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How survival and food intake of tri-spine horseshoe crabs, *Tachypleus tridentatus* respond to thermal variation: implications for understanding its distribution limit

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ABSTRACT

Temperature appears to have pervasive effects on larval development, feeding and movement patterns of tri-spine horseshoe crabs, *Tachypleus tridentatus*. To investigate how temperature determines their geographic distribution range, we examined the survival rate (SR), survival time (ST) and food intake of subadult *T. tridentatus* under different temperature levels in a seven-day experiment, followed by a three-day recovery to room temperature at 25°C. Significantly lower SR and ST of subadults were found at 40°C after the end of the seven-day experiment. Meanwhile, SR and ST of individuals at 0°C were negatively affected when the temperature was brought back to 25°C. Mean food intake of the subadults was statistically lower at 0–20°C and 35–40°C compared to that at 25°C and 30°C. After the subsequent three-day recovery to 25°C, only the individuals previously exposed to 15°C and 20°C had recovered to their normal level of feeding activities, but not the other treatment groups. These findings may provide preliminary data on how thermal tolerance determines the present distribution of *T. tridentatus*, which are found in large numbers around Beibu Gulf (annual temperature variation: 22–30°C), while considerably lower population densities are observed in the colder southern Sea of Japan and warmer seas of Southeast Asia.

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Horseshoe crab; thermal tolerance; distribution; temperature; recovery

Introduction

Gradients in environmental temperature commonly govern the latitudinal patterning of marine species distributions, particularly for ectothermic species with body temperatures that vary according to seasonal and tidal cycles (Helmuth 1999; Somero 2002). Sunday et al. (2012) examined 142 marine ectotherms and found that their geographic ranges conform closely to their thermal tolerance limits, which correspond to the highest summer and coldest winter temperatures. For instance, the marine copepod *Tigriopus californicus* has little potential for tolerance adaptation to an elevated temperature (Kelly et al. 2012). This finding, apart from being driven by temperature itself,

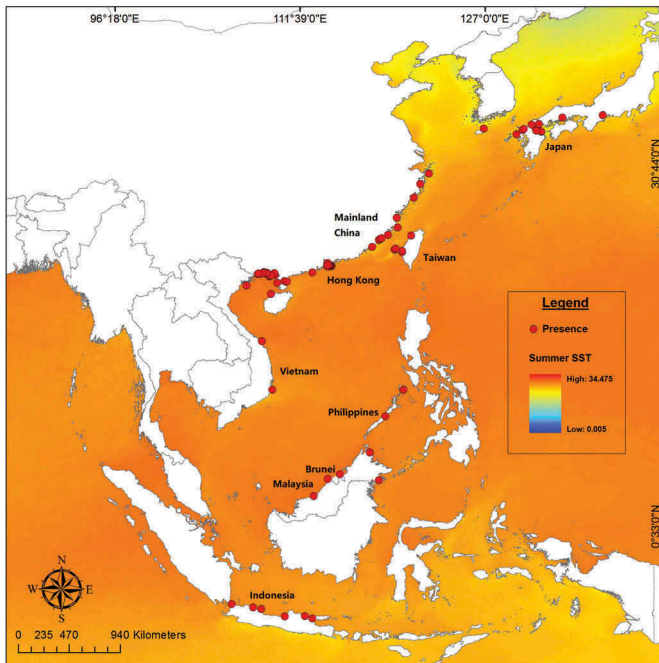
may also be due to other environmental factors that are closely related to the thermal condition, such as dissolved oxygen availability (Portner and Knust 2007).

Often referred to as marine 'living fossils', horseshoe crabs have undergone little morphological evolution since approximately 485 million years ago (Rudkin and Young 2009; Van Roy et al. 2010; Fortey 2011). Understanding the relationship between thermal tolerance limit and distribution of horseshoe crabs is paramount, particularly as populations have already decreased substantially across Asia-Pacific regions (Hsieh and Chen 2015; Kwan et al. 2016), and their responses to anthropogenic climate warming remain largely unknown (Faurby et al. 2010). The global distribution of tri-spine horseshoe crab, *Tachypleus tridentatus* is constrained to Asia-Pacific regions with varying water temperature: South Temperate (Japan, Zhejiang Province of China; 10–28°C), North Subtropical (Hong Kong, Taiwan, Fujian and Guangdong Provinces of China; 16–30°C), South Subtropical (Beibu Gulf, Vietnam; 22–30°C) and Tropical (Malaysia, Indonesia; 26–32°C). These regions are generally warm in the summer (Figure 1(a)), but winter water temperatures vary on a latitudinal basis (Figure 1(b)). Populations of *T. tridentatus* in the northern range, including Japan and China, are historically massive and widely distributed, whereas the populations in the southern range in Malaysia, Indonesia and Philippines are small and discrete. The area around Beibu Gulf is the centre of their distribution, and presently accommodates the largest populations (Brockmann and Smith 2009; Weng et al. 2012; Liao et al. 2019).

We have little understanding of how temperature affects distribution patterns of horseshoe crabs. Loveland et al. (1996) regarded horseshoe crabs as environmental generalists, but their current distribution worldwide indicates that they are not capable of surviving everywhere. Sekiguchi and Shuster (2009) concluded that continental temperature regimes and geomorphology are the two key factors which limit the widespread distribution of horseshoe crabs. Mayer (1914) reported the difference in temperature tolerance between two populations of the American horseshoe crab, *Limulus polyphemus*. *Limulus polyphemus* from Florida could not tolerate colder temperatures; conversely, those from Massachusetts suffered in warmer southern water temperatures. Similar to *L. polyphemus*, temperature appears to have a considerable impact on the embryonic development of *T. tridentatus* (Carmichael and Brush 2012). Among these studies, Hong (2011) found that egg incubation at a water temperature above 30°C could yield an impressively high survival rate (98%) and shorter hatching period (40 days). The juveniles were also noted to be actively feeding on intertidal flats during low tide in the summer, after hibernation in the winter and early spring (Chiu and Morton 2004; Hu et al. 2009). Wada et al. (2016) investigated the year-round movement patterns and residency of *T. tridentatus*, and concluded that water temperature was the determining factor that controls the locomotion and migration of the species. However, systematic studies reporting the effects of varying temperature levels on *T. tridentatus* are limited.

The present study investigates the survival rate, survival time and feeding of *T. tridentatus* under short-term exposure to various temperatures. Despite the fact that other physiological factors, such as changes in heart function, heat-shock protein expression, mitochondrial respiration and energetic cost, may be able to explain the temperature tolerance of *T. tridentatus*, this study aimed to select the two vital factors (i.e. survival

a) Summer



b) Winter

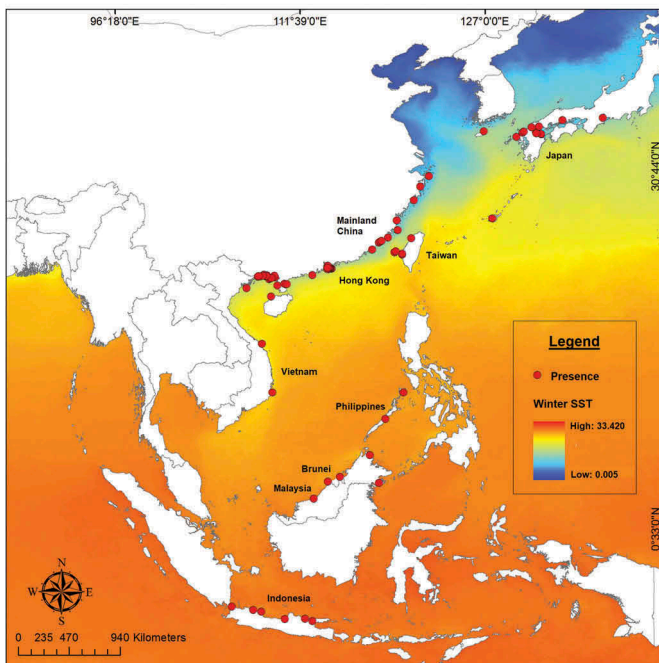


Figure 1. Global distribution of *T. tridentatus* as reported by previous literature, and the regional changes of sea surface temperature (SST) in (a) summer and (b) winter. Note the three occurrence reports outside their geographic range: (1) a non-native population from Chubu Region, Central Japan; (2) an escape population from Okinawa Prefecture, Japan due to broken nets during transportation; (3) a sighting of single female from Jeju, South Korea, which was not associated with a known spawning or juvenile nursery habitat.

and feeding) in providing preliminary information on the understanding of how thermal tolerance determines their present distribution patterns.

Methods

Sixteenth-instar subadult horseshoe crabs (in total 189 individuals, average mean prosomal width: 14.5 cm) were collected in August from the Dongnan Sea area of Leizhou Bay, China using a gill net. The subadults were transported back to the laboratory within an hour and allowed to acclimatise for 7 days under the following environmental conditions: temperature 25–28°C, salinity 28–30‰, pH 7.55–8.65, dissolved oxygen 6.3–7.0 mg O₂ L⁻¹ and photoperiod 12-h light:12-h dark cycle. The water quality was monitored three times a week before feeding, and water was entirely changed three times weekly or whenever water ammonia concentration was above 0.10 mg L⁻¹. None of the subadults showed signs of behavioural abnormalities or parasites on the carapace, gills and appendages during the one-week acclimatisation period.

The first experiment lasted for 7 days. Seven subadults were randomly assigned to each of the 27 aquarium tanks (55 L; three replicate tanks for each of the nine temperature levels) set at temperatures ranging from 0°C to 40°C with 5°C intervals. The water temperature was regulated using a chiller or thermostatically controlled heater. All tanks were filled with filtered natural seawater collected from Zhanjiang Sea. The water depth of each experimental tank was 25 cm to keep all subadults completely submerged. The individuals were fed 20 g of fresh cuttlefish (*Sepia aculeata*) daily at 19:00. Cuttlefish were purchased from a local fish market and cut into pieces (approximately 0.5–1.0 cm²) before the feeding, as described in Gao et al. (2003). After a three-hour feeding period, any uneaten feed was removed and reweighed. The water temperature was restored to 25°C for another 3 days when the seven-day treatment ended.

Survival rate (SR) and survival time (ST) of subadults were monitored every 12 h, and their relative food intake (I) was determined daily. The average of all the seven individuals in an aquarium tank was considered as a replicate. The calculations of SR, ST and I (Gao et al. 2003) were based on the below formulae:

$$SR = (N_s/N_i) \times 100\%,$$

where N_s is the total number of surviving horseshoe crabs from a particular temperature treatment after the experiment, and N_i is the initial number of horseshoe crabs.

$$ST = \frac{1}{N} \sum_{i=1}^N X_i$$

where N is the total number of experimental individuals, and X_i is the surviving hours of a particular subadult through the experiment.

$$I = (F/M) \times 100\%$$

where F is the total daily food intake, and M is the total body mass of individuals before the experiment.

Data were first examined for normality and homogeneity of variance using Shapiro–Wilk and Levene's tests, respectively. Since percentage data for subadult SR, ST and I failed to meet the normality requirements despite all possible arithmetic transformations, the

parameters were tested using non-parametric Kruskal–Wallis tests, followed by Mann–Whitney U tests with Bonferroni correction if a significant difference was detected between groups. The difference was considered significant at $p < 0.05$. All statistical analyses were conducted using SPSS software version 22.0 (International Business Machines Corp., Armonk, NY, USA).

Results

Survival rates and survival times among different treatments ranged from 0% to 100% and 72–168 h, respectively at the end of the first seven days of the experiment. No mortality was observed for treatments at temperatures of 0–35°C. Results of Kruskal–Wallis tests showed that significant differences were found in subadult SRs (Chi-square = 26.000, $p = 0.001$) and STs (Chi-square = 25.893, $p = 0.001$) among treatments. Mann–Whitney U post hoc tests with Bonferroni correction revealed significantly lower SRs and STs at 40°C. After the temperature was brought back to 25°C for 3 days, SRs and STs of experimental subadults exposed to temperatures of 0–35°C were in the range of 0–100% and 38–72 h, respectively. All individuals at 0°C died after the temperature restoration with a mean survival time of 38 h. Significant differences in SRs and STs were found after the three-day recovery (Kruskal–Wallis test, SRs: Chi-square = 23.000, $p = 0.002$; STs: Chi-square = 22.879, $p = 0.002$). Results of Mann–Whitney U post hoc tests with Bonferroni correction showed significantly lower subadult SRs and STs at 0°C after the three-day recovery period.

For mean food intake, the highest was recorded at 25°C (3.85%), followed by 30°C (3.12%) and 35°C (1.66%) after the seven-day treatment ([Figure 2\(a\)](#)). No feeding activity by the experimental individuals was observed at 0°C to 10°C or at 40°C. The percentage of mean food intake increased from 15°C to 20°C, peaked at 25–30°C, and then gradually decreased in the temperature range of 35–40°C. Kruskal–Wallis tests demonstrated significant differences in subadult food intake among different temperature levels (Chi-square = 51.479, $p = 0.000$). Results of Mann–Whitney U post hoc tests with Bonferroni correction showed that mean food intake at 25°C and 30°C was statistically higher than that at 15°C, 20°C and 35°C ([Figure 2\(a\)](#)). From Day 8 onwards, the individuals recovered to 25°C. Feeding activity was then noted in most treatment groups, except those exposed to extreme temperatures of 0°C and 40°C ([Figure 2\(b\)](#)). Significant differences in mean food intake were detected after the three-day recovery (Kruskal–Wallis test, Chi-square = 24.557, $p = 0.002$). The mean food intake of those under temperature treatments of 15°C and 20°C recovered from 0.28% to 0.86% to 5.68–5.93% ([Figure 2\(b\)](#)). However, negative and irreversible effects on the feeding of subadults, as reflected by their significantly lower mean food intake after the three-day recovery period, were found in the groups which were initially exposed to 5°C, 10°C and 35°C ([Figure 2\(b\)](#)).

Discussion

How temperature sets geographic range limits of marine species remains one of the most long-standing puzzles in ecology. Sunday et al. (2012) found that marine ectotherms occupy suitable habitats within latitudinal boundaries on the basis of their thermal tolerance. Sekiguchi and Shuster (2009) reported that the distribution of the four extant species of

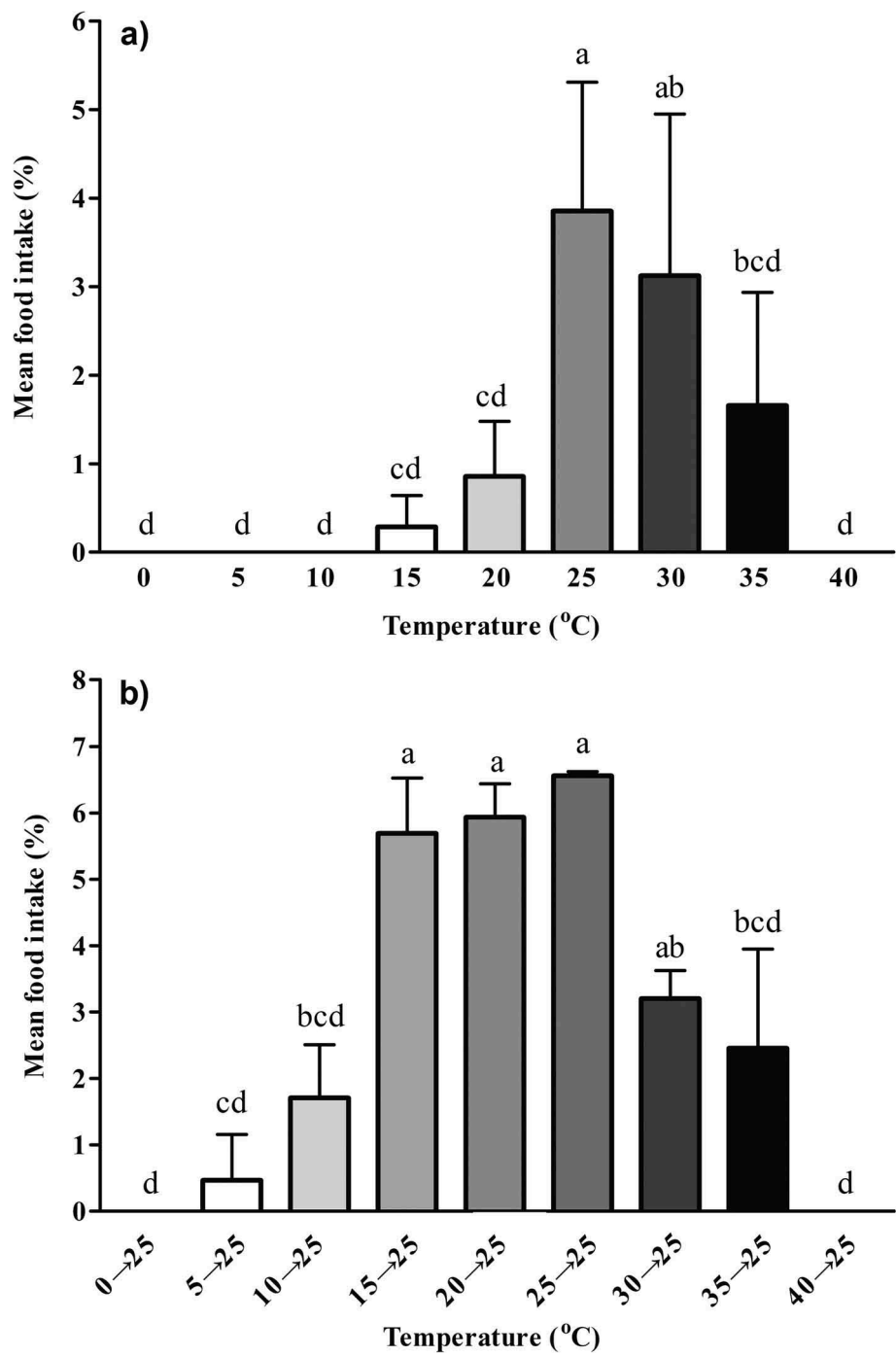


Figure 2. Effects of (a) different temperature levels for 7 days and (b) the subsequent recovery at 25°C for 3 days on the mean intake rate (%) of juvenile *T. tridentatus*. Values represent the mean + SD. Mann-Whitney U tests with Bonferroni correction with statistical difference between temperature levels and their recovery ($p < 0.05$) are presented in different lowercase letters.

horseshoe crabs is possibly constrained in their northern range, which implies that their tolerance to extreme winter temperatures may be limited. This study demonstrated that subadult *T. tridentatus* had little tolerance to temperatures outside the range of 25–30°C. Although the subadults were shown to survive in the temperatures of 5°C to 35°C for 7 days, the feeding activity of subadults previously exposed to 0–20°C and 35–40°C was still adversely affected after their three-day recoveries at room temperature. Similar to their American counterparts, *L. polyphemus*, foraging activity of *T. tridentatus* was optimum in the range of 25–30°C (Reynolds and Casterlin 1979). These findings are also consistent with the previous observations that juveniles actively foraged on intertidal flats covered with sea-grass during low tides in summer when the average water temperature was 25–29°C (Chiu and Morton 2004). A laboratory study by Lee and Morton (2009) also reported that no juvenile emerged from substratum under simulated tidal cycles when the water temperature was below 20°C. The decreased foraging activity due to extreme temperatures over an extended period can stimulate ‘hunger response’, resulting in a continuous decline in plasma glucose and increase in cholesterol, lipase and triglyceride (Hu et al. 2010).

The present findings, supported by the previous studies, may explain the current distribution of *T. tridentatus* which is concentrated mainly in southern China near Beibu Gulf (17°47′–21°28′ N), where the temperature variation is in the range of 22–30°C. The historical population of *T. tridentatus* was unusually high, up to 1.4 million individuals per year, in the 1980s (Liang 1985; Liao and Li 2001). Local fishers along the coast of northern Beibu Gulf indicated that the mean daily harvest of adult *T. tridentatus* offshore was circa 50–1,000 individuals in the 1990s (Liao et al. 2019). A density of 0.02–3.19 juvenile individuals per 100 m² was found on nursery grounds within northern Beibu Gulf (Chen et al. 2015; Hu et al. 2015), which is considerably higher than that found in Hong Kong (0.08–2.19 juvenile individuals per 100 m²; Kwan et al. 2016) when adopting a similar sampling method. However, *T. tridentatus* population densities in the colder northern China and the southern Sea of Japan are relatively lower (Seino et al. 2003; Weng et al. 2012; Nishida et al. 2015). The *T. tridentatus* population in China does not extend north to the Yellow Sea (Liao and Li 2001). The Yellow Sea is a gulf located between mainland China and the Korean Peninsula (Figure 1), where winter sea surface temperature can drop below 9°C due to the northerly monsoonal wind that brings in cold and dry air from the continent from late November to March (Xie et al. 2002). Similarly, the Sea of Japan, particularly the northern part, loses a huge amount of heat to the atmosphere in winter when the northwestern monsoon wind brings cold and dry air that lower the sea surface temperature (below 11°C) in the coastal areas of central and northeastern Japan (Seung and Yoon 1995). These suggest that the low temperature in winter generally governs the northern boundaries of *T. tridentatus* distribution (Sekiguchi and Shuster 2009). Coupled with coastal development and habitat degradation, *T. tridentatus* populations might be extirpated locally in Japan and Chinese coastal region around Yangtze Estuary, as predicted by the previous studies (Seino et al. 2003; Weng et al. 2012). Another possible explanation for the population’s distribution limit may be the lack of suitable spawning/nursery habitats associated with mangrove fringes (Chen et al. 2015; Fan et al. 2017). Highly productive mangrove ecosystems are found within tropical and subtropical areas between 30°N and 30°S (Kuenzer et al. 2011) in which the distribution is also strictly controlled by temperature regimes (Orth et al. 2000; Krauss et al. 2008). Despite the fact that the ecological relationship between mangrove and horseshoe crab population

remains unclear, it is noted that the northern boundaries of mangrove distribution perfectly overlap with the northern range limit of *T. tridentatus* populations. Our study provides a foundation from which future studies can examine further other physiological traits, such as heart function, heat-shock protein expression and changes in energetics, which potentially contribute to the distribution patterns of *T. tridentatus* population.

Conclusion

In conclusion, subadult *T. tridentatus* demonstrated characteristics of warm-water species in marine environments, namely that a) their optimal mean food intake occurs at 25–30°C, and b) they can cope with short-term temperature variations in the narrow range of 15–30°C given that their survival and feeding activities are not negatively affected. Our findings can thus serve as preliminary data explaining *T. tridentatus* distributional patterns where a higher abundance of the population is found in the northwestern South China Sea (22–30°C), but a considerably lower number of individuals are recorded in the colder southern Sea of Japan (10–28°C) and warmer Southeast Asia (26–32°C).

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Disclosure statement

No potential conflict of interest was reported by the authors.

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