunnel was  
140 raised and lowered at 8 °C h<sup>-1</sup> from the same 14°C starting point. Air temperature in the field at



141 HMS rarely exceeds 30°C, even on the warmest days, so all wind tunnel experiments used a  
142 maximum air temperature 30°C.

143 The recirculating wind tunnel was used to quantify survival of *L. gigantea* at high  
144 temperatures. Each lethal temperature trial used 5 *L. gigantea*, and each temperature trial was  
145 replicated three times. Maximum body temperatures in these trials were 30, 32, 34, 36 or 38°C.  
146 Following a heat stress exposure, experimental limpets were returned to running seawater for 24  
147 h. If the limpet could not maintain a grip on the substratum after 24 h, it was scored as  
148 “ecologically dead” (Wolcott 1973).

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

161 The number of surviving limpets in each trial was expressed as a fraction  $S$  of the total  
162 number of limpets in the trial.  $S$  was averaged for the three replicates at each temperature. The

163 lethal temperature,  $T$ , at which 50% of the limpets in a trial died (LT50) was estimated by a  
164 fitting a sigmoidal curve to the survival data:

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

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

### 168 *Sublethal stress*

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

182 Hsp70 assays were carried out using a protocol similar to that of Hofmann and Somero  
183 (1995). Each limpet was partially thawed and a small portion of the pallial gills and mantle tissue  
184 (~1 – 2 mg) was dissected from the edge of the mantle (Fisher 1904). Tissues were homogenized  
185 in buffer by boiling for 5 min followed by 2 min. in an automatic homogenizer (Tissuelyser,  
186 Qiagen, Valencia, CA, USA). The samples were boiled and homogenized twice more before  
187 centrifugation at 14,000 g for 15 min. The supernatant was pipetted to a new microfuge tube and  
188 stored at -20°C.

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

198 SDS-PAGE gel electrophoresis was used to separate proteins for western blotting. After  
199 boiling for 3 minutes at 100°C and centrifuging at 14,000 g for one minute, 7 µg of total protein  
200 from each limpet, along with Laemli sample buffer and distilled water, were loaded into a lane of  
201 a 10% Tris-HCl pre-cast gel (Ready-Gel Polyacrylamide gel, Bio-Rad Laboratories, Hercules,  
202 CA, USA). In addition to the heat-shocked samples, a molecular weight standard was loaded  
203 (Precision Plus Protein Standard, Bio-Rad Laboratories, Hercules, CA, USA), along with 30 ng  
204 of human Hsp70 in another lane (NSP-555, StressGen – Assay Designs, Ann Arbor, MI, USA).

205 Gels were submerged in Tris-glycine running buffer with 0.1% SDS. The gels were placed in a  
206 4°C cold room and electrophoresed at 200 V for 50 min.

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

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

222 The exposed x-ray film was digitally scanned on a transparency scanner. The image files  
223 were transferred to the image analysis program Image-J (Rasband 1997-2009) for densitometry.  
224 The density of each sample band at 70 kDa was measured relative to the density of the 30 ng  
225 human Hsp70 standard on each gel. The presence of the human standard on all gels allowed for  
226 comparison of relative density values across multiple western blots. Data were log transformed

227 to better conform to assumptions of normality and tested for homogeneity of variances using  
228 Cochran's test. The results for the limpets collected at high tide in the field were not included in  
229 the statistical analysis due to the difference in protocol, since they were not acclimated in the lab  
230 or handled during experiments.

### 231 *Limpet upper limits*

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

### 239 *Heat-budget modeling*

240 The heat-budget model of Denny and Harley (2006) was used to produce hindcasts of *L.*  
241 *gigantea* body temperatures on a number of hypothetical shore positions at HMS. The model,  
242 written and run using Matlab software, used the same parameters as the previously published  
243 model. An expanded 7 year set of historical weather data collected at HMS, from 1 August 1999  
244 through 31 July 2006, was used to produce a time series of body temperatures at 10 min intervals  
245 for the seven year period. The modeled limpet was 42 mm long, and was positioned in one of 5  
246 orientations: on a horizontal surface, or on sloped surfaces elevated above horizontal in 10°  
247 increments, facing different directions (north, east, south etc) around the compass in 22.5° steps.  
248 The limpets were modeled at elevations of 0.5 m to 2.0 m above Mean Lower Low Water

249 (MLLW) in 0.5 meter increments on a simulated wave-protected shore (i.e., no wave splash).  
250 These conditions were meant to produce a “worst-case scenario” for *L. gigantea* on the shore at  
251 HMS.

252 The predictions from the heat-budget model were used to calculate the frequency and  
253 severity of high temperature stress events over a 7 year period at HMS. Additionally, the  
254 conditions on days leading up to stress events were examined to address the potential for short-  
255 term physiological acclimation to high temperatures. We identified 130 days in the 7 year  
256 temperature record where the body temperature of a limpet on a southwest-facing sloped rock at  
257 2.0 m above MLLW was predicted to exceed 28°C. For each day predicted to raise body  
258 temperatures above this threshold, the temperature profiles from the previous two weeks were  
259 extracted from the data. The maximum temperature on each of the 14 days prior to each high  
260 temperature event was sampled, and the mean difference between the daily maximum body  
261 temperature and the 28°C threshold was calculated for each day.

## 262 RESULTS

### 263 *Lethal temperature trials*

264 When *L. gigantea* were subjected to a 7 h aerial exposure regime at 100% relative  
265 humidity, the median lethal temperature (LT50) was 37.4°C (Fig. 2). Limpets died at maximum  
266 temperatures as low as 34°C, and all limpets died when the maximum temperature was 42°C.

267 Survival rates in the recirculating wind tunnel at a more realistic 50-60% relative  
268 humidity were lower than the high-humidity treatments (Fig. 2). The LT50 for limpets in the  
269 low-humidity trials was 32.5°C, with mortality beginning to occur at 30°C.

270 *Sublethal temperature trials*

271 The primary antibody used in these experiments resolved one band of 70 kDa that  
272 showed low levels of constitutive expression and was strongly induced by temperature stress  
273 (Fig. 3a). There was a significant effect of temperature treatment on Hsp70 expression (ANOVA,  
274  $F_{4,20} = 4.66, P = 0.008$ ) for limpets stressed in the recirculating wind tunnel. The mean density of  
275 the Hsp70 band increased in treatments with increasing temperature up to 32°C, beyond which  
276 expression declined (Fig. 3b). Field control limpets exhibited the lowest levels of inducible  
277 Hsp70 expression.

278 *Limpet upper limits*

279 The upper limit of *L. gigantea* differed significantly among the four shore directions  
280 surveyed (Kruskal-Wallis test,  $X^2_{0.05,3} = 17.94, p < 0.001$ ). Sites facing northwest and northeast  
281 had significantly higher upper limits than southwest facing sites (post-hoc comparison, Zar  
282 1999), with southeast sites falling in between the two groups (Fig. 4).

283 *Heat-budget model*

284 Using the results from the sublethal and lethal stress experiments, two threshold  
285 conditions were chosen to define “stressful” conditions in the heat-budget model. The threshold  
286 for the onset of Hsp70 expression was defined as any day where the limpet body temperature in  
287 the model was predicted to exceed 28°C for more than 3.5 h. This corresponded to the minimal  
288 exposure regime in the recirculating wind tunnel that produced a significant increase in Hsp70  
289 relative to control conditions. The mortality threshold used in the heat-budget model was defined  
290 as any day where limpet body temperature was predicted to exceed 32°C for more than 2.5 h,  
291 based on the lowest temperature conditions conservatively expected to produce mortality in the

292 recirculating wind tunnel trials. The cumulative number of stress events varied with the altitude,  
293 azimuth, and shore height of the modeled substratum (Fig. 5), with southwest-facing sites angled  
294 between 40 and 50° above horizontal producing the largest number of high temperature days,  
295 and higher shore heights producing higher peak temperatures. The greatest number of mortality  
296 events, six events in 6 years, occurred at a south-southwest facing slope elevated 50° above  
297 horizontal at 2.0 m above MLLW. Results are shown for 1.0, 1.5 and 2.0 m above MLLW (Fig.  
298 5), while the number of sublethal stress and mortality events at 0.5 m above MLLW approached  
299 zero.

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

310         An analysis of the conditions during the 2 weeks preceding a day where body  
311 temperature was predicted to exceed 28°C for a limpet on a southwest slope at 2.0 m above  
312 MLLW is shown in Fig. 7a. The mean difference between the maximum temperature on each  
313 day leading up to the stress event and a 28°C threshold ( $\pm$  95% confidence limits) was  
314 calculated. Average daily maximum temperatures were on average 2.6°C cooler than the 28°C



315 threshold during the 2 weeks prior to a high temperature event, but this difference decreased as  
316 the target date drew closer. The 1 to 2 days preceding a day where limpet body temperature  
317 exceeded 28°C often consisted of maximum body temperatures that equaled or exceeded the  
318 temperature on the target day, indicating that many stress events could be multi-day occurrences  
319 rather than isolated events. We must include the caveat that the mean differences and confidence  
320 limits were calculated as if each day leading up to a high temperature event was an independent  
321 event, but there may be some autocorrelation among conditions on adjacent days. Two  
322 representative periods leading up to hot days are shown in Fig. 7b-c. Conditions preceding a  
323 28°C day may be consistently mild (Fig. 7b) or may consist of repeated days where body  
324 temperature reaches or exceeds the 28°C threshold (Fig. 7c)

## 325 DISCUSSION

### 326 *Thermal stress and limpet distributions*

327 The rocky intertidal zone is commonly acknowledged as one of the most stressful habitats  
328 on earth. The steep gradients in conditions along both horizontal and vertical axes can create  
329 greatly different conditions within microhabitats separated by only 10's of centimeters. For  
330 mobile organisms, or during the initial settlement period for sessile organisms, the choice of  
331 microhabitat can mitigate or intensify the severity of abiotic stresses, influencing survival and  
332 fitness (Williams & Morritt 1995, Davison & Pearson 1996, Bertness et al. 1999, Gray &  
333 Hodgson 2004).

334 For *L. gigantea*, upper distributional limits on vertical walls or sloped northeast-facing  
335 microhabitats at HMS should not be limited by thermal stress anywhere in the 0.5 to 2.0 m  
336 vertical zone modeled here. The *L. gigantea* used in the wind tunnel experiments survived

337 temperature and desiccation stress levels well beyond the most stressful conditions predicted by  
338 the heat-budget model for these microhabitats during the study period. Northwest-facing slopes  
339 were also generally benign, with steeply sloped habitats producing 0 – 10 sublethal-stress events  
340 and no mortality events at upper shore levels during the seven year timeseries.

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

354 Occasional acute temperature and desiccation stresses have been reported to cause  
355 mortality and potentially alter the upper limits of intertidal species (Sutherland 1970, Foster  
356 1971, Tsuchiya 1983, Wethey 1983, Bertness et al. 1999, Harley 2008). The study of these  
357 events is often complicated by their rarity, driven by the need for a confluence of ocean and  
358 weather conditions sufficient to raise temperatures to extreme levels (Denny et al *in press*). Calm  
359 seas, a mid-day low tide, and warm, sunny conditions must occur simultaneously. At HMS, these

360 conditions occur only a few times per year, and the frequency of potentially lethal high  
361 temperatures is therefore very low within a given year. However, *L. gigantea* is a long-lived  
362 species, with individuals living for more than 20 years (Fenberg 2008), which increases the  
363 chances of experiencing these rare stress events. Our observations of *L. gigantea* distributions at  
364 HMS agree with the predictions of the heat-budget model and physiology experiments. *L.*  
365 *gigantea* that live on open rock faces tend to cover a larger vertical range on northwest- and  
366 northeast-facing slopes compared to southwest and southeast-facing slopes. However, these  
367 patterns in the upper limit of *L. gigantea* cannot be solely attributed to the influence of acute  
368 thermal stress events. The intertidal zone at HMS is primarily oriented northward and eastward,  
369 with ocean swells approaching from the north, so that the total area available for habitation  
370 should be greater on rocks of these aspects. The high relief of the granite topography also  
371 provides many microhabitats that could serve as refuges from thermal stress by shading  
372 organisms and concentrating wave splash (O'Donnell & Denny 2008), allowing *L. gigantea* to  
373 extend their territories further up the shore.

374         The upper limits of limpet distributions can also be potentially set by other abiotic and  
375 biotic factors. Both competitive and facilitative interactions with neighboring species can  
376 influence the suitability of a patch of rock. Other grazers may reduce the standing crop of algae  
377 (Castenholz 1961), while sessile organisms such as macroalgae, barnacles and mussels may  
378 occupy potential limpet territories (Stimson 1970, 1973). Alternatively, neighboring algae and  
379 mussels could provide beneficial shade and retain moisture, ameliorating temperature and  
380 desiccation stresses at small scales (Bertness & Leonard 1997, Bertness et al. 1999). At higher  
381 shore heights, the time of emersion and higher solar irradiation may limit the growth of the

382 microalgal food supply of *L. gigantea*, making the habitat unsuitable for occupation (Castenholz  
383 1961).

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

402       Care must be taken in any generalization of our predictions from the heat-budget model.  
403 The frequency, intensity, and duration of thermal and desiccation stress events in the intertidal  
404 zone are intimately associated with the particular microhabitat of an organism (Grainger 1969,

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

#### 415 *Applying laboratory data to the field*

416         Accurately predicting how intertidal organisms will cope with prolonged aerial exposure  
417 during low tide requires experimental methods that reasonably recreate conditions in the field  
418 (Newell 1976, Branch 1981, Helmuth et al. 2006, Jost & Helmuth 2007). Simplified laboratory  
419 stress protocols have traditionally been used for comparative physiological studies, creating  
420 difficulties in the transition from idealized conditions in the laboratory to the extremely variable  
421 conditions of the real world. These difficulties raise a number of concerns that must be addressed  
422 before we can produce meaningful measures of thermal and desiccation tolerance for intertidal  
423 species. Much of the existing literature on thermal stress in intertidal organisms relies on lethal  
424 or sublethal experimental data derived from high humidity trials in closed chambers or tests in  
425 which organisms were submerged in seawater (Broekhuysen 1940, Orr 1955, Fraenkel 1968,  
426 Stirling 1982, McMahon & Britton 1985, Cleland & McMahon 1986, McMahon 1991, Sanders  
427 et al. 1991, Tomanek & Somero 1999, Clarke, Mill & Grahame 2000, Tomanek & Somero 2000,

428 McMahon 2001, Sorte & Hofmann 2005, Davenport & Davenport 2005, Berger & Emlet 2007).  
429 The prevalence of these protocols is understandable from a logistical standpoint, as the high-  
430 humidity or submerged trials can be carried out with simple water baths. Because much of the  
431 work on thermal tolerance in intertidal organisms has been focused on comparisons across  
432 species, these standardized methods allow researchers to remove much of the variability of the  
433 natural system. However, these methods have the potential to be misleading when the goal is to  
434 predict performance in the field. In the current study, *L. gigantea* were able to survive much  
435 higher temperatures when kept in saturated air (Fig. 2), a condition that is extremely unlikely to  
436 occur in the field at HMS. The median lethal temperatures for the 100% and 50% relative  
437 humidity trials decreased by nearly 5°C in the lower humidity trial. Clearly the method by which  
438 lethal temperature determinations are made will have substantial effects on the application of the  
439 data to species performance in the field. The practice of exposing intertidal organisms to thermal  
440 stress in seawater could complicate comparisons with both 100% RH and low-humidity aerial  
441 exposures due a number of factors. Submerged respiration rates and aerial respiration rates may  
442 differ markedly (Carefoot 1987). In air, the inability to dispose of metabolic waste products can  
443 create changes in the osmolality of both internal and external water stores, while desiccation will  
444 further concentrate solutes in the remaining water at low humidity, enhancing physiological  
445 stress (Segal & Dehnel 1962, Wolcott 1973, Newell 1976, Lowell 1984). For these reasons,  
446 caution is warranted when trying to apply results from submerged stress trials to emersion in the  
447 field, as they may overestimate the tolerance of the organism to stressful emersed conditions.  
448 Although the lethal temperatures differed greatly between the treatments used here, the onset  
449 temperature for the heat shock response of *L. gigantea* (24 - 28°C) was similar to previously  
450 published values for related limpets stressed in water (23 – 29°C, Sanders et al 1991) and for

451 *Chlorostoma (Tegula)* snails stressed in water (24-27°C, Tomanek and Somero 1999) or at low  
452 tide in the field (24-27°C, Tomanek and Sanford 2003).

453         The time and temperature profile of stress exposures used here represents only a small  
454 fraction of the large variety of potential field conditions that a limpet might experience. The  
455 maximum temperatures used in this study encompass the likely range of high temperatures that  
456 occur in the field at HMS. However, based on the results from the heat-budget model, there is  
457 wide latitude in the length of aerial emersion during low tide (Fig. 6). During calm ocean  
458 conditions, *L. gigantea* living in the upper portion of their range at HMS might reasonably  
459 remain emersed for 12 h or more, potentially causing even more severe physiological stress than  
460 the conditions used in this study. In addition to the time of exposure, the rate of heating may  
461 impact the extent of the heat shock response, potentially by damaging cellular processes before  
462 the onset of heat shock protein production. The rate of heating has been demonstrated to affect  
463 the thermal limits of insects (Chown et al 2009), but unfortunately studies on the effects of  
464 heating rate in intertidal organisms are rare. Tomanek & Somero (2000) observed a pronounced  
465 increase in the heat shock response of low-shore *Chlorostoma brunnea* when they were heated  
466 relatively slowly in air compared to being immersed directly in hot water, but the related mid-  
467 shore snail *Chlorostoma funebris* showed no difference in Hsp expression under the same  
468 heating conditions. These results again demonstrate that comparisons between submerged and  
469 emersed stress protocols should be approached with caution, as body temperature can respond  
470 much more quickly to changes in water temperature than air temperature due to the thermal  
471 conductivities of these fluids. Measuring the temperature of a water bath used to heat a container  
472 full of air may give an inaccurate estimation of body temperature of the animal in the container,  
473 so heating rate in air should be tied directly to the organism body temperature (as in the current

474 study). Model results from this study indicate that the rate of heating for *L. gigantea* in the field  
475 may vary between approximately 2 and 10°C h<sup>-1</sup> on warm days (Fig. 6). Slower heating rates  
476 must necessarily be accompanied by prolonged exposure times in order to reach stressful  
477 temperatures, and the effects of this combination of conditions on *L. gigantea* are currently  
478 unknown.

#### 479 *Acclimatization*

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

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



496 other hand, the cumulative effects of multi-day stress events could cause *L. gigantea* to succumb  
497 to lower maximum temperatures.

#### 498 *Conclusions*

499 Studies of the type described here represent progress towards the integration of  
500 biomechanical and physiological techniques with the goal of testing ecological hypotheses (Huey  
501 1991, Chown & Terblanche 2007). We can combine physiological performance data, gathered  
502 under realistic conditions, with computer simulations of organismal temperatures in the field to  
503 make predictions about where and when the distribution of an ecologically dominant species  
504 may be limited by extreme temperature events. This study and others like it provide ecologists  
505 with a robust set of tools to examine questions on the importance of thermal stress and  
506 microhabitat selection in determining the identity of species that comprise the community at a  
507 site. As with terrestrial species (Kearney & Porter 2004, Buckley & Roughgarden 2006,  
508 Pincebourde et al 2007, Mitchell et al 2008, Kearney et al 2008), a growing number of  
509 biophysical models for important intertidal species such as the limpets, mussels, and algae (Bell  
510 1995, Helmuth 1999, Denny & Harley 2006, Gilman et al 2006), coupled with increasingly  
511 detailed studies of the physiology of these organisms (Gracey et al 2008) will form the basis for  
512 mechanistic models to study species interactions and make predictions about future community  
513 development, especially in the face of an uncertain climatic future (Helmuth et al 2006).

#### 514 ACKNOWLEDGEMENTS

515 We are grateful to G. N. Somero for providing lab space and expertise for the heat shock  
516 protein work. M. I. Seabra, J. G. Sanders, M. Jelenic, and L. Tomanek were also helpful in fine-  
517 tuning protocols. The comments of L. Tomanek and two anonymous reviewers helped to  
518 improve the manuscript. This work was supported by a NSF grant OCE 9985946 to M. W.

519 Denny. This is contribution number XXX from PISCO, the Partnership for Studies of Coastal  
520 Oceans funded by the Gordon and Betty Moore Foundation and David and Lucile Packard  
521 Foundation. All animals were handled in accordance with university and government  
522 regulations.

## 523 REFERENCES

524 Bazterrica, M.C., Silliman, B.R., Hidalgo, F.J., Crain, C.M. & Bertness, M.D. (2007)  
525 Limpet grazing on a physically stressful Patagonian rocky shore. *Journal of Experimental*  
526 *Marine Biology and Ecology*, **353**, 22-34.

527 Bell, E.C. (1995) Environmental and morphological influences on thallus temperature and  
528 desiccation of the intertidal alga *Mastocarpus papillatus* Kutzing. *Journal of Experimental*  
529 *Marine Biology and Ecology*, **191**, 29-55.

530 Berger, M.S. & Emlet, R.B. (2007) Heat-shock response of the upper intertidal barnacle  
531 *Balanus glandula*: thermal stress and acclimation. *Biological Bulletin*, **212**, 232-241.

532 Bertness, M.D., Crain, C.M., Silliman, B.R., Bazterrica, M.C., Reyna, M.V., Hildago, F. &  
533 Farina, J.K. (2006) The community structure of western Atlantic Patagonian rocky shores.  
534 *Ecological Monographs*, **76**, 439-460.

535 Bertness, M.D. & Leonard, G.H. (1997) The role of positive interactions in communities:  
536 lessons from intertidal habitats. *Ecology*, **78**, 1976-1989.

537 Bertness, M.D., Leonard, G.H., Levine, J.M. & Bruno, J.F. (1999) Climate-driven  
538 interactions among rocky intertidal organisms caught between a rock and a hot place. *Oecologia*,  
539 **120**, 446-450.

540 Branch, G.M. (1981) The biology of limpets: physical factors, energy flow, and ecological  
541 interactions. *Oceanography and Marine Biology: an Annual Review*, **19**, 235-380.

542 Breen, P.A. (1972) Seasonal migration and population regulation in the limpet *Acmaea*  
543 (*Collisella*) *digitalis*. *The Veliger*, **15**, 133-141.

544 Broekhuysen, G.J. (1940) A preliminary investigation of the importance of desiccation,  
545 temperature and salinity as factors controlling the vertical distribution of certain intertidal marine  
546 gastropods in False Bay, South Africa. *Transactions of the Royal Society of South Africa*, **28**,  
547 255-291.

548 Buckley, B.A., Owen, M.-E. & Hofmann, G.E. (2001) Adjusting the thermostat: the  
549 threshold induction temperature for the heat-shock response in intertidal mussels (genus *Mytilus*)  
550 changes as a function of thermal history. *Journal of Experimental Biology*, **204**, 3571-3579.

551 Buckley, L. B. & J. Roughgarden (2006). Climate, competition, and the coexistence of  
552 island lizards. *Functional Ecology* **20**: 315-322.

553 Campbell, G.S. & Norman, J.M. (1998) *An Introduction to Environmental Biophysics*.  
554 Springer-Verlag, New York.

555 Carefoot, T. H. (1987). Gastropoda. *Animal Energetics*. T. J. Pandian and F. J. Vernberg.  
556 New York, Academic Press Inc. Vol 2: 89-172.

557 Castenholz, R.W. (1961) Effect of grazing on marine littoral diatom populations. *Ecology*,  
558 **42**, 783-794.

559 Chown, S. L. and J. S. Terblanche (2007). Physiological diversity in insects: ecological  
560 and evolutionary contexts. *Advances in Insect Physiology*. S. J. Simpson. Amsterdam, Elsevier.  
561 **33**: 50-152.

562 Chown, S. L., K. R. Jumbam, J. G. Sørensen and J. S. Terblanche (2009). Phenotypic  
563 variance, plasticity and hertibility estimates of critical thermal limits depend on methodological  
564 context. *Functional Ecology* **23**: 133-140.

565 Clarke, A.P., Mill, P.J. & Grahame, J. (2000) Biodiversity in *Littorina* species (Mollusca:  
566 Gastropoda): A physiological approach using heat-coma. *Marine Biology*, **137**, 559-565.

567 Cleland, J.D. & McMahon, R.F. (1986) Upper thermal limit of nine intertidal gastropod  
568 species from a Hong Kong rocky shore in relation to vertical distribution and desiccation  
569 associated with evaporative cooling. *Proceedings of the Second International Marine Biological*

570 *Workshop: The Marine Flora and Fauna of Hong Kong and Southern China, Hong Kong, 1986*  
571 (ed B. Morton), pp. 1141-1152. Hong Kong University Press, Hong Kong.

572 Connell, J. H. (1961). Influence of interspecific competition and other factors on the  
573 distribution of the barnacle *Chthamalus stellatus*. *Ecology* **42**(4): 710-723.

574 Davenport, J. & Davenport, J.L. (2005) Effects of shore height, wave exposure and  
575 geographical distance on thermal niche width of intertidal fauna. *Marine Ecology Progress*  
576 *Series*, **292**, 41-50.

577 Davison, I.R. & Pearson, G.A. (1996) Stress tolerance in intertidal seaweeds. *Journal of*  
578 *Phycology*, **32**, 197-211.

579 Denny, M.W. & Blanchette, C.A. (2000) Hydrodynamics, shell shape, behavior and  
580 survivorship in the owl limpet *Lottia gigantea*. *Journal of Experimental Biology*, **203**, 2623-  
581 2639.

582 Denny, M.W. & Harley, C.D.G. (2006) Hot limpets: predicting body temperature in a  
583 conductance-mediated thermal system. *Journal of Experimental Biology*, **209**, 2409-2419.

584 Denny, M.W., Miller, L.P. & Harley, C.D.G. (2006) Thermal stress on intertidal limpets:  
585 long-term hindcasts and lethal limits. *Journal of Experimental Biology*, **209**, 2420-2431.

586 Denny, M. W., L. J. H. Hunt, L. P. Miller & C. D. G. Harley (*in press*). On the prediction  
587 of extreme ecological events. *Ecological Monographs*.

588 Dong, Y., L. P. Miller, J. G. Sanders & G. N. Somero (2008). Heat-shock protein 70  
589 (Hsp70) expression in four limpets of the genus *Lottia*: interspecific variation in constitutive and  
590 inducible synthesis correlates with *in situ* exposure to heat stress. *Biological Bulletin* **215**: 173-  
591 181.

592 Feder, M.E. & Hofmann, G.E. (1999) Heat-shock proteins, molecular chaperones, and the  
593 stress response: evolutionary and ecological physiology. *Annual Review of Physiology*, **61**, 243-  
594 282.

595 Fenberg, P. B. (2008). The effects of size-selective harvesting on the population biology  
596 and ecology of a sex-changing limpet species, *Lottia gigantea*. PhD thesis, Biology, University  
597 of California, San Diego. 114 pgs.

598 Finke, G.R., Navarrete, S.A. & Bozinovic, F. (2007) Tidal regimes of temperate coasts and  
599 their influences on aerial exposure for intertidal organisms. *Marine Ecology Progress Series*,  
600 **343**, 57-62.

601 Fisher, W.K. (1904) The Anatomy of *Lottia gigantea* Gray. *Zoologische Jahrbucher*.  
602 *Abteilung fur Anatomic und Ontogenie der Tiere*, **20**, 1-66.

603 Foster, B.A. (1971) On the determinants of the upper limit of intertidal distribution of  
604 barnacles (Crustacea: Cirripedia). *Journal of Animal Ecology*, **40**, 33-48.

605 Fraenkel, G. (1968) The heat resistance of intertidal snails at Bimini, Bahamas; Ocean  
606 Springs, Mississippi; and Woods Hole, Massachusetts. *Physiological Zoology*, **41**, 1-13.

607 Galbraith, R.T. (1965) Homing behavior in the limpets *Acmaea digitalis* and *Lottia*  
608 *gigantea*. *American Midland Naturalist*, **74**, 245-246.

609 Gates, D.M. (1980) *Biophysical Ecology*. Springer-Verlag, New York, USA.

610 Gilman, S.E. (2006) Life at the edge: an experimental study of a poleward range boundary.  
611 *Oecologia*, **148**, 270-279.

612 Gilman, S. E., D. S. Wetthey and B. S. T. Helmuth (2006). Variation in the sensitivity of  
613 organismal body temperature to climate change over local and geographical scales. *Proceedings*  
614 *of National Academy of Sciences, USA* **103**(25): 9560-9565.

615 Gracey, A. Y., M. L. Chaney, J. P. Boomhower, W. R. Tyburczy, K. Connor & G. N.  
616 Somero (2008). Rhythms of gene expression in a fluctuating intertidal environment. *Current*  
617 *Biology* **18**(19): 1501-1507.

618 Grainger, J.N.R. (1969) Factors affecting the body temperature of *Patella*. *Verhandlungen*  
619 *der Deutschen Zoologischen Gesellschaft*, **3**, 479-487.

620 Gray, D.R. & Hodgson, A.N. (2004) The importance of a crevice environment to the  
621 limpet *Helcion pectunculus* (Patellidae). *Journal of Molluscan Studies*, **70**, 67-72.

622 Halpin, P.M., Sorte, C.J., Hofmann, G.E. & Menge, B.A. (2002) Patterns in variation in  
623 levels of Hsp70 in natural rocky shore populations from microscales to mesoscales. *Integrative  
624 and Comparative Biology*, **42**, 815-824.

625 Harley, C. D. G. (2003). Abiotic stress and herbivory interact to set range limits across a  
626 two-dimensional stress gradient. *Ecology* **84**(6): 1477-1488.

627 Harley, C. D. G. (2008). Tidal dynamics, topographic orientation, and temperature-  
628 mediated mass mortalities on rocky shores. *Marine Ecology Progress Series* **371**, 37-46.

629 Harley, C.D.G. & Helmuth, B.S.T. (2003) Local- and regional-scale effects of wave  
630 exposure, thermal stress, and absolute versus effective shore level on patterns of intertidal  
631 zonation. *Limnology and Oceanography*, **48**, 1498-1508.

632 Helmuth, B. (1999) Thermal biology of rocky intertidal mussels: quantifying body  
633 temperatures using climatological data. *Ecology*, **80**, 15-34.

634 Helmuth, B.S.T. & Denny, M.W. (2003) Predicting wave exposure in the rocky intertidal  
635 zone: Do bigger waves always lead to larger forces? *Limnology and Oceanography*, **48**, 1338-  
636 1345.

637 Helmuth, B.S.T. & Hofmann, G.E. (2001) Microhabitats, thermal heterogeneity, and  
638 patterns of physiological stress in the rocky intertidal zone. *Biological Bulletin*, **201**, 374-384.

639 Helmuth, B.S.T., N. Mieszowska, P. Moore & S. J. Hawkins (2006). Living on the edge of  
640 two changing worlds: forecasting the responses of rocky intertidal ecosystems to climate change.  
641 *Annual Review of Ecology, Evolution, and Systematics* **37**: 373-404.

642 Hofmann, G.E. & Somero, G.N. (1995) Evidence for protein damage at environmental  
643 temperatures: Seasonal changes in levels of ubiquitin conjugates and hsp70 in the intertidal  
644 mussel *Mytilus trossulus*. *Journal of Experimental Biology*, **198**, 1509-1518.

645 Hofmann, G.E. & Somero, G.N. (1996) Protein ubiquitination and stress protein synthesis  
646 in *Mytilus trossulus* occurs during recovery from tidal emersion. *Molecular Marine Biology and*  
647 *Biotechnology*, **5**, 175-184.

648 Huey, R. B. (1991). Physiological consequences of habitat selection. *The American*  
649 *Naturalist* **137**: S91-S115.

650 Hutchinson, G. E. (1957) Concluding remarks. Populations studies: animal ecology and  
651 demography. Cold Spring Harbor Symposia on Quantitative Biology. **22**: 415-427

652 Jost, J. & Helmuth, B. (2007) Morphological and ecological determinants of body  
653 temperatures of *Geukensia demissa*, the Atlantic ribbed mussel, and their effects on mussel  
654 mortality. *Biological Bulletin*, **213**, 141-151.

655 Kearney, M. and W. P. Porter (2004). Mapping the fundamental niche: physiology,  
656 climate, and the distribution of a nocturnal lizard. *Ecology* **85**(11): 3119-3131.

657 Kearney, M., B. L. Phillips, C. R. Tracy, K. A. Christian, G. Betts and W. P. Porter (2008).  
658 Modelling species distributions without using species distributions: the cane toad in Australia  
659 under current and future climates. *Ecography* **31**: 423-434.

660 Kensler, C.B. (1967) Desiccation resistance of intertidal crevice species as a factor in their  
661 zonation. *Journal of Animal Ecology*, **36**, 391-406.

662 Lewis, J. R. (1954). Observations on a high-level population of limpets. *Journal of Animal*  
663 *Ecology* **23**: 85-100.

664 Lindberg, D. R., J. A. Estes & K. I. Warheit (1998). Human influences on trophic cascades  
665 along rocky shores. *Ecological Applications* **8**(3): 880-890.

666 Lowell, R. B. (1984). Desiccation of intertidal limpets: effects of shell size, fit to  
667 substratum, and shape. *Journal of Experimental Marine Biology and Ecology* **77**: 197-207.

668 Markel, R.P. (1974) Aspects of the physiology of temperature acclimation in the limpet  
669 *Acmaea limatula* Carpenter (1864): an integrated field and laboratory study. *Physiological*  
670 *Zoology*, **47**, 99-109.

671 McMahon, R.F. (1991) Upper critical temperature limits, tissue temperatures during  
672 emersion and evaporative cooling at elevated temperatures in Western Australian intertidal  
673 gastropods: Evidence for a physiological barrier at the high tide mark. *Proceedings of the Third*  
674 *International Marine Biological Workshop: The Marine Flora and Fauna of Albany, Western*  
675 *Australia* (eds F.E. Wells, D.I. Walker, H. Kirkman & R. Lethbridge), pp. 661-673. Western  
676 Australia Museum, Perth.

677 McMahon, R.F. (2001) Acute thermal tolerance in intertidal gastropods relative to latitude,  
678 superfamily, zonation and habitat with special emphasis on the Littorinoidea. *Journal of Shellfish*  
679 *Research*, **20**, 459-467.

680 McMahon, R.F. & Britton, J.C. (1985) The relationship between vertical distribution,  
681 thermal tolerance, evaporative water loss rate, and behavior on emergence in six species of  
682 mangrove gastropods from Hong Kong. *The Malacofauna of Hong Kong and Southern China. II,*  
683 *Vol. 1 and 2. Second International Workshop: Hong Kong, Hong Kong, Apr. 6-24, 1983.* (eds B.  
684 Morton & D. Dudgeon), pp. 563-582. Hong Kong University Press, Hong Kong.

685 Mitchell, N. J., M. R. Kearney, N. J. Nelson & W. P. Porter (2008). Predicting the fate of a  
686 living fossil: how will global warming affect sex determination and hatching phenology in  
687 tuatara? *Proceedings of the Royal Society B* **275**: 2185-2193.

688 Newell, G.E. (1964) Physiological aspects of the ecology of intertidal molluscs.  
689 *Physiology of Mollusca* (eds K.M. Wilbur & C.M. Yonge), pp. 59-81. Academic Press, New  
690 York, USA.

691 Newell, R.C. (1976) Adaptations to intertidal life. *Adaptation to Environment: Essays on*  
692 *the Physiology of Marine Animals* (ed R.C. Newell), pp. 1-82. Butterworths, London, UK.

693 O'Donnell, M.O. & Denny, M.W. (2008) Hydrodynamic forces and surface topography:  
694 Centimeter-scale spatial variation in wave forces. *Limnology and Oceanography*, **53**, 579-588.

695 Orr, P.R. (1955) Heat Death. I. Time-temperature relationships in marine animals.  
696 *Physiological Zoology*, **28**, 290-294.



697 Pincebourde, S., H. Sinoquet, D. Combes & J. Casas (2007). Regional climate modulates  
698 the canopy mosaic of favourable and risky microclimates for insects. *Journal of Animal Ecology*  
699 **76**: 424-438.

700 Porter, W.P. & Gates, D.M. (1969) Thermodynamic equilibria of animals with  
701 environment. *Ecological Monographs*, **39**, 228-244.

702 Raffaelli, D.G. & Hughes, R.N. (1978) The effects of crevice size and availability on  
703 populations of *Littorina rudis* and *Littorina neritoides*. *Journal of Animal Ecology*, **47**, 71-83.

704 Rasband, W.S. (1997-2009) ImageJ. U. S. National Institutes of Health, Bethesda,  
705 Maryland, USA.

706 Roberts, D.A., Hofmann, G.E. & Somero, G.N. (1997) Heat-shock protein expression in  
707 *Mytilus californianus*: acclimatization (seasonal and tidal-height comparisons) and acclimation  
708 effects. *Biological Bulletin*, **192**, 309-320.

709 Sanders, B.M., Hope, C., Pascoe, V.M. & Martin, L.S. (1991) Characterization of the  
710 stress protein response in two species of *Collisella* limpets with different temperature tolerances.  
711 *Physiological Zoology*, **64**, 1471-1489.

712 Segal, E. and P. A. Dehnel (1962). Osmotic behavior in an intertidal limpet, *Acmaea*  
713 *limatula*. *Biological Bulletin* **122**(3): 417-430.

714 Somero, G.N. (2002) Thermal physiology and vertical zonation of intertidal animals:  
715 Optima, limits, and costs of living. *Integrative and Comparative Biology*, **42**, 780-789.

716 Sorte, C. J. B. & G. E. Hofmann (2005). Thermotolerance and heat-shock protein  
717 expression in Northeastern Pacific *Nucella* species with different biogeographical range. *Marine*  
718 *Biology* **146**: 985-993.

719 Southward, A.J. (1958) The zonation of plants and animals on rocky sea shores. *Biological*  
720 *Reviews*, **33**, 137-177.

721 Stephenson, T.A. & Stephenson, A. (1949) The universal features of zonation between  
722 tide-marks on rocky coasts. *Journal of Ecology*, **37**, 289-305.

723           Stillman, J. H. & G. N. Somero (2000). A comparative analysis of the upper thermal  
724 tolerance limits of eastern Pacific porcelain crabs, genus *Petrolithses*: Influences of latitude,  
725 vertical zonation, acclimation, and phylogeny. *Physiological and Biochemical Zoology* **73**(2):  
726 200-208.

727           Stimson, J. (1970) Territorial behavior of the owl limpet *Lottia gigantea*. *Ecology*, **51**, 113-  
728 118.

729           Stimson, J. (1973) The role of the territory in the ecology of the intertidal limpet *Lottia*  
730 *gigantea*. *Ecology*, **54**, 1020-1030.

731           Stirling, H.P. (1982) The upper temperature tolerance of prosobranch gastropods of rocky  
732 shores at Hong Kong and Dar ES Salaam, Tanzania. *Journal of Experimental Marine Biology*  
733 *and Ecology*, **63**, 133-144.

734           Sutherland, J.P. (1970) Dynamics of high and low populations of the limpet, *Acmaea*  
735 *scabra* (Gould). *Ecological Monographs*, **40**, 169-188.

736           Tomanek, L. (2002) The heat-shock response: Its variation, regulation, and ecological  
737 importance in intertidal gastropods (genus *Tegula*). *Integrative and Comparative Biology*, **42**,  
738 797-807.

739           Tomanek, L. (2005) Two-dimensional gel analysis of the heat-shock response in marine  
740 snails (genus *Tegula*): interspecific variation in protein expression and acclimation ability.  
741 *Journal of Experimental Biology*, **208**, 3133-3143.

742           Tomanek, L. & Sanford, E. (2003) Heat-shock protein 70 (Hsp 70) as a biochemical stress  
743 indicator: an experimental field test in two congeneric intertidal gastropods (Genus: *Tegula*).  
744 *Biological Bulletin*, **205**, 276-284.

745           Tomanek, L. & Somero, G.N. (1999) Evolutionary and acclimation-induced variation in  
746 the heat-shock responses of congeneric marine snails (Genus *Tegula*) from different thermal  
747 habitats: implications for limits of thermotolerance and biogeography. *Journal of Experimental*  
748 *Biology*, **202**, 2925-2936.

- 749 Tomanek, L. & G. N. Somero (2000). Time course and magnitude of synthesis of heat-  
750 shock proteins in congeneric marine snails (Genus *Tegula*) from different tidal heights.  
751 *Physiological and Biochemical Zoology* **73**(2): 249-256.
- 752 Tsuchiya, M. (1983) Mass mortality in a population of the mussel *Mytilus edulis* L. caused  
753 by high temperature on rocky shores. *Journal of Experimental Marine Biology and Ecology*, **66**,  
754 101-111.
- 755 Wethey, D.S. (1983) Geographic limits and local zonation: the barnacles *Semibalanus*  
756 (*Balanus*) and *Chthamalus* in New England. *Biological Bulletin*, **165**, 330-341.
- 757 Williams, G.A. & Morrill, D. (1995) Habitat partitioning and thermal tolerance in a  
758 tropical limpet, *Cellana grata*. *Marine Ecology Progress Series*, **124**, 89-103.
- 759 Wolcott, T.G. (1973) Physiological ecology and intertidal zonation in limpets (*Acmaea*) -  
760 critical look at limiting factors. *Biological Bulletin*, **145**, 389-422.
- 761 Zar, J. H. (1999). *Biostatistical Analysis* - 4th ed. Prentice-Hall, New Jersey. 929 pgs.

762 FIGURE LEGENDS

763 Fig. 1. Total elapsed time of all trials was 7 h.

764 Fig. 2. .

765 Fig. 3.

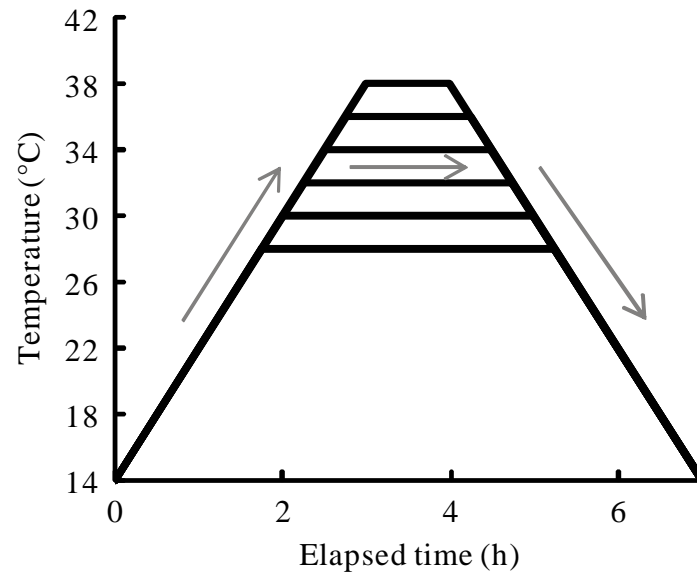
766 Fig. 4.

767 Fig. 5.

768 Fig. 6.

769

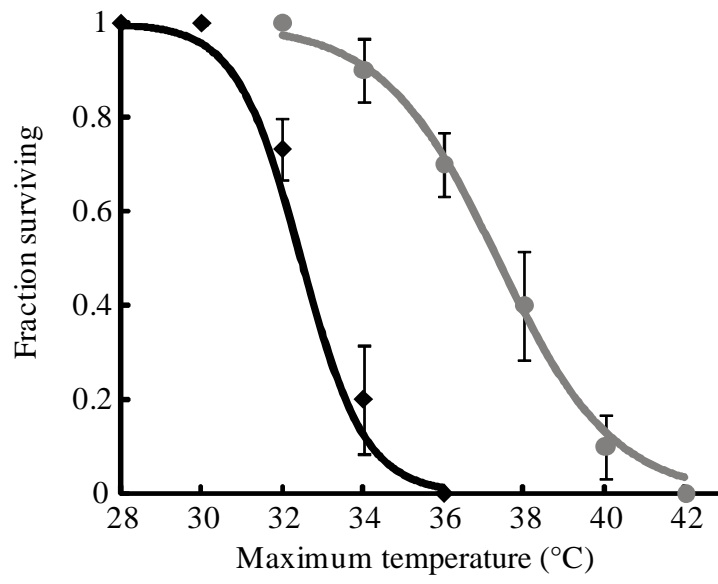
770



771

772

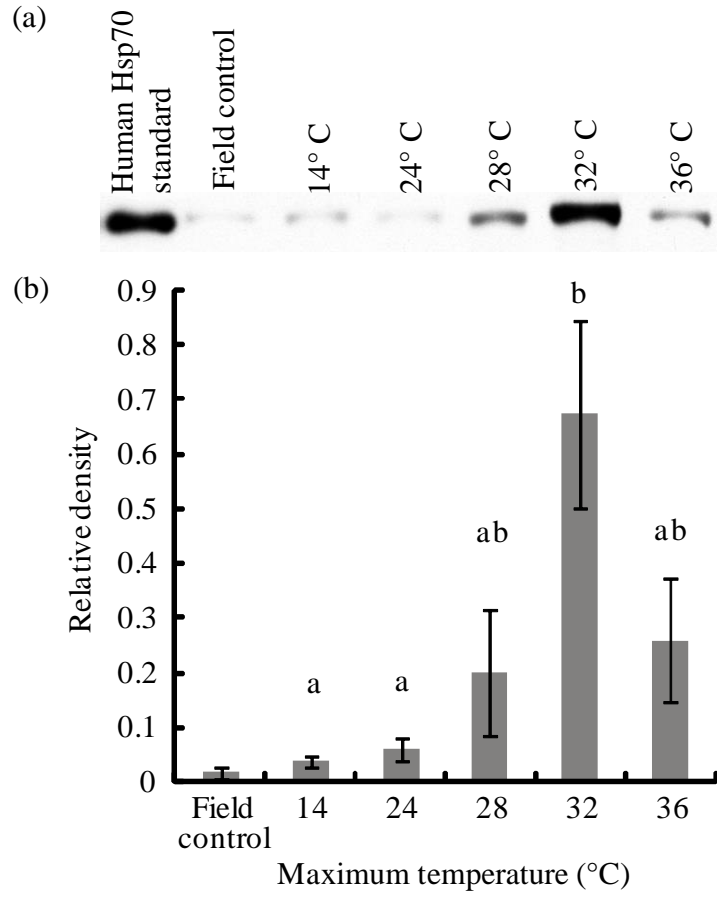
**Fig. 1.**



773

774

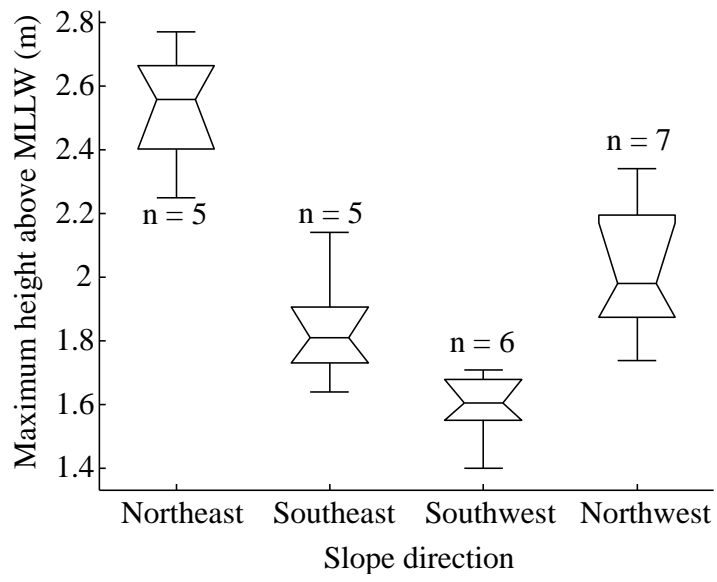
Fig. 2.



775

776 **Fig. 3.**

777

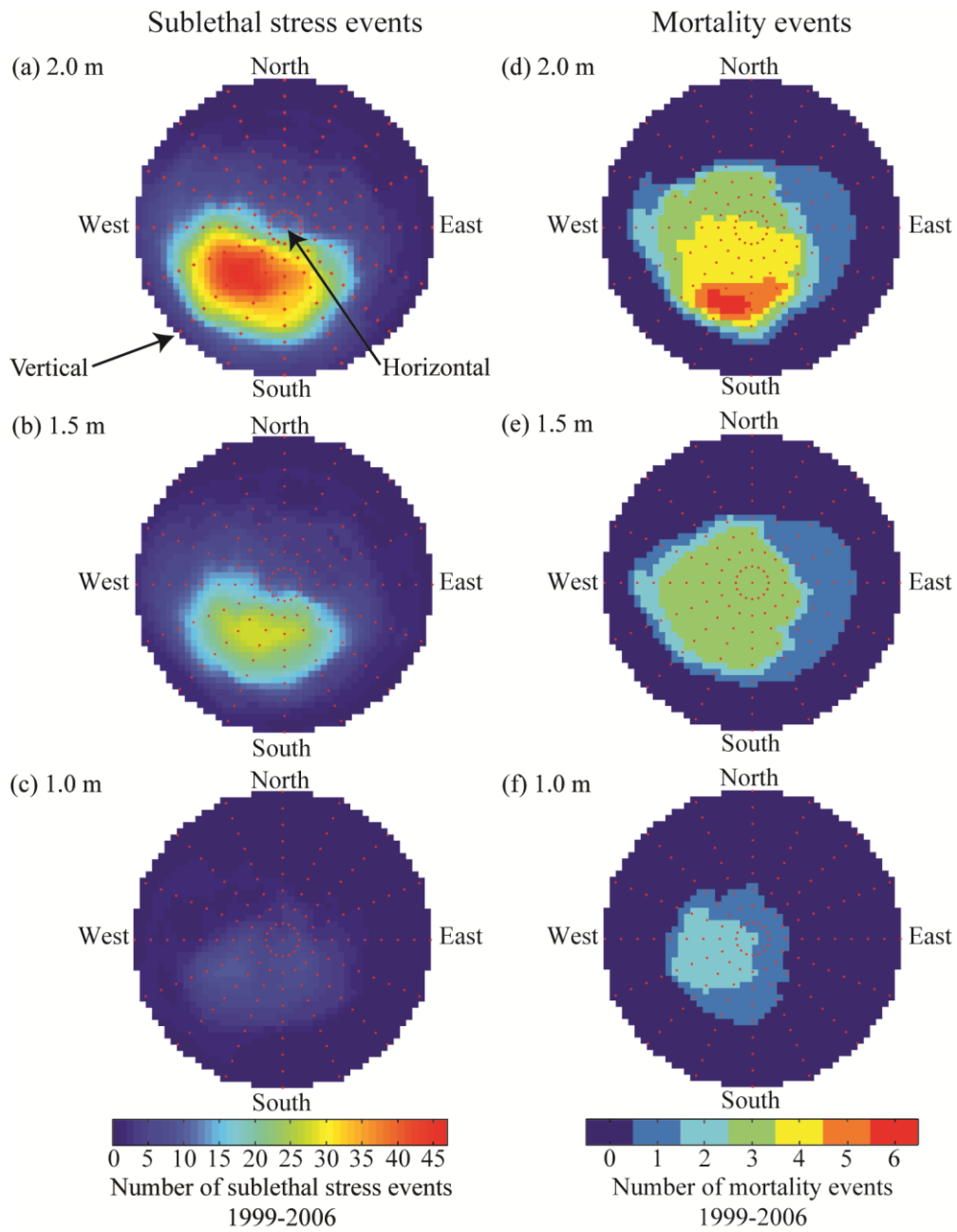


778

779

**Fig. 4.**

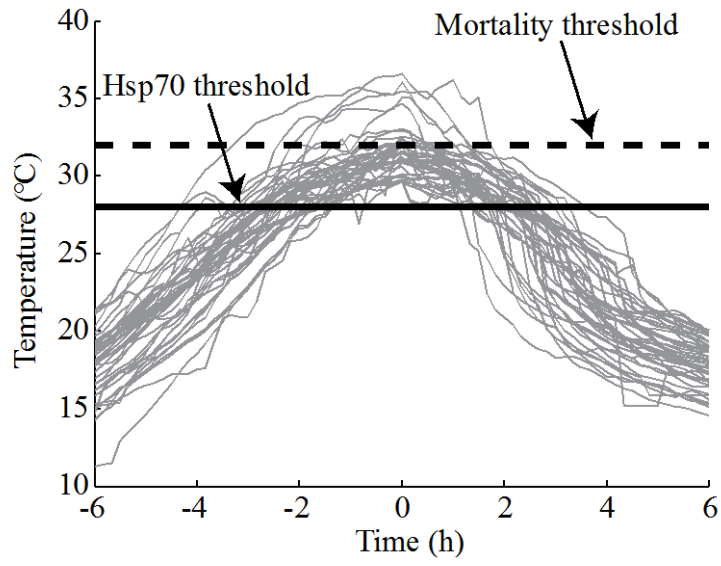




780

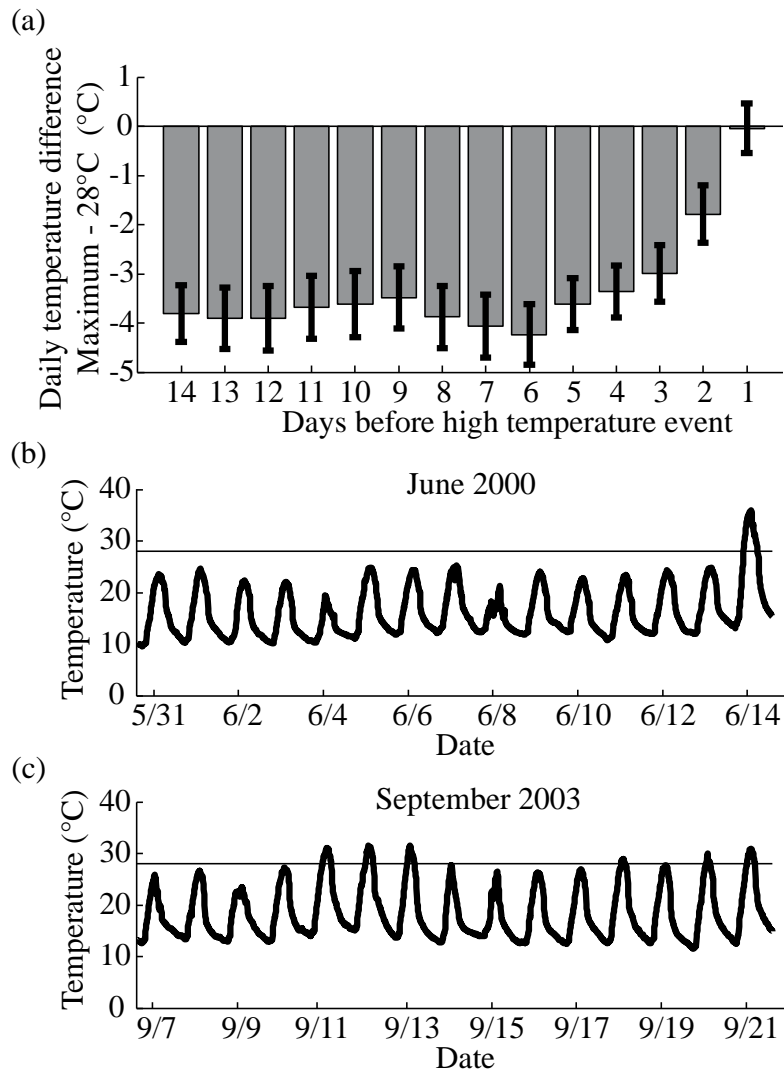
781

**Fig. 5.**



782

783 **Fig. 6.**



784

785

Fig. 7.

786