

A Comparative Analysis of the Upper Thermal Tolerance Limits of Eastern Pacific Porcelain Crabs, Genus *Petrolisthes*: Influences of Latitude, Vertical Zonation, Acclimation, and Phylogeny

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Accepted 12/2/99

ABSTRACT

Marine intertidal organisms are subjected to a variety of abiotic stresses, including aerial exposure and wide ranges of temperature. Intertidal species generally have higher thermal tolerance limits than do subtidal species, and tropical species have higher thermal tolerance limits than do temperate species. The adaptive significance of upper thermal tolerance limits of intertidal organisms, however, has not been examined within a comparative context. Here, we present a comparative analysis of the adaptive significance of upper thermal tolerance limits in 20 congeneric species of porcelain crabs, genus *Petrolisthes*, from intertidal and subtidal habitats throughout the eastern Pacific. Upper thermal tolerance limits are positively correlated with surface water temperatures and with maximal microhabitat temperatures. Analysis of phylogenetically independent contrasts (from a phylogenetic tree on the basis of the 16s rDNA gene sequence) suggests that upper thermal tolerance limits have evolved in response to maximal microhabitat temperatures. Upper thermal tolerance limits increased during thermal acclimation at elevated temperatures, the amount of increase being greater for subtidal than for intertidal species. This result suggests that the upper thermal tolerance limits of some intertidal species may be near current habitat temperature maxima, and global warming thus may affect the distribution limits of intertidal species to a greater extent than for subtidal species.

Introduction

Temperature is arguably the most important abiotic stress that ectothermic organisms experience because it pervasively affects biological processes at all levels of biological organization, from the whole organism to molecular processes (Hochachka and Somero 1984; Somero 1997). To elucidate the evolutionary responses of organisms to different habitat temperatures, comparative studies typically have focused on species distributed along latitudinal gradients (e.g., Vernberg 1959a, 1959b, 1962; Vernberg and Tashian 1959; Vernberg and Costlow 1966; Graves and Somero 1982). In contrast, relatively few studies have been devoted to the microgeographic evolutionary adaptation of organisms to temperature on local gradients (e.g., Nevo 1997). Local thermal gradients can be formed by fine-scale variation in, for example, altitude, solar exposure, or precipitation (Nevo 1997).

The marine intertidal zone is characterized by especially sharp spatial and temporal gradients in temperature as a result of alternating exposure to aquatic and terrestrial conditions during the tidal cycle. Intertidal organisms are routinely exposed to large variations in temperature that do not impact subtidal species (Vernberg and Vernberg 1972; Newell 1979). A species living in the midintertidal zone at one site experience a wider temperature range than does a subtidal species at the same site, even though mean body temperatures, which are determined largely by seawater temperature, may be similar for the two species (Stillman and Somero 1996 and references therein). Intertidal organisms have evolved differing levels of physiological tolerance to these abiotic stresses, and these tolerance adaptations are important in determining the maximal vertical distributions of intertidal species (Connell 1961; Edney 1961; Jensen and Armstrong 1991; Stillman and Somero 1996).

Here we present an evolutionary analysis of the upper thermal tolerance limits of a group of widely distributed intertidal and shallow subtidal congeneric crabs in the genus *Petrolisthes* (Anomura: Porcellanidae), within the conceptual framework of the comparative method (Harvey and Pagel 1991; Garland and Adolph 1994). The genus *Petrolisthes* is large, with >100 species worldwide. Approximately 45 species of *Petrolisthes* are distributed across both latitudinal and vertical gradients in the eastern Pacific (Haig 1960; Carvacho 1980; Romero 1982; Weber Urbina 1986; Jensen and Armstrong 1991). Species are grouped in four main biogeographic regions: north and south

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temperate, the northern Gulf of California, and throughout the Tropics (Carvacho 1980). Within each of these locations, species are distributed on a vertical gradient, such that some species inhabit the mid- to high-intertidal zones and other species are distributed in the low-intertidal and subtidal zones (Romero 1982; Weber Urbina 1986; Jensen and Armstrong 1991; Stillman 1998).

The distributions of eastern Pacific *Petrolisthes* result in a wide range of average and maximal body temperatures, as well as a variety of daily and seasonal body temperature fluctuations. Temperate low-intertidal species (e.g., *P. eriomerus*) likely experience an annual temperature range of 8°–16°C, whereas temperate mid- to high-intertidal species (e.g., *P. cinctipes*) can have annual temperature ranges from 0° to 32°C (Stillman and Somero 1996). Similar to temperate subtidal species, tropical subtidal species have a narrow annual temperature range, from 26° to 30°C. Although tropical high-intertidal species (e.g., *P. tridentatus*) can experience body temperatures >40°C (J. Stillman, personal observation), their annual temperature ranges are smaller than those of temperate intertidal congeners, from 26° to 40°C. In contrast, the subtropical fauna of the northern Gulf of California experience a large annual thermal range because water temperatures vary from 28° to 30°C in the late summer to 10°–15°C in the winter (Robinson 1973). Body temperatures of high-intertidal species living in the northern Gulf of California (e.g., *P. gracilis*; Romero 1982) can be >40°C during summer months (J. Stillman, personal observation) and may reach temperatures below 10°C during winter low tide periods.

Measurement of the thermal limits of heart rate in two species of *Petrolisthes*, *P. cinctipes* and *P. eriomerus*, indicates that species have thermal tolerance limits that correspond to their respective microhabitat conditions (Stillman and Somero 1996). *Petrolisthes cinctipes* is distributed in the upper intertidal zone and *P. eriomerus* in the low-intertidal to subtidal zones throughout their distribution ranges from central California to northern British Columbia (Jensen and Armstrong 1991). Measurement of the thermal limits of heart function in these two species indicates that *P. cinctipes* is able to tolerate both higher and lower temperatures than *P. eriomerus* (Stillman and Somero 1996). In addition, the upper thermal tolerance limit of *P. cinctipes* is similar to the maximal microhabitat temperature measured for this species (Stillman and Somero 1996).

We have determined the upper thermal tolerance limits for 20 species of *Petrolisthes* from throughout the aforementioned thermal microhabitats. The phenotypic plasticity of upper thermal tolerance limits was investigated by laboratory acclimation to different temperatures. The extent to which the upper thermal tolerance limits reflect evolutionary adaptation to microhabitat temperature was examined by analyzing the results of these studies within a phylogenetic context by application of independent contrasts analyses (Felsenstein 1985). Application of phylogenetic independent contrasts generates a set of data that is independent of phylogenetic relationships under a

Brownian-motion evolutionary model. The phylogenetic tree used to generate independent contrasts was constructed on the basis of molecular sequence data for the 16s rDNA gene as presented in detail elsewhere (Stillman 1998).

Our results show a strong correlation between maximal habitat temperature and upper lethal temperature, and suggest that in comparison with subtidal species, intertidal species can have body temperatures nearer to their upper lethal limits and have a more restricted ability to increase these limits.

Material and Methods

Specimen Collection and Maintenance

Specimens were collected from intertidal locations and held submerged at ambient water temperatures ($\pm 1.0^\circ\text{C}$ from collection location) until the time of study. Adult crabs of similar size were selected for each species, and no freshly molted specimens were used. For examination of thermal tolerance limits of field specimens, 20 species were collected and held under constant conditions for short periods. Collection location, date of collection, and date of thermal tolerance assay for each species are as follows: *P. cinctipes*, *P. manimaculis*, Monterey Bay, California (36°36'N, 121°53'W), collected January 21, 1996, assayed January 25–26, 1996; *P. cabrilloi*, La Jolla, California (32°51'N, 117°16'W), collected January 19, 1996, assayed January 26, 1996; *P. eriomerus*, Cape Arago, Oregon (43°21'N, 124°19'W), collected July 22, 1997, assayed July 26, 1997; *P. armatus*, *P. gracilis*, *P. sanfelipensis*, *P. hirtipes*, *P. crenulatus*, Puerto Peñasco, Sonora, Mexico (31°39'N, 113°15'W), collected August 15–16, 1997, assayed August 17–18, 1997; *P. granulatus*, *P. laevigatus*, *P. violaceus*, *P. tuberculatus*, *P. tuberculosus*, Las Cruces, Chile (33°33'S, 71°36'W), collected October 14–15, 1997, assayed October 21–22, 1997; *P. tridentatus*, *P. armatus*, *P. galathinus*, *P. platymerus*, *P. agassizii*, *P. holotrichus*, *P. haigae*, Naos Island, Pacific Panama (8°50'N, 79°8'W), collected March 25–29, 1998, assayed April 2–6, 1998.

The response of upper thermal tolerance limits to thermal acclimation was examined in three species. Specimens of *P. cinctipes*, *P. manimaculis* (collected at Monterey Bay, California, September 3, 1997), and *P. eriomerus* (collected at Cape Arago, Oregon, July 22, 1997) were held submerged in aquariums with a continuous supply of ambient water from Monterey Bay (12°–14°C). On September 20, 1997, healthy-looking specimens of each species were divided evenly into two groups and were acclimated at 8° or 18°C for a period of 10 wk. After the acclimation period, specimens from each group ($n = 16$) were used for determinations of upper thermal tolerance limits. During the acclimation period, crabs were held in recirculating temperature-controlled aquariums and fed on alternate days with pulverized fish pellets. Two-thirds of the water in each aquarium was changed once a week with fresh filtered seawater at ambient temperature from Monterey Bay. Acclimation tem-

peratures were only altered by 4°–6°C for a period of approximately 60 min during the water-change process.

Microhabitat Characterization

To define the microhabitat conditions for each species studied, we conducted thermal transects of crab microhabitats by flipping over stones and immediately measuring the temperature of crabs, the underside of the rocks, and the substratum. Underrock temperatures for each stone were used only when crabs were present. We also measured the seawater temperature at low tide. All temperature measurements were made by using thermocouple probes (Omega Instruments, K-type wire probes) connected to a digital thermometer (Omega Instruments, HH 82), which are accurate to 0.1°C and were calibrated against a mercury thermometer. Transects throughout the intertidal zone were made with the intent to assess the maximal temperatures that crabs experienced. Temperatures were measured from underneath 15–20 rocks in each vertical zone shortly before the incoming tide submerged the rocks. Rocks that were likely to have high underside temperatures (see Stillman and Somero 1996) were preferentially selected because the

goal was to assess maximal temperatures, not average temperatures, in the underrock microhabitat. All temperature measurements were made during late spring or summer and during periods of low spring tides that occurred during the middle of the day.

The maximal vertical position for each species was categorized into one of five vertical zones on the basis of data from the literature (Romero 1982; Weber Urbina 1986; Jensen and Armstrong 1991; Stillman 1998) and from our observations as follows: high (~mean high tide [MHT]), middle high (between MHT and mean water level [MWL], ~low high tide), middle (MWL), middle low (between MWL and mean low water level [MLWL]), and low (below MLWL).

Measurement of Thermal Tolerance Limits

The upper thermal tolerance limit of each species was determined by the following protocol, adapted from the procedure used for measurement of upper thermal tolerance limits of heart rate in *P. cinctipes* and *P. eriomerus* (Stillman and Somero 1996). Individual crabs were placed into small plastic containers, each containing approximately 100 mL of seawater taken

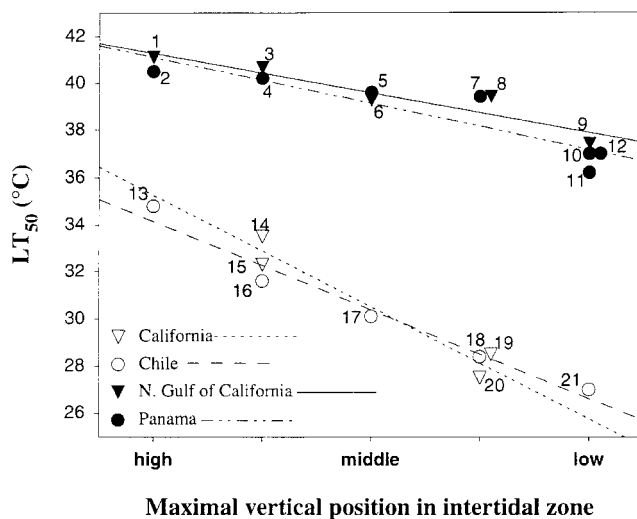


Figure 1. Thermal tolerance limits of *Petrolisthes* along latitudinal and vertical gradients. LT_{50} 's of species from four different locations are plotted for maximal vertical distribution of each species. Collection locations are as described in the text. Previously collected data on the vertical distribution of *Petrolisthes* for the north temperate zone in California, Oregon, and Washington (Jensen and Armstrong 1991); for the northern Gulf of California (Romero 1982); for the south temperate zone in central Chile (Weber Urbina 1986); and for Panama (Stillman 1998) were used to simplify the upper vertical positions of each species in the following categorization: 5 = high intertidal (below the splash zone) to 1 = subtidal (intertidal only during the lowest spring tides). Each point represents the LT_{50} of one species, coded as follows: 1, *P. gracilis* ($n = 18$); 2, *P. tridentatus* ($n = 16$); 3, *P. armatus* ($n = 24$, northern Gulf of California); 4, *P. armatus* ($n = 16$, Panama); 5, *P. holotrichus* ($n = 16$); 6, *P. hirtipes* ($n = 10$); 7, *P. platymerus* ($n = 16$); 8, *P. crenulatus* ($n = 18$); 9, *P. sanfelipensis* ($n = 16$); 10, *P. agassizii* ($n = 16$); 11, *P. haigae* ($n = 16$); 12, *P. galathinus* ($n = 16$); 13, *P. granulosus* ($n = 18$); 14, *P. cabrilloi* ($n = 18$); 15, *P. cinctipes* ($n = 3$ trials of 16); 16, *P. laevigatus* ($n = 16$); 17, *P. violaceus* ($n = 18$); 18, *P. tuberculatus* ($n = 18$); 19, *P. manimaculis* ($n = 3$ trials of 16); 20, *P. eriomerus* ($n = 24$); 21, *P. tuberculatus* ($n = 10$). Regression lines have the following parameters: northern Gulf of California, $y = 42.12 - 0.85x$, $r^2 = 0.88$; Panama, $y = 42.09 - 0.99x$, $r^2 = 0.83$; California, $y = 37.64 - 2.38x$, $r^2 = 0.94$; Chile, $y = 36.02 - 1.88x$, $r^2 = 0.97$. Overlapping symbols (8, 12, 19) have been jittered for display purposes only.

from the acclimation aquarium. These plastic containers were suspended in a water bath that was controlled to the nearest 0.1°C. The temperature of the water bath was set to the acclimation temperature. After placement of the crabs in the plastic containers, the acclimation temperature was maintained for 30–60 min. Thereafter, the temperature was increased at a rate of 1°C/15 min. Every 15 min, the water bathing the crabs was aerated with an air stone, the temperature in each container was checked (with thermocouple probes), and the sensory antennule activity of each crab was visually monitored. If no sensory antennule activity was noted, the mouthparts of the crab were gently prodded with the thermocouple probe. If no responsiveness to the prodding was noticed, the specimen was considered to be dead.

Between 10 and 24 specimens of each species were simultaneously incubated in the above conditions, and the percentage of specimens alive at each temperature was calculated (actual sample sizes are given in the legend of Fig. 1). To make the proportion of surviving crabs linear for temperature, this percentage was transformed by the arcsine square root function and expressed in radians. Linear-regression analysis was then used to find the slope of the line, from which the temperature at which 50% of the crabs had died (0.785 radians) was calculated. This temperature is used as the measure for upper thermal tolerance limits and is referred to as the LT_{50} .

The LT_{50} datum for each species does not have an associated variance because the LT_{50} is calculated from the proportion of specimens alive at each temperature. Because of constraints of time and specimen availability, it was impossible to repeat LT_{50} measurements for every species, but in two species, *P. cinctipes* and *P. manimaculis*, there was <0.5°C variation among three

separate determinations of the LT_{50} for each species ($n = 16$ for each). Thus, although the measurement of LT_{50} only once for each species does not allow for parametric statistical analyses to be used, regression analysis of the relationship between LT_{50} and an environmental variable can be done with LT_{50} 's of multiple species.

Independent Contrasts Analyses

Molecular sequence data of the 16s rDNA mitochondrial gene (~450 base pairs) were obtained by PCR amplification from whole genomic extracts, by using the universal primers 16SAR and 16SBR (Palumbi et al. 1991) and cycle sequencing (for details see Stillman 1998). Phylogenetic analysis was done by neighbor joining of bootstrapped distance matrices generated by the PHYLIP software package (Felsenstein 1989) as described in Stillman (1998). Phylogenetically independent contrasts (Felsenstein 1985) of LT_{50} and maximal habitat temperatures were generated by using the CAIC software package (Purvis and Rambaut 1995) and the results of PHYLIP analyses. These phylogenetically independent contrasts were used in linear-regression analyses, where the regression was forced through the origin, as is required for analyses of independent contrasts (Purvis and Rambaut 1995).

Results

Microhabitat Characteristics

Seawater temperatures during the times of temperature measurements ranged from 8° to 12°C in California and the Pacific Northwest, from 14° to 16°C in Chile, from 28° to 29°C in the

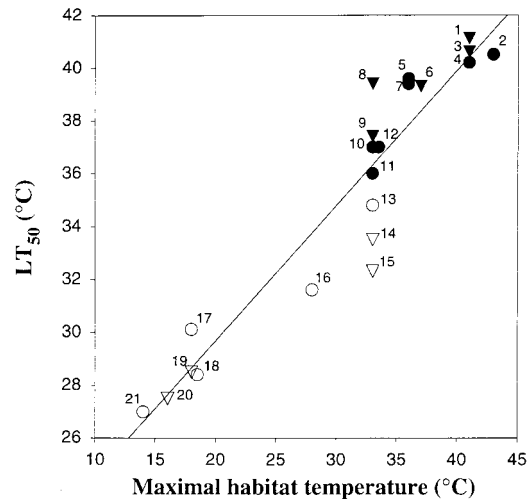


Figure 2. Relationship between LT_{50} and maximal habitat temperature. Each point represents the LT_{50} from one species. Regression coefficients are $y = 19.41 + 0.51x$, $r^2 = 0.88$. Symbols and species codes are as in Figure 1. Overlapping symbols (12, 19) have been jittered for display purposes only.

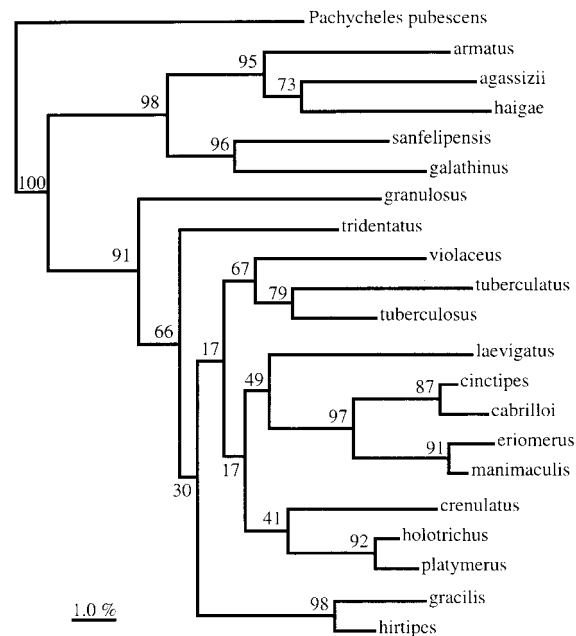


Figure 3. Phylogenetic tree on the basis of 16S rDNA gene sequence data of *Petrolisthes* for which LT_{50} data were collected. This tree was generated from a matrix of distances generated with a maximum likelihood model and constructed by neighbor-joining analysis. Values on the nodes are bootstrap values of a total of 100 replicate data sets. The tree was rooted with the out-group species *Pachycheles pubescens*, although this terminal node was not used to generate an independent contrast. Branching order and branch lengths from this tree were used for the construction of phylogenetic independent contrasts of thermal tolerance limits and microhabitat characteristics.

northern Gulf of California, and from 26° to 28°C in Panama. Maximal microhabitat temperatures for low-intertidal species were never more than 4°C above ambient water temperature at tropical field sites or 6°–8°C above ambient water temperature at temperate field sites. Maximal microhabitat temperatures for high-intertidal species ranged from 40° to 43°C in the northern Gulf of California during summer (mid-August) and in Panama during the hot, dry season (late March–early April). In the temperate regions, maximal temperatures were as high as 31.2°C (Stillman and Somero 1996) in Oregon during spring and as high as 28°C in Chile during late spring. Maximal temperatures recorded in Chile may not reflect absolute maximal temperatures because measurements were not made on cloudless calm days. Thus, the approximate range of temperatures encountered during low tide in temperate zone midintertidal species is about 18°–20°C above ambient seawater temperature, whereas tropical midintertidal species only encounter temperatures of 10°–12°C above ambient seawater temperature.

Upper Thermal Tolerance Limits

During thermal tolerance experiments, specimens exhibited a series of consistent behavioral responses. With increasing temperature, in sequence, the specimens became increasingly active, periodically but reversibly lost balance, irreversibly lost balance,

and last, ceased all movement. During loss of balance, specimens often made spasmodic, jerky movements. Specimens generally died in a clenched contortion, where the appendages were held beneath the body.

Upper thermal tolerance limits of field-acclimatized specimens were strongly correlated with maximal vertical position in the intertidal zone (Fig. 1). Comparisons of species within each of the four field sites showed that LT_{50} 's were linear for maximal vertical intertidal position. In addition, species from the two tropical habitats had similar LT_{50} 's at similar vertical positions, as did species from the two temperate zone habitats. The differences between the highest and lowest LT_{50} 's within a field site were greater in temperate zone species than in tropical species (Fig. 1), reflecting the larger differences in microhabitat temperature extremes in temperate microhabitats. Regression analyses indicated that the difference in LT_{50} relative to vertical position of temperate zone species (slopes of 2.38, 1.88) was about twice that of tropical species (slopes of 0.85, 0.99; Fig. 1 legend).

Across species, LT_{50} 's are strongly and positively correlated with maximal habitat temperature (Fig. 2). However, the LT_{50} 's of tropical low-intertidal species (Fig. 1, species 7–12) are greater than the LT_{50} 's of temperate high-intertidal species (Fig. 1, species 13–16), even though maximal microhabitat temper-

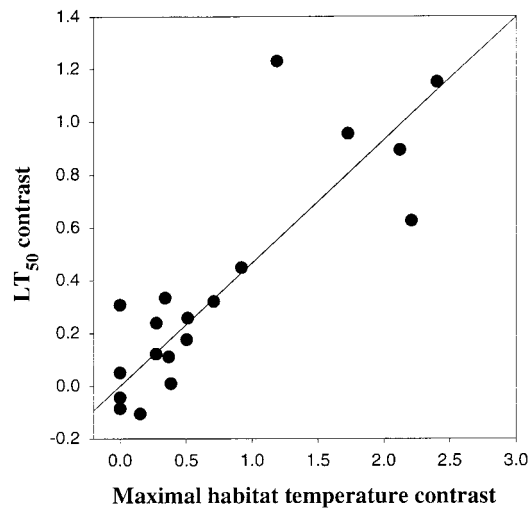


Figure 4. The relationship between LT_{50} and maximal habitat temperature for 20 species of *Petrolisthes* plotted as phylogenetically independent contrasts. Regression coefficients are $y = 0.4655x$, $r^2 = 0.72$.

atures are similar in both groups (Fig. 2, compare temperate [13–15] and tropical [8–12] species at $\sim 33^\circ\text{C}$).

Independent Contrasts Analyses

The phylogenetic tree used for generation of phylogenetic independent contrasts is shown in Figure 3. There was a strong, positive correlation between phylogenetic independent contrasts of LT_{50} and maximal microhabitat temperature (Fig. 4). Contrasts were small in most cases, but for five different nodes, there were large differences in LT_{50} and maximal habitat temperature contrasts (Fig. 4). Thus, phylogenetic independent contrast analysis suggests that there has been a strong evolutionary adaptive response of LT_{50} for microhabitat temperature.

Acclimation of Thermal Tolerance Limits

The LT_{50} 's of *P. cinctipes*, *P. eriomerus*, and *P. manimaculis* were higher after acclimation at 18°C than after acclimation at 8°C (Fig. 5). LT_{50} values for *P. cinctipes* were 32.1° and 34.0°C at 8° and 18°C , respectively (Fig. 5). In contrast, the LT_{50} 's of *P. manimaculis* and *P. eriomerus* changed by about twice as much after acclimation: LT_{50} 's of *P. manimaculis* acclimated at 8° and 18°C were 27.6° and 31.6°C , respectively, and for *P. eriomerus* they were 27.9° and 31.4°C , respectively (Fig. 5).

Discussion

Thermal Tolerance Limits of Congeners of *Petrolisthes*: Conclusions and Caveats

Analyses of upper thermal tolerance limits of congeneric species of *Petrolisthes* from different biogeographic regions and differ-

ent vertical positions in the subtidal to intertidal gradient within each region indicate that species have adapted their upper thermal tolerance limits to coincide with microhabitat conditions. In 20 species of *Petrolisthes*, maximal thermal tolerance limits reflected microhabitat conditions for both vertical distribution and maximal microhabitat temperatures (Figs. 1, 2). Assessment of upper thermal tolerance limits by determining the LT_{50} produced results similar to those seen when the thermal tolerance limits of heart function were measured (Stillman and Somero 1996). Arrhenius break temperatures of heart rate, temperatures above which further increases in temperature led to reductions in heart rate, were 31.5°C for *P. cinctipes* and 26.6°C for *P. eriomerus* (Stillman and Somero 1996), and these species had LT_{50} 's of 32.3° and 27.5°C , respectively.

The relation of thermal tolerance to habitat temperature and vertical zonation was examined within a phylogenetic context to determine the extent to which the observed patterns might derive strictly from phylogenetic relatedness among the species. *Petrolisthes* of the eastern Pacific are divided into two main clades (Stillman 1998). In the clade consisting of *P. agassizii*, *P. galathinus*, and others, upper thermal tolerance limits, vertical distribution limits, and thermal microhabitat characteristics are relatively similar in most species (Figs. 1–3). However, two species of this clade have different thermal habitats from the rest of the species in this clade. One of these two species, *P. armatus*, is found in the mid- to high-intertidal zone and has upper thermal tolerance limits that are about 3° – 4°C higher than those of the closest subtidal living relatives, *P. agassizii* and *P. haigae* (LT_{50} of 40.5° vs. 36° – 37°C). The other species to deviate from the common habitat of this clade is *P. desmarestii*, a subtidal south temperate species (Haig 1960) for which no thermal tolerance data are available.

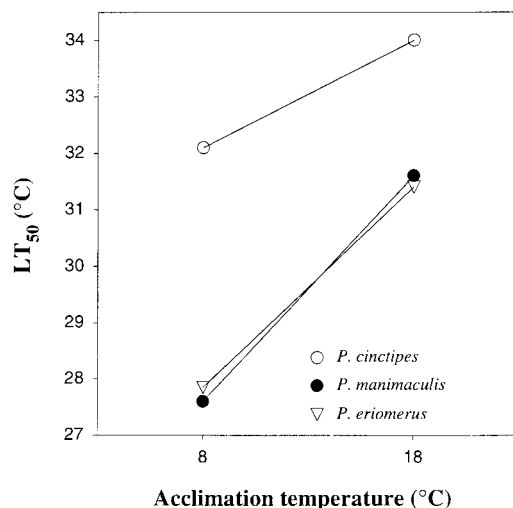


Figure 5. Acclimation of thermal tolerance limits in *Petrolisthes cincipes*, *P. eriomerus*, and *P. manimaculis*. Acclimation to 8° or 18°C was for a period of 10 wk. $n = 16$ for the determination of each data point in the figure.

The other clade includes members that have a greater diversity in microhabitat preference and upper thermal tolerance limits (Figs. 1–3). LT_{50} values among these species range from 27.5° to 41.1°C. In these species, upper thermal tolerance limits match the present microhabitat conditions, and sister species do not necessarily occupy similar thermal microhabitats (Fig. 3).

Phylogenetic independent contrasts analysis indicates that there has been an adaptive response of upper thermal tolerance limits to microhabitat temperature, as indicated by the positive correlation ($r^2 = 0.72$, $P < 0.05$) between the independent contrasts of LT_{50} and maximal microhabitat temperature (Fig. 4). The necessity of having an upper thermal tolerance limit higher than maximal microhabitat temperature is apparent for survival. Yet, high-intertidal species have upper thermal tolerance limits that are only slightly higher than maximal observed microhabitat temperatures (Fig. 2). In addition, although low-intertidal and subtidal species generally have LT_{50} 's that are considerably higher than maximal microhabitat temperatures, the actual LT_{50} 's are lower than what ancestral, intertidal species may have had (Fig. 3; e.g., *P. eriomerus* and *P. manimaculis* likely had an ancestor that inhabited an intertidal microhabitat). These observations suggest that, in the absence of selection for heat tolerance, this ability may be lost or that there may be physiological costs involved with maintaining an elevated LT_{50} .

Although the foregoing analysis of upper lethal temperatures in congeners of *Petrolisthes* is consistent with the hypothesis that genetically based differences account for interspecific variation in heat tolerance, it is conceivable that certain types of phenotypic changes could contribute to the patterns observed. One potentially confounding factor in the measurements of

upper thermal tolerance limits presented here is that specimens were used for experiments as soon as 48 h after collection. Previous studies have shown that crustaceans can have dramatically different upper thermal tolerance limits, depending on the acclimation conditions immediately preceding the measurement. For example, in *Artemia franciscana*, a brief heat shock (similar to what an intertidal crab might experience during a low tide period) can induce an increased thermal tolerance during subsequent thermal stress (Miller and McLennan 1988). The protective effects of the first heat shock wear off, however, and after <19 h after the initial heat shock, protection decreased by 60% in *A. franciscana* (Miller and McLennan 1988). If *Petrolisthes* have similar heat shock effects, then 48 h of acclimation of subtidal and intertidal congeners from a given location to identical conditions should have been long enough to ensure that the measured upper thermal tolerance limits do not include an environmentally induced transient adjustment.

The extent to which genotypic differences in thermal tolerance are obfuscated by acclimatization effects requires additional clarification. Had it been possible at field sites in Chile and Panama to acclimate specimens for longer time periods, some of the interspecific differences noted in the field-acclimatized specimens may have been reduced or eliminated. However, the findings that (1) the effects of acclimation on three temperate zone species did not abolish interspecific differences (Fig. 5) and (2) interspecific differences in LT_{50} were larger than acclimation-induced effects on LT_{50} (Figs. 2, 5) suggest that the diversity of thermal tolerance limits among congeners was caused at least in part by genotypic differences.

More difficult to test is the hypothesis that some type of parental effect influenced the results, for instance, a thermal acclimatization undergone by a female that led to altered ther-

mal tolerance in the next generation (e.g., Crill et al. 1996). Testing this possible basis for differences in LT_{50} would involve a common garden breeding experiment, an approach not yet used in the study of these species.

Acclimation of Thermal Tolerance Limits: Differences between Subtidal and Intertidal Species of Petrolisthes

Studies of differently thermally adapted congeneric ectotherms have shown that warm-adapted species may have a more limited ability to increase heat tolerance than cold-adapted species. For instance, a study of thermal acclimation of temperate and tropical fiddler crabs, genus *Uca*, found that only temperate zone species exhibited acclimation-induced adjustment in their thermal tolerance limits (Vernberg and Tashian 1959). The tropical species, because of the high-microhabitat temperatures routinely experienced, were hypothesized to have upper thermal tolerance limits that were as high as could be reached through acclimatization, so that no further acclimation was possible. Studies of laboratory-evolved *Drosophila melanogaster* also found that the ability to increase heat tolerance varied between cold- and warm-adapted organisms. Cavicchi et al. (1995) found that induced heat tolerance of adult *D. melanogaster* was significant for flies evolved at 18° and 25°C but absent for flies evolved at 28°C.

Acclimation of upper thermal tolerance limits in three species of temperate *Petrolisthes* (Fig. 5) suggests that the more warm-adapted intertidal species *P. cinctipes* may not be able to adjust its upper thermal tolerance limits to the same extent as the more cold-adapted subtidal species *P. eriomerus* and *P. manimaculis*. In general, intertidal species may currently be living nearer to their thermal maxima and may have reduced abilities to increase their upper thermal tolerance limits than subtidal species. This result suggests that, in the event of global warming, the distributions of intertidal *Petrolisthes* may be more impacted than those of subtidal congeners.

Acknowledgments

We thank G. Jensen for his assistance with specimen collection and measurement of vertical distributions and P. Fields and C. Fang for their assistance in acclimation studies. We also thank two anonymous reviewers for their constructive critiques of this manuscript. Support for this research was granted by the National Science Foundation through grants IBN 9206660 to G.N.S. and by a dissertation improvement grant IBN 9700701 to J.H.S. and G.N.S.

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