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CONTRIBUTED PAPER

Collective effects of rising average temperatures and heat events on oviparous embryos

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Article impact statement: Rising average temperatures and heat events collectively determine the embryonic survival under climate change.

Abstract

Survival of the immobile embryo in response to rising temperature is important to determine a species' vulnerability to climate change. However, the collective effects of 2 key thermal characteristics associated with climate change (i.e., rising average temperature and acute heat events) on embryonic survival remain largely unexplored. We used empirical measurements and niche modeling to investigate how chronic and acute heat stress independently and collectively influence the embryonic survival of lizards across latitudes. We collected and bred lizards from 5 latitudes and incubated their eggs across a range of temperatures to quantify population-specific responses to chronic and acute heat stress. Using an embryonic development model parameterized with measured embryonic heat tolerances, we further identified a collective impact of embryonic chronic and acute heat tolerances on embryonic survival. We also incorporated embryonic chronic and acute heat tolerance in hybrid species distribution models to determine species' range shifts under climate change. Embryos' tolerance of chronic heat (T-chronic) remained consistent across latitudes, whereas their tolerance of acute heat (T-acute) was higher at high latitudes than at low latitudes. Tolerance of acute heat exerted a more pronounced influence than tolerance of chronic heat. In species distribution models, climate change led to the most significant habitat loss for each population and species in its low-latitude distribution. Consequently, habitat for populations across all latitudes will shift toward high latitudes. Our study also highlights the importance of considering embryonic survival under chronic and acute heat stresses to predict species' vulnerability to climate change.

Liang Ma, Dan-Yang Wu, and Yang Wang contributed equally to this work.

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INTRODUCTION

Climate change threatens biodiversity across the planet (Barnosky et al., 2011). Predicting species' vulnerabilities to climate change and resulting shifts in species' distributions is crucial for biodiversity conservation but requires a comprehensive understanding of the species responses to projected climate change across geographic clines (Bonebrake et al., 2018). Heat tolerance metrics (e.g., critical thermal maximum) describe the resistance of animals to heat stress and thus facilitate predictions of species' responses to climate change (Deutsch et al., 2008), especially considering rising average temperatures and frequent heat events in recent years (Witze, 2022). However, most studies on physiological tolerance to heat stress have primarily focused on adults (Sunday et al., 2011, 2014), without considering the immobile embryonic stage. However, embryos of oviparous ectotherms (e.g., lizards) may be more vulnerable to heat stress than later life-history stages because of the limited ability of embryos to behaviorally thermoregulate (Taylor et al., 2020; Telemeco et al., 2016). Consequently, understanding embryonic heat tolerance and its relation to current and future nest temperatures is critical to predicting a species' vulnerability to climate change (Angilletta et al., 2013; Carlo et al., 2018; Hall & Warner, 2019; Sun et al., 2021). For instance, although no latitudinal variation in embryonic heat tolerance was observed for a widespread North American lizard (*Sceloporus undulatus*) (Angilletta et al., 2013), integrating future nest temperatures with embryonic heat tolerance metrics revealed that species survival decreases at low latitudes under climate change (Levy et al., 2015). Moreover, combining latitudinal plasticity of embryonic heat tolerance with hybrid species distribution models (SDMs) showed that the northern grass lizard (*Takydromus septentrionalis*) is more vulnerable at low latitudes under climate change (Sun et al., 2021). Therefore, understanding and adding embryonic heat tolerance to existing research frameworks (e.g., Williams et al., 2008) for assessing species' vulnerability to climate change can improve the accuracy of model predictions and account for local extinctions due to embryonic death (Angilletta et al., 2013; Carlo et al., 2018; Hall & Warner, 2019).

Recently, researchers have considered how multiple metrics of embryonic heat tolerance can be formulated and applied under fluctuations in environmental temperature at various temporal scales (Hall & Sun, 2021). Accordingly, embryonic heat tolerance can be classified theoretically based on 2 primary metrics: embryonic tolerance of chronic heat (T-chronic) and embryonic tolerance of acute heat (T-acute). Embryonic T-chronic indicates embryonic tolerance to rising average temperatures over the entire development period and predicts vulnerability to general warming (Gao et al., 2014; Liang et al., 2015), which can affect hatching success and species persistence. Embryonic T-acute indicates the resistance of embryos to a brief exposure to extremely high temperatures (e.g., heat waves and maximum daily temperatures) (Angilletta et al., 2013), which determines their survival in acute heat events (Levy et al., 2015; Sun et al., 2021). Therefore, T-chronic and T-acute reflect embryonic tolerance to different characteristics of the thermal environment (Hall & Sun, 2021): rising average temperatures and increasing extreme heat events, which are 2 major challenges for species under climate change (Hansen et al., 2012).

However, predicting the integrative impact of rising average temperatures and extremely high temperatures on oviparous embryos is challenging and has rarely been achieved (but see Bourne et al. [2020]). Several obstacles need to be overcome before this critical knowledge gap can be bridged. First, T-chronic and T-acute determine embryonic survival at different time scales. The baseline hatching success in response to the average temperature across the incubation period is determined by T-chronic, and T-acute determines survival at daily extreme temperatures (Hall & Sun, 2021). Second, the T-acute is subject to developmental plasticity in some species and may exhibit significant latitudinal variation (Sun et al., 2021). Accordingly, embryonic survival during the brief and extreme temperatures is a function of not only the daily maximum temperature, but also the incubation temperatures the embryo experienced since oviposition. Third, oviparous species lay eggs across reproductive seasons with various lengths and geographic ranges of various extents. This exposes eggs to various temperature profiles across time and space (Carlo et al., 2018; Hall & Sun, 2021; Levy et al., 2015; Sun et al., 2021).

To address these challenges, we applied an integrative model that accounted for both T-chronic and T-acute. We investigated 5 populations of 3 lizard species in the genus *Takydromus* (Figure 1), which are distributed across a large latitudinal gradient in East Asia, and utilized empirical measurements of embryonic heat tolerance, biophysical models, and hybrid SDMs to explore how chronic and acute heat stress independently and collectively determine the embryonic survival of an ectotherm across latitudes. Specifically, we measured the T-chronic and T-acute of 5 populations from 3 species and then estimated embryonic survival under climate change with a biophysical model that included both T-chronic and T-acute to reveal the independent and collective effect of chronic and acute heat stress on embryonic survival across latitudes. To validate our survival estimates, we incubated eggs under current and future nest temperatures, predicted survival with our model, and compared our predictions to the empirical results. Finally, we used a hybrid SDM to predict species' range shifts under climate change with and without embryonic survival. We tested the hypotheses that an increased number of acute heat events are more detrimental to embryonic survival than rising average temperature due to the accumulative effect of repetitive acute heat
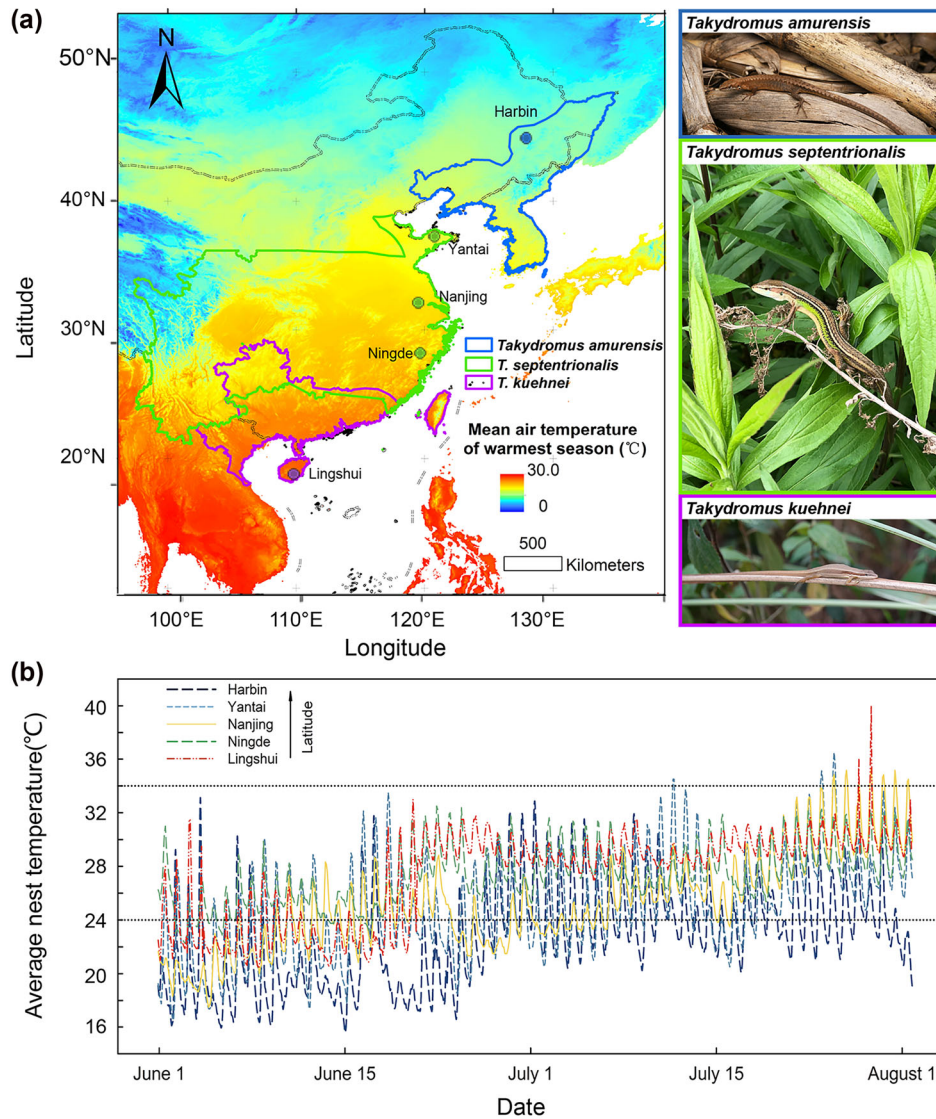
FIGURE 1 For *Takydromus amurensis*, *Takydromus septentrionalis*, and *Takydromus kuehnei*, (a) distribution and sampling sites (blue lines, *T. amurensis* distribution; green lines, *T. septentrionalis* distribution; purple lines, *T. kuehnei* distribution; circles, sample sites [color key as for distribution]; warmest season, duration of the reproductive season) and (b) average nest temperatures from June to August in the field (lines, average temperatures in natural nests from each location; top dotted line, maximum incubation temperature used; bottom dotted line, minimum incubation temperature used). Average nest temperatures calculated based on 2, 4, 3, 5, and 3 natural nests for the sample sites of Lingshui, Ningde, Nanjing, Yantai, and Harbin, respectively.

events and that the impact of climate change on embryos varies across latitudes because of the latitudinal variation in embryonic thermal tolerance.

METHODS

Study system

The grass lizards (*Takydromus*) are a genus of oviparous lizards distributed across latitudes in mainland China, East Asia, and Southeast Asia (Zhao & Adler, 1993). Their distribution encompasses tropical, subtropical, and temperate regions (Figure 1a). Accordingly, the environmental temperatures (average temperature and extreme temperature) experienced by different species or populations of grass lizards in China vary consid-

erably (Hao et al., 2020; Sun et al., 2013, 2021). As such, T-acute varies across *Takydromus* species (Hall & Sun, 2021). Mean values and developmental plasticity of T-acute vary across latitudes in the northern grass lizard, *T. septentrionalis* (Sun et al., 2021). In addition, each species demonstrates latitudinal shifts such that the activity time and metabolic rates of adults and embryonic incubation period decrease gradually toward high latitudes in the Amur grass lizards (*Takydromus amurensis*), northern grass lizards (*T. septentrionalis*), and Kuhne's grass lizards (*Takydromus kuehnei*) (Mi et al., 2022). Therefore, *Takydromus* lizards constitute an excellent system to integrate environmental temperatures with metrics of embryonic survival under chronic and acute heat stress (i.e., T-chronic and T-acute) to predict species' vulnerabilities to climate change across latitudes.

Lizard collection and egg incubation

We selected 5 populations from 3 grass lizard species across latitudes. Amur grass lizards (*T. amurensis*) were collected from Harbin and represent the highest latitude population (N45.20°, E127.95°). The high-, medium-, and low-latitude populations of northern grass lizards (*T. septentrionalis*) were collected from Yantai (N37.26°, E121.74°), Nanjing (N32.07°, E119.06°), and Ningde (N26.67°, E119.54°), respectively, which include intermediate latitudes. The Kuhne's grass lizards (*T. kuehnei*) were collected from Lingshui (N18.66°, E109.92°), which is the lowest latitude included in our study. Their habitats are mixtures of shrubland and grassland (Liu et al., 2022) and cover a latitudinal range of approximate 26.5° and approximately 3385 km from Harbin to Lingshui (Figure 1a).

Lizards were collected by lasso or hand during the beginning of the reproductive season in 2016 and 2021. We collected 45 gravid females and 25 males for each population of *T. septentrionalis* (i.e., Ninde, Nanjing, and Yantai), among which 35 females and 20 males were collected from mid-April to early May of 2016 (Sun et al., 2021) and 10 females and 5 males were collected in early May of 2021. We also collected 35 females and 20 males of *T. kuehnei* in early April and *T. amurensis* in early May of 2021, respectively. During collection for each population or species, females with oviductal eggs were used to locate natural nests. We found the natural nests where females laid eggs by monitoring their nesting behaviors. Then, we set data loggers (i-Buttons, DS1921; Maxim Integrated Products) in their natural nests to monitor the nest temperatures following published methods (Sun et al., 2021). The nest temperatures were collected in the reproductive season from early June to August for each location (Appendix S1). We found 4, 3, and 5 natural nests for the populations from Ningde, Nanjing, and Yantai, respectively, from which to collect nest temperatures for *T. septentrionalis* in 2016, and 2 and 3 nests in Lingshui and Harbin, respectively, to monitor nest temperatures of *T. kuehnei* and *T. amurensis*, respectively, in 2021.

After collection, the lizards were transferred to our laboratory in Beijing, where they were measured (within approximately 0.1 mm), weighed (within approximately 0.001 g), individually marked, and housed. Every 4 females and 2 males from each population were housed in a terrarium (600 × 450 × 400 mm) for breeding. The terraria were kept in a temperature-controlled room at 21–23°C. We used a heat lamp to create a thermal gradient from 22 to 40°C for thermoregulation from 08:00 to 20:00 each day. Food (larvae of *Tenebrio molitor* and crickets dusted with vitamins and minerals) and water were provided ad libitum. We housed the lizards and moved females with oviductal eggs to individual egg-laying terraria (350 × 210 × 200 mm). The egg-laying terraria were the same as for lizard husbandry (Sun, Ma, et al., 2018). Females started to lay eggs around 1 week after they were transferred to the laboratory (Sun et al., 2021).

We searched for freshly laid eggs 3 times daily (09:00, 13:00, and 18:00). Once found, eggs were immediately weighed (to the 0.001 g) and placed in plastic jars (55 × 70 mm [diameter × height]) with moist vermiculite (−220 kPa) for incubation. Because constant temperature and fluctuating temperatures (within 3°C) did not differentially affect the embryonic thermal tolerance (Sun et al., 2021), we incubated eggs of each population at 6 constant temperatures (24, 26, 28, 30, 32, and 34°C) with incubators (KB240; Binder). These incubation temperatures spanned the range of mean nest temperatures for most populations in the wild (Figure 1b). Eggs from the same female were randomly and evenly assigned to different incubation temperatures (i.e., split-clutch design). To estimate incubation period and T-chronic in response to mean nest temperatures, we incubated 194, 145, 164, 141, and 184 eggs of *T. amurensis*, Yantai, Nanjing, Ninde population of *T. septentrionalis*, and *T. kuehnei*, respectively, across the 6 incubation temperatures (Table 1). To estimate T-acute as well as plasticity in T-acute, we furtherly incubated 51, 56, 53, 59, and 64 eggs for *T. amurensis*, Yantai, Nanjing, Ninde population of *T. septentrionalis*, and *T. kuehnei*, respectively, across the 6 incubation temperatures. Ethics approval and protocol (IOZ-IACUC-2023-153) for the collection, handling, and husbandry of the study animals were given by the Animal Ethics Committees at the Institute of Zoology, Chinese Academy of Sciences.

Incubation period and developmental rate

We calculated the embryonic developmental rate of each population and species at each constant temperature (i.e., 24, 26, 28, 30, 32, and 34°C). No embryo from the Yantai population of *T. septentrionalis* or *T. amurensis* hatched successfully at 34°C, so we only estimated rates from 24 to 32°C for those groups. Toward the end of incubation, we checked the incubators 3 times per day for new hatchlings. Once the hatchling appeared, we calculated the incubation period as the number of days from oviposition to hatching and converted the incubation period to hours. The developmental rate (percentage of development per hour) at each temperature was calculated as the inverse of the incubation period (number of hours). Then, we created equations for calculating hourly developmental rates across temperatures by fitting linear models (model predictions in Appendix S2) with the hourly developmental rate as the dependent variable and the incubation temperature as the independent variable (statistics and model parameters in Appendix S3). We assumed embryos suspend development when temperatures were at or below the developmental zero temperature, which was calculated by solving the equations when developmental rates are equal to zero. Because the eggs from the same mother were randomly and evenly assigned to the different incubation temperatures and female identification did not significantly influence any trait in preliminary analysis, we did not use female identification as a random factor in statistical analyses of incubation period, T-chronic, or T-acute (Appendix S4).

Embryonic survival in response to chronic heat stress

We estimated embryonic T-chronic by performing logistic regressions of hatching success across constant incubation

TABLE 1 Number of eggs and hatchlings at each incubation temperature and incubation period for 5 populations of 3 *Takydromus* lizards.
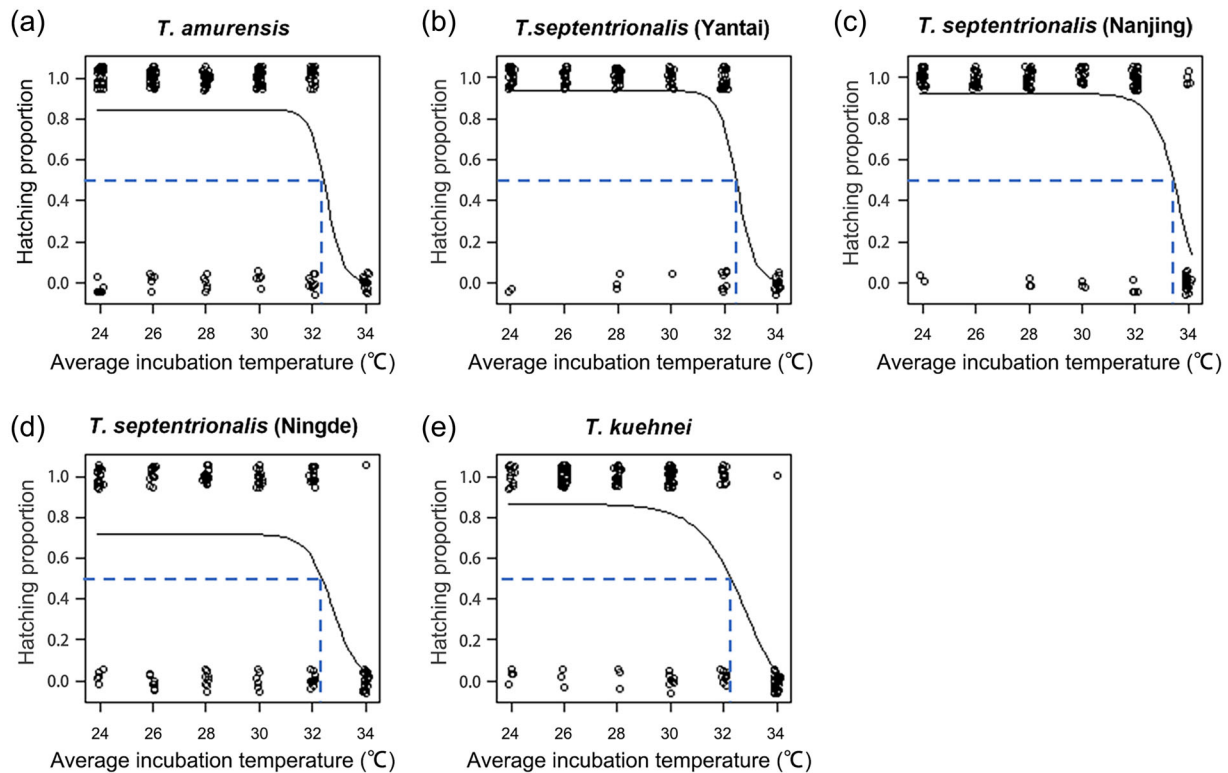
Species and population	Incubation temperature (°C)	Hatchlings, eggs	Incubation period (days)			
			Mean	SD	Min	Max
T. kuehnei; Lingshui	24	14, 18	53.86	3.57	49	60
	26	42, 45	44.38	2.63	39	49
	28	19, 22	38.32	1.67	36	42
	30	33, 43	32.06	1.43	28	36
	32	14, 23	31.14	2.03	29	35
	34	1, 33	35.00	3.57		
T. septentrionalis; Ningde	24	21, 26	41.19	1.97	38	46
	26	13, 20	35.54	1.51	33	37
	28	18, 27	31.39	0.92	30	33
	30	16, 22	26.81	1.28	25	29
	32	15, 25	25.33	1.23	23	28
	34	1, 21	25.00			
T. septentrionalis; Nanjing	24	28, 30	43.18	2.12	40	46
	26	20, 20	36.10	0.91	34	38
	28	30, 33	31.93	1.08	30	34
	30	17, 20	26.88	1.41	22	28
	32	31, 35	25.68	0.83	24	27
	34	5, 26	25.60	1.82	23	28
T. septentrionalis; Yanai	24	24, 26	44.29	3.68	37	52
	26	19, 19	34.89	0.99	33	36
	28	26, 29	30.69	0.79	30	33
	30	19, 20	26.95	1.43	25	30
	32	26, 34	24.62	0.94	23	26
	34	0, 17				
T. amurensis; Harbin	24	31, 37	41.77	1.93	38	47
	26	33, 38	37.15	1.09	35	39
	28	30, 37	32.83	1.12	31	36
	30	33, 38	29.03	0.73	28	32
	32	23, 32	27.61	0.89	27	30
	34	0, 12				

temperatures. We assigned binary values of hatching success to each egg (0, unhatched; 1, hatched) for each species and population. We fit multiple common dose–response models (e.g., 3-parameter vs. 4-parameter log-logistic regression, quadratic, Weibull) in which hatching success was the dependent variable and incubation temperature was the independent variable (function drm in R package drc) (Hall & Sun, 2021; Ritz et al., 2015). We then selected the best model for each population according to the Akaike's information criterion (selected models and model parameters in Appendix S5). The best model for *T. amurensis* and Yantai and Ningde populations of *T. septentrionalis* was a 3-parameter log-logistic model: fct = LL.3. The best model for Nanjing population of *T. septentrionalis* and *T. kuehnei* was a Weibull model: fct = W1.3 (Figure 2). We determined the T-chronic as the incubation temperature at which hatching

success declined to 50% (Hall & Sun, 2021). When estimating the integrative embryonic survival (i.e., combining T-chronic with T-acute, see below), we used these dose–response models to calculate embryonic survival in response to mean nest temperatures for each clutch of eggs.

Embryonic survival in response to acute heat stress

We estimated the T-acute as the temperature at which the embryo exhibits cardiac arrest (Angilletta et al., 2013; Taylor et al., 2020) based on an established method (Sun et al., 2021). Specifically, T-acute was measured halfway through the incubation period (within 2 days) for each incubation temperature; the

FIGURE 2 Fitted curves of hatching success as a function of constant (i.e., chronic) incubation temperature for the 3 species (5 populations) (open circles, raw data jittered around 1 or 0; solid lines, model predictions; blue broken lines, embryonic tolerance of chronic heat).

halfway point was estimated using published incubation periods and our previous records (Liu et al., 2022; Sun et al., 2021). Thus, embryos were approximately at developmental stage 36 when T-acute was determined (Sun et al., 2021). We weighed and acclimated the eggs at 28°C for 2 h in a programable incubator (KB240; Binder) and then heated them at a rate of 6°C/h, which is an approximately maximum heating rate detected in a natural nest (Ningde population) (Sun et al., 2021). While heating, the real-time embryonic heart rates were monitored with a digital egg monitor (Buddy; Vetronic Services). To prevent desiccation of eggs during measurement, 4 wet cotton balls were set around the egg to keep the air inside the heart rate monitor moist. Once the heart rate of an embryo could not be detected for 10 s (egg monitor showed 0 for heart rate), we immediately measured the core temperature of the egg with a calibrated thermocouple (Angilletta et al., 2013; Sun et al., 2021). This temperature was recorded as the T-acute. Then, we dissected the egg to confirm mortality. We eliminated the data from the embryos that were still alive on dissection. We analyzed T-acute in linear mixed models (lmer in the lmerTest package in R 3.3.1); population and incubation temperatures were main factors (Sun et al., 2021).

To estimate survival probabilities under acute heat stress, we formatted the T-acute data so that each egg was given a value of 0 or 1 (died vs. survived, respectively) at each acute temperature from 28 to 52°C in 1°C increments. For example, if an egg died at 44°C, it was assigned a value of 1 for each temperature from 28 to 43°C and a value of 0 from 44 to

52°C. We selected 52°C because no embryo could be alive at the surrounding temperature of 52°C. Using this data set, we calculated embryonic survival of acute heat stress with fitted generalized additive models (GAMs) with the acute temperature (i.e., 28–52°C) and the constant incubation temperature each egg experienced prior to T-acute measurement as independent variables. Survival was a binary response variable. We used the resulting equations to estimate egg survival in response to daily maximum nest temperatures while considering mean nest temperature (i.e., considering T-acute and plasticity in T-acute). Sample sizes and restricted maximum likelihood scores for the fitted GAMs (Wood, 2003) are in Appendix S6.

For the model fitting, we created a table of daily maximum temperatures, average incubation temperatures, and corresponding heat-stress survival results (alive or dead). We used the constant temperature the eggs were incubated at as the average incubation temperature. We then calculated the survival of acute temperature (S_{acute}) as the mathematic product for daily survivals of acute heat stress:

$$S_{\text{acute}} = \prod_{i=\frac{1}{3} \text{ of incubation}+1}^{\frac{2}{3} \text{ of incubation}} f\left(T_{\text{dmax}_i}, T_{\text{mean}}\right), \quad (1)$$

where T_{dmax_i} is the maximum temperature on day i during incubation and T_{mean} is the average incubation temperature. Because we measured only eggs' survival at daily extreme temperatures

for eggs in the middle one third of their incubation periods, we only consider this subset of incubation periods when calculating S_{acute}. In other words, eggs in their first and last one third of incubation were assumed to be unaffected by daily maximum temperatures.

Embryonic survival estimated by the integrative survival model

To understand how current and future nest temperatures influence embryonic survival, we modeled the developmental rate and survival of eggs laid from 1 June to 31 July by combining our laboratory data with temperature data from national weather stations (1437 in total) in each species' range. All 5 populations lay more than half of their yearly eggs during this period per our observations in seminatural enclosures (details in Appendix S1). For *T. septentrionalis*, we repeated the modeling with traits of the 3 populations (Yantai, Nanjing, and Ningde) because the 3 populations showed different developmental rates and embryonic heat tolerances (both T-chronic and T-acute), and the climate stations in the distribution range of *T. septentrionalis* were considered when projecting each of its 3 populations. We calculated survival of chronic heat stress and acute heat stress, and integrative survival of eggs laid from 00:00 1 June to 23:00 31 July, based on the assumption that the eggs were laid in an open area at a depth of 5 cm (Liu et al., 2022; Sun et al., 2021) and that the number of eggs laid each hour is consistent through time. When calculating survival for eggs laid at a specific hour, we estimated the incubation period by accumulating hourly developmental rates until the completion of development with the developmental rate models fitted above and extracted the hourly nest-temperature profile experienced by these eggs. We calculated survival in response to chronic stress by using the mean nest temperature in the fitted dose–response models. We calculated survival of acute stress by inserting daily maximum nest temperatures during the middle one third of the incubation period and the mean nest temperature into Equation (1). We calculated integrative survival as the product of the survival in response to chronic heat stress and survival of acute heat stress.

Model validation based on experiments in seminatural enclosures

We validated the integrative survival model against known values of embryonic survival by conducting an incubation experiment with the Nanjing population of *T. septentrionalis*. Specifically, we collected eggs from the Nanjing population and incubated them in artificial nests (depth of 5 cm) in seminatural enclosures. The enclosures were set to mimic the present climate and warming climate by controlling the greenhouse effect (Liu et al., 2022; Sun, Wang, et al., 2018). In brief, 3 enclosures (3 × 3 × 0.7 m for each) were half-covered with a shade net to mimic the natural shade of the present climate, and 3 enclosures were half-covered with plastic film to increase the greenhouse effect. We cut holes in the plastic film to allow rain

to pass through (20 holes on a 1.5 × 3-m plastic film with a diameter of 5 cm each), keeping the soil moisture between the 2 types of enclosures approximately equal (see figure 3S in Liu et al. [2022]). We incubated 89 eggs in the present climate enclosures and 127 eggs in the warming climate enclosures starting in late May. Incubation temperatures (i.e., nest temperatures) were recorded hourly with i-Buttons. The average nest temperature (from late May to early Sep) in the warming climate (28.45°C [SE 0.17], range 20.0–49.5°C) was 1.76°C higher than that in present climate (26.69°C [0.26], range 19.5–38.5°C), which was similar to the projected temperatures under a moderate warming climate change scenario (shared socioeconomic path SSP1-2.6, 1.3−2.4°C) (IPCC, 2021). Toward the end of incubation, we moved the eggs to incubators (KB240; Binder) set at 27°C with daily fluctuation of 3°C (from 24 to 30°C). We checked 3 times a day for hatchlings and recorded hatching success for each egg. We performed model validation by comparing hatching success from the experiment with predictions of hatching success generated based on recorded incubation temperatures with the integrative development model.

Range shifts under climate change in hybrid SDMs

To evaluate how embryonic integrative survival affects populations' and species' vulnerability to climate change across their ranges, we calibrated process-based hybrid SDMs with integrative embryonic survival and used climate variables as predictors. We compared the range shifts (percentage of the gain or loss of distribution area) in the future relative to the current distribution predicted by the hybrid SDMs with those predicted by SDMs with only climate predictors. Accordingly, the difference in range shifts between those predicted by hybrid SDMs and those predicted by SDMs was calculated as the difference caused by additional consideration of embryonic integrative survival.

We collected occurrence records of *T. amurensis* (initial $n = 46$, after thinning $n = 35$), *T. septentrionalis* (initial $n = 218$, after thinning $n = 131$), and *T. kuehnei* (initial $n = 30$, after thinning $n = 15$) from the distribution database of *Takydromus* lizards (Mi et al., 2022). We thinned collected occurrence records with a distance threshold of 20 km^2 to avoid spatial autocorrelation. Pseudoabsences were created by randomly sampling 1000 locations in the current range of each species (Roll et al., 2017). Bioclimate variables were downloaded from Chelsa 1.2 at a resolution of 30 arc seconds (Karger et al., 2017). To reduce multicollinearity among climate variables, 4–5 climate variables were selected for modeling according to the variance inflation factor (VIF < 3) (Appendix S7). These bioclimate variables represented the climate conditions at present (1979–2013) and in the future (2061–2080) (SSP1-2.6, 1.3−2.4°C). For future climates, we averaged projections of 5 general circulation models (BCC-CSM1.1, CSIRO-Mk3-6-0, FIO-ESM, GFDLESM2M, and MRI-CGCM3), which are thought to perform well in predicting historical climate change in China (Jia et al., 2019), to account for uncertainty. We created layers (present and future)

of overall embryonic survival for each of the 3 species by running the integrative survival model for all climate stations and then applying spatial interpolation using the kriging' method (Pebesma, 2004). Because *T. septentrionalis* is widely distributed and its 3 populations differ in their embryonic heat tolerance (Appendices S5 & S6), we first created layers for each of its 3 populations and then combined these layers by assigning sites values from the closest population by latitude.

In the processes of running the SDMs and hybrid SDMs, we implemented an ensemble approach with 8 algorithms: generalized linear model, generalized boosting model (i.e., boosted regression trees), classification tree analysis, artificial neural network, surface range envelop (i.e., BIOCLIM), flexible discriminant analysis, random forest, and maximum entropy with the biomod2 package (Thuiller et al., 2020) in R software (R Core Team, 2020). We used a 5-fold cross-validation procedure for all models; 80% of the data were for model training and residual 20% were for validating. We assembled predictions from individual models by estimating the weighted mean using True Skill Statistic (TSS score). Models with a TSS score <0.6 were excluded from the ensemble model.

Finally, we projected present and future (2060–2080) habitat with calibrated models. We transformed the presence probabilities to binary form (presence or absence) with the thresholds that maximized the TSS scores (Liu et al., 2005). Because small lizards have very short annual dispersal distances (usually <50 m, recorded maximum 420 m) (Calsbeek, 2009), we trimmed predicted habitat (presence) with the distribution range polygon for all 3 *Takydromus* species (Roll et al., 2017) (a 1° [≈111 km] buffer was considered to account for potential dispersals).

RESULTS

Incubation periods

The incubation period of *Takydromus* lizards decreased as the incubation temperature increased ($F_{4, 570} = 1992.5, p < 0.0001$). Among the 3 species, *T. kuehnei* had the longest incubation period, whereas *T. septentrionalis* had the shortest and *T. amurensis* was in between ($F_{2, 580} = 707.6, p < 0.0001$) (Table 1). Furthermore, among the 3 populations of *T. septentrionalis*, the medium-latitude population (Nanjing) had the longest incubation period, whereas the high-latitude population (Yantai) had the shortest ($F_{2, 308} = 5.081, p = 0.007$) (Table 1).

Embryonic survival in response to chronic heat stress

The survival rate for embryos of all *Takydromus* lizards decreased as incubation temperature increased (Figure 2; Appendix S8). At 34°C, no embryo of the Yantai population of *T. septentrionalis* (17 fertilized eggs) or *T. amurensis* (12 fertilized eggs) hatched. The dose–effect models fitted well with survival at 6 incubation temperatures (Appendix S5). The Ningde population of

T. septentrionalis exhibited lower survival (<0.8) than other populations and species at temperatures that did not significantly depress the survival rate (Figure 2). The Nanjing population of *T. septentrionalis* had the greatest embryonic T-chronic (33.7°C, 95% confidence interval [CI]: 33.4–34.0). The other populations had similar T-chronic values: Ningde (32.7°C, 95% CI: 32.1–33.6), Yantai (32.5°C, 95% CI: 32.2–32.7), *T. kuehnei* (32.8°C, 95% CI: 32.2–33.4), and *T. amurensis* (32.6°C, 95% CI: 32.2–33.1). Thus, the Nanjing population of *T. septentrionalis* was the most robust to chronic heat stress.

### Embryonic survival in response to acute heat stress

Embryonic T-acute differed considerably across species and populations and increased as latitude increased ($F_{4, 253} = 39.623, p < 0.0001$). The Yantai and Nanjing populations of *T. septentrionalis* and *T. amurensis* exhibited significantly higher T-acute compared with the Ningde population of *T. septentrionalis* and *T. kuehnei* (Yantai[a] > Harbin[ab] > Nanjing[b] > Ningde[c] > Lingshui[c]) (Figure 3).

The T-acute was also influenced by the incubation temperature and showed a thermally plastic response. Embryonic survival rates were thus affected by extreme temperature and the pervasive incubation temperature. Regardless of incubation temperature (except for those >32.5°C), embryos of the Yantai population of *T. septentrionalis* died when the daily maximum temperature reached 42.5°C (i.e., no plasticity, flat pattern). The Ningde population of *T. septentrionalis* showed a decreasing pattern of T-acute as incubation temperature increased, whereas *T. kuehnei* showed an increasing pattern. In contrast, *T. amurensis* and the Nanjing population of *T. septentrionalis* showed a bell-shaped pattern of T-acute; the highest T-acute was at intermediate incubation temperatures (Figure 4; Appendix S6).

Integrative survival rate

Acute heat stress had a larger effect on the integrative survival of *Takydromus* lizards than chronic heat stress under current and future climate conditions (2070), despite that only the middle one third of the incubation period was considered when calculating survival in response to acute temperatures (Figure 5). The average survival under chronic heat stress for all populations and species was >65% under current climate conditions and was not <45% for all populations and species in 2070. In contrast, survival in response to acute heat stress was much lower under both current and future climate conditions for all populations and species. Across the 5 populations of the 3 species, survival considering only T-acute and considering both T-chronic and T-acute lower at lower latitudes. Alarmingly, under a moderate climate change scenario (SSP1-2.6, 1.3−2.4°C), the integrative survival for all populations and species was predicted to decrease to levels below 25% by 2070, with the exception of *T. amurensis* (highest latitude population).
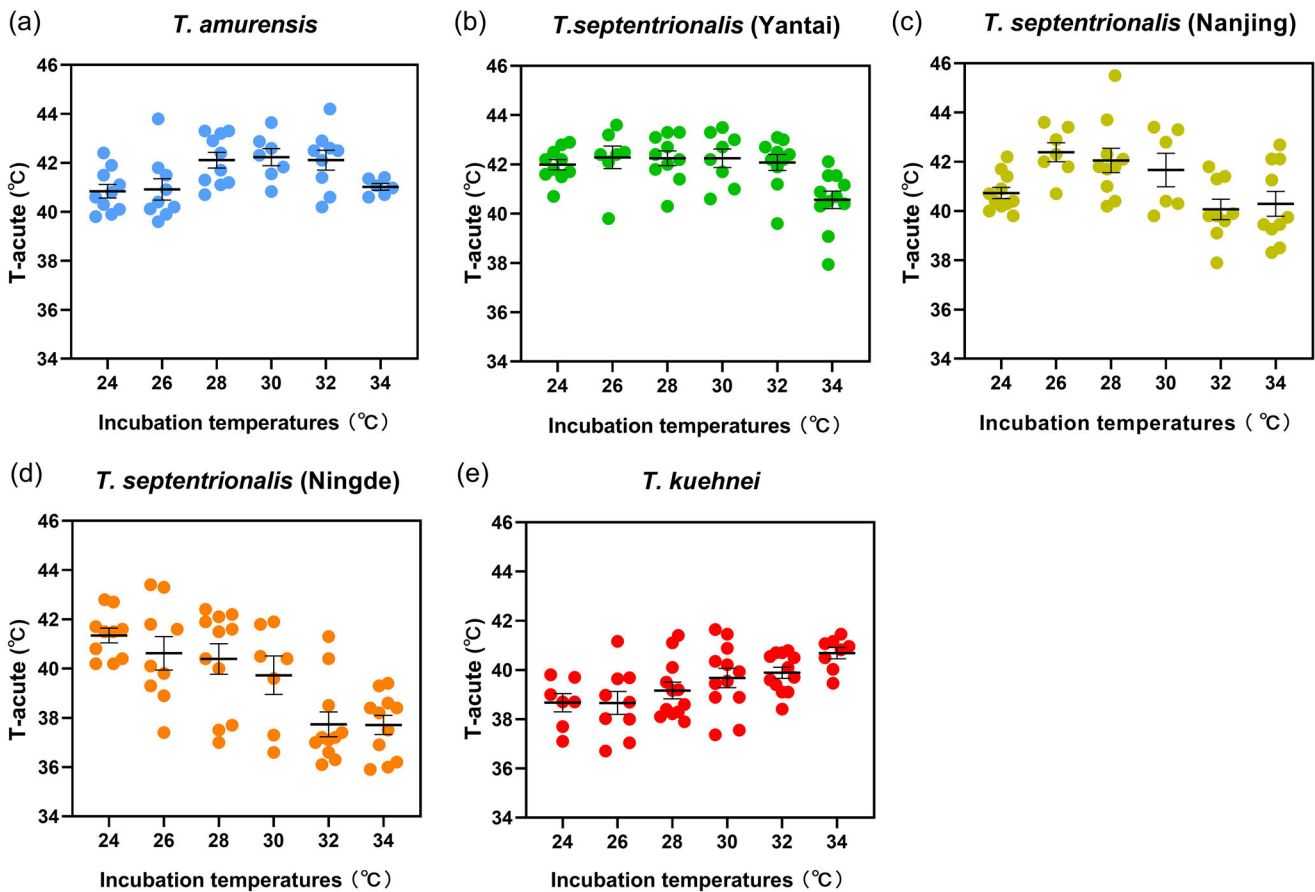
FIGURE 3 Embryonic tolerance of acute heat (T-acute) at different incubation temperatures from 5 populations of 3 *Takydromus* lizards (colored circles, T-acute of an embryo; horizontal lines, mean; bars, SE).

Model validation

The validation experiment demonstrated that the development model we used performed well in predicting the hatching success of eggs in the field. Specifically, all eggs that hatched were accurately predicted to survive, and 69.57% of unhatched eggs were predicted to die (Appendix S9).

Distribution range shifts under climate change

The hybrid SDMs, which incorporated climatic variables and embryonic integrative survival as predictors, outperformed SDMs that contained only climatic predictors for all 5 populations of 3 species (Appendix S7). Under a moderate climate change scenario (SSP1-2.6, 1.3−2.4°C), the hybrid SDMs predicted greater habitat loss in 2070 compared with SDMs for all 3 *Takydromus* lizards (Figure 6). Hybrid SDMs that contained embryonic survival predicted less habitat at present and in 2070 across all species (Figure 6), primarily due to greater loss of habitat in the southern parts of their distribution in the future. Specifically, for the high-latitude species, *T. amurensis*, the hybrid

SDMs predicted a 22% greater contraction of the distribution range than models with only climate predictors. Similarly, the predicted contractions were 3.6% and 17.5% greater with hybrid SDMs for *T. septentrionalis* and *T. kuehnei*, respectively. However, the predicted gain in habitat was similar to hybrid SDMs and SDMs that contained only climatic variables (difference <3.3%) (Figure 6).

DISCUSSION

Species' vulnerability under climate change has been universally connected to the thermal constraints on their performance and survival (Dahlke et al., 2020; Sun et al., 2022). Being exposed to stress-inducing warm temperatures at a relatively vulnerable stage (i.e., embryonic stage) may catastrophically induce population decline and local extinction (Carlo et al., 2018; Dahlke et al., 2020; Levy et al., 2015; Sun et al., 2021). By using a combination of empirical study and niche modeling, we predicted how embryonic T-chronic and embryonic T-acute independently and collectively determined the survival and range shifts of lizards across latitudes under climate change.
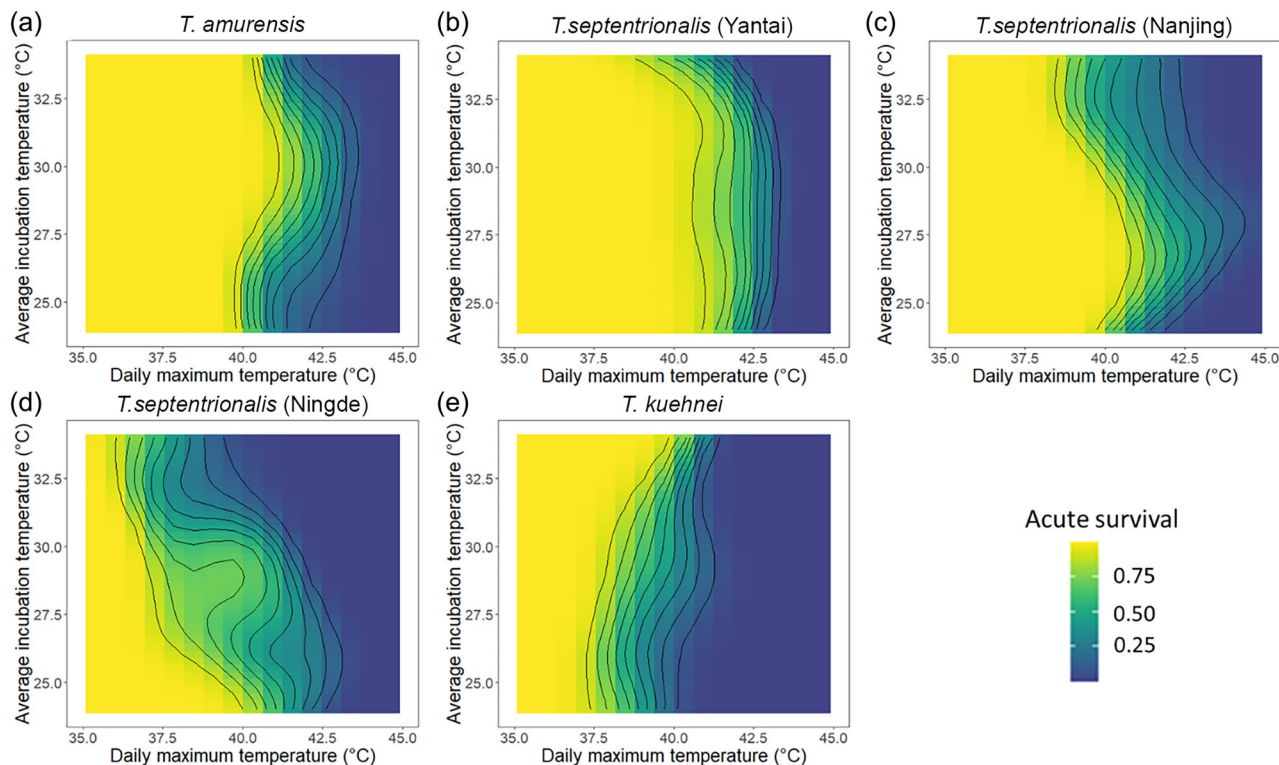
FIGURE 4 Egg survival as a function of constant incubation temperature and daily maximum temperature for 5 populations of 3 *Takydromus* lizards (acute survival, survival of acute high temperature; contour breaks, 0.1).

Latitudinal variation of T-chronic and T-acute

We investigated the latitudinal variation of T-chronic and T-acute in *Takydromus* lizards to determine how embryos may respond to increasing average temperatures and heat events across latitudes independently and interactively (Hall & Sun, 2021). Our findings showed that T-chronic was conserved across *Takydromus* lizards from different latitudes. In contrast, T-acute increased as latitude increased across *Takydromus* species, which was also found among populations of *T. septentrionalis* (Sun et al., 2021). The differing patterns in T-chronic and T-acute across latitudes indicated that these thermal metrics may evolve independently. In summary, our results suggest that the evolutionary responses of *Takydromus* lizards to climate change may differ across latitudes; northern populations may evolve greater T-acute and southern populations may evolve greater T-chronic.

Higher T-chronic and T-acute allow greater heat resistance in embryos, leading to improved hatching success (Angilletta et al., 2013; Dahlke et al., 2020; Levy et al., 2015; Sun et al., 2021) and, consequently, higher species fitness under climate change (Carlo et al., 2018; Gao et al., 2014). However, similar T-chronic values across latitudes did not predict equal survival rates for each population and species, due to higher average nest temperatures at low latitudes (Figure 1b) (Mi et al., 2022). Therefore, it is likely that low-latitude populations of *Takydromus* lizards will experience greater negative impacts from climate change than their high-latitude counterparts (Figure 5a). This latitudinal pattern

mirrors one recently revealed in *Takydromus* adults: the thermal safety margin is lower for species at low latitudes under climate change, even though the critical thermal maximum of *Takydromus* adults is similar across latitudes (Mi et al., 2022). Therefore, the combined effects of heat stress at multiple life stages may further depress the survival of low-latitude populations.

We found in a high-latitude (temperate) species, *T. amurensis*, that T-acute was enhanced when embryos were incubated at 28–32°C but decreased at 34°C (Figure 4a). This result means that T-acute of high-latitude species could be sensitive (i.e., plastic) to incubation temperature. The climate variable hypothesis predicts that phenotypic plasticity should be observed in fluctuating thermal environments, such as high-latitude regions (Ghalambor et al., 2006; Janzen, 1967; Sun et al., 2022). Thus, the observed sensitivity of T-acute in high-latitude *T. amurensis* supports this hypothesis. However, our results contradict previous findings, which undermined the hypothesis by showing low phenotypic plasticity in thermal tolerance for adult ectotherms from high latitudes (Magozzi & Calosi, 2015; Stillman, 2003). Additionally, we found that T-acute in a low-latitude (tropical) species, *T. kuehnei*, was enhanced as incubation temperatures increased from 24 to 34°C (Figure 4e), indicating beneficial plasticity in T-acute that can facilitate heat resistance if extreme heat events increase in conjunction with warming average temperatures (Agrawal, 2001; Han et al., 2020; Mousseau et al., 2009). *Takydromus kuehnei* provides the first instance of adaptive plasticity in T-acute to incubation temperatures. Although further instances are necessary to establish a general pattern of plasticity
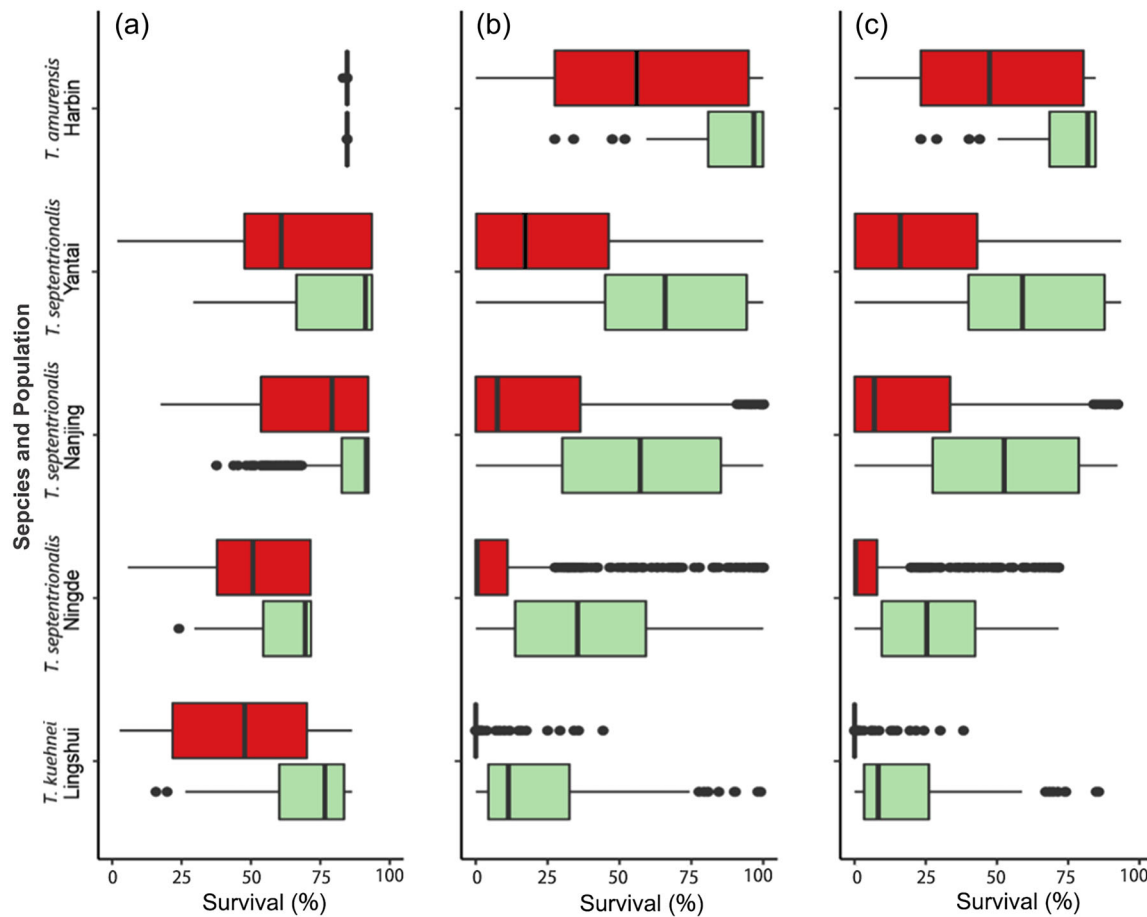
FIGURE 5 Average values of embryonic survival in response to (a) chronic heat stress (T-chronic), (b) acute heat stress (T-acute), and (c) integrative survival of embryos incubated across the reproductive season at climate stations in the species' distribution range (green, climate conditions in 2016; red, climate change scenario SSP1-2.6 for 2070; bar in the box, median value; left and right hinges, first and third quartiles; left and right whiskers, smallest and largest value no farther than 1.5-fold interquartile range from the hinge; points beyond the end of the whiskers, outliers).

in T-acute, this adaptive plasticity could benefit embryos during climate change (Hall & Sun, 2021). With adaptive plasticity in T-acute, embryos, populations, and species may have more time to adapt to rising temperatures through genetic assimilation and rapid microgeographic evolution (Arietta & Skelly, 2021; Campbell-Staton et al., 2021).

T-acute weight versus T-chronic weight in determining embryonic survival

One of our most significant findings was that T-acute had a greater impact than T-chronic on embryonic survival rates. This was supported by the lower survival rates observed when including T-acute data across all latitudes in both present and future (2070) climates (Figure 5). This pattern can be attributed to the distinct mechanisms by which thermal stress affects embryos through acute or chronic exposure to warm temperatures as well as the likelihood of experiencing chronic versus acute heat stress. First, the average temperatures in the nests are much lower than lizards' T-chronic (Gao et al., 2014; Liang et al.,

2015). Specifically, average nest temperatures from 1 June to 31 July (egg-laying peak) ranged from 27.3°C (Lingshui) to 22.7°C (Harbin) at present, which are much lower than the T-chronic (which are >32°C) of *Takydromus*. As a result, increasing average temperatures pose no serious threat at present or in the near future (Figure 5). The mortality induced by chronic heat stress is likely due to the accumulation of negative effects on physiological and biochemical processes. High average temperatures can accelerate the consumption of energy resources in eggs, reducing energy stores for embryonic development (Angilletta et al., 2006), and may potentially suppress immunity and antimicrobial functions, leading to infections and subsequent mortality (Bézy et al., 2020). These negative effects accumulate over time, but do not immediately result in embryonic mortality with short-time exposure to chronic heat stress. In contrast, future nest temperatures exceed the T-acute more frequently. The T-acute reflects resistance to periodic heat events, such as daily maximum temperatures that can cause mortality of embryos within seconds to hours (Angilletta et al., 2013; Hall & Sun, 2021; Hall & Warner, 2019; Sun et al., 2021). For instance, the highest daily maximum temperature across the season in this study was 40°C

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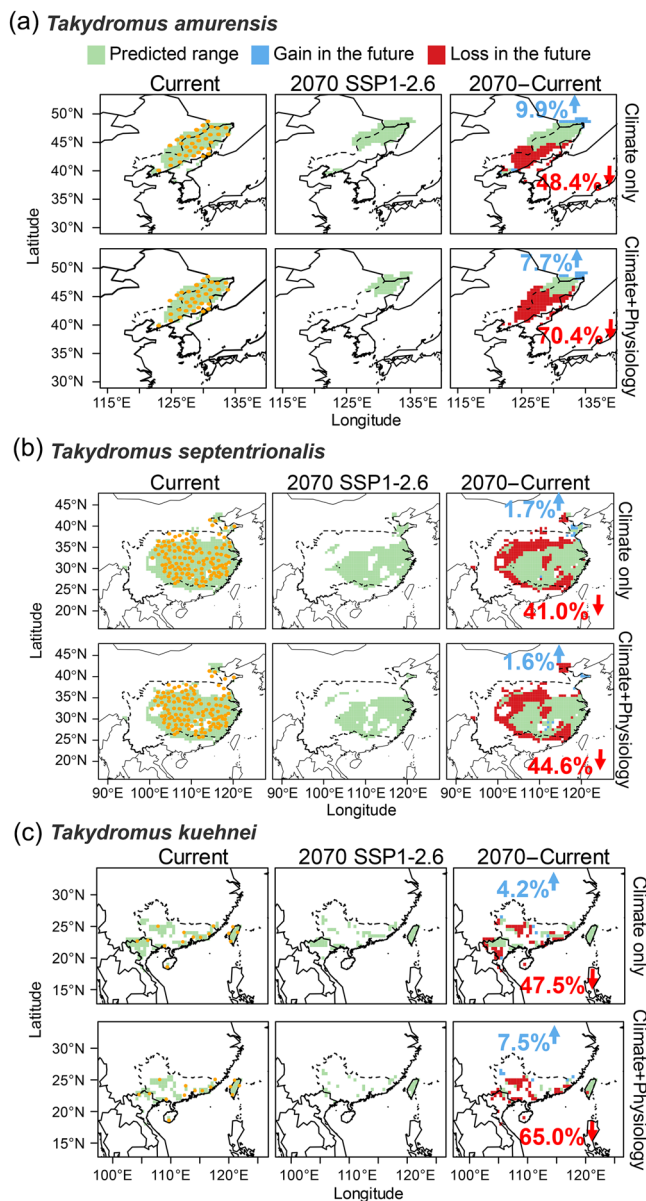
FIGURE 6 Habitat extent (green), gain (blue), and loss (red) at present and in 2070 for (a) *Takydromus amurensis*, (b) *Takydromus septentrionalis*, and (c) *Takydromus kuehnei* projected by species distribution models (SDMs) with only climatic variables and with hybrid SDMs with both climatic and physiological variables (orange points, existing occurrence records in the current projections for each species; scenarios of climate change, SSP1-2.6 for all species; habitat range in 3 types: current climate, 2070, and difference between 2070 and current climate).

(at Lingshui), which can directly kill embryos with a T-acute lower than 40°C. Heat events or daily maximum temperatures can induce the necrosis of organs via cell death (Angilletta et al., 2013; Li et al., 2014), functional loss of organelles (Dong et al., 2018), and degeneration of critical protein and enzymes of organisms (Dong et al., 2018; Liao et al., 2019), or can disrupt the interactions between the supply and demand of oxygen (Pörtner & Farrell, 2008; Portner et al., 2007). These suppressions can immediately lead to the mortality of organisms within seconds to hours (Gangloff & Telemeco, 2018).

Another significant finding in this study is that the survival of *Takydromus* is expected to decrease significantly under climate change at all latitudes. However, the integration of T-acute and T-chronic shows that embryonic survival would be highest at high latitudes (Figure 5). Notably, the mechanisms that reduce survival under heat stress are different for embryos from low and high latitudes. In low-latitude populations (*T. kuehnei*, Ningde population of *T. septentrionalis*), mortality is induced by both chronic and acute exposure to heat stress under climate change (Figure 5), whereas in high-latitude populations (Nanjing and Yantai populations of *T. septentrionalis*, *T. amurensis*), mortality primarily results from acute exposure to heat stress (Figure 5). Therefore, for high-latitude populations of *Takydromus*, more frequent exposure to acute heat stress would significantly decrease survival rates, but increasing average nest temperatures would not. This discrepancy highlights the interaction between the thermal environment and organism responses to climate change in determining species' vulnerabilities (Huey et al., 2012; Logan et al., 2013; Williams et al., 2008).

Range shifts under climate change based on embryonic survival

In recent years, it has been widely acknowledged that SDMs have evolved and become more sophisticated. It is believed that hybrid SDMs, which integrate behavioral and physiological responses of organisms, can provide more accurate projections of species' range shifts under climate change compared with SDMs that solely consider climatic variables (Kearney & Porter, 2009; Kearney et al., 2009). Recent studies have demonstrated that hybrid SDMs that incorporate both climatic variables and embryonic heat stress provided more accurate projections of species' range shifts under climate change than traditional SDMs (Carlo et al., 2018; Levy et al., 2015; Sun et al., 2021). In this study, we integrated embryonic survival, which reflects the impact of both average temperature during incubation and daily temperature extremes, in our hybrid SDMs. Using this robust model, we predicted more severe range contractions in the southern (i.e., low-latitude) parts of the species' ranges, with smaller range expansions in the northern (i.e., high-latitude) parts, when compared with climate-only models, except for *T. septentrionalis* (Figure 5). The relatively higher average temperatures at low latitudes and greater temperature fluctuations at high latitudes may have contributed to these patterns (Sun et al., 2021).

Complications of determining embryonic survival and future directions

Although this study successfully integrated embryonic survival in SDMs to predict range shifts under climate change, there are still opportunities for improvement. The latitudinal pattern of embryonic heat tolerance (both T-chronic and T-acute) and embryonic survival under climate change was produced

with empirical data from 5 populations of 3 *Takydromus* lizards (Figures 2 & 3), rather than from different populations of a single species. This may limit confidence in the pattern we observe, even though lizards in the genus *Takydromus* are generally good model systems of physiological patterns across latitudes (e.g., Mi et al., 2022; Sun et al., 2021, 2022). Therefore, some of the observed effects may be attributable to species-level differences rather than latitude, per se. In future studies, including embryonic heat tolerance (both T-chronic and T-acute) from more populations of a species could enhance confidence in the current latitudinal pattern. According to our model validation, our development model could not perfectly predict embryonic mortality in nature. This was likely due to the fact that we modeled only temperatures and did not account for other intrinsic and extrinsic factors that cause mortality, such as egg quality and bacterial infections. Thus, survival estimates from the model were somewhat inflated, and egg survival in the wild would be lower due to various sources of mortality affecting the embryos. Accordingly, incorporating additional physiological and ecological factors could enhance the accuracy of the predictions. For instance, hypoxia (Hall & Warner, 2021), flooding (Ishimatsu & Graham, 2011), drought (Santidrián Tomillo et al., 2015), and light conditions (Zhang et al., 2016) are also important abiotic factors that could depress the embryonic survival. Gravid females may adjust the location (Li et al., 2018), timing (Telemeco et al., 2009), and depth (Refsnider & Janzen, 2010) of nesting to mitigate the impacts of climate change and improve embryonic survival. However, incorporating these factors in hybrid SDMs can be challenging because they require species-specific traits. To expand predictions to more taxonomic groups and greater geographic scales, it would be necessary to assemble a database of embryonic heat tolerance for a more diverse range of species. Such a data set could then be used to develop more accurate and reliable models for predicting the impacts of climate change on oviparous species (Hall & Sun, 2021; Sun et al., 2021).

AUTHOR CONTRIBUTIONS

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.