Effect of thermal acclimation on thermal preference, resistance and locomotor performance of hatchling soft-shelled turtle

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Abstract The significant influence of thermal acclimation on physiological and behavioral performance has been documented in many ectothermic animals, but such studies are still limited in turtle species. We acclimated hatchling soft-shelled turtles Pelodiscus sinensis under three thermal conditions (10, 20 and 30°C) for 4 weeks, and then measured selected body temperature (Tsel), critical thermal minimum (CT Min) and maximum (CT Max), and locomotor performance at different body temperatures. Thermal acclimation significantly affected thermal preference and resistance of P. sinensis hatchlings. Hatchling turtles acclimated to 10°C selected relatively lower body temperatures and were less resistant to high temperatures than those acclimated to 20°C and 30°C. The turtles’ resistance to low temperatures increased with a decreasing acclimation temperature. The thermal resistance range (i.e. the difference between CT Max and CT Min, TRR) was widest in turtles acclimated to 20°C, and narrowest in those acclimated to 10°C. The locomotor performance of turtles was affected by both body temperature and acclimation temperature. Hatchling turtles acclimated to relatively higher temperatures swam faster than did those acclimated to lower temperatures. Accordingly, hatchlings acclimated to a particular temperature may not enhance the performance at that temperature. Instead, hatchlings acclimated to relatively warm temperatures have a better performance, supporting the “hotter is better” hypothesis [Current Zoology 59 (6): 718–724, 2013].

Keywords Pelodiscus sinensis, Thermal acclimation, Thermal resistance, Locomotor performance

Temperatures may vary considerably in natural environments, and impose pervasive impacts on almost every aspect of an organism’s life (Huey, 1982; Angilletta et al., 2002). For ectothermic animals, the ability to resist temperature extremes is normally limited, and prolonged exposure to extremely low or high temperatures may result in death (Cowles and Bogert, 1944; Lutterschmidt and Hutchinson, 1997). Even within the range of viable temperatures, changes in environmental temperature can alter the body temperatures, and thus physiological and behavioral performances (Huey and Stevenson, 1979; Huey, 1982). Meanwhile, organisms also can alter the effects of such temperature variations by thermal acclimation (Huey and Berrigan, 1996; Angilletta et al., 2002). Thermal acclimation, the process in an individual organism adjusting to a change in temperature, may occur to a number of physiological and behavioral traits including thermal resistance, thermal preference, and locomotor performance (Angilletta et al., 2002; Lagerspetz, 2006). Thermal acclimation of these physiological and behavioral traits has been widely studied both in the field (e.g. seasonal and geographic acclimatization) and the laboratory in various organisms from insects to mammals (Chaffee and Roberts, 1971; Wilson et al., 2000; Lagerspetz and Vainio, 2006; Gvoždík et al., 2007; Lachenicht et al., 2010).

Thermal acclimation may affect thermal preference and resistance. The relationship between acclimation temperature and preferred body temperature (or selected body temperature, T sel) appears to be diverse. For example, after acclimation to warmer temperatures, some fish species tend to prefer warmer temperatures, but other species do not change or may even decrease the preferred temperatures (Johnson and Kelsch, 1998). It has been predicted that species that experienced long and high-amplitude thermal cycles will exhibit a posi-
tive relationship between acclimation temperature and preferred temperature, whereas species that experienced short or low-amplitude thermal fluctuations have no relationship, or negative relationship, between these two variables (Johnson and Kelsch, 1998; Lagerspetz and Vainio, 2006). In most ectothermic animals, heat acclimation often enhances high-temperature resistance, while cold acclimation enhances low-temperature resistance (Rajaguru and Ramachandran, 2001; Lagerspetz and Vainio, 2006).

Thermal acclimation of locomotor performance has been widely investigated in ectotherms, because locomotor performance, which is closely related to the animal’s ability to forage and avoid predators, is an important determinant of the organism’s fitness (Arnold, 1983; Leroi et al., 1994). For example, under laboratory conditions, many species of fish acclimated to constant temperatures will alter their thermal sensitivity of locomotor performance (Johnson and Bennett, 1995; Wilson et al., 2007; Grigaltchik et al., 2012). Nevertheless, some small crustaceans and insects fail to acclimate their locomotor performance after experiencing predictable or stochastic cooling (Schuler et al., 2011; Niehaus et al., 2012). Several hypotheses have been proposed to explain the thermal acclimation effects on locomotor performances or fitness of animals (Wilson and Franklin, 2002; Deere and Chown, 2006). For example, the beneficial acclimation hypothesis predicts that acclimation to a particular temperature should enhance animal performance or fitness at that temperature (Leroi et al., 1994), whereas the hotter is better hypothesis predicts that animals acclimated to high temperatures should have a better performance or higher fitness across all temperatures than those acclimated to intermediate or low temperatures (Huey and Berrigan, 1996).

The ability for thermal acclimation of behavioral and physiological traits may vary among different species, populations, or even at different ontogenetic stages (Wilson et al., 2000; Grigaltchik et al., 2012). It has been predicted that thermal acclimatory responses of animals in aquatic habitats will be more easily observed than those in terrestrial habitats, because thermally variable environments may select for thermally independent physiological performances, resulting in reduced acclimatory abilities (Wilson et al., 2000). Acclimatory changes in thermal preference, thermal resistance and whole-animal performance have been investigated in reptiles, mainly including terrestrial taxa such as lizards and snakes (Kaufmann and Bennett, 1989; Huang et al., 2006, 2007; Yang et al., 2008; Li et al., 2009; Wang et al., 2013). However, studies addressing thermal acclimatory responses in aquatic reptiles such as most turtles are still limited (Wood et al., 1978; Hammond et al., 1988; Williamson et al., 1989; Tamplin and Cyr, 2011). To verify the prediction of divergent thermal acclimatory responses between species from different habitats in reptiles, it is necessary to collect more data from other species, particularly from aquatic reptiles.

The Chinese soft-shelled turtle *Pelodiscus sinensis* is a freshwater turtle species that widely distributed in central and southern China and south-eastern Asia (Zhao and Adler, 1993). This species is currently widely cultured in China, for food and traditional medicine. Previous studies have indicated that thermal environments during embryonic development can affect hatching traits, including body size, locomotor performance, post-hatching growth (Du and Ji, 2003), thermal tolerance, and thermal dependence of locomotor performance in hatching turtles (Sun et al., 2002). In the present study, we acclimated hatching *P. sinensis* under three constant temperature conditions for 4 weeks to assess the effects of thermal acclimation on thermal preference, resistance and swimming performance, particularly, to test for the above-mentioned hypothesis. The beneficial acclimation hypothesis would be supported if the locomotor performance of turtles from different thermal treatments is maximized at their own acclimated temperatures respectively, whereas the hotter is better hypothesis would gain support if a better locomotor performance is observed in turtles from the high acclimated temperature. On the basis of previous results on thermal effects on thermal preference and tolerance, we predict that hatching turtles acclimated to high temperatures would be more resistant of high temperatures but less resistant of low temperatures, and select higher body temperatures than do those acclimated to low temperatures.

1 Materials and Methods

1.1 Animal collection and maintenance

All juvenile turtles used in the present study were obtained from the eggs incubated in our laboratory. In mid-May 2012, we collected 188 fertilized eggs, for which maternal identity was not known, from a private hatchery in Hangzhou (Zhejiang, eastern China), and transferred them to our laboratory at Hangzhou Normal University, where they were weighed to the nearest 1 mg on a Mettler balance. The eggs were randomly allo-
cated into plastic containers (25 × 20 × 10 cm³) filled with moist vermiculite (~12 kPa, Du and Ji, 2003). All containers were put into one FPQ incubators (Ningbo Life Science and Technology Ltd., China), in which the temperature was set at 28 ± 1°C. About 2 months later, a total of 164 turtles were hatched. After hatching, the turtles were housed in twelve 60 × 45 × 30 cm³ aquaria (13–15 individuals in each aquarium) in a 15 cm depth of water. Water temperature was controlled at 28 ± 1°C. Pieces of tiles and layers of plastic plates were placed in the aquaria to provide shelters for the turtles. Throughout the experiment, turtles were fed an excess amount of commercial food daily (food composition: 10% water, 60% proteins, 5% lipids, 5% carbohydrates and 20% minerals).

In mid-July, hatching turtles were randomly divided into three groups, each of which was assigned to one of the three temperature treatments: 10°C (n = 55); 20°C (n = 54); and 30°C (n = 55). Juvenile *P. sinensis* usually hibernate at temperatures lower than about 10°C, and achieve a maximum growth rate at 30°C (Niu et al., 1999). Each group of animals was evenly housed in four aquaria before being transferred to three temperature-controlled rooms set at the experimental temperatures, respectively. The fluorescent tubes in the rooms were switched on at 07:00 h and off at 18:00 h. Turtles were acclimated at the designated temperatures for 4 weeks. During the acclimatization period, five hatching turtles (3 at 10°C and 2 at 20°C) died.

1.2 Experimental process

A total of 72 hatching turtles (10°C, n = 22; 20°C, n = 22; 30°C, n = 28) were used to measure for selected body temperature (T<sub>sel</sub>), critical thermal minimum (CT<sub>Min</sub>) and maximum (CT<sub>Max</sub>) in October. The CT<sub>Max</sub> and CT<sub>Min</sub> were used to assess thermal resistance, which were defined as the upper and lower temperatures, respectively, at which the organisms lose the ability to escape from lethal conditions (Cowles and Bogert, 1944; Paladino et al., 1980). Turtles were fasted for one day prior to testing. The experimental sequence was T<sub>sel</sub>, CT<sub>Max</sub> and CT<sub>Min</sub> at one-week intervals. All turtles were maintained in their housed aquaria during the intervals of trials, to minimize potential interference between trials.

We assessed the T<sub>sel</sub> in 200 × 20 × 25 cm³ tin cages with 10 cm depth water and pieces of gravel, which were placed in a temperature-controlled room set at 18°C. A thermal gradient, ranging from 18 to 50°C, was created by placing an electric stove below one end of the cage so that turtles could regulate body temperature within their voluntary range. Turtles were introduced from the cold side into the cage at 07:00 h. T<sub>sel</sub> measurements on each trial day began at 15:00 h and ended within 2 h. The cloacal temperature of each animal was measured with a UT-325 electronic thermometer (Uni-Trend Group Ltd., Shanghai, China). Each turtle was measured twice within 3 days, and the mean value was used for statistical analysis.

We used the dynamic method to determine CT<sub>Min</sub> and CT<sub>Max</sub> (Kour and Hutchison, 1970, Lutterschmidt and Hutchison, 1997). Trials were conducted in the FPQ incubators during 10:00–15:00 h. We cooled or heated the turtles from their acclimation temperatures at a rate of 0.3°C min⁻¹, and more slowly (0.1°C min⁻¹) when temperatures were lower than 5°C or higher than 35°C. Body temperatures associated with a transient loss of the righting response (right themselves after being placed on the backs) were considered as the endpoints for CT<sub>Min</sub> and CT<sub>Max</sub>, respectively (Qu et al., 2011). All turtles were recovered after testing, and no deaths occurred during the month following the termination of the tests.

The remaining 87 turtles (10°C, n = 30; 20°C, n = 30; 30°C, n = 27) were measured for locomotor performance at four body temperatures ranging from 15 to 33 (±0.5) °C (The temperature of 9°C was not included, because most turtles did not swim in the bath at that temperature). The trial sequence was randomized across test temperatures. Locomotor performance was evaluated by swimming speed inside a bath (120 × 10 × 20 cm³) with 10 cm depth water. The water temperature was maintained at the test level during trials. Body temperatures of turtles were achieved by placing them into an incubator at the corresponding temperatures for approximately 1 h prior to each trial. Turtles were placed into the bath, and then gently tapped on the mid-body with a paintbrush to encourage them to swim. We filmed them with a Panasonic HDC-HS900 digital video camera (Panasonic Co., Japan), and examined tapes with a computer using MGI VideoWave III software (MGI Software Co., Canada) for the maximal speed in 25 cm interval and average speed over 100 cm. To minimize the possible influence of diel variation in swimming performance, measurements on any given day started at 13:00 hr (Beijing time) and ended within 2 h.

1.3 Statistical analyses

We used Statistica 6.0 (StatSoft, Tulsa, USA) to analyze data. Data were tested for normality using Kolmogorov-Smirnov tests, and for homogeneity of variances using Bartlett’s test. Because the gender of hatching
turtles was difficult to determine, this factor was ignored in all analyses. We used one-way ANOVA to test the effects of acclimation temperature on $T_{sel}$, $CT_{Min}$ and $CT_{Max}$, and repeated-measures ANOVA to test the effects of acclimation temperature and body temperature on locomotor performance. Multiple comparisons were performed using Tukey’s test. Throughout the present paper, values were presented as mean ± SE and range, and the significance level was set at $\alpha = 0.05$.

2 Results

$T_{sel}$ and $CT_{Max}$ in turtles acclimated to 20°C was not different from those acclimated to 30°C, but significantly greater than those acclimated to 10°C ($T_{sel}$, $F_{2, 69} = 14.49, P < 0.0001$; $CT_{Max}$, $F_{2, 69} = 111.10, P < 0.0001$). $CT_{Min}$ shifted upward as acclimation temperature increased, with $CT_{Min}$ shifting from 2.7 to 4.8°C at the change-over of acclimation temperature from 10 to 30°C ($F_{2, 69} = 71.31, P < 0.0001$). Thermal resistance range (i.e., the difference between $CT_{Max}$ and $CT_{Min}$, TRR) was greatest in turtles acclimated to 20°C and smallest in those acclimated to 10°C, with those acclimated to 30°C in between ($F_{2, 69} = 6.81, P < 0.01$, Fig. 1).

Within each temperature treatment, the swimming capacity of turtles was independent of body size (mass) (linear regression analysis, all $P > 0.05$). Swimming speed of turtles increased with increasing body temperature within the range of 15 to 33°C (average speed, $F_{3, 252} = 132.98, P < 0.0001$; maximal speed, $F_{3, 252} = 70.50, P < 0.001$, Fig. 2). Swimming speed was also affected by acclimation temperatures, with the turtles acclimated to high temperature swimming faster than those acclimated to low temperature (average speed, $F_{2, 84} = 13.00, P < 0.001$; maximal speed, $F_{2, 84} = 12.48, P < 0.001$, Fig. 2). The interaction of body temperature and acclimation temperature had no significant effect on swimming speed (average speed, $F_{6, 252} = 1.93, P = 0.077$; maximal speed, $F_{6, 252} = 1.32, P = 0.247$).

3 Discussion

As found in other ectotherms (Wilson et al., 2000; Gvoždík et al., 2007; Yang et al., 2008), our data showed that temperature acclimation affected thermal preference, thermal resistance and locomotor performance of hatchling turtles. Despite widespread thermal acclimation across various lineages of ectotherms, the patterns of such acclimation effects may vary among different species (Rajaguru and Ramachandran, 2001; Huang et al., 2007; Li et al., 2009).

Selected body temperatures ($T_{sel}$), that ectotherms try to maintain the ideal body temperature for satisfying multiple aspects of physiological or behavioral proc-

![Fig. 1 Selected body temperature, critical thermal minimum, critical thermal maximum and thermal resistance range of Pelodiscus sinensis hatchlings acclimated to different temperatures](image-url)
esses, are often measured in a laboratory thermal gradient, and used as the preferred temperature (Licht et al., 1966). Hatchling turtles acclimated to the lowest temperature (10°C) selected low temperatures in the thermal gradient than did those acclimated to higher temperatures (20 and 30°C). Turtles acclimated to 30°C did not, however, select higher body temperatures than those acclimated to 20°C. That Tsel is not maximized at higher acclimation temperatures might be the result of a trade-off between costs and benefits of thermoregulatory behavior (Li et al., 2009), because thermoregulation in a given environment enables ectotherms to maintain their body temperatures within an optimal range, but it also entails several costs, such as an increase in energy demand and predation risk (Sartorius et al., 2002). Similar results were also found in other reptile species. For example, three Eremias lizards acclimated to three constant temperatures all had the maximized Tsel at the intermediate temperature rather than at the highest temperature (Li et al., 2009). Moreover, in other lizard species as well as in other ectotherms (Graham and Hutchison, 1979; Lagerspetz and Vainio, 2006), Tsel did not vary as acclimation temperatures changed (Licht, 1968), or even decreased with increasing acclimation temperatures (Wilhoft and Anderson, 1960).

Consistent with our prediction, the lower (CTMin) and upper (CTMax) limits of thermal resistance ability of P. sinensis hatchlings increased as acclimation temperature increased. Hatchling turtles acclimated to low temperatures might be more resistant of low temperatures but less resistant of high temperatures, whereas those acclimated to high temperatures might be more resistant of high temperatures but less resistant of low temperatures. Such results have been found in nearly all studied species of ectothermic vertebrates. Nonetheless, it is noteworthy that CTMax is not always maximized at the highest acclimation temperatures in some lizard species. For example, CTMax of E. argus individuals acclimated to 33°C was greater than that of those acclimated to 28°C or 38°C (Li et al., 2009); the western fence lizards Sceloporus occidentalis acclimated to 25°C had greater CTMax than those acclimated to 15°C or 35°C (Kour and Hutchison, 1970). In addition, the acclimation response ratio (ARR, the tolerance change per change in acclimation temperatures) was used to denote the physiological response of animals to a given temperature change (Claussen, 1977). Chatterjee et al. (2004) predicted that the magnitude of change of CTMin or CTMax should be reduced, and tend to be close towards zero as acclimation temperatures gradually approach such thermal limits. Fitting well with this prediction, the ARR value of CTMin (0.12) was lower than that of CTMax (0.25) at acclimation temperatures between 10 and 20°C, but slightly higher at acclimation temperatures between 20 and 30°C in P. sinensis hatchlings (0.09 for CTMin 0.04 for CTMax). In most studied species including fish, amphibians and reptiles, the ARR values of CTMax are generally relatively lower than those of CTMin at acclimation temperatures approaching the upper thermal limits (Kour and Hutchison, 1970; Chatterjee et al., 2004; Huang et al., 2006, 2007; Wang et al., 2008, 2013). Whether the ARR values of CTMin are lower than that of CTMax at acclimation temperatures approaching the lower thermal limits is, however, far from conclusive, probably because the magnitude of tolerance response to thermal acclimation varies considerably among different species. This may reflect inter-species differences in the ability to expand thermal tolerance under different thermal environments. In our study, TRR of P. sinensis hatchlings was maximized at an acclimation temperature of 20°C. The widest TRR at the intermediate acclimation temperature was also found in other types of species, such as the southern catfish Silurus...
**meridionalis**, northern grass lizard *Takydromus septentrionalis* and Taiwanese pit-viper *Trimeresurus gracilis* (Huang et al., 2007; Wang et al., 2008; Yang et al., 2008). These consistent results suggest that many ectothermic animals have greater thermal resistance ability under mild thermal conditions that are closer to the environmental temperatures in their natural habitats. Nevertheless, TRR can also be maximized at relatively low acclimation temperatures in several species. For example, the widest TRR was exhibited at an acclimation temperature of 10°C rather than at 20 or 30°C in two snake species, the brown spotted pit-viper *T. marmoratus* and Chinese green tree pit-viper *T. s. stejnegeri* (Huang et al., 2007).

At the test temperatures from 15 to 33°C, the swimming speed of hatchling turtles increased as acclimation temperature increased. Such results suggest that acclimation to relatively high environmental temperatures should be propitious to improve locomotor performance of *P. sinensis* hatchlings. Accordingly, our study may support the hotter is better hypothesis, which predicts that animals acclimated to high temperatures have a better performance, or higher fitness, across all temperatures than do those acclimated to low or intermediate temperatures, but not support the beneficial acclimation hypothesis (Huey and Berrigan, 1996).

Thermal acclimation effects on locomotor performances of animals vary among different taxa or even among different ontogenetic stages within a single species (Johnson and Bennett, 1995; Kaufmann and Bennett, 1989; Wilson et al., 2000, 2007). For example, in some species of amphibians, a significant effect of thermal acclimation on swimming performance was found before metamorphosis, but not after metamorphosis (Wilson et al., 2000). These among-species and ontogenetic differences in thermal acclimatory ability of locomotor performance in ectotherms may be related to temperature variations in their natural environments. Animals from habitats with large daily temperature fluctuations often have reduced thermal acclimatory abilities, whereas those from thermally stable habitats display obvious acclimatory responses (Wilson et al., 2000). Thermal acclimatory changes in locomotor performance are associated with the changes in muscle contractile properties and enzyme activities, which have been demonstrated in several species of fish and amphibian (Johnson and Bennett, 1995; Wilson et al., 2000; Johnston and Temple, 2002). For example, acclimatory change of escape performance in the goldfish *Carassius auratus* is tightly coupled to thermal acclimation of myosin isoform expression, myofibrillar ATPase activity and contractile kinetics, whereas a reduced acclimatory response in the killifish *Fundulus heteroclitus*, which often experiences relatively large thermal fluctuations, is accompanied by only minor changes in muscle properties (Johnson and Bennett, 1995).

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