

Thermal stress and morphological adaptations in limpets

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Summary

1. On thermally stressful rocky shores, small, slow-moving ectotherms such as limpets exhibit morphological characteristics such as high-spined and heavily ridged shells which may reduce the likelihood of reaching stressful or lethal body temperatures.
2. The effects of shell height and shell surface area on predicted limpet body temperatures were tested with a previously developed heat budget model. The model was parameterized with morphological data from three species (*Lottia gigantea*, *Patella vulgata* and *Siphonaria gigas*), which differ dramatically in their morphology and in the body temperatures they are likely to reach in the field.
3. Limpet models and standard cones with higher height : length ratios lost heat to convection more readily than models with lower spired shells.
4. Heavily ridged shells lost heat to convection more readily than smoother shells, but this effect was only pronounced at high wind velocities.
5. When the heat budget model parameters were applied to a real environmental data set, the model predicts that maximum body temperatures and cumulative thermal stress vary among species. These differences are related primarily to the height : length ratio of the shell, and to a lesser extent to the presence of ridges.
6. These results suggest that some intra- and interspecific variation in limpet morphology may be phenotypic or evolutionary responses to variation in environmental temperatures. Our findings are supported by observed patterns of limpet morphological variation across natural thermal gradients.

Key-words: body temperature, heat budget model, *Lottia gigantea*, morphological adaptation, *Patella vulgata*, rocky intertidal zone, shell morphology, *Siphonaria gigas*, thermal stress

Introduction

Environmental temperature influences a variety of organismal processes in ectotherms, including growth, reproduction and survival (Hochachka & Somero 2002). Because body temperature is often a strong correlate of fitness (Huey & Berrigan 2001), organisms have evolved a variety of strategies for regulating their body temperatures. In ectotherms, these strategies fall into three broad categories: behavioural regulation, physiological regulation and morphological regulation. Behavioural options such as movement (active microhabitat selection) and body reorientation are effective means of avoiding potentially damaging body temperatures, and these strategies are commonly used by a variety of ectotherms (Heath 1970). To achieve physiological regulation, animals can alter metabolic rate, heart rate and the flow of blood towards or away from

heat sources or sinks (Seebacher & Franklin 2005). Morphological regulation refers to non-behavioural changes in an organism's overall appearance (broadly defined to include both shape and colour), which can influence rates of heat gain and heat loss (e.g., Vermeij 1973; Etter 1988).

Although behavioural and physiological mechanisms of body temperature control are better studied than morphological mechanisms, many of the sessile and slow-moving invertebrate ectotherms that predominate on intertidal shores may be unable to effectively utilize them. For these species, behavioural strategies such as microhabitat selection often cannot be employed over relevant time scales (e.g. over the course of a 6-h low tide). Even behavioural control of evaporative cooling [e.g., gaping in barnacles and bivalves, raising of the shell (mushrooming) in limpets] may be of little consequence in typically small-bodied intertidal animals with limited reservoirs of water (Denny & Harley 2006). The small size of most intertidal animals may also preclude meaningful thermal

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Fig. 1. Photograph of representative shell morphologies for *Lottia gigantea* (left), *Patella vulgata* (centre), and *Siphonaria gigas* (right). The upper panel is the dorsal view, and the lower panel is the posterior view. Scale bar = 2 cm.

control via reductions in metabolic rate and shifting patterns of blood flow. Thus, many intertidal species may emphasize morphological strategies to influence their body temperatures.

Adaptation to thermal stress is particularly important on rocky intertidal shores, where substratum temperatures can increase from 10 °C (sea water temperature) to over 40 °C during a single low tide on temperate shores (Harley & Helmuth 2003) and exceed 50 °C on tropical shores (Williams & Morritt 1995). Thermal stress can result in dramatic mortality events via direct impacts on individual organisms (Frank 1965; Sutherland 1970; Tsuchiya 1983). Thermal stress may also lead to mortality indirectly via increased susceptibility to predators (e.g., Frank 1965) and disease (Harvell *et al.* 2002). Finally, sublethal thermal stress can reduce fitness by incurring physiological costs associated with protection and repair of cellular components (Somero 2002). Given the limitations of behavioural and physiological stress avoidance mechanisms, there may be important temperature-related selection on organismal morphology on rocky shores.

Intertidal limpets are an excellent model system for addressing the role of morphology in moderating thermal stress. All limpet shells are variations on a simple geometric shape – the cone. However, within this conical theme, there is broad variation in shell size, the shape of the aperture, the height : length ratio, and architectural features on the shell's surface. Some aspects of shell morphology appear to correspond to the limpet's thermal environment. For example, *Patella vulgata* and *P. aspera* living high on the shore tend to be higher spired than conspecifics living lower on the shore (Orton 1933; Ebling *et al.* 1962). Shell morphology is plastic in this genus, and *P. vulgata* transplanted from warmer microhabitats to a cooler microhabitat exhibited a change in their pattern of shell growth from high-spined to low-spined (Moore 1934). Among-species patterns of shell morphology are also apparent; generally speaking, high-shore and tropical limpet species tend to have higher spires and more shell architecture (ridges, etc.) than low-shore and temperate species (Vermeij 1973).

Vermeij (1973) hypothesized that these morphological characteristics were adaptations to thermally stressful conditions. For a limpet of a given volume, an individual with a taller shell will have a smaller area in contact with the substratum, thus reducing conduction when the limpet is sitting on a hot surface. Relatively tall shells may also project up into faster wind velocities, facilitating convective cooling. The addition of bumps, ridges, or other features on the shell will increase convective surface area and increase the rate at which excess heat is lost via convection (Johnson II 1975). Although these hypotheses are intuitive, the actual thermal significance of morphological variation in limpets has not been rigorously tested.

In this study, we investigate the relationship between limpet shell morphology and body temperature during aerial exposure at low tide. We compare three species that differ in both thermal niche and in morphological characteristics: *Lottia gigantea*, *P. vulgata*, and *Siphonaria gigas*. *L. gigantea* is very low-spined and lacks architectural features on the shell (Fig. 1). *Patella vulgata* and *S. gigas* are both high-spined, but they differ in shell architecture. Whereas *P. vulgata* possesses small and in many cases very faint radial ridges, *S. gigas* may possess dramatic raised ridges that run from the apex down the length of the shell (Fig. 1). The use of these three species allowed us to explicitly analyze the effectiveness of two morphological strategies: increased spire height, and architectural features that increase shell surface area. We used the morphological characteristics of these three species to parameterize a recently developed heat budget model (Denny & Harley 2006) and thereby to determine the importance of various morphological attributes in controlling predicted body temperature.

Methods

SPECIES CONSIDERED

To examine the role of shell morphology on predicted body temperature, we chose three species with contrasting shell characteristics:

L. gigantea, *P. vulgata* and *S. gigas*. We recognize that these three species do not represent all possible limpet morphologies; they do, however, represent opposing ends of the spectrum for our focal morphological comparisons (low- vs. high-spined and smooth vs. ribbed shells). Both *L. gigantea* and *P. vulgata* are prosobranch gastropods. *Siphonaria gigas* is a pulmonate gastropod; thus, although it has a conical shell, it is not a true limpet. Despite their taxonomic and physiological differences, the same biophysical principles apply to all three species. Ecologically, all three species are abundant, and all three are known to exert important top-down control of algal biomass on rocky shores (Levings & Garrity 1984; Lindberg *et al.* 1998; Jenkins *et al.* 1999). All three species also exhibit homing behaviour as adults; the snug fit of a limpet's shell to the substratum at the home scar reduces desiccation stress (Lowell 1984).

Lottia gigantea ranges from northern California to Baja, Mexico, and is characterized by a low-spined, relatively smooth shell. In Pacific Grove, CA, *L. gigantea* is predominantly found on steeply sloping surfaces and vertical walls, which remain cooler at low tide than gently sloping and horizontal surfaces (Denny *et al.* 2006). The LT_{50} of *L. gigantea* from Pacific Grove was 36.7 °C when limpets were exposed to a 3.5 h thermal ramp followed by an immediate return to seawater temperature, and 32.5 °C when limpets were exposed to a 3.5 h thermal ramp followed by a gradual 3.5 h cooling period (Denny *et al.* 2006; Miller 2008).

Patella vulgata occurs from Norway to Portugal, possesses a higher spined shell than *L. gigantea*, and can have weakly developed ribs or ridges. Morphological plasticity is well known in this species; individuals living higher on the shore and in warmer, more desiccating microhabitats have more highly spined shells than those animals living lower on the shore or in cooler, damper microhabitats (Orton 1933; Ebling *et al.* 1962). Experimental shading enhances *P. vulgata* grazing rates, which suggests that sublethal thermal stress may be important in this species (Thompson *et al.* 2004). *Patella vulgata* body temperatures can exceed 36 °C in the field (Davies 1970). Although this field maximum is well below the 42 °C lethal limit of the species, it approaches the temperature (37–38 °C) at which *P. vulgata* enters a heat coma (Evans 1948).

Siphonaria gigas is native to the tropical East Pacific from Mexico to northern Peru. *Siphonaria gigas* has a very high-spined shell, and often has well-developed ridges running from the shell's apex down to the aperture. Shell morphology in this species is also plastic; shells from a wave exposed area in Columbia were thicker and marginally ($P = 0.06$) shorter than shells from a nearby protected area (Giraldo-López & Gómez-Schouben 1999). *Siphonaria gigas* is able to maintain a body temperature lower than that of the substratum, and is also able to occupy more stressful microhabitats than sympatric species with smaller and lower spined shells (Garrity 1984). The thermal tolerance of *S. gigas* is not known, but body temperatures of 40 °C have been measured in the field (Garrity 1984). Like *L. gigantea*, *S. gigas* is found preferentially in cooler microhabitats such as crevices and vertical walls (Levings & Garrity 1984).

FIELD ESTIMATES OF BODY TEMPERATURE

Field estimates of organismal body temperatures can be obtained by placing temperature data loggers within objects, which mimic the morphological characteristics of the organism of interest. We constructed mimics for *L. gigantea*, *P. vulgata* and *S. gigas*, and deployed them in the species' native habitats. Mimics were constructed by embedding an iButton temperature data logger (Dallas Semiconductor) within an empty adult limpet shell using silicone, a technique that has been used with success previously (Helmuth &

Hofmann 2001). Limpet shells were obtained from live animals or, when empty shells in excellent condition were available, from the drift. All limpet shells used for mimics exhibited morphological characteristics (e.g., size, shape, colour, degree of erosion) similar to live limpets in the same habitat. Mimics were attached to the rock by the adhesive nature of the silicone, plus a small amount of marine epoxy (Zspar Splash Zone Compound), where necessary. All mimics were placed in the intertidal zone near living representatives of each target species. At all three outplant sites, structure-forming sessile organisms (upright algae, mussels, or large barnacles) were sparse and did not shade limpets or limpet mimics. Temperature loggers were set to record at 20-min intervals over the course of 4 weeks. *Lottia gigantea* mimics ($n = 8$) were deployed in Pacific Grove, CA, from 13 May to 6 June 2003. For this species, we collected simultaneous temperature point samples from adjacent limpets by inserting a thermocouple thermometer under the shell between the foot and the mantle. *Patella vulgata* mimics ($n = 4$) were deployed in Mothcombe, Devon, UK from 11 July to 1 August 2007. *Siphonaria gigas* mimics ($n = 3$) were deployed on Naos Island, Panama, from 11 July to 8 August 2004. Due to summer fog and the timing of low tides, May is among the hottest months for intertidal organisms in Pacific Grove (Helmuth *et al.* 2002). July is typically the hottest month of the year in southwestern England. Temperatures in Panama vary by only a few degrees over the course of the year.

THE HEAT BUDGET MODEL

The body temperature of an ectotherm can be modelled as the balance of several sources and sinks for heat (Denny & Harley 2006). Briefly, solar radiation absorbed by an animal will increase its body temperature. Heat can be gained or lost via long-wave radiation to the sky (or nearby objects) and via conduction between the organism and the substratum. Convection can also increase or decrease body temperature, but because maximum organismal temperatures are generally well in excess of air temperature, convection typically acts to cool an organism when it is hot. Although evaporative cooling can serve to reduce body temperatures of organisms exposed to air, limited body water may preclude this from being overly important in limpets (Denny & Harley 2006, but see Williams *et al.* 2005). Metabolic heat production is considered to be a trivial source of body heat and is ignored in the model. For a full description of the model and a validation of its predictions, see Denny & Harley (2006) and Denny *et al.* (2006).

Several morphological parameters determine the equilibrium temperature of a limpet. For example, shell colour determines the proportion of incoming solar radiation that is reflected by the animal. Conduction is proportional to the area of the foot, which in turn is proportional to the length and width of the shell. Convection varies with the surface area of the shell, which is determined by both its basic dimensions (length, width, height) and any surface architecture (e.g., ridges or bumps). To examine their importance to the equilibrium temperature of a limpet, these parameters can be modified in the heat budget model.

MODEL PARAMETERIZATION

We collected four to five shells for each of our three species. *Lottia gigantea* shells ($n = 5$) were collected in Pacific Grove, CA. *Patella vulgata* shells were collected from Porthcawl ($n = 2$) and Plymouth ($n = 2$), UK. *Siphonaria gigas* shells were collected from Toboga Island ($n = 2$) and Naos Island ($n = 3$), along the Pacific coast of

Panama. There were no readily apparent between-site differences in *P. vulgata* or *S. gigas* shells. The absorptivity of the shells was measured by a spectroradiometer with an integrating sphere (Li-Cor model 1800). Length, width and height were measured to the nearest of 0.1 mm with digital callipers. Limpet volume (the space enclosed by the shell plus the volume of the shell itself) was calculated by weighing solid casts of each shell (see below) and dividing by the density of the casting material (sterling silver; 10.4 g cm^{-3}). Surface area was measured by carefully covering the surface of a shell with a single layer of aluminium foil and calculating the area based on the weight of the foil. Four such estimates were made for each shell, and the results were averaged.

In order to calculate the importance of convection, it was necessary to measure a shell's heat transfer coefficient. To do this, we created sterling silver casts of each shell; silver was used because its high thermal conductivity ensured that any thermal gradients within the replica were negligible. The heat transfer coefficient was calculated from the rate at which a heated replica cooled (see Denny & Harley 2006 for methodological details) at windspeeds ranging from *c.* 0.25 – 5.2 ms^{-1} . These windspeeds were generated in a wind tunnel, the floor of which was tiled with plaster casts of a rough granite surface to mimic the boundary-layer turbulence likely to be experienced in the field. To investigate the importance of basic shell shape (i.e., the height : length ratio) in determining among-species variation in heat transfer coefficients, we also measured heat transfer coefficients for a series of standard copper cones (diameter = 25 mm) at wind speeds ranging from 0.25 to 5.0 ms^{-1} . The cones ($n = 5$) spanned the natural range of limpet shell height : diameter ratios. Heat transfer coefficients were measured once per cone (or limpet cast) per windspeed.

We characterized the effects of morphology and windspeed on heat loss by calculating two dimensionless numbers: the Reynolds number (Re), and the Nusselt number (Nu). The Reynolds number is the ratio of viscous to inertial forces on a body moving relative to a fluid such as air or water. It is a factor of the object's size and the velocity, density and viscosity of the moving fluid. For an object of a given size in a fluid of a given density and viscosity, higher Re indicates higher velocity. The Nusselt number is the ratio of heat transfer by convection to heat transfer by conduction. Higher values of the Nusselt number reflect greater rates of convective heat loss to a moving fluid. In general, the faster the flow speed, the higher the rate of convective heat loss. Thus, the Re : Nu relationship is expected to have a positive slope.

The dimensions of representative limpets ($n = 4$ – 5 per species) were used in the model. Mean (\pm SD) *L. gigantea* lengths and heights were 43.1 ± 6.7 (range: 35.5 – 51.8) and 12.3 ± 2.4 (range: 9.6 – 15.1) mm, respectively. *Patella vulgata* lengths and heights were 34.4 ± 1.5 (range: 33.0 – 36.2) and 14.2 ± 2.0 (range: 12.6 – 16.8) mm, respectively. *Siphonaria gigas* lengths and heights were 42.4 ± 1.9 (range: 40.2 – 44.8) and 21.7 ± 3.5 (range: 18.0 – 25.6) mm, respectively. Because we were interested in variation in shape rather than in shell colour, we standardized shell absorptivity in the model across individuals and species. To facilitate comparisons with earlier work, we chose a mean absorptivity value for *L. gigantea* (0.679 ; see Denny & Harley 2006). It should be noted, however, that colour can be highly variable in some species; individual *S. gigas* shells were both the most and least absorptive of any measured limpet shell (Harley, unpublished data), which may reflect selection for crypsis in non-thermally stressful microhabitats (Etter 1988).

Other model inputs include environmental conditions (wind speed, solar irradiance, and air and water temperatures), thermal properties of the granite substratum, and location and orientation

within the intertidal zone (e.g. vertical shore level, substrate orientation, etc.). Environmental data were provided by a 5-year time series from the Hopkins Marine Station in Pacific Grove, CA (see Denny *et al.* 2006 for details). We ran the model for a limpet on a horizontal surface at 1.5 m above mean lower low water for conditions representative of a wave protected shore. Full consideration of variation in wave exposure, vertical position on the shore, and limpet orientation is provided elsewhere (Denny *et al.* 2006). For each model run, we recorded the maximum temperature as well as the cumulative number of hours spent above $28 \text{ }^\circ\text{C}$. A threshold of $28 \text{ }^\circ\text{C}$ was chosen because this temperature is probably reached by all three species in the field (see below), and because it approximates the heat shock induction temperature for several Californian limpets (Sanders *et al.* 1991; Miller 2008).

STATISTICAL ANALYSES

All statistical analyses were performed in JMP 5.1 (SAS Institute). Cumulative exposure to temperatures above $28 \text{ }^\circ\text{C}$ was log transformed to improve the distribution of the residuals. Reynolds and Nusselt numbers were ln transformed by convention.

Results

GEOGRAPHIC THERMAL COMPARISONS

Field temperatures of limpet mimics

Horizontal *L. gigantea* mimics in California reached temperatures up to $35.5 \text{ }^\circ\text{C}$ (Fig. 2a). However, most *L. gigantea* at this site (and therefore the majority of mimics) were found on steeply sloping substrates (slope $> 45^\circ$) where they remain considerably cooler. Typical daily maximum temperatures, averaged across mimics, ranged from 15.6 on a cool day to $22.6 \text{ }^\circ\text{C}$ on a warm day. *Patella vulgata* mimics reached temperatures as high as $31 \text{ }^\circ\text{C}$, and daily maxima averaged across mimics ranged from 15.9 to $29.8 \text{ }^\circ\text{C}$ (Fig. 2b). *Siphonaria gigas* mimics in Panama reached much higher temperatures (Fig. 2c). The maximum mimic temperature recorded in the field was $48.0 \text{ }^\circ\text{C}$. Daily maximum temperatures averaged across mimics ranged from 28.5 to $42.8 \text{ }^\circ\text{C}$.

The *L. gigantea* mimics were good predictors of live *L. gigantea* body temperatures (linear fit: $T_{\text{mimic}} = 1.22; T_{\text{limpet}} - 4.00; F_{1,45} = 1184, P << 0.001, R^2 = 0.963$). However, mimics tended to slightly underestimate limpet body temperatures at low temperature and slightly overestimate limpet body temperature at high temperature (e.g., mimic temperatures were *c.* $0.7 \text{ }^\circ\text{C}$ too low when body temperatures were $15 \text{ }^\circ\text{C}$, and *c.* $3.7 \text{ }^\circ\text{C}$ too high when body temperatures were $35 \text{ }^\circ\text{C}$). This suggests that a thermal gradient developed between the rock and the position of the iButtons embedded within the mimics; in live limpets, blood circulation promotes the transport of heat and prevents the establishment of large thermal gradients within the animal. We were not able to ground-truth the accuracy of the *P. vulgata* or *S. gigas* mimics in the field. However, the iButtons were approximately the same distance from the substratum in both types of mimics, and the insulating effect of the silicon was likely similar for these species as well.

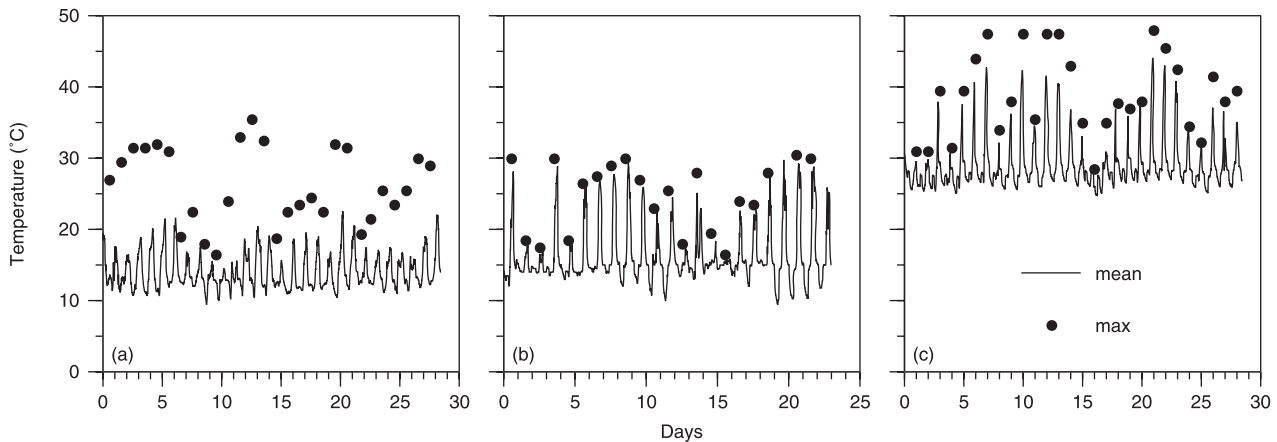


Fig. 2. Temperature of limpet mimics in the field. (a) *Lottia gigantea* mimics ($n = 8$) deployed in Pacific Grove, California, USA. (b) *Patella vulgata* mimics ($n = 4$) deployed near Plymouth, England. (c) *Siphonaria gigas* mimics ($n = 3$) deployed on Naos Island, Panama. The solid lines represent the mean temperature of all limpets, recorded once every 20 min. The solid circles represent the highest daily maximum temperature recorded across all mimics.

Using the relationship measured for *L. gigantea* for all three species, we can estimate that *L. gigantea* and *P. vulgata* reached body temperatures up to 32.4 and 28.7 °C during the measurement period, respectively, whereas *S. gigas* reached temperatures as high as 42.6 °C. Using the same corrections, typical daily maximum body temperatures during the measurement period would have ranged from 16.1 to 21.8 °C in *L. gigantea*, 16.3 to 27.7 °C in *P. vulgata*, and 26.6 to 38.4 °C in *S. gigas*.

THE IMPORTANCE OF SHELL MORPHOLOGY

Heat loss from standard cones

In standard cones, the heat transfer coefficient (h_c) varied significantly with both wind velocity and the cone height : diameter ratio (multiple regression: wind velocity effect $F_{1,32} = 1020$, $P < 0.0001$; $h : d$ ratio effect $F_{1,32} = 56.1$, $P < 0.0001$). Together, these two variables explained virtually all of the variation in h_c ($R^2 = 0.969$). All cones lost heat more rapidly at higher wind velocities, as would be expected (Fig. 3a). Among the different cone shapes, the heat transfer coefficient increased with increasing height : diameter ratio to a certain point (height : diameter ratio of 0.63), after which no additional increase in the heat transfer coefficient was observed (Fig. 3a).

A similar pattern was observed in the $Re : Nu$ relationship. The effects of $\ln(Re)$ and cone shape were both significantly related to $\ln(Nu)$ (ANCOVA: cone shape $F_{4,44} = 209$, $P < 0.0001$; $\ln(Re)$ $F_{1,44} = 17\,200$, $P < 0.0001$). Higher spired cones lost heat via convection more effectively (as indicated by a higher Nu) at any given size/wind speed (as indicated by variation in Re) (Fig. 3b). The combination of cone shape and $\ln(Re)$ explained virtually all of the variation in $\ln(Nu)$ ($R^2 = 0.997$).

Interspecific morphological variation

The height : length ratio of the shell varied significantly among species (ANOVA, $F_{2,11} = 23.2$, $P = 0.0001$; Fig. 4a), with

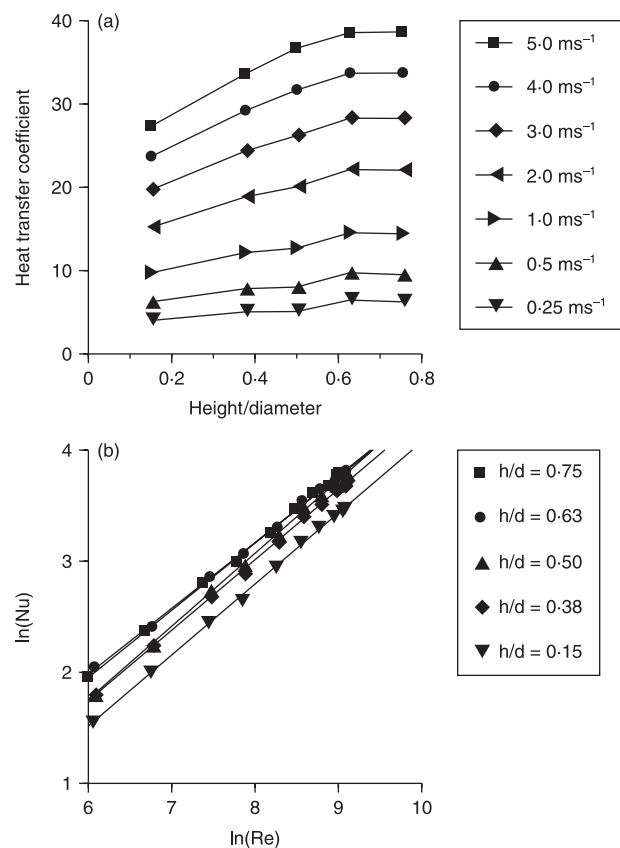
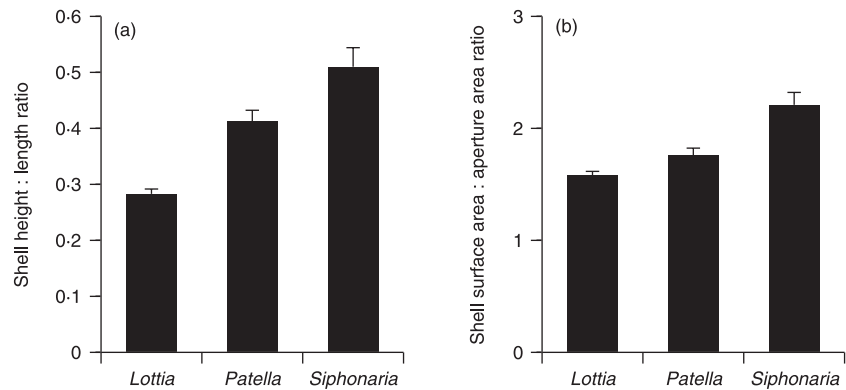


Fig. 3. Relationship between heat loss and wind speed for five standard cones that vary in their height : diameter ratio. (a) Heat transfer coefficients vs. wind velocity. (b) Nusselt number vs. Reynolds number.

L. gigantea having the lowest ratio (i.e. the lowest spired shells), and *S. gigas* having the highest ratio (i.e. the highest spired shells). *Siphonaria gigas* shells were significantly higher spired than *P. vulgata* shells, which in turn were significantly higher spired than *L. gigantea* shells (Tukey HSD). The

Fig. 4. Morphological differences among species. (a) The ratio of shell height to shell length, which is an index of how low- or high-spired the shell is. (b) The ratio of shell surface area to aperture area, which is an index of the degree of architectural complexity (e.g. ridges) present on the shell's surface. Error bars are standard error.



surface area to aperture area ratio also varied among species (ANOVA, $F_{2,11} = 16.6$, $P = 0.0005$; Fig. 4b). *Siphonaria* shells had a greater relative surface area than either *L. gigantea* or *P. vulgata* (Tukey's HSD). The latter two species were statistically indistinguishable.

Morphology and heat loss

As expected, the $Re : Nu$ relationship was positive for all limpet shells, indicating that higher wind speeds were correlated with more rapid rates of heat loss. However, the $Re : Nu$ relationship depended on species identity (Fig. 5). Although Nusselt numbers overlap broadly among the three species at low wind velocities, *S. gigas* shells lose heat slightly faster than other species at higher wind velocities (i.e. the highest Reynolds numbers). The slope of the $Re : Nu$ relationship varied among species (ANCOVA, species $\times \ln(Re)$ interaction: $F = 5.06$, $P = 0.0086$), being steepest for *S. gigas*. However, the relationship between species identity and convective heat loss was not strong at low-to-moderate wind velocities that are likely to accompany high body temperatures in the field.

Predicted temperatures

We used a heat-budget model (Denny & Harley 2006) and 5 years of environmental data from Pacific Grove, CA (Denny *et al.* 2006), to hindcast limpet body temperatures for the environment at the Hopkins Marine Station. The maximum temperature attained over the 5-year period differed among species (ANOVA, $F_{2,11} = 11.0$, $P = 0.0024$; Fig. 6a). *Lottia gigantea* were predicted to reach significantly higher temperatures than *S. gigas*, with *P. vulgata* being intermediate and statistically indistinguishable from either (Tukey's HSD). Species also differed in cumulative time spent above 28 °C (Fig. 6b; ANOVA, $F_{2,11} = 15.0$, $P = 0.0007$). *Lottia gigantea* was exposed to temperatures exceeding 28 °C for longer than the other two species, which were statistically similar (Tukey's HSD).

Predicted body temperatures were largely determined by shell morphology. Across species, shells with lower spires (i.e., lower height : length ratios) were predicted to reach higher temperatures (Fig. 7a), as were the shells with lower surface area : aperture area ratios (Fig. 7b). Once either of these

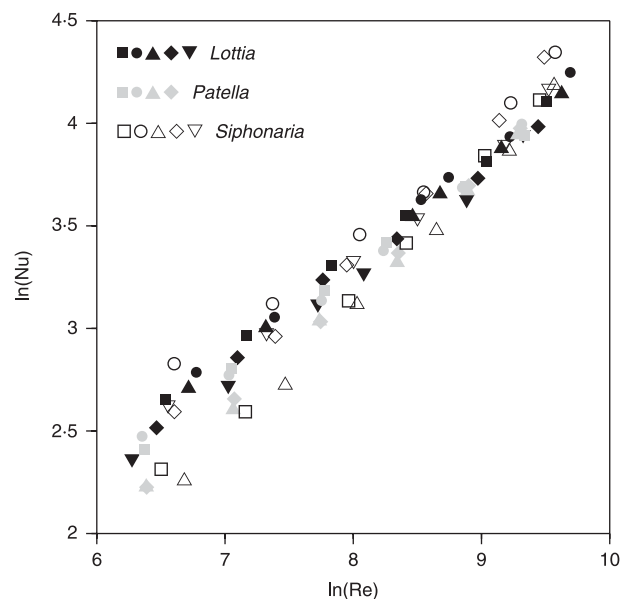


Fig. 5. Interspecific comparisons of the Reynolds number: Nusselt number relationship, which is related to the relative effectiveness of convective heat loss. Individual symbols represent individual shells. Note that *Siphonaria gigas* has the highest $Nu : Re$ ratio at high Reynolds numbers (i.e. high wind speeds), but that all species are similar at low Reynolds numbers (i.e. low wind speeds).

morphological metrics was included as a covariate, the main effect of species was no longer significant (Table 1). Similar patterns were seen for cumulative time spent above 28 °C; lower spires and lower surface area : aperture area ratios were correlated with longer cumulative exposure times (Fig. 7c,d). Again, the effect of species was not significant after these morphological metrics had been incorporated into the statistical model (Table 1). If the non-significant effect of species is ignored, the height : length ratio explains most of the variance in maximum temperature (linear regression, $R^2 = 0.914$) and time spent above 28 °C ($R^2 = 0.889$). The surface area : aperture area ratio also is highly correlated with maximum temperature and time spent above 28 °C, but the relationships were somewhat weaker (linear regression, $R^2 = 0.836$ and 0.773 , respectively).

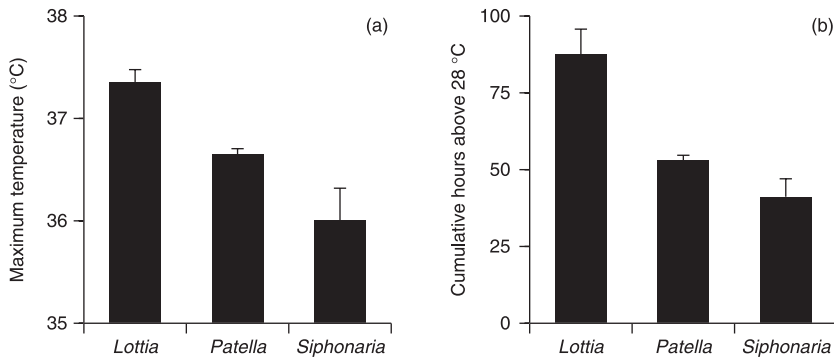


Fig. 6. Predicted thermal patterns across the 5-year environmental time series. (a) Maximum temperature reached. (b) Cumulative number of hours spent above 28 °C. Error bars are standard error.

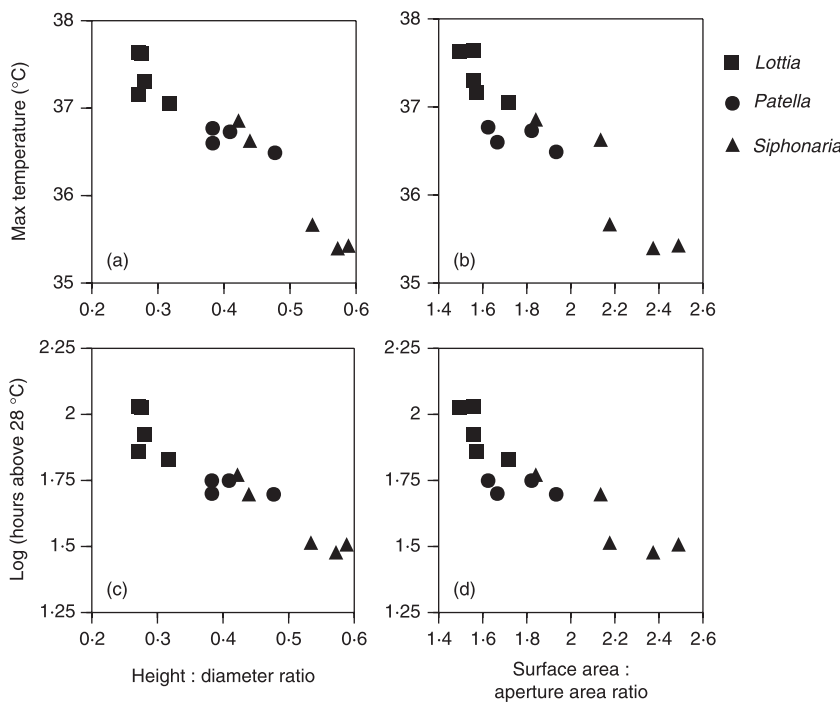


Fig. 7. Relationship between morphological parameters and patterns of predicted body temperatures. (a) Shell height : length ratio vs. maximum predicted temperature. (b) Shell surface area : aperture area ratio vs. maximum predicted temperature. (c) Shell height : length ratio vs. predicted time spent above 28 °C over 5 years. (d) Shell surface area : aperture area ratio vs. predicted time spent above 28 °C over 5 years.

Table 1. Results of ANCOVAs comparing species identity and shell morphology with maximum predicted temperature and predicted time spent above 28 °C. Significant results are highlighted in bold

Dependent variable	Independent variables	d.f.	F	P
Maximum temperature	Species	2	0.95	0.419
	Height : length ratio	1	39.7	< 0.0001
Maximum temperature	Species	2	1.85	0.208
	Surface area : aperture area ratio	1	20.1	0.0012
Log (cumulative hours above 28 °C)	Species	2	0.13	0.881
	Height : length ratio	1	16.9	0.0021
Log (cumulative hours above 28 °C)	Species	2	3.92	0.055
	Surface area : aperture area ratio	1	12.8	0.0050

Discussion

LIMPET ADAPTATIONS TO THERMAL STRESS

The rocky intertidal zone features some of the most extreme thermal conditions on the planet, and the global distribution of limpets spans habitats from arctic sites that drop to well

below freezing during the winter to tropical sites where rock temperatures can exceed 50 °C. Thermal stress in general, and high temperature extremes in particular, are likely to be a strong selective force on limpets. Indeed, limpet mortality following periods of thermal stress have been observed on tropical and temperate shores around the world (Lewis 1954; Frank 1965; Sutherland 1970; Kohn 1993; Williams *et al.*

2005; Harley 2008). To cope with extreme high temperatures, limpets have evolved a variety of behavioural, physiological and morphological strategies.

In some cases, limpets are able to take advantage of behavioural strategies. In Hong Kong, *Cellana grata* migrates up to one vertical meter with the advance and retreat of the tide, thus remaining within the swash zone for a greater proportion of the day (Williams & Morrill 1995). Many limpets also tend to occupy crevices and vertical substrata, which remain cooler than flat, gently sloping surfaces (Levings & Garrity 1984; Williams & Morrill 1995; Gray & Hodgson 2004). When stranded at low tide in an unfavourable thermal microhabitat, some limpets will 'mushroom' by raising the margin of their shell off the substratum and exposing their soft tissues to the air (Garrity 1984). This behaviour is thought to be an emergency attempt to evaporatively cool; it effectively lowers the body temperature of *C. grata* by c. 2 °C for 1–2 h before water loss becomes severe and evaporative cooling is no longer effective (Williams *et al.* 2005). Mushrooming may also change the heat transfer coefficient by raising the shell into faster windspeeds. Finally, some limpets contract their foot to reduce the area of conductive heat transfer when the substratum becomes hot (Garrity 1984). The relative importance of these postural changes to limpet body temperature remains unexplored.

Limpets also respond to the thermal environment via physiological tolerance mechanisms. *Patella* spp. have a higher thermal tolerance in the summer than in the winter (Evans 1948). This shift may reflect higher constitutive levels of heat shock proteins during hotter times of the year, as has been shown for *L. digitalis* (Halpin *et al.* 2002). Limpets from high shore populations have higher maximum thermal tolerance than conspecifics from lower on the shore (Hardin 1968; Wolcott 1973). This may also relate to constitutive heat shock protein levels; *L. digitalis* exhibits higher constitutive levels of Hsp70 in high-shore individuals than mid-shore individuals (Halpin *et al.* 2002). The existence of among-species variation in upper thermal tolerance (e.g., Evans 1948; Wolcott 1973) indicates that longer-term adaptation and evolution of physiological pathways is important with regards to thermal stress (see, e.g., Sanders *et al.* 1991). A comparison of available data on the upper thermal limits in the three species studied here (*S. gigas* > *P. vulgata* > *L. gigantea*; e.g., Evans 1948; Garrity 1984; Denny *et al.* 2006) provides further evidence that physiological tolerances reflect the thermal environment of these species.

Two types of morphological adaptations have also been postulated to result in lower body temperature on hot days: (i) high-spined shells and (ii) architectural features such as bumps and ridges (Vermeij 1973). It is evident that both mechanisms operate, but that only one offered any real protection from extreme body temperatures. Animals with the highest spined shells were predicted to remain the coolest (Fig. 7), and the ratio of shell height to shell length explained > 91% of the variation in maximum predicted body temperature regardless of species identity. As predicted, shell architecture, in this case ridges on *S. gigas* shells, did increase rates of convective heat loss; this has also been demonstrated in a

comparison of lightly ribbed *L. austrodigitalis* to more heavily ribbed *L. scabra* (reported as *Acmaea digitalis* and *Acmaea scabra*, respectively) (Johnson II 1975). However, we found that this mechanism was only effective at the highest wind speeds. Because high winds generally prevent body temperatures from rising much above air temperature, windy days are unlikely to be particularly thermally stressful. The ineffectiveness of ridges as a cooling mechanism on potentially stressful days (i.e. at low to moderate wind speeds) may explain why *S. gigas*, which has ridges that can be extremely striking, was not predicted to reach lower long-term thermal maxima than *P. vulgata*, which has small ridges if any.

OTHER CONSTRAINTS ON MORPHOLOGY

The evolution of limpet shell morphology is guided by several selective forces in addition to temperature, such as desiccation, hydrodynamic stress and biological interactions. Limpets can reduce water loss by reducing the circumference of their aperture, for example, by having a more circular aperture and a higher spire (Lowell 1984). Spire height also influences drag and lift, suggesting that limpet species that occupy exposed shorelines may have shells that minimize the hydrodynamic forces felt by the animal. However, *L. gigantea*, which is often found on very exposed shores, has a shell that is far from optimally shaped if wave force were the main selective force at work (Denny 2000; Denny & Blanchette 2000). Higher spired shells may also be more susceptible to predation by birds, which often peck at a shell's margin to overturn a limpet, and crabs, which cannot effectively grip low-spined shells (Lowell 1986). In addition to considerations relevant to the transmission of force (i.e. from beaks or claws) to limpet shells, higher spired limpets also have smaller attachment areas relative to their size and thus are less resistant to prying predators (Lowell 1986). The success of crab predation is often negatively dependent upon limpet shell thickness (Lowell 1986), and heavy ribs could reinforce the shells of species such as *S. gigas* and thus reduce mortality due to predation. Shell architecture may also influence vulnerability to fish predation; in South Africa, clingfish select smoother-shelled limpet species in laboratory trials (Lechanteur & Prochazka 2001). In general, differences in shell strength between tropical and temperate limpets reflect the different forces they are likely to experience when attacked by predators (Lowell 1987). Finally, inter- and intra-specific interactions among limpets may also be important. *Lottia gigantea* defend their territories against other limpets by repeatedly ramming them. The strength with which they can push an object is presumably proportional to the attachment area of the foot. Thus, the low-spined morphology of *L. gigantea* may in part reflect selection for a large foot area to body size ratio (Denny & Blanchette 2000).

IMPLICATIONS

It is well established that the morphology of ectotherms can influence their heat budget, and that organisms can influence their body temperatures via behavioural changes in posture

(Heath 1970). Morphologies that are not behaviourally plastic (e.g. molluscan shells) are also under selective pressure from the thermal environment. Patterns of limpet shell morphology across latitudinal and intertidal (vertical) gradients suggest that thermal stress selects for taller shells and for the development surface architecture (Vermeij 1973). Our heat budget models support both of these hypotheses, although the benefits of surface architecture for convective cooling are likely to accrue only at relatively high wind speeds and are therefore questionable.

Limpets are not the only organisms for which these results may pertain. Barnacles are also roughly conical and have large areas of attachment to the substratum, which facilitate conductive heat exchange. Although the morphology of intertidal barnacles is known to vary with interactions with predators and competitors (Lively 1986; Bertness, Gaines & Yeh 1998), the extent to which barnacles are under morphological selective pressure via the thermal environment remains unexplored. Encrusting organisms – essentially cones with a height of zero – are also appropriately described by the heat budget model used here (Denny & Harley 2006). In the Northeast Pacific, the crustose coralline alga with the highest intertidal distribution (*Pseudolithophyllum neofarlowii*) is light in colour, which presumably reflects incoming solar radiation, and possesses a rugose surface which could enhance convective cooling. As with barnacles, the extent to which encrusting organisms' morphology represents an adaptation to the thermal environment remains unclear.

The establishment of a link between limpet morphology and maximum body temperatures suggests that there may be adaptive scope with regards to thermal stress, particularly for those species that do not currently have high spires. Along with physiological and behavioural adaptations, the plastic or evolved changes in shell morphology may allow limpets to offset some of the predicted increases in emersed body temperature due to global climate change. However, these changes, if they occur, may be limited by countervailing ecological and genetic constraints.

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