Thermal Physiology and Vertical Zonation of Intertidal Animals: Optima, Limits, and Costs of Living[1]

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SYNOPSIS.   Temperature's pervasive effects on physiological systems are reflected in the suite of temperature-adaptive differences observed among species from different thermal niches, such as species with different vertical distributions (zonations) along the subtidal to intertidal gradient. Among the physiological traits that exhibit adaptive variation related to vertical zonation are whole organism thermal tolerance, heart function, mitochondrial respiration, membrane static order (fluidity), action potential generation, protein synthesis, heat-shock protein expression, and protein thermal stability. For some, but not all, of these thermally sensitive traits acclimatization leads to adaptive shifts in thermal optima and limits. The costs associated with repairing thermal damage and adapting systems through acclimatization may contribute importantly to energy budgets. These costs arise from such sources as: (i) activation and operation of the heat-shock response, (ii) replacement of denatured proteins that have been removed through proteolysis, (iii) restructuring of cellular membranes ("homeoviscous" adaptation), and (iv) pervasive shifts in gene expression (as gauged by using DNA microarray techniques). The vertical zonation observed in rocky intertidal habitats thus may reflect two distinct yet closely related aspects of thermal physiology: (i) intrinsic interspecific differences in temperature sensitivities of physiological systems, which establish thermal optima and tolerance limits for species; and (ii) 'cost of living' considerations arising from sub-lethal perturbation of these physiological systems, which may establish an energetics-based limitation to the maximal height at which a species can occur. Quantifying the energetic costs arising from heat stress represents an important challenge for future investigations.

INTRODUCTION

The latitudinal and vertical patterning of species' distributions commonly reflects gradients or discontinuities in environmental temperature. For example, along the subtidal to intertidal gradient, species replacements are common, and there are many examples of one species in a genus replacing a congener over relatively small vertical ranges where thermal gradients exist (Gowanlock and Hayes, 1926; Broekhuysen, 1940; Evans, 1948; Wolcott, 1973; Newell, 1979; Garrity, 1984; Tomanek and Somero, 1999; Stillman and Somero, 2000). Because of the pervasive effects of temperature on physiological systems, the tight linkage observed between environmental temperature and species' distribution patterns is to be expected, especially for ectothermic species whose body temperatures may vary widely on seasonal and tidal cycle time scales (Helmuth, 1998, 1999). Virtually all biological structures and processes are affected by temperature, and, for this reason, temperature-adaptive variation that establishes different thermal optimal and thermal limits among species is widespread (Hochachka and Somero, 2002). Much of our initial knowledge about this adaptive variation came from studies of species that are widely divergent phylogenetically. Although studies of widely diverged species have revealed major adaptive patterns, they generally have not provided evidence concerning the role of adaptation to temperature in establishing fine-scale patterns of zonation, such as that seen along the subtidal-intertidal gradient. To address the latter question, it has proven useful to compare closely related species, especially congeners that exhibit temperature-related patterns of zonation. Studies of congeners allow adaptive variation to be delineated clearly, independent of effects of phylogeny (see Stillman and Somero, 2000). The chief objective of this review is to illustrate how the study of congeneric species found at different tidal heights along the subtidal-to-intertidal axis has provided especially clear insights into the roles of temperature adaptation in fine-scale spatial patterning, including the establishment of the upper limits of distribution in rocky intertidal habitats.

In this review of the thermal physiology of rocky intertidal organisms, two distinct, yet closely related, issues related to the role of temperature in establishing vertical zonation will be addressed. First, I examine temperature-adaptive physiological differences among species, which are instrumental in establishing their thermal optima and thermal tolerance limits and, thereby, their different vertical zonations. A variety of physiological systems are examined, including systems that are genetically fixed and not subject to acclimatization (seasonal or diurnal) and others that are phenotypically plastic and can be adaptively modified during the individual's lifetime, in some cases during the course of a single tidal cycle (Williams and Somero, 1996). Second, I consider how thermal damage to temperature-sensitive structures and processes, followed by its repair, and the acclimatization of phenotypically plastic systems affect the 'cost of living' faced by organisms

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with different vertical zonations. I show that, whereas we have made considerable progress in delineating the adaptive modifications of physiological, biochemical, and molecular traits that may be responsible for establishing thermal optima and thermal limits, a high level of uncertainty remains about the role that thermal effects on bioenergetics plays in vertical patterning. Although it is not possible at present to quantify the bioenergetic costs of thermal stress, there is increasing evidence that these costs play important roles in establishing patterns of zonation. Thus, the long-standing hypothesis that predicts a pivotal role for abiotic factors like temperature in restricting upper distributions limits (see Connell, 1961) will be seen to be supported by many of the recent findings discussed in this review.

In my analysis, I will take a reductionist approach to illustrate how differences observed at the levels of the whole organism, organ function, cellular activity, organelle function, biochemical pathways, and molecular-level phenomena all reflect temperature-adaptive differences among congeneric species. This review of adaptive variation is designed to at once show the scope of temperature-adaptive change and pinpoint many of the sites of thermal perturbation that are likely to incur substantial costs in energy for repair or adaptive modification. As indicated above, I will focus on congeneric species that differ in their vertical distributions and allow an especially clear analysis of adaptive variation related to temperature. Importantly for the goal of developing general principles about thermal effects and distribution patterns, the many cases in which replacement of one congener by another is found across the subtidal to intertidal gradient allow one to conduct a taxonomically broad analysis. This review will examine congeneric groups of snails, mussels, crabs, abalone, and fish, in an effort to reach general conclusions that may be applicable across the animal kingdom and, for many ubiquitous physiological traits, for other types of organisms as well.

WHOLE ORGANISM THERMAL TOLERANCE CORRELATES WITH VERTICAL POSITION ALONG THE SUBTIDAL TO INTERTIDAL GRADIENT

Measuring heat death of animals is technically quite simple. However, because the intensity of heat stress is a function of several variables, including the past thermal history of the specimens, the magnitude of the increase in temperature, the rate at which heating occurs, and the duration of exposure to heat, valid comparisons among studies often are difficult if not impossible to make. However, when consistent experimental treatments are employed, striking patterns relating adaptation (or acclimation) temperature and heat tolerance are observed, as has been emphasized by other contributors to this symposium (Stillman, 2002; Tomanek, 2002). Figure 1 presents data on three groups of marine invertebrates for which congener replacement patterns along the subtidal to intertidal gradient are found. In all cases, there is a strong corre-
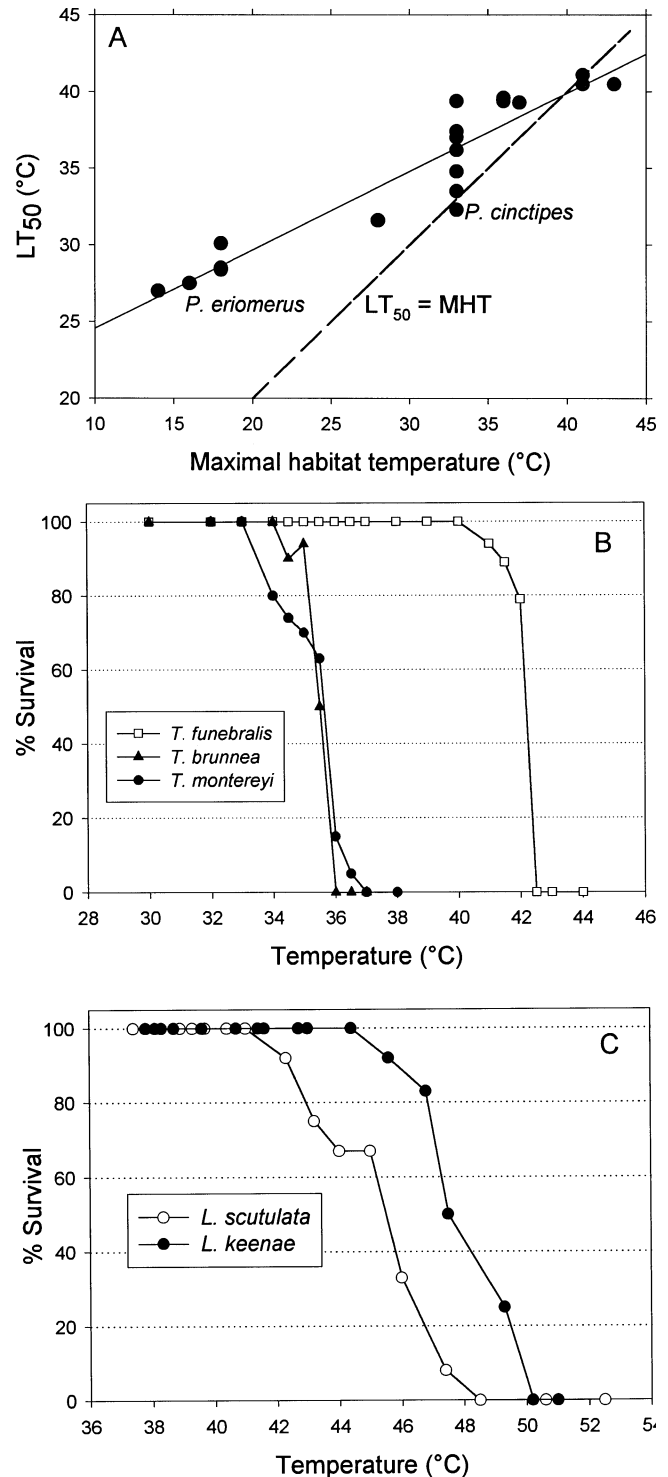

FIG. 1. Differences in whole organism heat tolerance between congeneric species found at different heights along the subtidal to intertidal gradient. Upper panel: temperatures at which 50 percent lethality occurred (LT_{50}) for 19 species of porcelain crabs (genus *Petrolisthes*) (Stillman and Somero, 2000). The dashed line labeled LT_{50} = MHT is a line for which lethal temperature equals the maximal habitat temperature (MHT). Middle panel: effects of heating at a rate of 1°C per 12 min on survival of congeners of *Tegula* (Tomanek and Somero, 1999). Lower panel: effects of thermal exposure (3 hr of exposure under immersion in seawater, followed by 45 min of recovery) on survival of congeners of *Littorina* (M. Phillips and G. N. Somero, unpublished data).
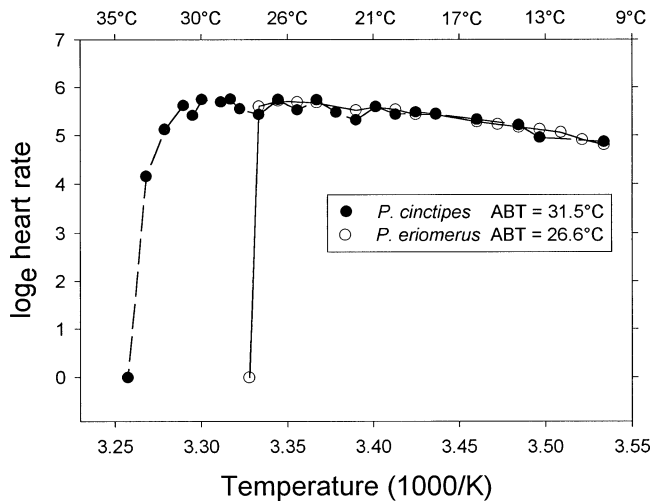
FIG. 2. Thermal limits of heart rate in *Petrolisthes cinctipes* and *P. eriomerus*. Temperatures of immobilized crabs were increased at a rate of 1°C per 15 min, a heating rate that mimics heating under extreme field conditions. Responses of single individuals of each species are shown. Arrhenius break temperature (ABT) values are averages of 6 individuals for each species. (Figure modified after Stillman and Somero, 1996).

lation between increasing tidal height and resistance to high temperature. For porcelain crabs (genus *Petrolisthes*), 19 species from different latitudes and vertical positions show a highly significant correlation between upper lethal temperature (LT_{50}, the temperature at which 50% mortality occurs) and maximal habitat temperature (MHT), a trend that is independent of phylogenetic influences (Stillman and Somero, 2000) (Fig. 1A). As shown by the line of equality between MHT and LT_{50}, those species of porcelain crabs that currently encounter the highest habitat temperatures generally are living nearer to their LT_{50} than congeners found in cooler habitats. Even in the case of a north temperate mid-intertidal species, *Petrolisthes cinctipes*, MHT approaches LT_{50} (see below). Species for which LT_{50} is close to MHT may be especially vulnerable to effects of global warming. The congruence between MHT and LT_{50} for several species of porcelain crabs contradicts earlier conjectures that intertidal animals, although differing in thermal tolerance in relation to their vertical zonation, do not encounter lethal thermal conditions under natural conditions (see Wolcott, 1973). In the context of physiological determinants of vertical zonation, note that many of the more cold-adapted porcelain crabs could not withstand the habitat temperatures experienced by more warm-adapted congeners. For instance, *P. eriomerus*, a low-intertidal to subtidal species whose latitudinal distribution overlaps that of *P. cinctipes*, dies at temperatures several degrees below the MHT of the latter, higher-occurring species.

For turban snails (genus *Tegula*) differences are observed between two subtidal to low-intertidal species (*T. montereyi* and *T. brunnea*) and a mid-intertidal species, *T. funebralis*, which have overlapping latitudinal

distributions (Tomanek and Somero, 1999) (Fig. 1B). As in the case of the two species of porcelain crabs just discussed, the two subtidal to low-intertidal species of *Tegula* are unable to withstand the habitat temperatures that may be experienced by their mid-intertidal relative during extremes of heat stress. For littorinid snails, which commonly are one of the highest-occurring intertidal invertebrates, thermal tolerance again is correlated with vertical position (Fig. 1C). The differences in thermal tolerance seen among these three sets of congeners, in conjunction with earlier studies that have shown correlations between vertical position and heat tolerance (Gowanlock and Hayes, 1926; Evans, 1948; Wolcott, 1973; Garrity, 1984), provide a strong basis for assigning an important role to temperature-adaptive physiological variation in establishing patterns of vertical zonation. Can we identify the temperature-sensitive physiological systems that underlie the whole organism effects just discussed and thereby explain mechanistically the strong correlation between thermal tolerance and zonation and the basis of heat death, especially in cases where LT_{50} and MHT closely coincide?

THERMAL LIMITS OF PHYSIOLOGICAL SYSTEMS: A MULTI-LEVEL SEARCH FOR "WEAK LINKS"

In view of the pervasive nature of thermal effects on biological structures and processes, there is a strong *a priori* basis for arguing that all links in the physiological chain are apt to be "weak," although some systems may prove more vulnerable to thermal stress than others (Hochachka and Somero, 2002). Surveys of diverse physiological systems, in a taxonomically varied range of species, have shown that this prediction is correct. Below, I present selected examples of thermally sensitive physiological systems, which offer especially clear cases of how thermal physiology contributes to patterns of zonation in rocky intertidal habitats. In some cases, the data help to explain mechanisms of acute heat death; in other cases, insights are presented concerning how sub-lethal effects of high temperature may lead to substantial increases in the organism's "cost of living."

Organ-level effects: heart function in porcelain crabs

The first example of temperature-adaptive variation among homologous physiological systems of congeners with different vertical positions involves the effect of acute temperature change on heart rate in two congeners of *Petrolisthes*. As shown in Figure 1A, *P. cinctipes* and *P. eriomerus* differ in LT_{50} by 5°C (32.3°C and 27.5°C, respectively), in accord with their different MHTs (approximately 33°C and 16°C, respectively) (Stillman and Somero, 1996, 2000). The responses of the hearts of these two congeners to rising experimental temperature differ in manners that suggest that heart function plays an important role in setting zonation patterns. For the less thermally tolerant species, *P. eriomerus*, heart rate falls dramatically at 26.6°C (Fig. 2). This is the Arrhenius "break" tem-

perature (ABT), the temperature at which a sharp discontinuity in slope occurs in an Arrhenius plot (log rate *vs* reciprocal of absolute temperature (K)). For the more heat-tolerant *P. cinctipes*, the ABT is several degrees higher (31.5°C). These data support three important conclusions about the role of physiological systems in vertical zonation. First, heart function of the lower-occurring *P. eriomerus* could not be sustained at the habitat temperatures encountered during hot periods by *P. cinctipes*. Thus, *P. eriomerus* could not invade the habitat of its higher-occurring congener. Second, for *P. cinctipes*, the ABT of heart function is essentially identical with the LT_{50}. Thus, heart failure may be one of the proximate causes of heat death in this species. Third, the close proximity of upper habitat temperature to the upper thermal limit of heart function suggests that this species may be living on the edge of ''heart failure'' in its native habitat. It remains to be established how plastic the ABT of heart function is during acclimatization. Whole organism thermal tolerance studies of crabs acclimated to 8°C and 18°C revealed that the LT_{50} increased by 4°C for *P. eriomerus* and 2°C for *P. cinctipes* (Stillman and Somero, 2000). Thus, crab hearts may be able to adjust their thermal tolerance (ABT) to at least some degree. Note that the more limited ability to warm acclimate observed for *P. cinctipes* is further evidence that this species lives near the limits of its thermal tolerance *in situ*. It is noteworthy that, even though *P. cinctipes* is more eurythermal than *P. eriomerus*, as shown by its greater ability to survive at low as well as at high temperatures (Stillman and Somero, 1996), it has a lower capacity for thermal acclimation to high temperatures than its more stenothermal congener.

Differences between these two congeners of *Petrolisthes* also were observed in cold tolerance of heart function: heart function of *P. cinctipes* is both more heat tolerant and more cold tolerant than that of *P. eriomerus* (Stillman and Somero, 1996). Thus, even though this review focuses primarily on heat stress, it is important to emphasize that tolerance of low temperature may also be important in establishing zonation along the subtidal-intertidal gradient. Species with the highest vertical distributions are apt to be more eurythermal than low-intertidal and subtidal species. As will be shown later in the contexts of protein thermal stability and membrane structure, the need for a mid- to high-intertidal species to function over a relatively wide thermal range may incur considerable physiological costs, which may serve to establish the upper limits of these species' distributions.

Cellular level effects: action potential generation

The nervous systems of animals are known to be especially sensitive to temperature change and to serve as critical sites in establishing upper and lower thermal limits (Cossins *et al.*, 1977; Cossins and Bowler, 1987; Hochachka and Somero, 2002). Although few data exist on the thermal sensitivities of neural function in differently adapted congeneric species found across
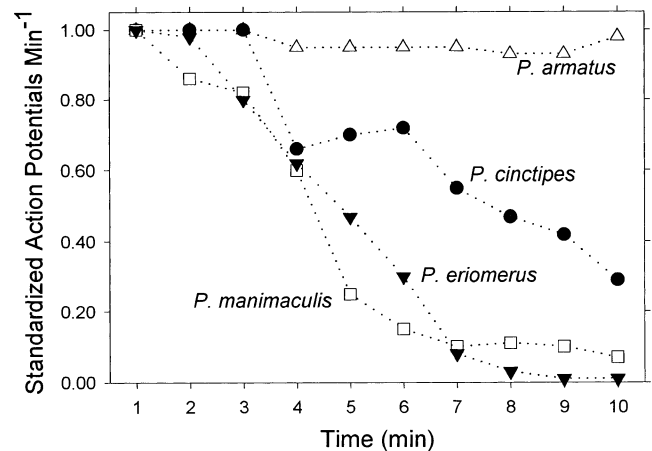

FIG. 3. Effects of incubation at 30°C on action potential generation in an abdominal mechanosensory nerve bundle for four congeners of *Petrolisthes*. *P. armatus* is an intertidal species found through the tropical Pacific; *P. cinctipes* is a cool temperate low- to mid-intertidal species; and *P. eriomerus* and *P. manimaculis* are cool temperate subtidal species. The number of action potentials per min are standardized to the number observed at the initiation of the 30°C exposure (Jessica Knape and GNS, unpublished data).

the subtidal-intertidal gradient, what information is available suggests the presence of significant adaptive variation related to habitat temperature. Recent studies of porcelain crabs have revealed interspecific variation that closely mirrors the differences seen for whole animal heat tolerance (Fig. 1A) and heart function (Fig. 2). One illustration of these effects is the influence of incubation temperature on rates of action potential generation in a mechanosensory nerve bundle (Fig. 3; Knape and Somero, unpublished observations; also see Stillman, 2002). At an experimental temperature of 30°C, the mechanosensory bundle of the subtropical species *P. armatus* shows stable firing over a longer period than was observed for the temperate mid-intertidal species *P. cinctipes*. In turn, *P. cinctipes* is less heat-sensitive than two subtidal to low-intertidal congeners, *P. eriomerus* and *P. manimaculis*, whose latitudinal distributions overlap with *P. cinctipes*. Although these initial studies of nerve function in differently thermally adapted congeners do not allow definitive conclusions to be drawn about the thermal limits of neural function, the trends shown in Figure 3 do provide a basis for concluding that temperature-adaptive variation in thermal sensitivities of nervous systems may contribute importantly to vertical zonation.

Organelle-level effects: mitochondrial respiration

Like the rate of heart beat, the rate of oxygen consumption by isolated mitochondria typically exhibits a ''break'' at some elevated temperature, the ABT, when values are displayed on Arrhenius plots. Figure 4 illustrates how the ABT of mitochondrial oxygen consumption varies with adaptation and acclimation temperature among a wide range of marine fishes and invertebrates (mollusks, annelids, and arthropods) (Dahl-
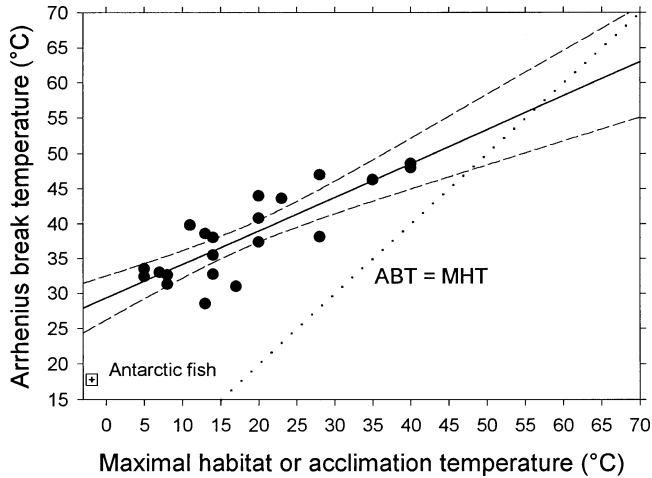
FIG. 4. Arrhenius break temperatures of respiration by mitochondria of diverse marine invertebrates and fishes. The data for the Antarctic fish *Trematomus bernacchii* are not included in the regression analysis. The dotted line is a line of equality between ABT and maximal habitat temperature. (Figure modified after Weinstein and Somero, 1998)

hoff and Somero, 1993; Weinstein and Somero, 1998). ABT increases with adaptation temperature among species and with acclimation temperature in single species (see below). However, the rise in ABT with increasing adaptation or acclimation temperature has a slope of less than unity, as shown by comparison of the regression line fit to available ABT data (solid line; the value for the Antarctic fish is not included in the regression analysis) and the line for which ABT = adaptation temperature (dashed line). The higher the temperature of adaptation, the nearer is the ABT of mitochondrial respiration to the species' adaptation temperature. The two lines intersect at a temperature between 55 and 60°C. This intersection suggests that, regardless of adaptation temperature, mitochondrial function cannot increase its thermal stability, as indexed by the ABT of respiration, above 55–60°C. These temperatures, then, may be the highest temperatures to which animals can adapt (Hochachka and Somero, 2002).

Within a species' normal (=physiological) range of body temperatures, the ABT of mitochondrial respiration may be subject to adaptive modification through acclimatization or acclimation. Phenotypic plasticity in the ABT of mitochondrial respiration has been observed in congeners of abalone (genus *Haliotis*) from different thermal habitats (Dahlhoff and Somero, 1993). Figure 5A illustrates variation in ABT among differently thermally acclimated individuals of four congeners that occur at different latitudes and at different vertical positions along the subtidal-to-intertidal gradient (see legend of Fig. 5 for distributions). When comparisons are made between field-acclimatized specimens (data not shown; see Dahlhoff and Somero, 1993), relatively warm-adapted species that occur into the intertidal zone, *e.g.,* green (*H. fulgens*) and black (*H. cracherodii*) abalone, have higher ABTs than more
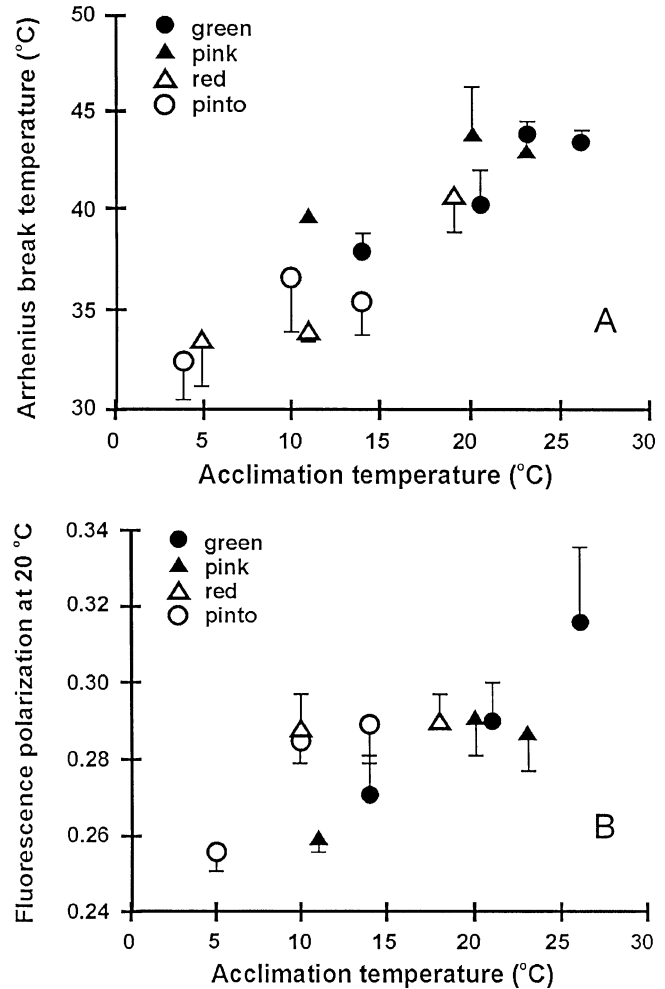

FIG. 5. (A) The effects of acclimation temperature on the Arrhenius break temperature of mitochondrial respiration for four species of abalone (genus *Haliotis*). Values are means ± 1 S.E.M. N = 4 for all species and temperatures except for pink abalone at 23°C and pinto abalone at 5C, where N = 3. Overlapping data points (red and pink @ 20°C and pinto and red @ 5°C) have been offset by 1°C for clarity. (B) The effects of acclimation temperature on mitochondrial membrane fluidity, as measured by the fluorescence polarization of the probe 1,6-diphenyl-1,3,5-hexatriene (DPH) for congeners of abalone. N = 3 for all species. The eurythermal green abalone (*H. fulgens*) occurs intertidally and encounters temperatures of 14–27°C over its biogeographic range. The pink abalone (*H. corregata*) occurs subtidally and encounters temperatures of 12–23°C over its biogeographic range. The red abalone (*H. rufesens*) is a cool temperate species with a habitat temperature range of 8–18°C. The pinto abalone (*H. kamtschatkana*) is the most northern-occurring species and encounters habitat temperatures between 4 and 14°C. See Dahlhoff and Somero (1993) for additional data on the distributions and thermal regimes of these species. (Figure modified after Dahlhoff and Somero, 1993)

cold-adapted species, *e.g.,* the pinto abalone (*H. kamtschatkana*), which is the northernmost species studied and which is found intertidally only at high latitudes (Gulf of Alaska and northern British Columbia). However, these interspecific differences are not fixed; all species examined (inadequate availability of black abalone precluded use of this species in acclimation studies) showed acclimatory plasticity in ABT. Following

acclimation to a common temperature, ABT values were similar among species. However, the thermal range over which ABT was modified was typically wider for more eurythermal species than for stenothermal species (compare green and pinto abalones).

Membrane-level effects: Alterations in bilayer static order

To determine the mechanistic basis of the observed differences in ABT among the differently acclimated abalone, the static order of the mitochondrial membranes was measured, using the technique of fluorescence polarization (Dahlhoff and Somero, 1993). This technique involves intercalation into the membrane bilayer of a fluorescent probe molecule such as 1,6-diphenyl 1,3,5-hexatriene (DPH). The static order of the bilayer determines the mobility of the probe, such that the strength of the polarization signal is directly related to the rigidity of probe's microenvironment. A more rigid (less fluid) microenvironment prevents the probe from moving freely, thereby enhancing the probe's ability to emit polarized light in an orientation that can be detected by the fluorescence spectrophotometer.

As shown in Figure 5B, there was a generally similar pattern in the responses of ABT and DPH polarization intensity to acclimation in the four species of abalone. Rising acclimation temperature led to increases in ABT and generally parallel increases in the rigidity of the membrane lipid bilayer (as shown by stronger DPH polarization signals). The term ''homeoviscous adaptation'' is commonly used to refer to the stability in membrane static order that is acquired during adaptation or acclimation to temperature (see Hazel, 1995). Broad interspecific comparisons, as well as studies of differently acclimated specimens, have shown that membrane static order is strongly conserved at normal body temperatures. There appears to be an optimal range of membrane fluidity and phase that allows such critical membrane functions as active transport, enzymatic activity, and exocytosis to be conserved at physiological temperatures (Hazel, 1995).

The changes in membrane static order, the homeoviscous adaptations, observed in mitochondria from differently acclimated abalone were likely the consequence of several types of alterations in membrane lipid composition. Comparisons of membrane lipids from animals adapted and acclimated to different temperatures have revealed pervasive changes in head group, acyl chain saturation, and chain length (see Hazel, 1995; Logue *et al.*, 2000). Alterations in membrane lipid composition may be the most common type of adaptation to temperature (Hazel, 1995).

In the case of rocky intertidal invertebrates, which may encounter changes in body temperature in excess of 25°C during a single tidal cycle (see Helmuth, 1998, 1999; Tomanek and Somero, 1999), rapid adjustments in membrane lipid composition may be needed to maintain the appropriate membrane static order. Indeed, studies of *Mytilus californianus* showed that, in addition to pronounced seasonal time-scale homeovis-
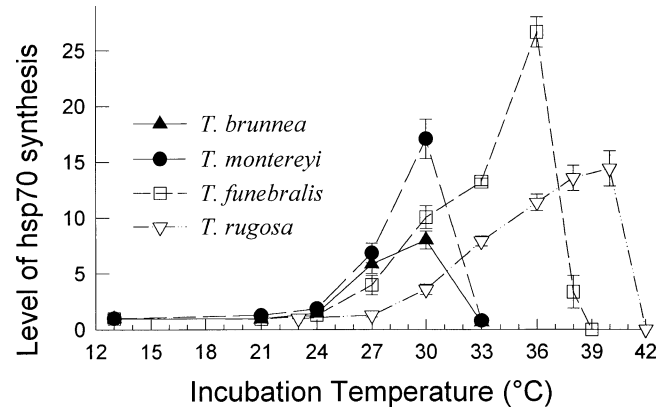

FIG. 6.   Induction of heat-shock protein 70 (hsp70) synthesis in four congeners of *Tegula* acclimated to 23°C. Gill tissue was incubated at a series of temperatures in seawater containing ^{35}S-labeled methionine and cysteine. Proteins were resolved by SDS-PAGE and radioactivity was quantified by autoradiographic and densitometric analysis (see Tomanek and Somero (1999) for protocols). The amounts of hsp70 synthesized during the incubation and recovery period are normalized to the levels of hsp70 synthesis observed in tissue exposed to 13°C. Values are means ± 1. S.E.M. N = 5 for all data points except for *T. funebralis* @ 33°C (N = 4) and *T. brunnea* @ 13°C. (Figure modified after Tomanek and Somero, 1999).

cous adaptation, mussels were able to alter their membrane lipid composition in response to temperature changes associated with a tidal cycle (Williams and Somero, 1996). The ability to alter membrane static order on time scales of hours was found in mussels from the high intertidal zone but not in conspecifics that occurred at low intertidal sites. These differences among conspecifics suggest that the exposure history of an organism may affect its ability to respond to environmental change, an issue that is addressed in the next section as well as in the concluding discussion of this review.

Biochemical systems: Protein synthesis and the heat-shock response

Variation among congeners in thermal optima and tolerance limits also has been observed in protein biosynthesis. For example, congeners of *Tegula* differ in heat tolerance of protein synthesis and in the patterning of heat-shock protein expression in response to heat stress (Tomanek, 2002; Tomanek and Somero, 1999, 2000, 2002). Congeners of the mussel genus *Mytilus* likewise have shown differences of these types (Hofmann and Somero, 1996a). As shown in Figure 6, 23°C-acclimated congeners of *Tegula* induce expression of heat-shock protein-70 (hsp70) at temperatures that reflect the species' widely different adaptation temperatures. The most warm-adapted species studied, *T. rugosa*, which occurs intertidally in the Gulf of California, did not induce hsp70 synthesis until 30°C and exhibited maximal induction at 36°C. Synthesis of hsp70 ceased by 42°C. At the other extreme, two temperate zone subtidal to low intertidal- species, *T. montereyi* and *T. brunnea*, induced hsp70 at 27°C

and showed maximal synthesis at 30°C. Synthesis of hsp70 ceased by 33°C. Although not shown in Figure 6, the maximal temperature of synthesis of hsp70 coincides with the upper temperature at which protein synthesis per se is possible in the four congeners (Tomanek and Somero, 1999). Temperate zone subtidal to low intertidal species of *Tegula* (*T. montereyi* and *T. brunnea*) are unable to synthesize proteins at temperatures that the mid-intertidal *T. funebralis* may encounter during emersion on hot, clear days. In turn, *T. funebralis* is unable to synthesize proteins at temperatures encountered by the subtropical species, *T. rugosa*.

One important difference between the thermal effects encountered by subtidal and intertidal species lies in the frequency with which the heat-shock response is likely to be induced. For *T. brunnea* and *T. montereyi*, hsp70 induction temperatures are several degrees higher than the maximal habitat temperatures these two subtidal species are likely to experience (Tomanek and Somero, 1999). In contrast, both mid-intertidal species studied, and *T. rugosa* in particular, are likely to experience regularly thermal conditions that activate hsp70 synthesis. These differences in frequency and intensity of synthesis of heat-shock proteins could result in significant differences in 'cost of living' between subtidal and intertidal animals, as discussed below.

Protein-level effects: Enzyme thermal stability

The activation of the heat-shock response is indicative of thermal denaturation of proteins during periods of heat stress (Feder and Hofmann, 2000). The observation that the temperatures at which the heat-shock response is activated correlate positively with adaptation temperature, as shown in Figure 6 for congeners of *Tegula*, suggests that species adapted to different temperatures have proteins with different intrinsic thermal stabilities. Such a trend between evolutionary adaptation temperature and intrinsic stability of orthologous proteins is common (see Hochachka and Somero, 2002, for review) and is illustrated by malate dehydrogenases (MDHs) from five species of *Tegula* and *Littorina* (Fig. 7). Thermal stabilities of malate dehydrogenase (MDH) increase regularly with increasing vertical position. Differences in protein thermal stability of the type observed for the MDHs of these molluscs thus may account in part for different onset temperatures of heat-shock protein expression.

The observation that proteins may denature at temperatures lying within the upper reaches of the physiological temperature range raises an interesting question about what might be regarded as an apparent "shortfall" of evolution. Proteins can be modified during evolution and, now, in the molecular biologist's laboratory to attain extremely high levels of intrinsic stability. Most remarkable are proteins from thermophilic members of the Domain Archaea, which are stable at temperatures in excess of 100°C (reviewed in Hochachka and Somero, 2002). Would it not be ben-
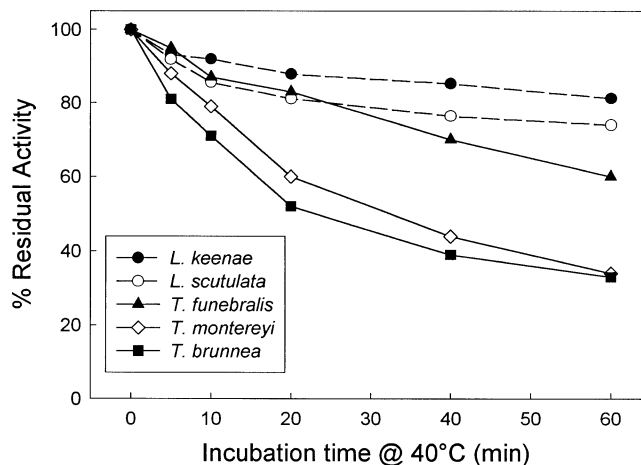

FIG. 7. Thermal stabilities of malate dehydrogenases (MDH) of congeners of *Littorina* and *Tegula*. Homogenates of foot tissue were prepared in 20 mM imidazole-Cl buffer (pH 7.0 @ 5°C) containing 1 mM EDTA and 5 mM DTT. Homogenates were centrifuged at 15,800 g for 30 min and supernatants were used for activity assays. Residual activity at various time points during incubation at 40°C was measured in a reaction mixture containing 80 mM imidazole-Cl buffer (pH 7.0 @ 20°C), 100 mM KCl, 150 μM NADH and 200 μM oxaloacetic acid. Loss of activity during incubation at 40°C was corrected for any loss of activity in a control sample kept on ice. (Data of Dr. Lars Tomanek (*Tegula*) and Michelle Phillips and the author (*Littorina*))

eficial to an intertidal invertebrate to possess proteins that are sufficiently rigid to avoid denaturation even at the highest body temperatures that are experienced during emersion on hot, sunny days?

The answer to this question involves an integrative perspective on protein structure and function, one in which the evolution of a single characteristic such as thermal stability is weighed against possible negative effects on other characteristics of the protein. In the case of enzymatic proteins, which generally alter their conformations during function, it is necessary to maintain an optimal balance between rigidity of structure, which allows persistence of the native folded state, and flexibility, which ensures that the conformational changes required for function can occur at appropriate rates at normal cell temperatures. In essence, this structure-function relationship dictates that the "moving parts" of proteins must reach a balance between stability and flexibility that is appropriate for the normal temperatures at which the proteins work (Fields and Somero, 1998). Proteins of cold-adapted species thus have a higher degree of conformational flexibility than proteins of warm-adapted species. In the case of eurythermal intertidal species, which face wide variation in temperature during alternate periods of immersion and emersion, enzymatic function must be retained over a wide range of cell temperatures. In fact, as discussed later, metabolic processes may be especially active during periods of immersion, when access to oxygen and food is greater than under conditions of emersion. Therefore, if a mid-intertidal species like *T. funebralis* were to evolve highly rigid enzymes to

avoid thermal unfolding at the highest temperatures it experiences during emersion, metabolic activity at the low temperatures characteristic of immersion might be compromised because these relatively rigid proteins would be incapable of undergoing rapid reversible changes in conformation in the cold. The need to have enzymes and other proteins that can function well at low temperatures during immersion requires that heat-tolerant, eurythermal species like *T. funebralis* face the consequences of thermal denaturation *in situ*. The repair and replacement of heat-damaged proteins thus may represent a significant and unavoidable energetic cost for intertidal species.

'COST OF LIVING' ISSUES: DO COSTS OF REPAIR AND REBUILDING LIMIT VERTICAL POSITION?

Marine ecologists have long regarded the stresses imposed by abiotic factors like temperature to play pivotal roles in establishing the upper limits to zonation in the intertidal zone (Connell, 1961; Newell, 1979; Newell and Branch, 1980). The foregoing sections that described the effects of temperature on a diverse suite of physiological traits provide strong support for this hypothesis. Although it is impossible at present to affix a firm number, *e.g.,* a percentage of total metabolic turnover, to the costs of coping with thermal stress, the data presented above do imply that an intertidal species that experiences extensive periods of emersion is apt to spend a considerable amount of metabolic effort to repair, replace, and restructure thermally sensitive biochemical components of the cell.

Protein replacement and repair

One of the best-understood aspects of thermal perturbation involves the damage done to proteins and the subsequent restoration of protein homeostasis. The heat-shock response may be strongly activated at physiological temperatures, at least in the case of intertidal species like *T. funebralis* and *T. rugosa* (Fig. 6) and mussels of the genus *Mytilus* (Hofmann and Somero, 1995, 1996a). Energy is required at several events in the heat-shock response, including the activation of transcription of heat-shock genes, the synthesis of hsps, and the ATP-requiring chaperoning by hsps. Further energy is required if proteins are irreversibly denatured and cannot be rescued by heat-shock proteins. Many denatured proteins undergo a process known as ubiquitination in which a small protein, ubiquitin, is bound covalently to the denatured target protein. Ubiquitination requires ATP, as does the subsequent proteolysis of the ubiquitinated protein through non-lysosomal proteolytic pathways. Heat-stressed mussels (*M. trossulus*) found in high-intertidal habitats contained significantly higher levels of ubiquitinated proteins than subtidal conspecifics (Hofmann and Somero, 1995), another indication that vertical position affects the energetic costs of life. Proteins that are degraded must, of course, be replaced, if metabolic activities are to be sustained. The energy-demanding process of protein biosynthesis, which may require synthesis of the machinery for biosynthesis, *e.g.,* ribosomal proteins, as well as synthesis of replacement proteins, thus may figure importantly in calculations of energy costs of heat stress.

Membrane-level effects: Temperature-dependent permeabilities and lipid restructuring to maintain static order

Two types of energetic costs can be envisioned in terms of the thermal effects on membrane function and structure that an intertidal species would face during alternate periods of emersion and immersion. Increases in temperature during emersion will tend to increase the passive permeability of membranes, thereby leading to a rise in uncontrolled flux of small ions and molecules (including water) through the membrane. Restoration of trans-membrane gradients will require energy, *e.g.,* more activity of the Na^+-K^+-ATPase (sodium pump). The restructuring of the membrane bilayer, which may occur during the tidal cycle, if body temperature fluctuates widely during emersion and immersion, represents a second energetic cost to intertidal species. Estimating the costs entailed in maintaining an appropriate static order is not currently possible and remains a challenge for future investigation.

Anaerobic metabolism: Another cost to intertidal species

Membrane-localized effects linked to emersion may lead to another important impact on energy budgets. Loss of cellular water across membranes may accompany emersion, especially if temperatures and wind velocities are high, adding a threat of desiccation to the cell. This threat may be met by a reduction in gas exchange across respiratory surfaces, as animals sequester these surfaces from the air during emersion. This response is common, as seen in the closures of mussel valves and opercular openings in snails during emersion. A consequence of this reduction in the threat of desiccation may be a shift away from efficient oxygen-based pathways of ATP generation to less efficient anaerobic ATP generating reactions, which are highly developed in many intertidal invertebrates (DeZwann and Mathieu, 1992).

If an invertebrate does undergo a shift towards anaerobic ATP generating pathways during emersion, it may only be during the subsequent period of re-immersion in oxygen-rich water that it will be able to generate sufficient ATP to support the costs of protein repair and replacement. In studies of the mussel *Mytilus californianus*, it was found that activation of the heat-shock response and ubiquitination of heat-damaged proteins occurred primarily after the animals were re-immersed following heat stress (Hofmann and Somero, 1996b). Thus, under conditions of heat stress, the longer the period of emersion relative to the time of immersion, the greater will be the amount of heat damage and the more limited will be the time for redressing this injury. Furthermore, if feeding is reduced during emersion, the energetic costs linked to repair of

thermal damage will be even more of a problem for the organism, as the limited energy available to the organism may need to be directed away from growth and reproduction to repair.

Shifts in gene expression

In the broad context of the costs of coping with abiotic stress such as that arising from temperature change, the extent and nature of the shifts in gene expression that are necessary for repair, replacement, and restructuring of the cell represent an important and, now, accessible frontier of study. To date, there has been no systematic study of the changes in gene expression, as shown by analysis of the transcriptome (the populations of mRNA produced through transcriptional activity), that occur as a result of temperature change in any intertidal species. However, there are data on the changes in gene expression that accompany the reduced access to oxygen that may occur during the tidal cycle. These data, obtained in studies of euryoxic intertidal and estuarine goby fish of the genus *Gillichthys*, indicate a large-scale re-organization of metabolism during the shift from normoxic to hypoxic conditions (Gracey *et al.*, 2001). In particular, genes encoding proteins involved in protein synthesis (*e.g.,* ribosomal proteins) and enhancement of cell proliferation are strongly down-regulated in response to hypoxia. Conversely, genes encoding enzymes that support anaerobic production of ATP and the provision of substrates for these pathways (*e.g.,* enzymes facilitating conversion of amino acids into glucose units) are strongly up-regulated. On-going studies in our laboratory are examining temperature-regulated shifts of gene expression as well, to determine how extensively gene expression is altered through the effects of the two environmental variables, temperature and oxygen availability, that are apt to play especially pivotal roles in stress during tidal cycles for intertidal species. It is, of course, premature to attempt even "back of the envelope" calculations of the energetic costs of these shifts in gene expression. Nonetheless, the costs of transcription and translation are apt to be large and to contribute in a measurable way to the "cost of living" in the intertidal zone.

Growth rates of intertidal and subtidal congeners of Tegula

With the foregoing analysis of energetic costs entailed in living in the intertidal zone as a backdrop, I consider briefly the relative rates of growth of congeners found at different vertical sites along the subtidal to intertidal gradient. Congeners of *Tegula* exhibit large (2–3-fold) differences in rates of growth in association with their vertical zonations (Fig. 8). *T. funebralis*, the highest-occurring species examined, grew most slowly; *T. brunnea*, was intermediate, and *T. montereyi*, the lowest occurring species, grew fastest (Frank, 1965; Watanabe, 1982). These data are consistent with, albeit they do not prove, that energetic costs are higher for species that experience the greater de-
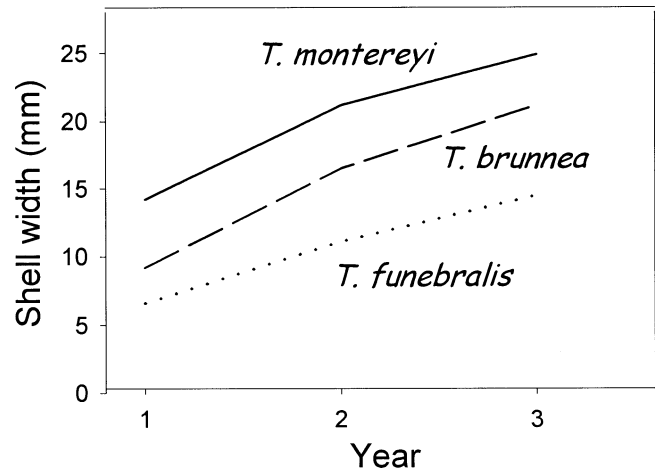

FIG. 8. Growth rates of congeners of *Tegula*. Data for *T. funebralis* are from Frank (1965) and data for *T. montereyi* and *T. brunnea* are from Watanabe (1982).

gree of thermal stress, plus the associated costs resulting from longer periods of anaerobiosis and shorter periods of feeding, found in the intertidal zone. Although the primary cause of the slower growth found in the higher-occurring congeners might not be a consequence of energetics costs from thermal perturbation of physiological systems, these costs remain an important missing piece in energy budgets and merit close study by ecological physiologists interested in vertical and latitudinal patterning of species.

SUMMARY

The widespread occurrence of adaptive variation in the thermal sensitivities of physiological traits shows that vertical zonation may be established and maintained in considerable measure by a wide suite of physiological adaptations. The thermal sensitivities of physiological traits also indicate that thermal stress is apt to create significant costs to the organism, notably in the contexts of repairing and replacing heat-denatured proteins and in the adaptive alterations of cellular structures and processes during acclimatization. The picture that is emerging from new studies that examine transcriptomes of environmentally stressed organisms is one in which widespread alterations in gene expression and, therefore, in metabolic organization occur in response to environmental changes. To date, we are likely to have observed only the tip of the iceberg of metabolic changes that occur in response to alterations in the environmental factors that most affect intertidal species, namely temperature and access to molecular oxygen. As we more fully elucidate the nature of thermal stress and the responses made by organisms to this stress, we are certain to gain important new insights into the interplay between physiology and vertical patterning along the subtidal to intertidal gradient.

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