Thermal tolerance for two cohorts of a native and an invasive freshwater turtle species

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Abstract. The ability to tolerate environmental stress may determine invasion success of alien species. Comparative data on physiological thermal tolerance between native and invasive vertebrates are quite limited. Here, we assessed the difference in thermal tolerance between a native (Mauremys reevesii) and an invasive (Trachemys scripta elegans) freshwater turtle species. We incubated eggs of M. reevesii and T. scripta elegans from different cohorts at 29 °C, and measured the critical thermal minimum (CTMin) and maximum (CTMax) of hatchlings. Our results preliminarily showed that the hatchlings of T. scripta elegans had a greater high-temperature tolerance and wider tolerance range than the hatchlings of M. reevesii; in the two-cohort system, individuals from the high-latitude cohort seemed to have greater low-temperature tolerance but similar high-temperature tolerance compared with those from the low-latitude cohort. Relatively greater thermal tolerance ability for T. scripta elegans might reflect its environmental adaptability to thermal stress.

Keywords. Mauremys reevesii, Trachemys scripta elegans, critical thermal limits, thermal tolerance ability, invasion success.

Introduced alien species must overcome several potential challenges due to local climatic and biotic conditions to become established and successfully invade a novel environment (Crowl et al., 2008; Kelley, 2014). Climatic constraints have been considered as the first barrier for restricting invasion success of alien species (Olyarnik et al., 2009). The ability of invasive species to withstand harsh environmental conditions, such as high salinity, drought and extreme temperatures, may affect their invasive potential (Lee, 2002; Bates et al., 2013; Kelley, 2014). It has been shown that many invasive species are often more tolerant to environmental stress than co-occurring native species (Nyangkondiwa et al., 2010; Lockwood and Somero, 2011; Zerebecki and Sorte, 2011; Weldon et al., 2016).

One of the most important environmental factors impacting species’ distributions is temperature (Johnston and Bennett, 2008; Gallien et al., 2010). Physiological thermal tolerance for a given species is limited (Angilletta, 2009). Eurythermal species can maintain physiological function over a wider temperature range and are expected to have a broader distribution range than stenothermal ones (Overgaard and Hoffmann, 2011; Gerick et al., 2014). Eurythermality may contribute to the successful invasion of alien species. Consequently, invasive species are expected to be eurythermal more often than native species (Zerebecki and Sorte, 2011; Kelley, 2014). A comparison of the physiological thermal tolerance between native and invasive organisms has been made in many species (e.g., Kimball et al., 2004; Kolbe et al., 2010; Lockwood and Somero, 2011; Zerebecki and Sorte, 2011; Yu et al., 2012; Ju et al., 2013; Urquhart and Koetsier, 2014; Davies et al., 2015; Barahonegoveia et al., 2016). These studies mainly focused on invertebrate species, and some
results were opposite to the above prediction (Human and Gordon, 1996; McMahon, 2002; Barahona-Segovia et al., 2016). For example, two species of aquatic invasive bivalves (*Corbicula fluminea* and *Dreissena polymorpha*) show lower physiological thermal tolerances than the correspondent native species, because they experience less selection pressure for evolution of thermal tolerance (McMahon, 2002).

The red-eared slider turtle (*Trachemys scripta elegans*) is native to southern United States and northern Mexico, and has been introduced in many countries of Africa, Asia and Europe as a pet, becoming invasive in many areas, and posing a serious threat to the survival of native turtle species (Lowe et al., 2000; Cadi and Joly, 2004; Pearson et al., 2015). Previous studies have shown that *T. scripta elegans* has a number of distinct competitive advantages over native turtle species, such as a more aggressive behavior (Cadi and Joly, 2003; Polo-Cavia et al., 2010, 2011; Pearson et al., 2015), wider niche breadth (Polo-Cavia et al., 2008; Wang et al., 2013) and greater thermal inertia (Polo-Cavia et al., 2009). However, none of these studies has still focused on interspecies differences in physiological thermal tolerance between invasive *T. scripta elegans* and native turtle species. *T. scripta elegans* was introduced to China in the 1980s, and spread into most southern provinces over the last decades (Liu et al., 2011). Since its introduction, the native Chinese three-keeled pond turtle, *Mauremys reevesii*, which is one of the most common and widespread turtle species in southern China (Zhao and Adler, 1993), has suffered a considerable decline, and started to be displaced by *T. scripta elegans* in its natural habitats (Liu et al., 2011). Our aim here was to compare the thermal tolerance of the invasive *T. scripta elegans* and the native turtle species *M. reevesii*. For this purpose, we incubated eggs of both species from two sites at different latitudes and measured the critical thermal minimum (CTMin) and maximum (CTMax) of hatchlings.

We collected a total of 80 fertilized eggs (20 eggs selected randomly from unidentified clutches (probably 6–8 clutches for *M. reevesii* and 5–6 clutches for *T. scripta elegans*) for each cohort of both cultured turtle species) that laid within a 2 to 4-day period from two private hatcheries in Haikou (Hainan province, southern China, 19°46’N, 110°19’E, hereafter the low-latitude cohort) on May 22, and Haining (Zhejiang province, eastern China, 30°19’N, 120°25’E, hereafter the high-latitude cohort) on June 2 of 2016, respectively. The low-latitude site had a higher annual mean air temperature with relatively less thermal variation than the high-latitude site (Fig. 1, http://data.cma.cn). In both hatcheries, cultured turtles were kept in outdoor artificial ponds (length × width × height: 20 × 15 × 1.5 m³) and thus were exposed to the local thermal environments.

The eggs were randomly allocated into plastic containers (25 × 20 × 10 cm³) filled with moist vermiculite (approximately −12 kPa water potential, 1 g dried vermiculite/2 g water, Du et al., 2007). The eggs were half-buried in the substrate, with the surface near the embryo exposed to air inside the container. The containers were placed into a FPQ incubator (Ningbo Life Science and Technology Ltd., China) and held at a temperature of 29 ± 1 °C (this temperature yields an approximate 1:1 offspring sex ratio for both species; Wibbels et al., 1998; Du et al., 2007, 2009). Every other day, water was added into the substrate to keep the water potential of the substrate relatively constant. The containers were daily moved among the shelves to minimize any effects of thermal gradients inside the incubator. When eggs were found to have pipped, they were moved individually into glass jars. Body mass of each turtle was measured once yolk sac was completely absorbed. Then hatching turtles were housed individually in plastic containers (20 × 15 × 12 cm³) with 3 cm water depth, which placed in a temperature-controlled room at 29 °C with an 11 h light: 13 h dark photoperiod.

Thirty-two hatchlings (8 individuals in each cohort of both species) were randomly selected to measure CTMin and CTMax following the procedures by Xu et al. (2015). The hatchlings were placed into the FPQ incubators that initially were set at 29 °C, and then cooled or heated at a rate of 0.3 °C/min (but more slowly, at a rate of 0.1 °C/min, when temperatures were lower than 5 °C or higher.
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than 35 °C). The body temperatures of hatchlings were measured using an electronic thermometer (UT-325, Uninet Group Ltd., China) when they lost righting response. All trials were run between 10:00 and 15:00. We first measured CTMin, and then CTMax a week later to minimize possible interactions between CTMin and CTMax measurement. The thermal tolerance range (TTR) was calculated by subtracting the CTMin from the CTMax for each individual (Xu et al., 2015; Gu et al., 2016).

We used linear regression analysis to examine potential relationships between turtle body size (mass) and CTMin, CTMax or TTR. We used nested analysis of variance (ANOVA) or analysis of covariance (ANCOVA) with cohort being nested within species to determine whether there were differences in body mass, CTMin, CTMax and TTR between species and between cohorts. Normality of data and assumption of homogeneity of variances was checked using Kolmogorov-Smirnov tests and Bartlett’s test, respectively. All statistical analyses were performed using SPSS 18.0 for PC. The sex effect was ignored in this study because of the difficulty in determining the sex of hatchling turtles.

There was no significant difference in hatchling body mass between different cohorts (F 2,28 = 2.35, P = 0.114). However, T. scripta elegans hatchlings were heavier than M. reevesii hatchlings (F 1,28 = 53.70, P < 0.001) (Table 1). CTMin differed significantly between different cohorts (F 2,28 = 7.32, P < 0.01) but not between species (F 1,28 = 0.26, P = 0.612), whereas CTMax differed between species (F 1,28 = 6.06, P = 0.020) but not between different cohorts (F 2,28 = 0.76, P = 0.479). Overall, the cohorts of both turtle species from the low-latitude site had higher mean values of CTMin than the cohorts from the high-latitude site (Fig. 2A). T. scripta elegans had higher mean values of CTMax than M. reevesii in both cohorts (Fig. 2B). TTR differed significantly between species (F 1,28 = 4.53, P = 0.042) and between cohorts (F 2,28 = 3.57, P = 0.042). TTR of T. scripta elegans was wider than that of M. reevesii; and TTR for the high-latitude cohorts was wider than that for the low-latitude cohorts (Fig. 2C). Linear regression analysis showed that CTMax and TTR (but not CTMin) was positively correlated with body mass of turtles (CTMin, r^2 = 0.03, F 1,30 = 0.86, P = 0.361; CTMax, r^2 = 0.28, F 1,30 = 11.81, P < 0.01; TTR, r^2 = 0.22, F 1,30 = 8.31, P < 0.01). Both between-species (CTMax, F 1,27 = 0.002, P = 0.967; TTR, F 1,27 = 0.02, P = 0.887) and between-cohort (CTMax, F 2,27 = 0.27, P = 0.763; TTR, F 2,27 = 2.50, P = 0.101) differences in CTMax and TTR were not significant after removing the effect of body mass of turtles.

Table 1. Mean hatchling body mass (± SE) of two turtle species (Mauremys reevesii and Trachemys scripta elegans) from two different cohorts.

<table>
<thead>
<tr>
<th>Species</th>
<th>Low-latitude cohort</th>
<th>High-latitude cohort</th>
</tr>
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<tbody>
<tr>
<td>Mauremys reevesii</td>
<td>6.24 ± 0.23 g</td>
<td>6.17 ± 0.23 g</td>
</tr>
<tr>
<td>Trachemys scripta elegans</td>
<td>7.69 ± 0.34 g</td>
<td>8.47 ± 0.19 g</td>
</tr>
</tbody>
</table>

Fig. 2. Mean values (± SE) for critical thermal minimum (A), critical thermal maximum (B) and thermal tolerance range (C) of hatchlings of Mauremys reevesii and Trachemys scripta elegans from two different cohorts.
Our study was limited by relatively small sample size (8 individuals in each cohort for both species) and by the lack of cohort replication (one cohort from each site), but it could allow us to provide a preliminary evaluation on between-species and between-cohort differences in thermal tolerance of hatching turtles. Except for slightly higher CTMax for hatchling *M. reevesii*, the values of CTMin and CTMax in this study fell within the ranges reported in previous studies on these turtle species (Xu et al., 2015; Gu et al., 2016). Both *M. reevesii* and *T. scripta elegans* are semi-aquatic species. Their critical thermal limits were similar to the values for other semi-aquatic turtle species, but seemed to be intermediate between primarily aquatic (CTMin: 2.7–4.8 °C for *Pelodiscus sinensis*, Wu et al., 2013; CTMax: 39–41 °C, Hutchison et al., 1966) and terrestrial turtle species (CTMax: 43–44 °C, Hutchison et al., 1966).

Despite resulting from a larger body size, higher CTMax and wider TTR for *T. scripta elegans* probably indicated that invasive *T. scripta elegans* had greater thermal tolerance to high temperature and to thermally variable habitats than native *M. reevesii*. Such abilities may enhance invasion success when an alien species expands its geographic range and frequently faces extreme environmental conditions, such as near-critical high temperatures (Dukes and Mooney, 1999; Zerebecki and Sorte, 2011; Kelley, 2014). Relatively greater thermal tolerance ability has been found in most studied invasive species (Lockwood and Somero, 2011; Yu et al., 2012; Ju et al., 2013; Lejeusne et al., 2014; Davies et al., 2015). Unfortunately, due to only one native turtle species in our study, more data from other native turtle species should be collected before drawing the conclusion that thermal tolerance ability of invasive turtles is greater than native turtles.

The mechanistic bases for difference in thermal tolerance between native and invasive species may lie in differences in the expression of some stress-related genes and thermal sensitivity of some enzymes (Lockwood et al., 2010; Kelley et al., 2011; Zerebecki and Sorte, 2011; Yu et al., 2012). For example, a high level of heat-shock protein (HSP) 70 and 24 expression in invasive species is believed to contribute to enhancing their high-temperature tolerances (Zerebecki and Sorte, 2011). The ability to stabilize enzymatic activity at relatively high temperature may be related to high-temperature tolerance of invasive species (Lockwood and Somero, 2011).

Thermal tolerance abilities differ between low- and high-latitude populations (or cohorts) of some ectothermic species (Fangue et al., 2006; Yang et al., 2008; Kelley et al., 2011; Gaitán-Espitia et al., 2013). Such differences were also showed in both *M. reevesii* and *T. scripta elegans*, without considering hatchery pond effects. However, it was too early to draw a final conclusion due to the limitation in terms of lack of cohort replication. Our results preliminarily suggested that high-latitude turtles had a greater low-temperature tolerance than low-latitude turtles. Geographic difference in thermal tolerance might result from local adaptation to thermal environments (Kelley, 2014; Gaitán-Espitia et al., 2014). The individuals living in thermally variable environments should have a greater ability to withstand extreme temperatures than those living in relatively stable environments (Kelley, 2014). Turtles from the high-latitude site would experience larger daily and seasonal thermal fluctuations than those from the low-latitude site. This might be the cause of greater thermal (especially low-temperature) tolerances for high-latitude turtles. Furthermore, thermal tolerance plasticity may play a role in enhancing invasion potential of alien species (Nyamukondiwa et al., 2010; Tepolt and Somero, 2014; Davies et al., 2015). Over similar ranges of acclimation temperatures, CTMin and CTMax varied by 1.9 and 2.6 °C for *M. reevesii* (acclimation temperatures from 17 to 33 °C, Xu et al., 2015), and by 2.5 and 2.1 °C for *T. scripta elegans* (from 16 to 32 °C, Gu et al., 2016), respectively. The greater acclimation response of CTMin in *T. scripta elegans* may confer a survival advantage over other native turtles when facing a novel environment. Surprisingly, the magnitude of acclimation change of CTMax in *T. scripta elegans* seemed to be lower than in *M. reevesii*. It might probably reflect that the ability to adapt quickly to a low-temperature environment is more important than to a high-temperature environment for successful invasion of species spreading to colder regions.

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