

Latitudinal embryonic thermal tolerance and plasticity shape the vulnerability of oviparous species to climate change

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Abstract. Heat tolerance at the immobile embryonic stage is expected to be critical in determining species vulnerability to climate change. However, how the mean and developmental plasticity of embryonic heat tolerance vary geographically, and how these geographic variations affect species' vulnerability under climate change remain unknown. We experimentally determined the mean and developmental plasticity of embryonic acute heat tolerance (EAHT, i.e., heat shock temperature at which embryonic heartbeats ceased) for three latitudinally distributed populations of an oviparous lacertid lizard. The experimental results suggested that the mean EAHT decreased with decreasing latitude and that the reaction norms of EAHT in relation to developmental temperatures showed “flat,” “bell-shaped,” and “decreasing” patterns at high, medium, and low latitudes, respectively. Based on the means and plasticity of EAHT and weather data across China, we project that the heat stress frequency would increase from the present to the future and increase toward low latitudes. Furthermore, heat stress becomes more extensive with the incorporation of developmental plasticity. Incorporating the mean EAHT during the embryonic development season, heat stress frequency, and climate variables in a species distribution model projects that suitable habitats could move northward in response to ongoing climate change and shrink due to the loss of southern habitat. Moreover, even lizards within the areas that are predicted to remain highly suitable are expected to experience increases in heat stress over time, particularly at medium and low latitudes. Our study reveals geographic variation in the mean and developmental plasticity of EAHT and highlights its importance for predicting species vulnerability and range shifts in response to climate change.

Key words: climate warming; embryo; heat stress; latitudinal variation; reptile; thermal tolerance.

INTRODUCTION

Ongoing climate change is characterized by more frequent and intense heat extremes that directly depress the functional performance of species (Hansen et al. 2012, IPCC 2013). The vulnerability of species to climate change largely depends on the frequency and magnitude of environmental heat extremes exceeding organismal upper thermal tolerance (heat tolerance) (Deutsch et al.

2008, Sunday et al. 2012, Sunday et al. 2014, Levy et al. 2015). The heat tolerance varies across ontogeny in species with complex life cycles, with early life stages such as embryos often having relatively lower heat tolerance and thus being more vulnerable to heat stress than adults (Bowler and Terblanche 2008, Radchuk et al. 2013, Du and Shine 2015, Dahlke et al. 2020). Accordingly, understanding embryonic acute heat tolerance (EAHT) is essential for predicting the climate-change vulnerability of species with complex life cycles (Radchuk et al. 2013, Levy et al. 2015, Carlo et al. 2018, Dahlke et al. 2020).

Geographic variation of heat tolerance may represent an adaptation to survive heat stress associated with maximum temperatures during the hot season

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(Chown et al. 2004, Ghalambor et al. 2006) and thus vulnerability to climate change (Stillman 2003, Sunday et al. 2011, 2012, 2014). For example, heat tolerances of adult lizards (i.e., CT_{max}) increase at higher latitudes (i.e., cooler climates) if they are more likely to occupy unshaded environments or spend more time basking, whereas a counter-gradient trend occurs for most other terrestrial and marine species (Hoffmann et al. 2002, Sunday et al. 2011, 2012). In addition, adults may exhibit thermal acclimatization/acclimation (i.e., plasticity) in heat tolerance (i.e., CT_{max} or lethal temperature) (Ghalambor et al. 2007, Charmantier et al. 2008, Bozinovic et al. 2011, Gunderson and Stillman 2015), which may either buffer or aggravate heat stress (Bozinovic et al. 2013, Magozzi and Calosi 2015). Compared with species occupying narrow or stable environments, species inhabiting a more variable environment can evolve higher heat tolerance at the cost of tolerance plasticity (e.g., Stillman 2003, Magozzi and Calosi 2015). The high heat tolerance may reduce the risk of acute heat shock in an unpredictable environment and induce selection advantages (Stillman 2003, Somero 2011, Sunday et al. 2011, 2014). In contrast to adults, geographic variation in acute heat tolerances of early life stages (e.g., EAHT) is largely unknown. Incorporating such geographic patterns is likely to be particularly critical for predicting vulnerabilities of species to climate change when the climate warming rate varies geographically (Kawecki 2000, Somero 2005, 2011, 2012, Sunday et al. 2012, 2014, Seebacher et al. 2015, Dahlke et al. 2020) or when species are broadly distributed (Huey et al. 2009, 2012, Seebacher et al. 2015, Dahlke et al. 2020). Ignoring geographic variation in EAHT may decrease the accuracy of the climate change vulnerability projections (Crozier et al. 2008, Deutsch et al. 2008, Levy et al. 2015, Telemeco et al. 2017).

As ectotherms, widespread oviparous lizards are an ideal system for predicting the vulnerability of species based on the geographic and plastic responses of embryos. In addition, embryos of oviparous lizards are sensitive to temperature and are usually without parental care during development (Huey et al. 2012, Du and Shine 2015, Noble et al. 2018). In this study, we incubated eggs from three latitudes representing the northern edge (i.e., high latitude, Yantai), center (i.e., medium latitude, Nanjing), and southern edge (i.e., low latitude, Ningde) of the distribution range of a widespread oviparous species, northern grass lizard *Takydromus septentrionalis*. From April to August, female *T. septentrionalis* lay multiple clutches underground at a depth of about 5 cm (Sun et al. 2013, 2018). The eggs from the high-, medium-, and low-latitude populations likely experience divergent potential nest temperatures (soil temperatures at 5 cm depth) differing in mean values (23.8°, 24.8°, and 27.8°C) and fluctuating ranges (9.8°, 9.9°, and 7.6°C). Accordingly, this system of different geographic populations in the northern grass lizard provides an

ideal opportunity for identifying the geographic variation in EAHT of widespread species and its importance in predicting species' vulnerability to climate change. More specifically, by experimentally measuring the EAHT of *T. septentrionalis* from different latitudes, developing a mechanistic model, and calibrating a hybrid process-based species distribution model (SDM), we aim to address the following questions:

How do the mean and developmental plasticity of EAHT vary with latitude?

Previous studies on adults demonstrated that high-latitude ectotherms have higher CT_{max} and lower plasticity because a constant high CT_{max} has selective advantages in unstable thermal environments (Stillman 2003, Huang et al. 2006, Deutsch et al. 2008, Sunday et al. 2014, Magozzi and Calosi 2015). This suggests the mean and developmental plasticity of EAHT may vary geographically. If embryos show similar latitudinal patterns of heat tolerances to adults (Stillman 2003, Deutsch et al. 2008, Somero 2011, Sunday et al. 2011, 2014), we hypothesize that embryos from high latitudes, which develop in variable thermal environments, would have high EAHT and show limited developmental plasticity of EAHT due to a trade-off between the absolute value and plasticity of heat tolerance. Conversely, embryos from low latitudes, which develop in stable thermal environments, would have low EAHT and express pronounced plasticity in EAHT. More specifically, in light of the plasticity of heat tolerance documented in adults, we hypothesize that EAHT could respond to developmental temperatures in one of the following reaction norms: (1) flat, EAHT is independent of developmental temperatures as seen in high-latitude adults (e.g., Stillman 2003, Overgaard et al. 2014); (2) increasing, EAHT enhances as developmental temperatures increase, as predicted by the beneficial plasticity hypothesis (e.g., Jiang et al. 2008, Han et al. 2020); (3) decreasing, EAHT decreases as developmental temperatures increase, as predicted by the maladaptive plasticity hypothesis (see details of *Halozetes marinus*, in Sinclair et al. 2016); (4) bell shape, EAHT enhances first and then decreases as developmental temperatures increase (e.g., Magozzi and Calosi 2015).

How does latitudinal variation in the mean and developmental plasticity of EAHT affect heat stress experienced by embryos?

We defined the situation that the soil temperature is higher than the EAHT of developing embryos, as a "heat stress event" (Fig. 1a). Correspondingly, a "heat stress day" can be defined as a single day with at least one heat stress event. The vulnerability of embryos at a certain site can therefore be quantified by heat stress frequency: the number of heat stress days through the embryonic development season.

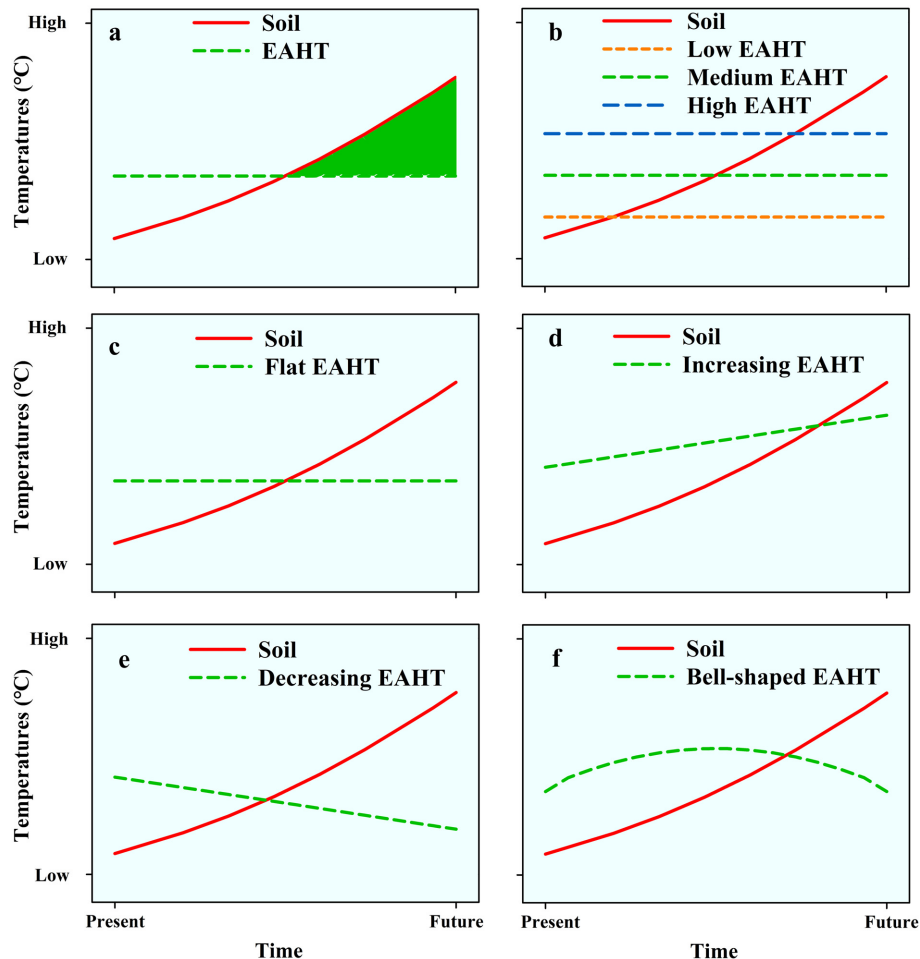


FIG. 1. A framework for determining the (a) heat stress of embryos based on the (b) mean value and (c–f) developmental plasticity of embryonic acute heat tolerance (EAHT). (a) Heat stress events (shaded) are defined as the soil temperature higher than the EAHT of embryos. (b) Different average values of EAHT along a geographic gradient and the EAHT (c) is independent of, (d) increases with, (e) decreases with, or (f) shows a bell-shaped response to developmental temperatures.

We integrated latitudinal variation in the mean and plasticity of EAHT to estimate the heat stress faced by embryos from different populations and their vulnerability to global warming. To facilitate addressing the impact of EAHT on species vulnerability, we assume that average soil (nest) temperatures are increasing from present to future as predicted by IPCC (IPCC 2013), and the mean and developmental plasticity (i.e., reaction norm) of the EAHT do not evolve from present to future, although such evolution is possible. When considering geographic variation in the mean value of EAHT, we predict that the high-latitude population with higher EAHT would have lower heat stress frequency under climate warming (Fig. 1b). When considering the developmental plasticity of EAHT, we predict that (1) if the EAHT does not respond to developmental temperatures, the increase of heat stress frequency would not be buffered or aggravated by plasticity (Fig. 1c). (2) If the EAHT increases with the increasing developmental

temperatures, the heat stress would be buffered by the developmental plasticity of EAHT (Fig. 1d). (3) If the EAHT decreases with the increasing developmental temperatures, the heat stress would be aggravated by the developmental plasticity (Fig. 1e). (4) If there is an optimal temperature for EAHT in response to developmental temperatures analogous to thermal performance curves, EAHT would possibly show a bell-shaped response to developmental temperatures. Accordingly, the heat stress would be buffered by the developmental plasticity in the near future but aggravated in the far future (Fig. 1f).

How do EAHT and heat stress frequency influence the prediction of range shifts under climate change?

We developed a process-based hybrid species distribution model (SDM) to integrate the EAHT, heat stress frequency, and distribution shifts. The model uses the

average EAHT of embryonic development season (i.e., seasonal average EAHT) and the heat stress frequency along with climate variables to predict the species' range shifts under climate change. We predicted that the process-based hybrid SDM would provide a more robust estimate for the suitable habitat because classic SDMs often ignore those essential biological processes like EAHT, and thus possibly overestimate the suitability of habitat for a species. Accordingly, we hypothesize that high-latitude populations would have more extended habitats under climate change since they have higher EAHT and would experience correspondingly less heat stress at the embryonic stage. They would therefore suffer less under heat events caused by climate warming. On the contrary, low-latitude populations would lose more habitats because they would suffer higher risk under climate warming due to their lower EAHT, and thus higher heat stress frequencies for embryos.

MATERIALS AND METHODS

Study system, lizard collection, husbandry and egg incubation

The northern grass lizard, *T. septentrionalis*, is a small oviparous lacertid lizard (up to 80 mm snout-vent length, SVL) with a wide distribution in the central and southern provinces of China. Adult *T. septentrionalis* from warmer environments prefer warmer temperatures (Du 2006). Also, the critical thermal minimum (CT_{min}), critical thermal maximum (CT_{max}), and thermal preferences of *T. septentrionalis* increase with the acclimation temperature (Yang et al. 2008). Under constant incubation temperatures, embryonic development rates are enhanced, but hatching success is depressed when the incubation temperature increases from 28° to 33°C (Du and Ji 2006).

From mid-April to early May of 2016, we collected gravid females from populations on the northern edge (high latitude, Yantai, 37°27'50.46" N, 121°26'52.8" E, warm temperate region), central area (medium latitude, Nanjing, 32°03'28.8" N, 118°47'45.6" E, northern subtropical region) and southern edge (low latitude, Ningde, 26°39'57.6" N, 119°32'52.8" E, southern subtropical region) of the distribution range of *T. septentrionalis*. Their habitat covers a latitudinal range of ~1,210 km from the high- to low-latitudinal population in a mixture of shrub and grasslands. They lay eggs in open grasslands (Appendix S1: Fig. S1). We collected 35 gravid females and 20 males from each population. Because *T. septentrionalis* has a limited home range (about 36 m² on average; Ji et al. 1994), we located nest sites by monitoring the nesting behaviors of gravid females to find where they burrow and lay eggs. We found four, three, and five natural nests where we placed temperature loggers (iButton, DS1921; Maxim Integrated Products, Sunnyvale, California, USA) for the low-, medium-, and high-latitude populations to collect nest temperatures

from April to September, respectively. The mean nest temperature decreased with increasing latitudes (24.5°, 24.9°, and 26.5°C for the high-, medium-, and low-latitude populations, respectively). The fluctuant range of nest temperatures was more extensive in the high- (5.8°C) and medium-latitude (6.7°C) sites than the low-latitude site (3.3°C; Fig. 2a). The average depth of nests for low-, medium-, and high-latitude populations were 5.05 ± 0.18, 4.33 ± 0.59, and 4.92 ± 0.42 cm, respectively, with an overall mean depth of 4.82 ± 0.23 cm.

The collected lizards were transferred to our laboratory in Beijing, where they were weighed (±0.01 g), measured (SVL, ± 0.5 mm), and individually marked. Populations were maintained in groups of five females and three males in terraria (600 × 450 × 400 mm) within a temperature-controlled room at 22° ± 1°C with a natural light cycle (14 h light:10 h dark). Full-spectrum heating bulbs (25 W) were suspended 50 mm above one end of the terraria to provide supplementary

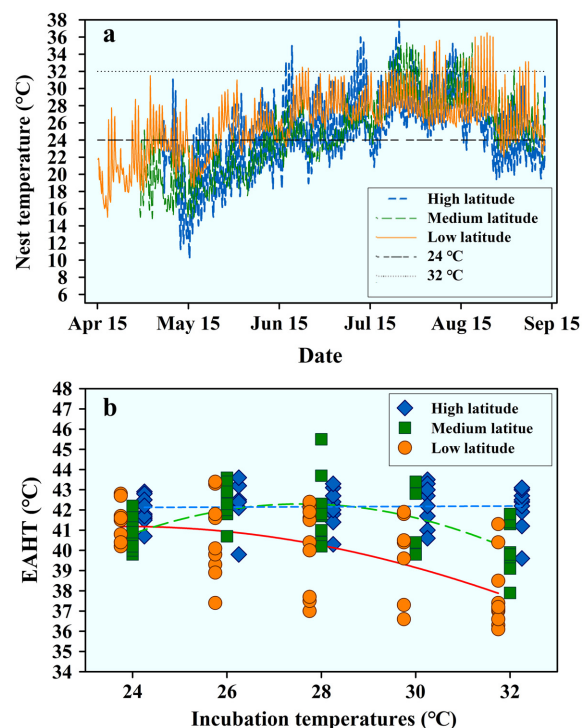


FIG. 2. Average nest temperatures of *Takydromus septentrionalis* from (a) different latitudes and (b) embryonic acute heat tolerance (EAHT) of *T. septentrionalis* from low- (Ningde), medium- (Nanjing), and high- (Yantai) latitude populations. (a) Blue, green, and orange lines are hourly average nest temperatures in high- (Yantai), medium- (Nanjing), and low- (Ningde) latitude populations, respectively. In order to illustrate the ecological relevance of our regimes in incubation temperature setting, 24° and 32°C are marked with two dotted lines. (b) Eggs were incubated at five constant temperatures (24°, 26°, 28°, 30°, and 32°C) and were measured for EAHT around halfway through incubation duration. Curves were fitted by regressing incubation temperature against EAHT using polynomials of multiple orders (1–4; with lm function in R 3.3.1). The best fit model was chosen by Akaike information criterion (AIC).

heating from 08:00 to 18:00, creating a thermal gradient in the terrarium from 22° to 38°C for lizards. Food (crickets mixed with vitamins and minerals) and water were provided ad libitum. We palpated females every other day and moved females with oviductal eggs to individual egg-laying terraria (350 × 210 × 200 mm) with 10 mm of moist vermiculite and shelters (Sun et al. 2013). The setup of the egg-laying terraria was the same as the ones for lizard husbandry.

Females laid eggs in 6.70 ± 0.30 , 3.67 ± 0.22 , and 6.86 ± 0.49 d for the high-, medium-, and low-latitude populations respectively, after being transferred to the laboratory. We checked for freshly laid eggs three times (morning, noon, and evening) daily. Once found, fertilized eggs were weighed (± 0.001 g) immediately and incubated in plastic boxes (160 × 115 × 40 mm) with moist vermiculite (-220 kPa) located in temperature-controlled incubators (KB240, Binder, Tuttlingen, Germany). Constant and fluctuating incubation temperature treatments with the same mean have similar effects on the developmental plasticity of embryonic and offspring traits (e.g., incubation duration, hatchling SVL, hatchling body mass, locomotion) in *T. septentrionalis* (Du and Ji 2006, Du and Feng 2008). To further compare the effects of constant temperatures and natural nest temperatures on EAHT, we incubated the eggs from a medium-latitude population near Nanjing, at constant temperatures of 24°, 28°, and 32°C vs. fluctuating temperatures of $24 \pm 3^\circ$, $28 \pm 3^\circ$, and $32 \pm 3^\circ$ C, which mimicking nest temperatures. Given that the EAHT did not differ between the eggs incubated at constant temperatures vs. fluctuating temperatures mimicking nest temperatures (Appendix S1: Table S1), we incubated eggs at five constant temperatures (24°, 26°, 28°, 30°, and $32 \pm 0.5^\circ$ C) to cover most of the temperature range in natural nests during the reproductive season (Fig. 2a). We used a split-clutch design to incubate fertilized eggs; the eggs from the same clutch were incubated at different temperatures.

Measuring EAHT in the high-, medium-, and low-latitude populations

To identify latitudinal variation and developmental plasticity in embryonic acute heat tolerance, we measured the EAHT at about halfway through incubation because embryonic heart rates used for evaluating EAHT are stable and easy to record during this period (e.g., Du et al. 2010, 2011). The halfway point of the incubations with an average developmental stage of 36 (Appendix S1: Table S2) were determined according to incubation periods of *T. septentrionalis* from different latitudes (Lin and Ji 1998, Du and Ji 2006, Du and Feng 2008; Appendix S1: Table S3). A total of 47, 46, and 50 eggs were used for measuring the EAHT in the high-, medium-, and low-latitude populations, respectively. We defined the EAHT as the temperature at which embryonic cardiac collapse and determined the EAHT with an

established method of “thermal ramps” with minor modifications (Angilletta et al. 2013, Hall and Sun 2020, Taylor et al. 2020). Before measurements, the eggs were weighed (± 0.001 g) and acclimated at 28°C for 2 h in a programmable temperature-controlled incubator (KB240, Binder, Tuttlingen, Germany). After acclimation, eggs were continuously warmed from 28°C at a rate of 6°C/h, corresponding to the maximum daily heating rate in a natural nest (5.5°C/h from 11:00 to 13:00 in one unshaded nest of the low-latitude population, on 22 August 2016). During the warming process, the real-time embryonic heart rates and the surface temperature of eggs were monitored synchronously. Each candidate egg was positioned on the sensor of the digital egg monitor (Buddy, Vetronic Services, Devon, UK), and a calibrated thermocouple (with two tips linked) was fixed on the monitor with one tip adhered to the egg surface. For each measurement, we set four wet cotton balls around the rubber sensor to provide moist air around the eggs. In the course of warming, if the egg monitor could not detect heart rates for 10 s (shown as “0” on egg monitor), we measured the EAHT as the core temperature of the egg where embryos were located with the other tip of the calibrated electronic thermocouple (Angilletta et al. 2013). Then we dissected the eggs to confirm embryonic mortality; if the embryo was still alive, we excluded its data from further analyses. Two eggs from high, three eggs from medium, and two eggs from low-latitude populations were then excluded. Correspondingly, the sample sizes of EAHT recorded for high-, medium-, and low-latitude populations were 45 (10, 7, 10, 8, and 10 at 24°, 26°, 28°, 30° and 32°C), 43 (11, 7, 10, 6, and 9 at 24°, 26°, 28°, 30° and 32°C) and 48 (10, 9, 11, 7, and 11 at 24°, 26°, 28°, 30° and 32°C), respectively.

Linear mixed models (function `lmer` in the `lmerTest` package; Kuznetsova et al. 2017) were implemented in R 3.3.1 (R Core Team 2019) to analyze how EAHT varies with population, incubation temperature, and their interactions as fixed factors and mother identification as a random factor. We used the `anova` function in `lmerTest` package to produce type III anova table for fixed-effect terms (contains *P* value for incubation temperatures and populations). The normality of the residual distribution was tested with Shapiro-Wilk Normality Test. We conducted pairwise comparisons among three populations with the `lsmeans` function in the `lsmeans` package (Lenth 2016). Reaction norms for EAHT variation as a function of incubation temperatures for each population were fitted with raw data as polynomials of multiple orders (1–4; with `lm` function in R 3.3.1). The best fit model was chosen by Akaike information criteria (AIC).

Predicting the EAHT and heat stress frequency for embryos

Soil temperatures at present and under climate change.— We purchased a data set of hourly soil temperature (at -5 cm) for 2016 at 2,168 Chinese national stations from

the China meteorological data service center (CMDC; available for purchase online).⁹ Stations with incomplete data (i.e., missing temperatures for any hours) were removed from the analyses, which resulted in 1,825 stations with available data (Appendix S1: Fig. S2). For each station, annual soil temperature data were trimmed to the embryonic developmental season of *T. septentrionalis*, which lasts from 1 April to 31 August. Because hourly soil temperature data for the future were not available for our study area (see a similar data set for North America: Levy et al. 2016), and the warming rates of the soil temperature at shallow depths (5–20 cm) are similar to that of air temperature (Fang et al. 2018), we estimated soil temperatures in 2050 and 2070 by adding predicted air temperature anomalies to hourly soil temperature in 2016. We downloaded the annual mean air temperature at present (estimated using available recorded data of 1960–1990), in 2050 and 2070 (Representative Concentration Pathway 6.0 [RCP 6.0]; predicted by BCC-CSM1-1) from the Worldclim 1.4 (Hijmans et al. 2005) in a resolution of 10 arc-minutes.

Predicting the EAHT and heat stress frequency in embryonic development season.—Using the hourly soil temperatures at present (2016) and in 2050 and 2070, we subsequently predicted the EAHT and the heat stress frequency of half-developed embryos that were assumed to exist in the nest at each hour of the embryonic developmental season (00:00 1 May to 23:00 31 August). When predicting the EAHT, we considered two scenarios: (1) the EAHT was independent of the developmental temperatures (no plasticity); we used the averaged EAHT across all incubation temperatures in the experiment as the fixed EAHT throughout the embryonic developmental season for each population (Table 1); (2) the EAHT was dependent on the developmental temperatures (with plasticity); we calculated the EAHT as a function of developmental temperatures. Specifically, for each hour in the embryonic developmental season, we assumed a half-developed embryo exists in the nest (–5 cm), and calculated its EAHT by two steps gradually: (1) we calculated how long it took for the embryo to complete 50% of development by accumulating developmental rate retrospectively. We regressed incubation temperature against 1/incubation period (day) to estimate the response of developmental rate to temperature for each population (with *lm* function in R 3.3.1). Using these fitted functions (Appendix S1: Table S4), we then estimated the developmental zero temperatures (D0) by solving for 1/incubation period = 0 (Telemeco et al. 2017). Subsequently, we developed functions for the effect of temperature on hourly developmental rate for each population (Appendix S1: Table S4). (2) We calculated the EAHT of the embryo by using the average soil temperature during the 50% development as the incubation temperature and inputting it into the reaction

norms derived from the experiments. Because no eggs can hatch with an incubation period over 60 d in *T. septentrionalis* (Du and Ji 2006, Du and Feng 2008, Sun et al. 2018), we arbitrarily set a limit of 30 d for embryos to accumulate 50% development. If an embryo cannot accumulate 50% development within 30 d before a certain time, we did not estimate EAHT or heat stress (see next paragraph) of this hour for the present, 2050, or 2070. For each station, we calculated the “seasonal average EAHT” by averaging the EAHT of half-developed embryos that are assumed to exist in the nest at hours throughout the embryonic developmental season (NAs are removed).

We defined a heat stress event as an hour that the soil temperature (at –5 cm) is higher than the EAHT of half-developed embryos existing in the nest. A heat stress day was correspondingly defined as a single day with at least one heat stress event. Then for each site and time period, we estimated heat stress frequency as the number of heat stress days in the embryonic developmental season (from 00:00 1 May to 23:00 31 August).

We ran the Kruskal-Wallis rank sum test to analyze the variation of heat stress frequency among levels of the time period (2016, 2050, 2070), population (high, medium, and low latitudinal) and with/without developmental plasticity considered. For each factor (time period, population, or developmental plasticity), the analyses were conducted repeatedly considering all combinations of levels of other factors. Pairwise-comparison was performed using the Dunn test (package FSA; Ogle et al. 2020).

Integrating EAHT and heat stress frequency into species distribution models

To explore how the geographic variation of EAHT and heat stress frequency could shape species' vulnerability and distribution under climate warming, we compared environmental suitability estimates between a standard species distribution model (SDM) using climate variables and a hybrid SDM that additionally incorporated seasonal average EAHT and heat stress frequency as physiological predictors.

We collected the presence coordinates of *T. septentrionalis* from the Global Biodiversity Information Facility ($N = 129$, <https://doi.org/10.15468/dl.vfg5lw>) and the literature ($N = 61$, Zhao et al. 1999). To prevent sampling bias, we kept only one presence coordinate per 20 km², and thus yielded 75 presence coordinates used for calibrating the SDMs (Appendix S1: Fig. S3). Pseudo-absence coordinates were created by randomly sampling 1,000 locations within the extent of mainland China (17°–54° N, 73°–136° E; Hijmans et al. 2013).

As climate predictors, we used the 19 standard bioclimatic variables (Appendix S1: Table S5) downloaded from the Worldclim 1.4 (Hijmans et al. 2005). These variables represent the climate conditions at present, in 2050, and 2070 (RCP 6.0, predicted by BCC-CSM1-1)

⁹ <http://data.cma.cn/en>.

TABLE 1. Estimates of embryonic acute heat tolerance (EAHT) from high- (Yantai), medium (Nanjing), and low- (Ningde) latitude populations.

Incubation temperature	<i>N</i>	High latitude (Yantai) EAHT (°C)	<i>N</i>	Medium latitude (Nanjing) EAHT (°C)	<i>N</i>	Low latitude (Ningde) EAHT (°C)
24°C	10	41.99 ± 0.21 (40.7–42.9)	11	40.73 ± 0.23 (39.8–42.2)	10	41.34 ± 0.30 (40.2–42.8)
26°C	7	42.29 ± 0.46 (39.8–43.6)	7	42.39 ± 0.38 (40.7–43.6)	9	40.62 ± 0.68 (37.4–43.4)
28°C	10	42.25 ± 0.30 (40.3–43.3)	10	42.05 ± 0.50 (40.2–45.5)	11	40.39 ± 0.62 (37.0–42.4)
30°C	8	42.25 ± 0.38 (40.6–43.5)	6	41.67 ± 0.68 (39.8–43.4)	7	39.73 ± 0.78 (36.6–41.9)
32°C	10	42.08 ± 0.33 (39.6–43.1)	9	40.06 ± 0.41 (37.9–41.8)	11	37.74 ± 0.50 (36.1–41.3)

Notes: Eggs were incubated at 24°, 26°, 28°, 30°, and 32°C, respectively. Data are shown as mean ± SE and minimum to maximum.

and cover global land area at a resolution of 30 arc seconds (~1 km). As physiological predictors, we determined EAHT and the seasonal average heat stress frequency for each station ($N = 1,825$) using the values for the population with the closest latitude. We then interpolated seasonal average EAHT and heat stress frequency to all localities on the map (17°–54° N, 73°–136° E) with the inverse distance weighted interpolation method.

We built SDMs with and without the physiological predictors. We implemented a multi-algorithm approach using eight algorithms: GLM (Generalized Linear Model), GBM (Generalized Boosting Model or usually called Boosted Regression Trees), CTA (Classification Tree Analysis), ANN (Artificial Neural Network), SRE (Surface Range Envelope or usually called BIOCLIM), FDA (Flexible Discriminant Analysis), RF (Random Forest), and Maxent (Maximum Entropy) with the biomod2 package (Thuiller et al. 2019) in R software (R Core Team 2019). We used a threefold cross-validation procedure for these models, using 80% of the data for model training and retaining 20% of the data for testing. We assembled predictions from individual models by estimating the weighted mean (by TSS score; Appendix S1: Table S6) (Chakraborty et al. 2019). Models with a TSS score < 0.7 were excluded from the ensemble model.

Finally, we projected the species distribution models for three periods (present, 2050, and 2070). Climate data for 2050 and 2070 were from the BCC-CSM1-1 model and the RCP 6.0 emissions scenario. Seasonal average EAHT and heat stress frequency data for 2050 and 2070 were determined and interpolated for all localities with the same method. We transformed the presence probabilities into binary form (presence/absence) using the thresholds that maximize the TSS scores. Because lizards have very short annual dispersal distances (usually < 50 m; recorded maximum = 420 m; Massot et al. 2003, Olsson and Shine 2003, Calsbeek 2009, Vercken

et al. 2012), we trimmed predicted habitat (presence) using the distribution range polygon for *T. septentrionalis* from IUCN Red List (a 1° [~111 km] buffer was added to account for potential dispersal).

RESULTS

The latitudinal patterns of the mean and developmental plasticity of embryonic acute heat tolerance (EAHT)

EAHT differed considerably across populations ($F_{2,130} = 6.418$, $P = 0.002$). The high-latitude population had higher average EAHTs ($42.2^\circ \pm 0.221^\circ\text{C}$ [mean ± SE]) than the medium- ($41.30^\circ \pm 0.226^\circ\text{C}$), and low- ($40.0^\circ \pm 0.214^\circ\text{C}$) latitude populations (Table 1, Fig. 2b). The incubation temperatures significantly affected the EAHT ($F_{1,130} = 14.653$, $P = 0.0002$), and interestingly, the population and incubation temperature interactively determined the EAHT ($F_{2,130} = 9.479$, $P = 0.0001$). In particular, for the high-latitude population, the EAHT did not vary among incubation temperatures ($F_{1,43} = 0.031$, $P = 0.861$, adjusted $R^2 = -0.023$), indicating a flat reaction norm (EAHT = $0.0086 \times T + 41.917876$ [where T is temperature; AIC = 127.195; $\Delta\text{AIC} = -1.392$ [from the next best model]). Moreover, in the medium-latitude population, the EAHT increased with developmental temperatures between 24° and 26°C, but decreased at temperatures between 28° and 32°C ($F_{2,40} = 9.827$, $P < 0.001$, adjusted $R^2 = 0.296$), indicating a bell-shaped reaction norm (EAHT = $-0.1152 \times T^2 + 6.3565 \times T - 45.3656$; AIC = 145.628; $\Delta\text{AIC} = -1.646$ [from the next best model]). Finally, in the low-latitude population, the EAHT decreased with increasing incubation temperatures ($F_{2,45} = 12.35$, $P < 0.0001$, adjusted $R^2 = 0.326$), indicating a decreasing reaction norm (EAHT = $-0.05227 \times T^2 + 2.51287 \times T + 10.98166$; AIC = 195.696; $\Delta\text{AIC} = -0.097$ [from the next best model]) (Table 1; Fig. 2b).

Prediction of embryonic heat stress frequency

Embryos are predicted to experience higher heat stress frequencies in the future for all populations because of increasing soil temperatures caused by climate warming, regardless of the developmental plasticity of the EAHT (all $P < 0.001$, Tables 2, 3). Besides, the magnitude of increase from the present to 2050 is higher than that from 2050 to 2070 (Fig. 3a). The predicted heat stress frequencies are highest for the low-latitude population, and lowest for the high-latitude population, with the medium-latitude population in between at all periods, no matter whether the developmental plasticity was considered (all $P < 0.001$, Tables 2, 3, Fig. 3b).

Because of the flat reaction norm, the incorporation of developmental plasticity did not alter the predicted heat stress frequencies for the high-latitude population during any period (i.e., using the mean of EAHT measured at multiple incubation temperatures; all $P > 0.05$, Tables 2, 3, Fig. 4a–c vs. Fig. 5a–c). However, considering the developmental plasticity of EAHT significantly raised heat stress frequencies at all periods for the medium-latitude population (with the bell-shaped reaction norm) and in 2050 and 2070 for the low-latitude population (with the decreasing reaction norm; all $P < 0.01$, Tables 2, 3, Fig. 3c, d, Fig. 4d–f vs. Fig. 5d–f, Fig. 4g–i vs. Fig. 5g–i).

Suitable habitats based on only climatic predictors vs. using both climatic and physiological predictors

The two types of SDMs performed equally well (Appendix S1: Table S6). However, the SDM with both climate and physiological parameters predicted less suitable habitat for *T. septentrionalis* at present (10.3%), in

2050 (13.46%), and 2070 (17.8%) compared to the SDM with only climate predictors (Appendix S1: Fig. S4). Projected rates of habitat decline were more remarkable for the SDM with both climate and physiological predictors relative to the SDM with only climate predictors: the total amount of suitable habitats for *T. septentrionalis* is projected to decline by 0.59% and 4.06%, respectively, until 2050, and by 10.33% and 17.80%, respectively until 2070 (Fig. 6). The greater decline in suitable habitat for the SDM with climate and physiological predictors is due to less northeastward expansion and more shrinkage in the southern part of present suitable habitats (Fig. 6c, d; Appendix S1: Fig. S4e, f). Furthermore, SDMs using only climatic predictors predicted that suitable habitats could dramatically expand toward the northeast, which may compensate for the habitat loss in the southwest, especially in 2070 (Fig. 6a, b; Appendix S1: Fig. S4c, d).

DISCUSSION

Geographic variation in the mean and developmental plasticity of EAHT

This study explicitly demonstrates a latitudinal pattern and developmental plasticity of thermal limits in reptile embryos (but see estimates of *Sceloporus* embryonic heat tolerance; Angilletta et al. 2013, Levy et al. 2015, Smith et al. 2015, Carlo et al. 2018). We found that the EAHT increased toward high latitude, similar to the latitudinal pattern in heat tolerance of adult lizards, but not in many other terrestrial and marine animals (Sunday et al. 2011). This suggests that low-latitude populations may be more vulnerable to climate change than high-latitude populations in terms of EAHT. In contrast, geographic variation in embryonic thermal limits was not significant in *Sceloporus* embryos (Angilletta et al. 2013). The discrepancy in latitudinal pattern between *T. septentrionalis* and *Sceloporus* lizards indicates more case studies are needed to assess whether geographic variation in embryonic thermal tolerance can be generalized across squamates.

The EAHT showed latitudinal variation in developmental plasticity in response to incubation temperatures, with flat, bell-shaped, and decreasing reaction norms at high, medium, and low latitudes, respectively (Table 1, Fig. 2b). Higher average EAHT across incubation temperatures would allow higher heat resistance of embryos at high latitude than those at medium and low latitudes. Moreover, the insensitivity of EAHT to incubation temperatures is adaptive in broad and fluctuating nest thermal environment, which is similar to the patterns found in post-hatching individuals (Stillman 2003, Magozzi and Calosi 2015, Sinclair et al. 2016). In contrast, EAHT of the medium- and low-latitude populations are more sensitive to developmental temperatures (bell-shaped and decreasing patterns, Fig. 1d, e). This phenomenon is consistent with the specialist prediction that

TABLE 2. Heat stress frequencies estimation at levels of times, latitudes, and plasticity.

	Heat stress frequency	
	lsmean	SE
Time		
Present	17.006	0.163
2050	20.753	0.198
2070	21.942	0.21
Latitudes		
Low	27.731	0.216
Medium	20.367	0.195
High	14.71	0.16
Developmental plasticity		
Without plasticity	19.319	0.148
With plasticity	22.552	0.174
Reaction norms		
Decreasing	30.372	0.33
Bell-shaped	22.628	0.299
Flat	14.658	0.226

Note: Data are listed by times, latitudes, considering plasticity, and reaction norms. Data are shown as lsmean and SE.

TABLE 3. Statistical results in heat stress frequency at levels of times, latitudes, and plasticity.

Time period	Combinations of levels of other factors		Medium		Low		2016		2050		2070	
	High χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>
With plasticity	436.68	<0.001	566.34	<0.001	669.69	<0.001						
Without plasticity	438.8	<0.001	469.53	<0.001	536.19	<0.001						
Population												
With plasticity							273.18	<0.001	548.34	<0.001	643.95	<0.001
Without plasticity							277.22	<0.001	361.85	<0.001	379.08	<0.01
With/without plasticity												
High							0.006	0.937	0.030	0.862	0.045	0.831
Medium							7.302	0.007	38.780	<0.001	62.224	<0.001
Low							2.176	0.14	58.205	<0.001	96.051	<0.001

Notes: The analyses were performed with the Kruskal-Wallis rank sum test by interactions of times, latitudes, and with/without developmental plasticity. Boldface type indicates results with $P < 0.05$. χ^2 indicates Chi-square.

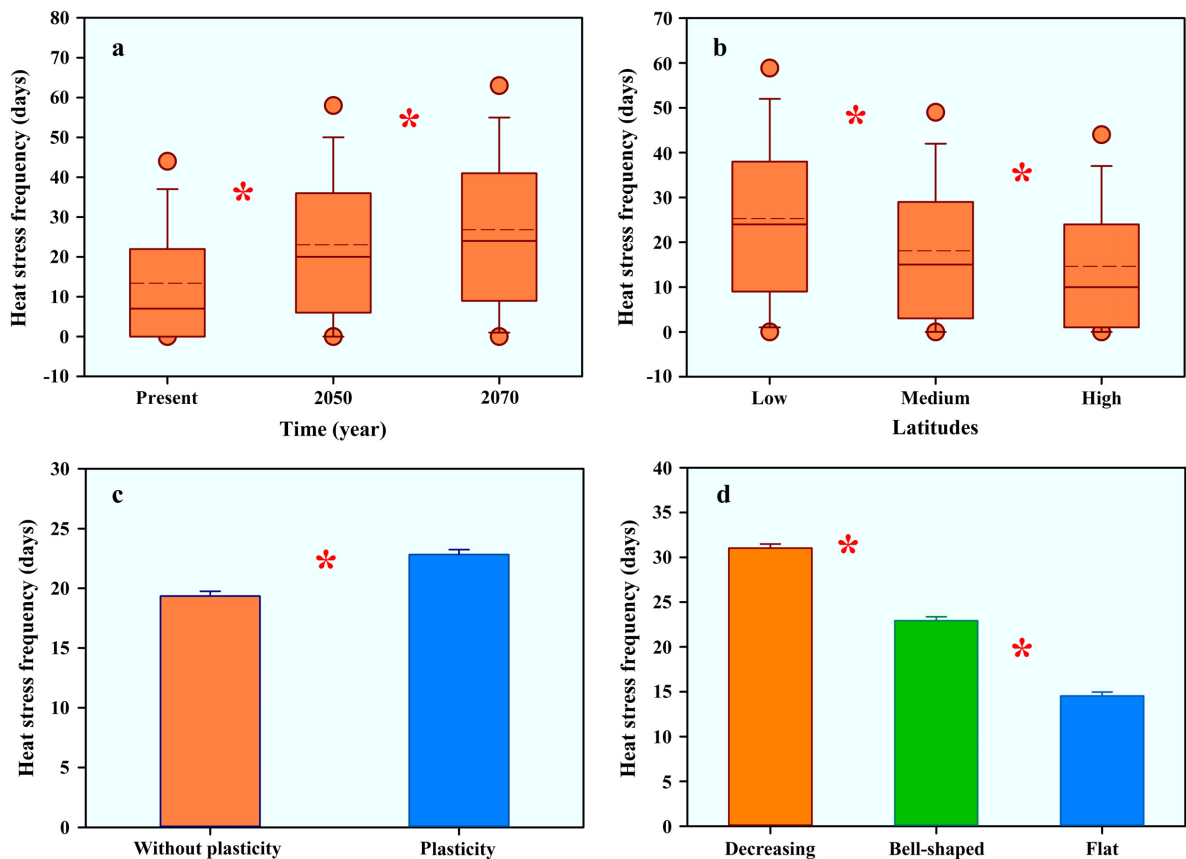


FIG. 3. Estimates of heat stress frequency experienced by embryos with considerations of (a) period, (b) latitude population, (c) developmental plasticity, and (d) reaction norms. In panels c and d, the data are expressed as mean and SE. In panels a and b, solid center lines indicate the median values, dashed center lines indicate the mean values, box edge indicates quartile, whiskers indicate SE, points indicate 5th/95th percentil. Asterisks indicate significant differences ($P < 0.05$) between groups in all panels.

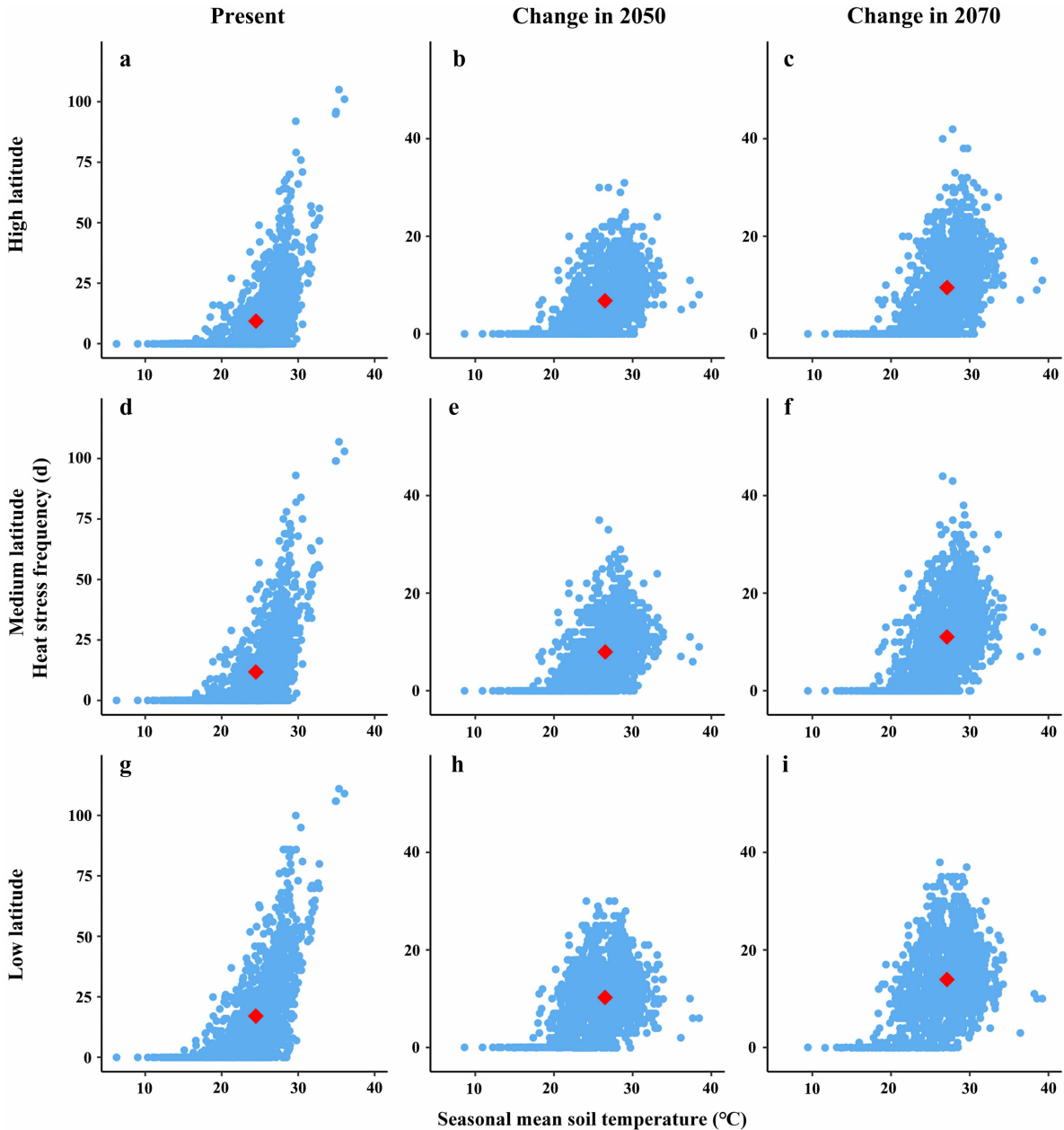


FIG. 4. Embryonic heat stress frequency of *Takydromus septentrionalis* as a function of seasonal mean soil temperature. Predictions are based on only the average EAHT of the high (Yantai, a–c), medium (Nanjing, d–f), and low (Ningde, g–i) populations (columns) and depict the present, change in 2050, and change in 2070, respectively (rows), under moderate climate warming scenarios (RCP 6.0). Blue dots indicate the heat stress frequency of each site, while the red dots indicate the average heat stress frequency. Embryonic heat stress frequency increase with mean soil temperatures of reproductive season.

physiological traits might be more sensitive to temperatures in stable thermal environments (e.g., Ghalambor et al. 2006). However, these developmental plasticities were non-beneficial or even detrimental for embryonic heat tolerance as developmental temperature increases, suggesting lizards could be more vulnerable to climate warming than we thought before (Angilletta et al. 2013).

Phenotypic plasticity has been found to be adaptive, neutral, or even maladaptive (Mousseau and Fox 1998b, Telemeco et al. 2017). The role of the EAHT plasticity in responding to climate warming depends on the interaction between gene expression for thermal tolerance and future warming conditions. The plasticity of EAHT could be beneficial under moderate warming condition,

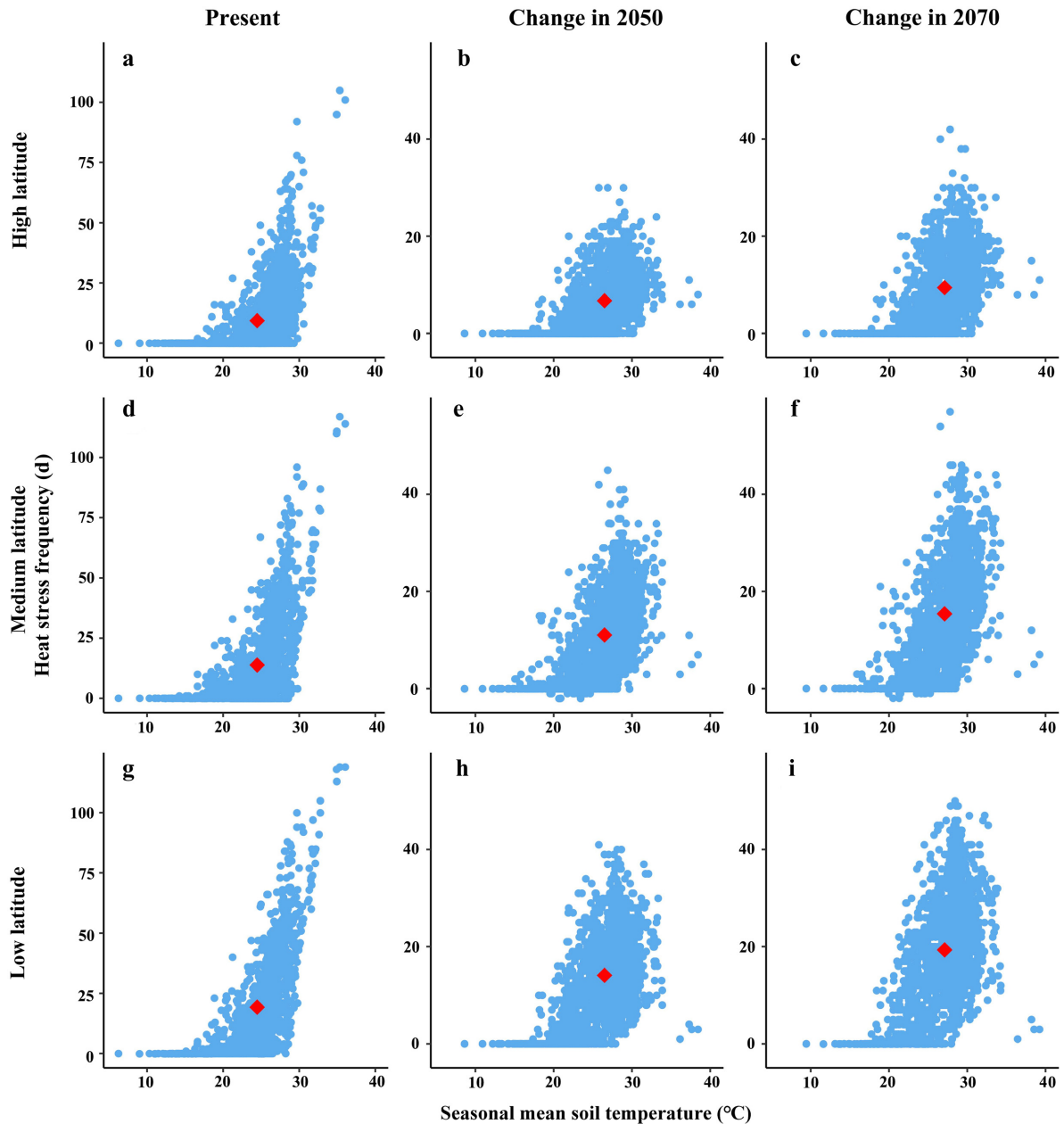


FIG. 5. Embryonic heat stress frequency of *Takydromus septentrionalis* as a function of seasonal mean soil temperature. Predictions are based on high EAHT with flat plasticity (Yantai, a–c), medium EAHT with bell-shaped plasticity (Nanjing, d–f), and low EAHT with decreasing plasticity (Ningde, g–i) and depict the present, change in 2050, and change in 2070 (columns) under moderate climate warming scenarios (RCP 6.0). Blue dots indicate the heat stress frequency of each site, while the red dots indicate the average heat stress frequency. Embryonic heat stress frequency increase with mean soil temperatures of reproductive season.

but non-beneficial under stressful warming conditions that are rarely experienced by organisms (Via et al. 1995, Hoffmann and Merilä 1999, Ghalambor et al. 2007, Hoffmann et al. 2017). Plasticity of EAHT from medium and low latitudes was non-beneficial, which is inconsistent with the conventional notion that phenotypic plasticity may help organisms to cope with the unpredictable environment by enhancing EAHT under high-

temperature environments (Fig. 1b; Mousseau and Fox 1998a, Agrawal 2001, Han et al. 2020). This non-beneficial plasticity would decrease embryonic resistance to intense heat events under climate change. More evidence is needed to determine whether this is a specific case or a universal phenomenon in embryos that is different from the general conclusion of adaptive phenotypic plasticity derived from post-embryonic individuals.

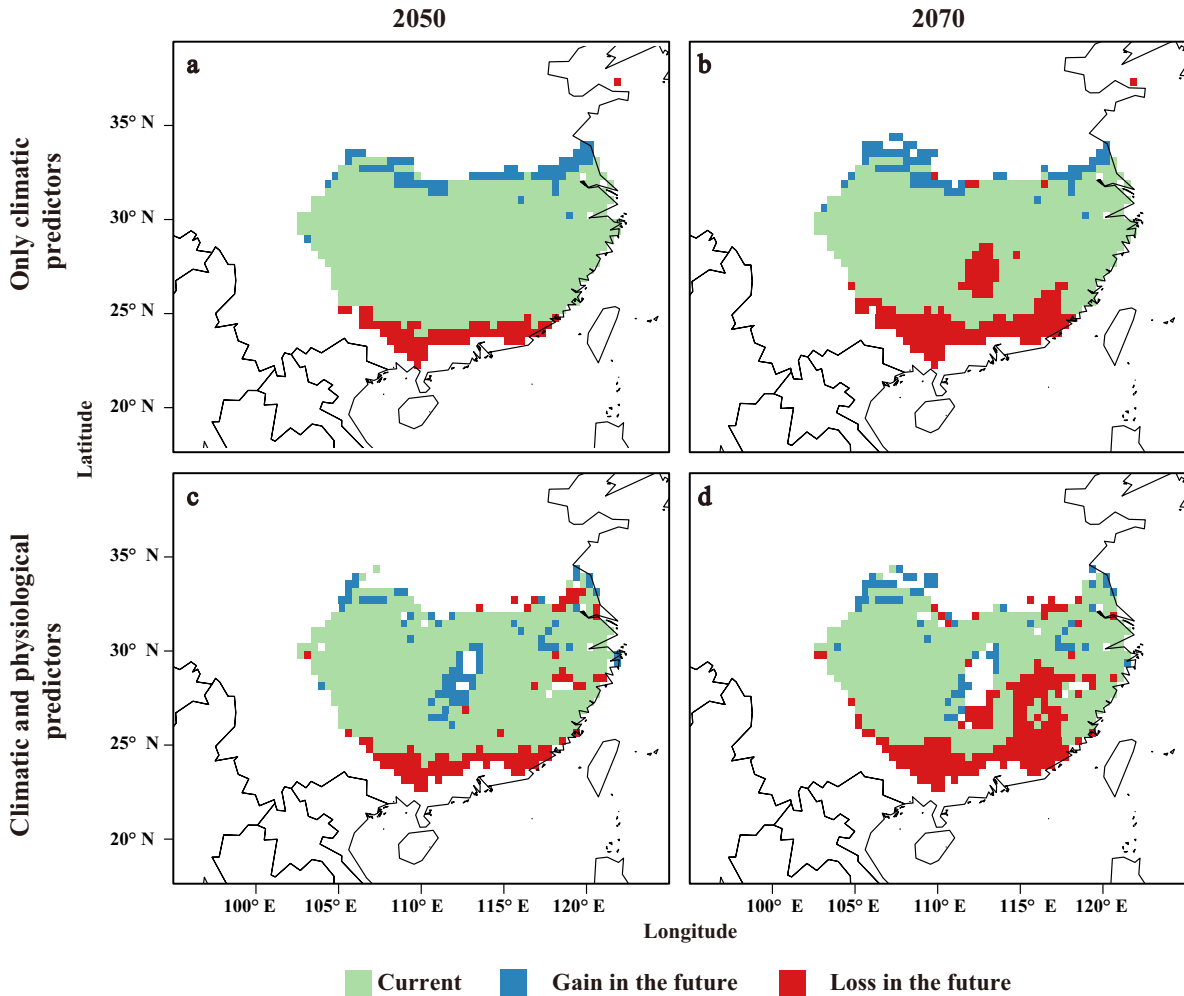


FIG. 6. Suitable habitats of *Takydromus septentrionalis* predicted by SDMs (a, b) with only climatic variables and (c, d) with both climatic and physiological variables for 2050 and 2070. Green regions indicate the current distribution area, while blue and red regions indicate the gain and loss of the suitable distribution, respectively.

In summary, the fitness consequence of phenotypic modification strongly depends on the adaptive capacity of genetic-expression modulation (DeWitt and Scheiner 2004, Somero 2005, Ghalambor et al. 2007, VanDerWal et al. 2013).

Incorporating the EAHT and heat stress frequencies altered the previously optimistic predictions by SDM

In contrast to lizards from tropical and subtropical regions, which are projected to suffer serious negative effects of climate warming on activity, growth, and reproduction, congeners from the temperate region have been expected to face positively increasing opportunities for growth and reproduction (Buckley 2008, Sinervo et al. 2010). Nevertheless, we found that heat stress will increase across the distribution of *T. septentrionalis* at all periods we considered. First, as latitude increases,

extreme heat events and warming rates are predicted to increase in the future (Root et al. 2003, Parmesan 2006), but EAHT did not show any “increasing” reaction norm (i.e., beneficial plasticity) among all studied populations (Figs. 1b, 2b). Second, if species’ distributions are determined by lethal limits (Jones et al. 2010), the embryonic stage might be a more stringent limit because we found that EAHT is lower than adult CT_{max} . These thermal bottlenecks in the life cycle are also documented in fish (Dahlke et al. 2020). The only consolation is that higher EAHT of the population from high latitude could slow the stress imposed by intense heat in the near future, which could not be accomplished by populations from medium and low latitudes.

After developmental plasticity was considered in the mechanistic model, we revealed that reaction norms of EAHT additionally aggravate the heat stress frequency of species (Fig. 5d–i). However, our findings are

insufficient to conclude that the reaction norms of EAHT vary in a consistent latitudinal pattern. We cannot separate the effects of average EAHT and the reaction norm types in predicting the heat stress. The embryos from the high-latitude population would be less vulnerable to intense heat events after both latitudinal EAHT and flat reaction norm are considered. First, high absolute EAHT would impose selection advantages in fluctuating thermal environments (e.g., Overgaard et al. 2014). Second, less plasticity might accelerate the adaptation of the EAHT to environmental change by allowing the phenotype to be more exposed to natural selection (e.g., Fox et al. 2019). Nevertheless, the flat EAHT reaction norms for the high-latitude population might limit the possibility of responding to climate warming by developmental plasticity, which might add the vulnerabilities to further intense heat events caused by climate change before achieving adaptation in EAHT (Stillman 2003, Sunday et al. 2012, Magozzi and Calosi 2015). Pessimistically, bell-shaped and decreasing reaction norms significantly aggravated heat stress frequency in response to increasing nest temperatures especially within 25°–35° N, which is the main distribution range predicted by SDM (Appendix S1: Fig. S4; Fig. 6). Depressed EAHT at high developmental temperatures under bell-shaped and decreasing reaction norms would decrease the heat resistance of embryos when they are exposed to high developmental temperatures, and therefore increase the risk to embryos under future climate change (Fig. 1d, e). Predictions using these two reaction norms indicated significant increases in heat stress frequency (Fig. 5). In the future, high average temperatures are predicted to be accompanied by an increased incidence of intense heat events (IPCC 2013). Bell-shaped and decreasing reaction norms of EAHT would impose additional negative effects on embryos. Accordingly, physiological functions and population growth of species would be suppressed by lower embryonic survival, even when the thermal environment is proper for adults (Radchuk et al. 2013, Woods 2013). Under even moderate warming (RCP 6.0), fatal effects were found for populations from medium and low latitudes, which incur the two disadvantages of lower absolute EAHT and non-beneficial plasticity (Figs. 4, 5, 6). In addition, several other species (e.g., *T. amurensis*, *T. wolteri*, *T. kuehnei*, *T. sexlineatus*) from *Takydromus* genus occur outside the distribution range of *T. septentrionalis* (Zhao et al. 1999). Our prediction beyond the distribution of *T. septentrionalis* may also help to understand the response of these congeners to climate change.

Our study found that SDMs with both climatic and physiological predictors would predict less suitable habitat in all periods (i.e., present and future). This prediction indicates that the SDM with only climate predictors may overestimate the area of suitable habitats because it fails to take the heat stress risk of embryos into account (Levy et al. 2015, Carlo et al. 2018). Moreover, we found that the SDM with only climate predictors would also

underestimate the potential of thermal refuges for the lizards with low EAHT. Around the distribution center, the lizards with low EAHT can survive if the thermal environments are mild (Fig. 6), and the heat stress is accordingly low (Hall and Sun 2020). This suggests that more vital biological information should be incorporated in hybrid SDMs in future studies to provide more reliable predictions of range shifts induced by climate change (e.g., Rodríguez et al. 2019, Gamliel et al. 2020).

The northward movement of suitable habitats as a response to climatic change has been reported for numerous taxa (Parmesan 2006, Pearson et al. 2010, Pöyry et al. 2010, Devictor et al. 2012). The models with consideration of climate predictors expected a northeastward movement in *T. septentrionalis*. The southern edge of the distribution is predicted to contract, while the northern edge of the distribution is predicted to expand, particularly within the range from 20° N to 30° N, where thermal environments are the most suitable at present (Fig. 6; Appendix S1: Fig. S4). Nonetheless, some biotic factors, in addition to climate variables, could limit the species' range shifts. First, lizards with limited dispersal ability are unlikely to catch up with rapid climate change by migration (e.g., Schloss et al. 2012, Schmaljohann and Both 2017). Second, geographical barriers (e.g., mountains and rivers) would also make migration difficult (Ghalambor et al. 2006). Third, recent studies revealed increased vulnerabilities under climate change by integrating embryonic heat stress into SDMs (Levy et al. 2015, Carlo et al. 2018). Our study also confirms that, after taking latitudinal variation in EAHT and heat stress frequency into account, future distributions might be further contracted than that predicted by the SDM using only climate predictors (Fig. 6).

It is noteworthy that our modeling projection is based on soil temperatures rather than nest temperatures. Although soil temperatures at 5 cm depth are a good proxy for nest temperatures, given that females lay eggs at this depth, females may be able to select specific nest sites with a suitable thermal environment for embryonic development. For example, the toad-headed agamid lizard (*Phrynocephalus przewalskii*) selects warm nests at open sites that may enhance developmental rate and success (Li et al. 2018); the water dragon, *Physignathus lesueurii*, selects more open sites in high-latitude populations, but more shady sites in low-latitude populations to compensate latitudinal variation in soil temperatures (Doody et al. 2006). In addition, females may change their nesting behaviors to buffer the impact of climate change on embryonic developments, including changing nesting time, nesting in cooler locations, or constructing deeper nests (Refsnider and Janzen 2010). For example, nesting lizards (*Bassiana duperreyi*) adjusted oviposition timing and nest depth to partly compensate for recent rising nest temperatures (Telemeo et al. 2009). The selection of cool nest sites by female lizards (*Anolis cristatellus*) can enhance embryonic survival for lizards facing heat stress associated with

urbanization (Tiatragul et al. 2020). Turtles can choose nest sites with particular shade cover (Refsnider and Janzen 2012), or change the timing of nesting depending on previous winter climate (Schwanz and Janzen 2008). Despite the buffering impact of maternal nest behavior, future climate change may impose greater impacts on the species than we predicted in the current study. First, repeated exposure to sublethal temperatures (i.e., close to but below the EAHT) may induce long-term negative effects on embryonic development and hatchling phenotypes (Carlo et al. 2018, Hall and Warner 2019a). Second, EAHT could be lower at earlier or later stages relative to at halfway (Hall and Warner 2019b), which may aggravate the heat stress frequency and thus the vulnerabilities to climate change on all populations.

Our study could inspire researchers to examine more complex embryonic responses to extreme heat events. In addition to the chronic negative effect of sublethal developmental temperatures on offspring fitness (Carlo et al. 2018), we found that embryonic heat tolerance (i.e., EAHT in this study) not only differs geographically but also is likely to impact embryonic survival and thus population dynamics. In the future, it would be necessary and important to know the underlying mechanisms of latitudinal variation and developmental plasticity of EAHT at multiple levels, such as physiological, biochemical, and genetic (Somero 2005, 2012). For example, a growing number of researchers suggest embryonic heat tolerance is related to oxygen limitation at extremely high temperatures in reptile and avian species (Vimmerstedt et al. 2015, Hall and Warner 2019a). Such mechanistic knowledge can help us to understand the proximate and evolutionary factors that results in the variations in EAHT, and what is more important to predict survival probability under ongoing climate warming with adaptation and evolution (Huey et al. 2012, Angilletta et al. 2013).

Moreover, intrinsic and extrinsic effects on EAHT are also worth considering in the context of climate warming, including developmental processes, nesting behavior, maternal effect, and other environmental factors, etc. (Angilletta 2009, Angilletta et al. 2013, Li et al. 2018, Noble et al. 2018). For example, we determined EAHT at halfway of incubation, but the EAHT at different embryonic stages may provide a detailed panoramic view of heat tolerance through embryonic development, which may help to predict the vulnerabilities of animals to climate warming (Angilletta et al. 2013, Hall and Warner 2019b, Hall and Sun 2020). Furthermore, although the EAHT did not differ between eggs incubated at constant vs. fluctuating temperature in our case; fluctuating temperatures mimicking nest temperatures are more ecologically relevant. Such an experimental setup is recommended to determine the effect of temperatures on embryonic development and offspring traits (e.g., Levy et al. 2015, Carlo et al. 2018). In the future, these intrinsic and extrinsic factors should be incorporated into the integrative models to provide a more

accurate prediction of species' vulnerability to climate change.

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SUPPORTING INFORMATION

Additional supporting information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecm.1468/full>

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Data (Sun et al. 2021) are available on the Dryad Digital Repository: <https://doi.org/10.5061/dryad.bk3j9kdbk>.