

A comparative analysis of plasticity of thermal limits in porcelain crabs across latitudinal and intertidal zone clines

Jonathon H. Stillman*

Department of Zoology, 152 Edmondson Hall, 2538 McCarthy Mall, University of Hawaii, Manoa, Honolulu, HI 96822, USA

Abstract. The effect of thermal acclimation on cardiac thermal performance limits was examined in congeneric species of porcelain crabs (genus *Petrolisthes*) from temperate and subtropical habitats. In vivo heart rate was monitored using impedance electrodes during thermal ramps (0.1 °C/min) where temperature either increased or decreased from an intermediate temperature to thermal extremes. Arrhenius plots were used to define upper and lower critical temperatures of cardiac function (CT_{max} and CT_{min} , respectively). Across species and acclimation conditions, CT_{max} ranged from approximately 28.4 to 41.7 °C and CT_{min} from approximately -1.3 to 11.3 °C. Thermal acclimation had the greatest effect on CT_{max} in species from the coolest thermal microhabitat and the smallest effect on CT_{max} in species from the hottest thermal microhabitat. The opposite effect was observed on CT_{min} . The ecological consequences of these results are counterintuitive, as the most heat-tolerant species are predicted to be most susceptible to global warming. The results of this study will form the foundation of future studies designed to elucidate the mechanistic bases of thermal plasticity and eurythermy in porcelain crabs. © 2004 Elsevier B.V. All rights reserved.

Keywords: Thermal tolerance; Thermal acclimation; CT_{max} ; CT_{min} ; Porcelain crabs; Acclimation capacity; Cardiac thermal limits; Global climate change

1. Introduction

For ectothermic organisms, habitat temperature is a critically important environmental factor because of the effects of temperature on all biological processes [1]. Evolutionary responses of organisms to changes in temperature have been shown at a wide range of levels of biological organization, from molecular to physiological to behavioural [1,2]. Changes in

* Tel.: +1 808 956 9821; fax: +1 808 956 9812.

E-mail address: stillman@hawaii.edu.

habitat temperature can occur on a wide temporal range, from fluctuations that occur in hours as a result of day–night or tidal cycles [3–6] to those that might occur over many years as a result of global climate change [7–9]. To understand how the distribution and abundance limits of organisms might change as a result of changes in daily habitat temperature fluctuations associated with climate change [4], we must understand how much the thermal sensitivity of organisms can change, or acclimatize. The magnitude of effects of global warming on species distribution ranges will be a result of the proximity of organismal thermal limits to maximal habitat temperatures, and the capacity of those organisms to adjust their thermal limits through acclimation [10].

Thermal tolerance ranges, the range of temperatures between upper and lower thermal thresholds to normal biological performance (here referred to as upper and lower thermal tolerance limits, CT_{max} and CT_{min} , respectively), can be shifted through thermal acclimation. The degree to which organisms are able to adjust CT_{max} and CT_{min} has been referred to as acclimation flexibility [2], and capacity for acclimation [10].

In this study, the effects of thermal acclimation on cardiac CT_{max} , and CT_{min} have been investigated in six species of porcelain crabs in the genus *Petrolisthes*. Porcelain crabs are an excellent group of organisms for comparative studies of temperature adaptation because species exist across a large range in thermal microhabitat conditions as a result of differences in latitudinal and vertical (intertidal zone) distribution patterns [11], and because phylogenetic information for these crabs [12] facilitates selection of closely related species that live in different thermal microhabitats. Previous studies have shown that in over 20 *Petrolisthes* species, upper thermal tolerance limits are evolutionarily correlated with maximal habitat temperature [3,10,11,13], and cold tolerance varies across species from different vertical zones [3,10]. Differences in capacity for thermal acclimation of cardiac performance limits have been shown in four species of *Petrolisthes* from different thermal microhabitats [10]. *Petrolisthes* from the hottest habitats had the highest CT_{max} , but they also had the lowest acclimation capacity of CT_{max} , while the opposite pattern was true for CT_{min} [10].

2. Materials and methods

2.1. Species selection

Six species of *Petrolisthes* were selected for study. Four of the study species, *Petrolisthes cinctipes*, *Petrolisthes cabrilloi*, *Petrolisthes manimaculis* and *Petrolisthes eriomerus*, are within one phylogenetic clade of northern temperate species with a most recent common ancestor of about 16 million years ago (mya) [12], and two, *Petrolisthes gracilis* and *Petrolisthes hirtipes*, fall within one phylogenetic clade of species endemic to the northern Gulf of California with a most recent common ancestor of about 8 mya, and these two clades shared a common ancestor about 25 mya [12]. Both temperate and Gulf of California species have distinct vertical zonation patterns: *P. cinctipes* and *P. cabrilloi* inhabit the middle–upper intertidal zone, *P. eriomerus* and *P. manimaculis* inhabit the low intertidal and subtidal zones, and *P. gracilis* and *P. hirtipes* inhabit the high and middle intertidal zones, respectively [14].

The two species from the northern Gulf of California live in an extreme thermal habitat, as water temperatures can vary from 15 to 30 °C annually, and during low tide

conditions, temperature fluctuations can be much greater. During low tide in the northern Gulf of California, under rock temperatures in *Petrolisthes* microhabitats have been measured at over 40 °C during summer months. In the cold north temperate, water temperatures fluctuate between 7 and 13 °C, and intertidal species of *Petrolisthes* experience fluctuating microhabitat temperatures (Fig. 1) that can be above 30 °C in summer months [3]. Thus, the species selected for study here are from a great range of thermal microhabitats, from *P. eriomerus*, which sees the coolest and least variable habitat temperatures, to *P. gracilis*, which sees the warmest and most variable thermal habitat.

2.2. Specimen collection

Specimens were collected by hand during low tide and transported to Hopkins Marine Station where they were maintained in flowing seawater at 12–15 °C (temperate species) or 25 °C (Gulf of California species) for approximately 5 weeks until the beginning of thermal acclimations. Crabs were fed SELCO-enriched *Artemia* and frozen copepods three times per week throughout the duration of the study.

2.3. Thermal acclimation experiments

Thermal acclimations were performed in recirculating aquaria with carbon and biological filtration (FLUVAL 304 canister filters) and weekly water changes. *P. cinctipes* and *P. eriomerus* were acclimated together for 8 weeks to 8, 13 and 18 °C, and *P. manimaculis* were also acclimated in the 8 and 13 °C tanks. *P. cabrilloi* were acclimated to 8 and 18 °C for 4 weeks. *P. cinctipes*, *P. hirtipes* and *P. gracilis* were acclimated together for 4 weeks to 15 and 25 °C (*P. eriomerus* cannot survive acclimation to 25 °C), and *P. gracilis* alone was acclimated to 35 °C (no other species in this study can survive acclimation to 35 °C). *P. cinctipes* were also acclimated to 22 and 4 °C, but none of the specimens survived for over 7 days at 4 °C, and no heart rate data were collected for those crabs.

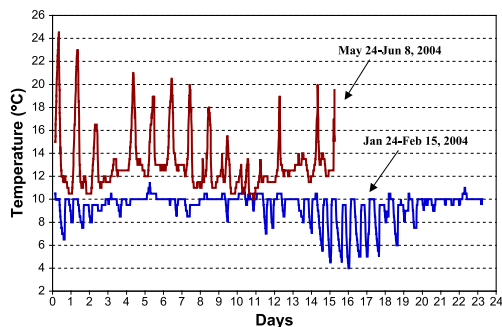


Fig. 1. Microhabitat temperatures of *P. cinctipes* at Cape Arago, OR during winter and summer months. Temperature data loggers ($n=5$; ibutton, Dallas Semiconductor) were placed underneath the same stones in winter and summer, 2004, and were set to collect temperature data every 15 min (summer) or 20 min (winter). Data shown is the average temperature of the recovered data loggers ($n=2$ (summer), $n=3$ (winter)), and variation between loggers was generally within 1–2 °C at any time-point.

2.4. Determination of cardiac thermal performance limits

Cardiac activity was monitored using impedance electrodes (as described elsewhere [10]) during thermal ramp experiments where temperature was increased (CT_{max}) or decreased (CT_{min}) at $0.1\text{ }^{\circ}\text{C min}^{-1}$. Briefly, crabs were suspended from a cork glued to their carapace in a water bath containing a heat exchanger connected to a recirculating temperature control device. Temperatures were held constant for 1 h prior to initiating the thermal ramp, and ramp experiments were generally completed within 5 h. Average heart rates (beats per minute) were calculated in bins of 30 s for the duration of the recording. Arrhenius break temperatures of heart rate (CT_{max} and CT_{min}) were determined from plots of the natural log of heart rate vs. inverse temperature by calculating the intersection point of regression lines fitted to the first 200 data points (before a transition in slope occurred) and the final 60 data points of each record (after the heart began to fail), as described elsewhere [10].

3. Results

3.1. Thermal acclimation of cardiac thermal performance limits

Thermal acclimation affected upper and lower thermal limits of cardiac function in all species; however, the magnitude of change of CT_{max} and CT_{min} differed among species. *P. eriomerus* and *P. manimaculis*, the temperate subtidal zone species, showed the greatest change in CT_{max} ($\sim 4\text{ }^{\circ}\text{C}$) and the smallest change in CT_{min} of all of the species tested (Fig. 2A). In *P. eriomerus* and *P. manimaculis*, CT_{max} changed by 2.1 and $3.5\text{ }^{\circ}\text{C}$, respectively, over the $10\text{ }^{\circ}\text{C}$ acclimation temperature range of $8\text{--}18\text{ }^{\circ}\text{C}$ (Fig. 2A). CT_{max} of *P. cinctipes* ranged from $32.6\pm 0.2\text{ }^{\circ}\text{C}$ (in $8\text{ }^{\circ}\text{C}$ acclimated crabs) to $33.9\pm 0.3\text{ }^{\circ}\text{C}$ (in $18\text{ }^{\circ}\text{C}$ and $22\text{ }^{\circ}\text{C}$ acclimated crabs), and thus changed by $1.3\text{ }^{\circ}\text{C}$ over the $10\text{ }^{\circ}\text{C}$ acclimation temperature range of $8\text{--}18\text{ }^{\circ}\text{C}$ (Fig. 2A). The CT_{max} of *P. cinctipes* changed the most between 8 and $18\text{ }^{\circ}\text{C}$ (the largest jump was from 8 to $13\text{ }^{\circ}\text{C}$), and changed little from 18 to $25\text{ }^{\circ}\text{C}$ (Fig. 2A). CT_{max} of *P. cabrilloi* was the same as *P. cinctipes* for $8\text{ }^{\circ}\text{C}$ acclimated crabs, but increased by $2.3\text{ }^{\circ}\text{C}$ in $18\text{ }^{\circ}\text{C}$ acclimated crabs (Fig. 2A).

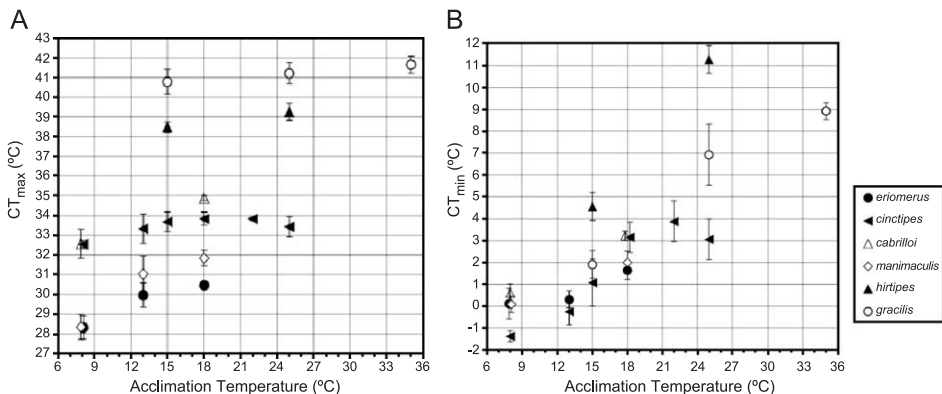


Fig. 2. Acclimation of cardiac upper thermal limits (CT_{max} , A) and lower thermal limits (CT_{min} , B) in six species of porcelain crabs (genus *Petrolisthes*) from different thermal habitats to a range of constant thermal regimes. Sample sizes are from $n=3$ to 11, and each point is the mean \pm one standard deviation.

In the warm-adapted crabs *P. gracilis* and *P. hirtipes* from the northern Gulf of California, small increases in CT_{max} were observed as the result of thermal acclimation over a 10 or 20 °C acclimation range (Fig. 2A). CT_{max} of *P. gracilis*, the high intertidal and most warm adapted species, increased by 0.4 °C from 15 °C acclimated crabs (40.8 ± 0.5 °C) to 25 °C acclimated crabs (41.2 ± 0.5 °C), and increased 0.4 °C further in 35 °C acclimated crabs (41.6 ± 0.4 °C). CT_{max} of *P. hirtipes* changed by 0.8 °C from 15 °C acclimated crabs (38.5 ± 0.2 °C) to 25 °C acclimated crabs (39.3 ± 0.4 °C) (Fig. 2A). *P. hirtipes* did not survive acclimation to 35 °C.

The interspecific differences in the effects of thermal acclimation on CT_{min} were opposite of those observed in CT_{max} . *P. gracilis* and *P. hirtipes*, the most warm adapted species, showed the largest change in CT_{min} , and *P. eriomerus* and *P. manimaculis*, the least warm adapted species, showed the smallest change in CT_{min} (Fig. 2B). CT_{min} of *P. gracilis* changed from 1.9 ± 0.5 °C in 15 °C acclimated crabs to 6.9 ± 1.3 °C in 25 °C acclimated crabs, a 5.0 °C change over this 10 °C temperature range, and increased by another 2 °C in crabs acclimated to 35 °C (Fig. 2B). In *P. hirtipes*, the change in CT_{min} was 6.7 °C over a 10 °C acclimation range, from 4.6 ± 0.5 °C in 15 °C acclimated crabs to 11.3 ± 0.4 °C in 25 °C acclimated crabs.

P. manimaculis and *P. eriomerus* had the same CT_{min} values across acclimation temperatures, and changed CT_{min} by 2 °C over the 10 °C acclimation temperature range from 8 to 18 °C (Fig. 2B). In *P. cinctipes*, CT_{min} changed by 4.3 °C from -1.4 ± 0.3 °C in 8 °C acclimated crabs to 3.9 ± 0.9 °C in 22 °C acclimated crabs, but did not increase further in crabs acclimated to 25 °C (Fig. 2B), and there was no significant difference between CT_{min} values of 18, 22 or 25 °C acclimated specimens of *P. cinctipes*. In *P. cabrilloi*, CT_{min} changed by 2.6 °C from 0.6 ± 0.4 °C in 8 °C acclimated crabs to 3.2 ± 0.2 °C in 18 °C acclimated crabs (Fig. 2B). CT_{min} values for *P. cinctipes* and *P. cabrilloi* were the same

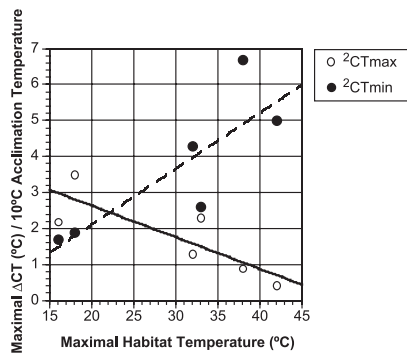


Fig. 3. Maximal plasticity of critical thermal limits of cardiac function during thermal acclimation in six species of porcelain crabs (genus *Petrolisthes*) from different thermal habitats. For each species, the maximal change in CT was calculated over a 10 °C acclimation temperature range using the above data for CT_{max} (Fig. 2A) and CT_{min} (Fig. 2B). From lowest to highest maximal habitat temperature, pairs of points for ΔCT are for *P. eriomerus*, *P. manimaculis*, *P. cinctipes*, *P. cabrilloi*, *P. hirtipes*, and *P. gracilis*. Linear regression indicated a significant relationship between ΔCT and maximal habitat temperature for both CT_{max} ($p=0.040$, $r^2=0.692$) and CT_{min} ($p=0.0396$, $r^2=0.694$). Maximal habitat temperature data from Ref. [13].

in 18 °C acclimated crabs, but in 8 °C acclimated crabs, *P. cabrilloi* had CT_{\min} values that were about 2 °C higher than those of *P. cinctipes*. This is the opposite pattern observed for CT_{\max} for these two species.

3.2. Maximal capacity for thermal acclimation of CT_{\max} and CT_{\min}

The maximal amount of change in CT_{\max} and CT_{\min} over a 10 °C acclimation range differs among species in a manner consistent with maximal microhabitat temperature ([10]; Fig. 3). The 10 °C range used was 8–18 °C for *P. eriomerus*, *P. cinctipes*, *P. cabrilloi*, and *P. manimaculis*, and 15–25 °C for *P. gracilis* and *P. hirtipes*. The change in CT_{\max} of *P. gracilis* was the same across the 15–25 and the 25–35 °C acclimation temperature interval, but the change in CT_{\min} was much greater across the 15–25 °C acclimation temperature interval for this species, and thus this interval was used in the analysis.

To remove the effects of phylogeny from these data, phylogenetic independent contrasts were generated following methods previously published for these crabs [13]. Independent contrasts of maximal habitat temperature were not significantly correlated with contrasts of ΔCT_{\max} ($p=0.53$) or contrasts of ΔCT_{\min} ($p=0.18$).

4. Discussion

4.1. Comparative analysis of thermal acclimation capacity

Functional analyses of the concomitant effects of thermal acclimation on both heat and cold tolerance have been made in many species (e.g., crabs [10], planarians [15], earthworms [16,17], flies [18], ticks [19], fish [20–23], crayfish [24], copepods [25]). However, few of these studies examined the relative effects of acclimation temperature on thermal tolerance in multiple species adapted to different thermal habitats. Thermal acclimation of three species of *Drosophila* from different thermal microhabitats had a greater affect on CT_{\min} than on CT_{\max} ; however, this pattern did not vary among species and acclimation capacity was not clearly related to thermal habitat [18]. In porcelain crabs from different thermal habitats, this study shows that CT_{\max} is inversely correlated with maximal habitat temperature, and the opposite is true for CT_{\min} (Fig. 3). Although phylogenetics-based transformation of these data does not clarify the evolutionary significance of this relationship, the results suggest an evolutionary and/or functional trade-off between achieving extreme tolerance limits and maintaining plasticity of tolerance limits.

4.2. Ecological significance of thermal acclimation capacity

To assess the ecological consequences of the relationship between acclimation capacity of CT_{\max} and maximal habitat temperature, it is necessary to also consider the proximity of thermal performance limits to maximal habitat temperatures. In the most warm adapted species, *P. gracilis*, CT_{\max} of summer acclimatized crabs (25–30 °C) is within 1 °C of its maximal habitat temperature [13], whereas in the least warm adapted species, *P. eriomerus*, CT_{\max} of summer acclimatized crabs (13 °C) is over 15 °C away from its maximal habitat temperature ([13]; Figs. 2A and 3). Since the acclimation capacity of CT_{\max} of *P. gracilis* is only 1 °C across a 20 °C range of acclimation temperature (Fig. 2A), if maximal habitat temperatures that this species experiences change by more than 2

°C as a result of global climate change, it is likely that there will be a decline in abundance of this species. Thus, it is the most heat-tolerant species that will be most susceptible to global warming, a result that is counterintuitive.

References

- [1] P.W. Hochachka, G.N. Somero, *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*, Oxford University Press, New York, 2002.
- [2] J.G. Kingsolver, R.B. Huey, Evolutionary analyses of morphological and physiological plasticity in thermally variable environments, *American Zoologist* 38 (3) (1998) 545–560.
- [3] J. Stillman, G. Somero, Adaptation to temperature stress and aerial exposure in congeneric species of intertidal porcelain crabs (genus *Petrolisthes*): correlation of physiology, biochemistry and morphology with vertical distribution, *Journal of Experimental Biology* 199 (Pt 8) (1996) 1845–1855.
- [4] B. Helmuth, et al., Climate change and latitudinal patterns of intertidal thermal stress, *Science* 298 (5595) (2002) 1015–1017.
- [5] B. Helmuth, How do we measure the environment? Linking intertidal thermal physiology and ecology through biophysics, *Integrative and Comparative Biology* 42 (4) (2002) 837–845.
- [6] J.E. Podrabsky, G.N. Somero, Changes in gene expression associated with acclimation to constant temperatures and fluctuating daily temperatures in an annual killifish *Austrofundulus limnaeus*, *Journal of Experimental Biology* 207 (2004) 2237–2254.
- [7] L. Hughes, Climate change and Australia: trends, projections and impacts, *Australian Ecology* 28 (4) (2003) 423–443.
- [8] L. Hughes, Biological consequences of global warming: in the signal already apparent? *Trends in Ecology and Evolution* 15 (2000) 56–61.
- [9] J.P. Barry, et al., Climate-related, long-term faunal changes in a California rocky intertidal community, *Science* 267 (5198) (1995) 672–675.
- [10] J.H. Stillman, Acclimation capacity underlies susceptibility to climate change, *Science* 301 (5629) (2003) 65.
- [11] J.H. Stillman, Causes and consequences of thermal tolerance limits in rocky intertidal porcelain crabs, genus *Petrolisthes*, *Integrative and Comparative Biology* 42 (4) (2002) 790–796.
- [12] J.H. Stillman, C.A. Reeb, Molecular phylogeny of eastern Pacific porcelain crabs, genera *Petrolisthes* and *Pachycheles*, based on the mtDNA 16S rDNA sequence: phylogeographic and systematic implications, *Molecular Phylogenetics and Evolution* 19 (2) (2001) 236–245.
- [13] J.H. Stillman, G.N. Somero, A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus *Petrolisthes*: influences of latitude, vertical zonation, acclimation, and phylogeny, *Physiological and Biochemical Zoology* 73 (2) (2000) 200–208.
- [14] C.G. Romero, *Sistemática, Biología y Ecología de Los Anomuros (Crustaceos: Decapodos) de Laguna Percebu, Alto Golfo de California [M.S.]*, Universidad Autónoma de Baja California, Ensenada, 1982.
- [15] H. Tsukuda, K. Ogoshi, Heat and cold tolerance of the planarian, *Dugesia japonica*, in relation to acclimation temperature, *Nippon Dobutsugaku Iho* 51 (1978) 70–78.
- [16] G.A. Nair, S.A. Bennour, Thermal reactions of the earthworm *Aporrectodea caliginosa* (Savigny 1826) (Oligochaeta: Lumbricidae), *Proceedings of the Indian National Science Academy, Part B* 63 (1977) 53–62.
- [17] M.M. Hanumante, Thermal relations of the tropical poikilotherm, *Perionyx excavatus* (Oligochaeta: Megascolecidae), *Geobios* 4 (1977) 21–26.
- [18] T. Ohtsu, C. Katagiri, M.T. Kimura, Biochemical aspects of climactic adaptations in *Drosophila curviceps*, *D. immigrans*, and *D. albicans* (Diptera: Drosophilidae), *Physiological and Chemical Ecology* 28 (1999) 968–972.
- [19] M.M. Hanumante, P.M. Patil, R. Nagabhushanam, Thermobiology of the ixodid tick *Hyalomma anatolicum anatolicum*, *Revista di Parasitologia* 42 (1981) 67–78.
- [20] R.M. Hernandez, R.L.F. Buckle, Temperature tolerance polygon of *Poecilia sphenops* Valenciennes (Pisces: Poeciliidae), *Journal of Thermal Biology* 27 (1) (2002) 1–5.
- [21] F.E.J. Fry, J.R. Brett, G.H. Clawson, Lethal temperature limits of young goldfish, *Revue Canadienne de Biologie* 1 (1942) 50–56.

- [22] J.R. Brett, Some principles in the thermal requirements of fishes, *Quarterly Review of Biology* 31 (1956) 75–87.
- [23] O.A.M. Al-Habbib, M.P. Yacoob, Effects of acclimation and experience to changing heat and cold shock temperature on lethal temperature and thermal tolerance of *Gambusia affinis* (Baird and Girard) (Poeciliidae), *Cybiurn* 17 (1993) 265–272.
- [24] J.R.J. Layne, M.L. Manis, D.L. Claussen, Seasonal variation in the time course of thermal acclimation in the crayfish *Orconectes rusticus*, *Freshwater Invertebrate Biology* 4 (1985) 98–104.
- [25] B.P. Bradley, Increase in range of temperature tolerance by acclimation in the copepod *Eurytemora affinis*, *Biological Bulletin* 154 (1978) 177–187.