Increasing salinity stress decreases the thermal tolerance of amphibian tadpoles in coastal areas of Taiwan

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Global warming is the main cause for the rise of both global temperatures and sea-level, both major variables threatening biodiversity. Rising temperatures threaten to breach the thermal limits of organisms while rising sea-level threatens the osmotic balance of coastal animals through habitat salinization. However, variations in thermal tolerance under different salinity stresses have not yet been thoroughly studied. In this study, we assessed the critical thermal maxima (CTmax) of amphibian tadpoles in different salinity conditions. We collected tadpoles of Duttaphrynus melanostictus, Fejervarya limnocharis and Microhyla fissipes from coastal areas and housed them in freshwater, low, and high salinity treatments for 7 days of acclimation. The CTmax, survival rate, and development rate of tadpoles in high salinity treatments were significantly lower than that of the two other treatments. Our results indicate that physiological performances and heat tolerances of tadpoles are negatively affected by salinization. Maximum entropy models showed that CTmax and sea-level rise are predicted to negatively affect the distribution of the three focal species. The present results suggest that global warming can lead to negative dual-impacts on coastal animals because of reduced thermal tolerances at elevated salinity. The impacts of global warming on anurans in coastal areas and other habitats impacted by salinization may be more severe than predicted and it is likely to cause similar dual-impacts on other ectotherms.

Global warming leads to the thermal expansion of seawater and melting of polar ice caps, both major contributors to the rise of global mean sea level1–3. Between the late nineteenth century and the early twenty-first century, global mean sea level rose by 195 mm at an average velocity of 1.44 mm per year4–6. In addition, the global mean sea level is projected to rise by at least 1 m by the end of the twenty-first century7,8. Changes in sea level will result in negative ecological impacts such as habitat loss and salinization of coastal areas9–12.

Thermal tolerance varies greatly among species13,14, and the critical thermal maximum (CTmax) is a commonly applied index to assess the sensitivity of organisms to high temperature15–25. Earlier studies have evaluated the impact of warming temperature on species across latitudes using the more accurate warming tolerance (WT) index, defined as the differences between CTmax and the highest habitat temperature Tmax13,14. Results of earlier studies suggest that the impacts of rising temperature are more severe on species living in tropical areas when compared to temperate areas due to a narrower WT13,14,26. In addition, species with a lower CTmax are exposed to a greater risk in the presence of warming ambient temperature because of their smaller WT13,26.

Changes in environmental factors such as temperature have a greater impact on ectotherms, such as amphibians, which are present in a great variety of both aquatic and terrestrial habitats15. Amphibians are sensitive to environmental changes due to their permeable skin and their poor osmoregulatory ability during larval

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stages, and especially so during the aquatic larvae life stage when they highly dependent on the availability of freshwater and humid environments. In addition, rising water temperature is challenging the thermal tolerance of tadpoles and affecting their physiological function and ecological performance, including survival and development.

Rising sea level can inundate land and cause saltwater to intrude into groundwater, resulting in habitat salinization, negatively affecting coastal amphibians. Earlier studies have reported that salinity exposure may decrease the survival and development of tadpoles. However, acclimation to increasing salinity enhances salt tolerance of tadpoles by increasing the expression of branchial Na+, K+ -ATPase (NKA). As a result, variation in salinity tolerance among amphibian species can directly influence the community composition, species abundance, and distribution. Hence, global warming can affect both temperature and habitat salinity and both of these factors can affect organisms independently, but we know little about their joint effects.

Amphibians living and breeding in coastal areas may be more severely affected by global warming. Although amphibians generally avoid inhabiting and breeding in brackish habitats, field surveys have revealed that some amphibian populations breed in this type of environment. In addition, global warming leads to rising sea level, resulting in habitat salinization, potentially affecting the thermal tolerance of amphibians. Earlier studies have demonstrated that CTmax decreased in highly saline environments in several non-amphibian species. However, it is still relatively unknown how atypical salinity affects thermal tolerance of animals and thus impacts their ecology, including the distribution of populations. In view of amphibians’ quality as early indicators of habitat condition changes and tadpoles' specific sensitivity to water quality changes, amphibians are a perfect model to study thermal physiology in relation to salinity stress.

In this study, we used three anuran species: Duttaphrynus melanostictus (Bufonidae), Fejervarya limnocharis (Microhylidae), and Microhyla fissipes (Microhylidae). All of these species can be found in coastal areas, and F. limnocharis is known to breed in brackish habitats. Based on the fact that salinity change has detrimental effects on thermal tolerance of vertebrates and invertebrates, we predicted that the CTmax of tadpoles will decrease as salinity increases. In addition, earlier findings indicated that salinity may decrease survival and development rate of tadpoles, and we consequently predict that the survival and development of tadpoles will be negatively affected by salinity.

**Materials and methods**

**Animal collection and study sites.** Tadpoles of Duttaphrynus melanostictus, Fejervarya limnocharis and Microhyla fissipes were used in this study. They are common species in lowlands of East Asia and breed from spring to summer, including July, the warmest month of the year. The monthly mean temperature, highest temperature, and lowest temperature recorded from nearest weather station were provided in the Supplementary Information (Supplementary Table S1; Supplementary Fig. S1). We collected tadpoles from coastal areas during summer, 2016. Tadpoles of F. limnocharis and M. fissipes were collected from the windbreak woodlands in Cheng-Xi village (23.041379° N, 120.080005° E) of Tainan County. The brackish tidal gullies where we collected tadpoles were covered with leaves and branches of Casuarina equisetifolia. The salinity fluctuation at that site depends on rainfall and evaporation. When a typhoon comes, high tides bring seawater, and salinity dramatically increases from about one to eight ppt (parts per thousand). Tadpoles of D. melanostictus were collected from Lim-Chu (23.669155° N 120.175966° E) in the Yun-Lin County. Brackish water in this area was created by the seawater intrusion into groundwater caused by land subsidence.

**Salinity acclimation.** We collected 30–50 tadpoles at Gosner stages 26–30 for each species and brought them back to the laboratory. Tadpoles were subjected to three different treatments: freshwater, low salinity, and high salinity, with salinity based on their maximum salinity tolerance. For D. melanostictus and F. limnocharis, the low and high salinity treatments were 3 and 5 ppt, respectively. For M. fissipes, the low and high salinity treatments were 3 and 5 ppt, respectively. The treatments for M. fissipes were based on a pilot study on salinity tolerance of tadpoles (see Supplementary Information). Tadpoles were randomly assigned to one of the three treatments (n = 10 to 15), with each tadpole reared separately in a 10.5 × 7.5 × 4.5 cm plastic container filled with 150 ml of the corresponding treatment solution. The freshwater solution used in this study was dechlorinated tap water, which was around 1 ppm. The salinity solution was a mixture of deionized water and Coralife sea salt (Energy Savers Unlimited, INC, Carson, CA, USA). Salinity was measured using an electronic salinity meter (Rixen, Model SM-10, Seoul, Republic of Korea) at room temperature. We did not use deionized water for control treatment due to the extreme low osmotic pressure, which may be harmful to tadpoles. Tadpoles were placed in an incubator and kept at 25°C on a 12:12L photoperiod.

**Effect of salinity on tadpole survival and development.** Acclimation lasted for seven days, generally following the methods from Wu et al. We recorded the survival of tadpoles and renewed water every day to prevent the misalignment of salinity caused by evaporation and the deterioration of water quality caused by feeding. According to the specificity of foraging behaviour in each species, tadpoles of F. limnocharis and D. melanostictus were fed cooked spinach, while tadpoles of M. fissipes were fed powdery fish food daily. The developmental stages of tadpoles were measured before and after salinity acclimation. The developmental stages were identified based on Gosner criteria. Each tadpole was isolated in a glass petri dish and the developmental stage was examined with a 10× hand lens and cold light.

**Critical thermal maximum measurement.** The critical thermal maximum (CTmax) is defined as “the temperature at which an animal loses its essential motor function.” CTmax of tadpoles was measured on the eighth day, right after acclimation. Each tadpole that survived during salinity acclimation was put into a beaker...
filled with 200 ml treatment water. The beaker was then put into a plastic box filled with water and then into
a temperature-control water bath (BH-230D, Yihder Technology Co., Ltd, Taiwan). Water was heated from ca.
25 °C at the rate of 0.25 °C/min\(^{30}\), while the water temperature was monitored by a probe thermal meter (nearest
to 0.1 °C). We used a blunt probe to assess the activity of the tadpole every degree at the beginning of the experi-
ment, and at increasing intervals every minute (ca. every 0.25° of increasing). We determined the CTmax as the
point when a tadpole became disoriented in locomotion and lost its ability to escape from the stimuli. Once
CTmax was reached, tadpoles were directly transferred into cool water for recovery.

The expected distribution and coastal flooding area. Presence points from survey data of *D. melanostictus*, *F. limnocharis*, and *M. fissipes* were used in maximum entropy models (MaxEnt 3.4.4)\(^{36}\) for each species in Taiwan. The distribution data were collected every three months (four times in a year) by well-trained
citizen scientists from 451 sites throughout Taiwan from 2006 to 2018, in which investigators did a 50 m transect
visual encounter survey for each site, and resulting in a total of 28,601 observations for *D. melanostictus*, 26,625
observations for *F. limnocharis*, and 12,672 observations for *M. fissipes*. Nineteen bioclimatic variables (Supple-
mentary Table S2) were used as predictor variables\(^{37}\) representing biologically meaningful climatic gradients. All
variables were retained in models as is commonly used in MaxEnt modelling for other species\(^{58-61}\). A bias layer
made from kernel density of all observation points was input into the model, and background points were set to
10,000. To reduce spatial bias, we selected the option in MaxEnt to remove duplicate presence points, effectively
reducing all observations taken within the environmental layer cell size to a single occurrence when training
the models. We used a regularization, or alpha, multiplier of three to smooth the responses of each species to
bioclimatic variables. Each model was run for ten bootstrap replicates with a random test percentage of 20%. We
used the averages of these replicates to represent the predicted suitability for each species across the Taiwan main
island, with values above 0.5 representing predicted presence within a presence-absence dichotomy.

As a baseline of effect of salinity on CTmax, we first mapped the current maximum temperature across Taiwan
using data from 29 weather stations from 2010 to 2018 (Central Weather Bureau of the Government of Taiwan);
then classified by the mean CTmax calculations at different levels of salinity for each species and overlaid with
current predicted distributions of each species. To create a uniform surface temperature layer across the study
region, we used the ‘EBK regression prediction’ (Empirical Bayesian Kriging Regression Prediction) tool in Arc-
GIS Pro ver. 2.8.1 (ESRI, Redlands, CA, USA) with a digital elevation model (DEM) as our explanatory variable
raster and maximum surface temperature at each georeferenced weather station as our dependent variable. In
a one-week observation, the water temperature was within the daily variant range of air temperature, but lower
the instant air temperature most of the time in study site Cheng-Xi village (see Supplementary Fig. S2).

To determine potential impact of climate change on CTmax was considered, we combined maps of 1 m sea level rise and predicted the maximum temperature for the year 2100 by increasing current temperature by 4.8 °C (Source: Airbus, USGS, NGA, NASA, CGIAR, GEBCO,
NLS, OS, NMA, Geodatastyrelsen, GSA, GSI and the GIS User Community). Extrapolations to future climate
change scenarios were created by adding projected increase in maximum temperature as constant values to our
present-day temperature layer through the raster calculator. The predicted maximum temperature was also
classified by CTmax (CTmax at no, low, and high salinity) and overlaid with current predicted distributions. All
maps in this study were generated by using ArcMap 10.6 (ESRI, Redlands, CA, USA).

Statistical analyses. The survival of tadpoles during acclimation was analyzed through a survival analysis
(Cox proportional hazard model and Kaplan–Meier survival curve). Kruskal–Wallis tests with Dunn posterior
tests were used for comparison between the developmental stage and CTmax of tadpoles acclimated in different
treatments. Spearman’s rank correlation was used to test the effects of development stage on CTmax of tadpoles
exposed to salinity treatments. Data analyses were conducted using R 3.1.0. Significance level was set as
\( \alpha = 0.05 \).

Ethical statement. The animal use protocol in this study were carried out in accordance with relevant
guidelines and regulations, and the experimental protocols have been reviewed and approved by the Institu-
tional Animal Care and Use Committees of Tunghai University (IACUC permit No. 103-16), and all experi-
ments were performed in accordance with relevant guidelines and regulations of ARRIVE Guidelines (https://
arriveguidelines.org). No other permits were required because these field studies were not conducted in a pro-
tected area nor were our subjects an endangered or protected species. In total, there were 113 animals used as
subjects in this study, 19 of them were sacrificed by the high salinity in the survival experiments, while there was
no animal sacrificed doing the CTmax experiments. The animals were released at their site of capture right after
finishing the experiments within 10–30 days of being captured.

Results Effect of salinity on survival and development. The survival analysis showed that the survival of
tadpoles in all three species was significantly different among treatments of salinity (Cox model, likelihood
ratio = 20.4, df = 2, \( p < 0.001 \) for *Duttaphrynus melanostictus*; likelihood ratio = 13.6, df = 2, \( p = 0.001 \) for *Fejervarya
limnocharis*; and likelihood ratio = 9.12, df = 2, \( p = 0.011 \) for *Microhyla fissipes*, Fig. 1). No mortality occurred in
freshwater and low salinity treatments in all three species. In the high salinity treatments, 57% of *F. limnocharis*,
60% of *D. melanostictus* and 64% *M. fissipes* tadpoles survived.

For all three species, the developmental stage of each species before acclimation was not significantly differ-
ent between treatments (Kruskal–Wallis test, *F. limnocharis*: \( \chi^2 = 0.69, df = 2, p = 0.710 \); *D. melanostictus*: \( \chi^2 = 1.38, df = 2, p = 0.502 \); *M. fissipes*: \( \chi^2 = 4.04, df = 2, p = 0.133 \); Fig. 2). For *F. limnocharis*, there were significant
Figure 1. The survival rate of tadpoles under salty water. Survival of *Duttaphrynus melanostictus* (left), *Fejervarya limnocharis* (middle), and *Microhyla fissipes* (right) tadpoles in freshwater (open square), low salinity (open circle), and high salinity treatments (x-mark). *Duttaphrynus melanostictus*: n = 15, 14, and 15, respectively; *F. limnocharis*: n = 12, 12, and 14, respectively; *M. fissipes*: n = 10, 10, and 11, respectively. (Photos were taken by M-F Chuang from Taichung City for *D. melanostictus*, and from Nantou County for *F. limnocharis* and *M. fissipes*.)

Figure 2. The development of tadpoles under salty water. The difference of developmental stage before and after salinity acclimation of *Duttaphrynus melanostictus* (left), *Fejervarya limnocharis* (middle), and *Microhyla fissipes* (right) tadpoles in freshwater (open square), low salinity (open circle), and high salinity treatments (x-mark). Values of Δ stage with different letters represent a statistical difference (Kruskal–Wallis tests with Dunn posterior tests, p < 0.05) among treatments. *Duttaphrynus melanostictus*: n = 12, 12, and 7, respectively; *F. limnocharis*: n = 12, 12, and 8, respectively; *M. fissipes*: n = 10, 10, and 7, respectively. Data presented as means ± SD.
differences in developmental stage between treatments after acclimation (Kruskal–Wallis test, $\chi^2 = 19.31, \text{df} = 2, p < 0.001$; Fig. 2 middle upper and bottom). Post hoc comparisons showed that development rate of tadpoles in freshwater was significantly faster than tadpoles in low ($p = 0.001$) and high salinity treatments ($p < 0.001$), and development rate of tadpoles in low salinity treatment was also significantly faster than in high salinity treatment ($p = 0.008$). Similarly, there were significant differences among treatments in development rate of *D. melanostictus* tadpoles (Kruskal–Wallis test, $\chi^2 = 16.14, \text{df} = 2, p < 0.010$; Fig. 2 left upper & bottom). Post hoc comparisons showed that there was no significant difference in development rates between freshwater and low salinity treatments ($p = 0.285$), and tadpoles in high salinity treatment developed significantly slower than those in freshwater ($p < 0.001$) and low salinity treatments ($p < 0.001$). The development rates of *M. fissipes* tadpoles were not significantly different among treatments (Kruskal–Wallis test, $\chi^2 = 19.31, \text{df} = 2, p = 0.600$; Fig. 2 right upper & bottom).

**Effect of salinity on CTmax.** Results showed that there were significant differences in CTmax among treatments in both *F. limnocharis* and *D. melanostictus* tadpoles (Kruskal–Wallis test, $\chi^2 = 26.6, \text{df} = 2, p < 0.001$; $\chi^2 = 20.58, \text{df} = 2, p < 0.001$, respectively). Post hoc comparison showed that the CTmax of *F. limnocharis* tadpoles in high salinity treatments was significantly lower than that of low salinity treatments ($p = 0.008$; Fig. 3 middle) and freshwater treatments ($p < 0.001$; Fig. 3 middle). Similarly, the CTmax of *D. melanostictus* tadpoles in high salinity treatments was significantly lower than that of low salinity treatments ($p = 0.004$; Fig. 3 left) and freshwater treatments ($p < 0.001$; Fig. 3 left), while the CTmax in low salinity treatments was significantly lower than that in freshwater treatments ($p = 0.015$; Fig. 3 left). In contrast, we did not find a significant effect of salinity on CTmax of *M. fissipes* tadpoles (Kruskal–Wallis test, $\chi^2 = 2.86, \text{df} = 2, p = 0.240$; Fig. 3 right). In summary, all tested species showed a consistent pattern where CTmax of tadpoles decreased as salinity increased.

In addition, we tested the effects of development stage on CTmax after exposed to salinity treatments. Results showed the CTmax of tadpoles was not in relation to development stage in *D. melanostictus* (Spearman’s $r = 0.34$, $n = 31$, $p = 0.061$; Fig. 4 left) and *M. fissipes* (Spearman’s $r = 0.33$, $n = 27$, $p = 0.096$; Fig. 4 right). In contrast, the
CTmax of *F. limnocharis* tadpoles was in relation to the development stage after exposed to salinity treatments (Spearman’s $r = 0.75$, $n = 32$, $p < 0.001$; Fig. 4 middle).

The expected distribution and coastal flooding area. MaxEnt models for the three species showed similar patterns of suitability across Taiwan (Fig. 5). Areas under the curve (AUC), or model fit, were 0.719, 0.734, and 0.771 for *D. melanostictus*, *F. limnocharis*, and *M. fissipes*, respectively. The area with a suitability over 0.5 covered 19,091 km² for *D. melanostictus*, 20,811 km² for *F. limnocharis*, and 15,340 km² for *M. fissipes*. Suitable climate for *D. melanostictus* and *F. limnocharis* were found in low to middle elevations, while *M. fissipes* was distributed more broadly at intermediate elevations.

Under the current climate, only *F. limnocharis* is predicted to occur in areas where Tmax is reaching CTmax for high salinity conditions (Fig. 6, top row). For year 2081–2100 predicted climate, however, high salinity CTmax
temperatures will be reached for all species, and low salinity CTmax temperatures will be reached for *M. fissipes* (Fig. 6, bottom row). These areas also overlap with predicted flooding due to sea level rise (Fig. 6).

**Discussion**

**Effect of salinity on survival and development.** Survival and development rate of tadpoles were lower in high salinity treatments, highlighting that salinity has a negative effect on tadpole fitness. In this study, all three species exhibited a similar trend: lower survivals and delayed developments under increased salinity. Similar results were also reported in the same species of *Fejervarya limnocharis* tadpoles, and also the tadpoles of *Litoria ewingii* and *Litoria aurea*. Although development was not delayed obviously in *Microhyla fissipes* tadpoles, we suggested this may be due to the use of lower salinity as treatments for this species. Living in high salinity habitats not only leads to low survival, the delayed development of tadpoles may also increase the risks of predation or desiccation and result in a decreased population fitness.
Effects of salinity on CTmax. The CTmax of tadpoles was lower when exposed to high salinity, suggesting that salinity reduces their ability to tolerate heat stress. The CTmax of *F. limnocharis* and *D. melanostictus* tadpoles acclimated in high salinity was significantly lower than that of tadpoles acclimated in freshwater, while there was no significant effect of salinity treatments on *M. fissipes* tadpoles. Our findings agree with results from earlier studies: CTmax of Antarctic collembolans (*Cryptopygus antarcticus*) acclimated in freshwater was 22.2 °C but dropped to 18.9 °C when acclimated in 200 ppt51. In addition, CTmax of yellow-fin sea breams (*Acanthopagrus latus*) maintained at 33 ppt was significantly lower than 0.3 and 15 ppt49, and CTmax of green sturgeons (*Acipenser medirostris*) acclimated in bay water (24 ppt) was significantly lower than those acclimated in freshwater 0–1 ppt60. However, the opposite pattern was found in three spine stickle back (*Gasterosteus aculeatus*), for which the CTmax of individuals acclimated in 20 ppt was significantly higher than that of individuals acclimated in 2 ppt64.

In contrast, the increase of salinity in hypotonic waters may increase CTmax. For instance, in the Andean toads (*Rhinella spinulosa*), froglets under 150 ppm (i.e. 0.15 ppt) salinity cannot tolerate more than 35 °C, whereas tadpoles in waters between 630 and 1800 ppm can tolerate temperatures higher than 36 °C65. As their results are opposite to ours, we assume there is an optimal salinity for animals, and CTmax will decrease in hypertonic or hypotonic solution65.

Mechanisms of the decline of development and CTmax under salinity stress. The negative effects of high salinity on tadpoles is probably due to energy acquisition and allocation50 and the dehydration induced by salinity stress51,67. Several observations showed that tadpoles in high salinity water fed less33,68,69, suggesting that salinity negatively affected foraging behaviors, and thus resulted in a decrease in energy uptake to maintain regular development rate or CTmax. Salinity may depresses the thyroid hormone in tadpoles50, an essential hormone affecting their development50,51. Furthermore, previous studies showed that tadpoles consumed more glucose69 and had higher expression of branchial Na+/K+-ATPase (NKA) at high salinity50,56, suggesting that tadpoles spent more energy for osmoregulation and consequently reduced their development and ability to tolerate to temperature increase. A study also found that starving tadpoles had a significantly lower CTmax52, indicating that energy limitation had negative effect on the CTmax of tadpoles. In our study, we found the CTmax of at least one species was affected by development stage. However, the CTmax of tadpoles were usually steady throughout the larvae period52. Further experiments will be needed to figure out the relationships between these variables. For example, we noticed the food in high salinity treatment was usually left untouched. Hence, a quantitative experiment will help us to know the relationship between food intake and CTmax by the tadpoles acclimated in different salinity.

The decrease of CTmax may also relate to the dehydration of animals. Earlier studies showed that exposure to salinity could lead to dehydration at the cellular level53,67. *Fejervarya limnocharis* tadpoles are known to follow the same pattern, gradually dehydrating when transferred to a higher salinity treatment54. Earlier studies in other taxa showed that CTmax could be negatively affected by dehydration73–75. A proposed explanation is that cellular dehydration leads to changes in membrane fluidity, affecting heat tolerance51.

Finally, another explanation for why CTmax decreased for tadpoles acclimated in high salinity relates to the induction of heat shock proteins (HSPs). For instance, the increased CTmax at high salinity in *G. aculeatus* was associated with the induction of HSPs64. The heat shock response may decrease with time due to changes in HSP expression in response to nutritional status76. In this study, tadpoles acclimated for seven days may have starved, and consequently, protein re-allocation during starvation may have depressed the expression of HSPs, explaining why tadpoles displayed a lower CTmax under the high salinity treatments.

The expected impact from simulation and the conservation issue. During the past two decades, the global amphibian decline has become an important conservation issue77, and numerous studies revealed that multiple causes contributed to the extirpation of amphibian population78–80. Our findings showed that the predicted impact of sea level rise in coastal regions may reduce suitable habitats due to inundation. In addition, underground saltwater intrusion, although not modeled in the present study, may further expand habitat loss of coastal freshwater habitats. Our findings that the CTmax of tadpoles declined in high salinity treatments has important implications for the effects of global warming on frog populations in coastal regions. Even though some amphibians are capable of surviving in brackish condition and adapted rapidly in several decades81, but species undergoing adaptive change may not do so at a pace to guarantee their persistence82. In regions where sea level rise is predicted to result in an increase in salinity levels of freshwater habitats, populations may become extirpated when the environmental salinity reaches the value of high salinity treatment, due to the reduction in survival and development as well as the reduction in thermal tolerance. Only the population of *M. fissipes* was predicted to be affected when environmental salinity reaches the value of low salinity treatment. However, the mean CTmax was fairly similar across the three treatments for *M. fissipes* suggesting that the species is not highly sensitive to salinization. Instead, the species is at greater risk due to its somewhat lower CTmax, regardless of salinity treatment. Also, the predictions based on air temperature may not accurately represent the real situation where tadpoles live, because tadpoles in deeper water, more shaded location, or by moderating temperature exposure behaviorally, may lower the impacts when the maximum air temperature reaches the organism’s CTmax.

In conclusion, the results suggest that global warming has a double impact on the biology of tadpoles. During the larval period, rising temperature due to global warming gradually approaches the upper thermal limits of tadpoles, which threatens their survivorship51. The salinity stress not only retards the growth and development but also reduces the abilities of tadpoles to tolerate high temperature. The distribution ranges of the three species in this study were also expected to shrink in the future. Global warming may negatively affect the survival and
Data availability

The datasets generated and/or analysed during the current study are available in the Supplemental Data.

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References

1. Root, T. L. et al. Fingerprints of global warming on wild animals and plants. Nature 421, 57–60 (2003).
2. Meehl, G. A. et al. How much more global warming and sea level rise?. Science 307, 1769–1772 (2005).
3. Stocker, T. F. et al. (Cambridge University Press, 2013).
4. Kopp, R. E. et al. Probabilistic 21st and 22nd century sea-level projections at a global network of tide-gauge sites. Earths Future 2, 383–406 (2014).
5. Church, J. A. & White, N. J. A 20th century acceleration in global sea-level rise. Geophys. Res. Lett. 33 (2006).
6. Church, J. A. & White, N. J. Sea-level rise from the late 19th to the early 21st century. Surv. Geophys. 32, 585–602 (2011).
7. Vermeer, M. & Rahmstorf, S. Global sea level linked to global temperature. Proc. Natl. Acad. Sci. 106, 21527–21532 (2009).
8. Horton, B. P., Rahmstorf, S., Engelhart, S. E. & Kemp, A. C. Expert assessment of sea-level rise by AD 2100 and AD 2300. Quatern. Sci. Rev. 84, 1–6 (2014).
9. Day, J. W., Pont, D., Hensel, P. F. & Ibáñez, C. Impacts of sea-level rise on deltas in the Gulf of Mexico and the Mediterranean: The importance of pulsing events to sustainability. Estuaries 18, 636–647 (1995).
10. Feagin, R. A., Sherman, D. J. & Grant, W. E. Coastal erosion, global sea-level rise, and the loss of sand dune plant habitats. Front. Environ. Sci. 3, 359–364 (2005).
11. Nicholls, R. J. Planning for the impacts of sea level rise. Oceanography 24, 144–157 (2011).
12. Hinkel, J. et al. Coastal flood damage and adaptation costs under 21st century sea-level rise. Proc. Natl. Acad. Sci. 111, 3292–3297 (2014).
13. Deutsch, C. A. et al. Impacts of climate warming on terrestrial ectotherms across latitude. Proc. Natl. Acad. Sci. 105, 6668–6672 (2008).
14. Duarte, H. et al. Can amphibians take the heat? Vulnerability to climate warming in subtropical and temperate larval amphibian communities. Glob. Change Biol. 18, 412–421 (2012).
15. Licht, P. & Brown, A. G. Behavioral thermoregulation and its role in the ecology of the red-bellied newt, Taricha rivularis. Ecology 48, 598–611 (1967).
16. Feder, M. E. & Pough, F. H. Temperature selection by the red-backed salamander, Plethodon cinereus (Green) (Caudata: Plethodontidae). Comp. Biochem. Physiol. Part A Physiol. 50, 91–98 (1973).
17. Keen, W. H. & Schroeder, E. E. Temperature selection and tolerance in three species of Ambystoma larvae. Copeia 1975, 523–530 (1975).
18. Hoppe, D. M. Thermal tolerance in tadpoles of the chorus frog Pseudacris triseriata. Herpetologica 318–321 (1978).
19. Cüpp Jr, P. V. Thermal tolerance of five salientian amphibians during development and metamorphosis. Herpetologica 234–244 (1980).
20. Howard, J. H., Wallace, R. L. & Staufer, J. R. Critical thermal maxima in populations of Ambystoma macrodactylum from different elevations. J. Herpetol. 17, 400–402 (1983).
21. Floyd, R. B. Ontogenetic change in the temperature tolerance of larval Bufo marinus (Anura: Bufonidae). Comp. Biochem. Physiol. A Physiol. 75, 267–271 (1983).
22. Floyd, R. B. Effects of photoperiod and starvation on the temperature tolerance of larvae of the giant toad, Bufo marinus. Copeia 1985, 625–631 (1985).
23. Manis, M. L. & Clausen, D. L. Environmental and genetic influences on the thermal physiology of Rana sylvatica. J. Therm. Biol. 11, 31–36 (1986).
24. Layne, J., Clausen, D. & Manis, M. Effects of acclimation temperature, season, and time of day on the critical thermal maxima and minima of the crayfish Orconectes rusticus. J. Therm. Biol. 12, 183–187 (1987).
25. Lutterschmidt, W. J. & Hutchison, V. H. The critical thermal maximum. History and critique. Can. J. Zool. 75, 1561–1574 (1997).
26. Simon, M. N., Ribeiro, P. L. & Navas, C. A. Upper thermal tolerance plasticity in tropical amphibian species from contrasting habitats: Implications for warming impact prediction. J. Therm. Biol. 48, 36–44 (2015).
27. Boutilier, R., Donohoe, P., Tattersall, G. & West, T. Hypometabolic homeostasis in overwintering aquatic amphibians. J. Exp. Biol. 200, 387–400 (1997).
28. Shoemaker, V. & Nagy, K. A. Osmoregulation in amphibians and reptiles. Annu. Rev. Physiol. 39, 449–471 (1977).
29. VierTEL, B. Salt tolerance of Rana temporaria: Spawning site selection and survival during embryonic development (Amphibia, Anura). Amphibia-Reptilia 20, 161–171 (1999).
30. Wu, C.-S. & Kam, Y.-C. Thermal tolerance and thermoregulation by Taiwanese rhabdophorid tadpoles (Buergerea japonica) living in geothermal hot springs and streams. Herpetologica 61, 35–46 (2005).
31. Gomez-Mestre, I. & Tejedor, M. Local adaptation of an anuran amphibian to osmotically stressful environments. Evolution 57, 1899–1899 (2003).
32. Christy, M. T. & Dickman, C. R. Effects of salinity on tadpoles of the green and golden bell frog (Litoria aurea). Amphibia-Reptilia 23, 1–11 (2002).
33. Wu, C.-S. & Kam, Y.-C. Effects of salinity on the growth, survival, development, and metamorphosis of Fejervarya limnocharis tadpoles living in brackish water. Zool. Sci. 26, 476–482 (2009).
34. Wu, C. S., Yang, W. K., Lee, T. H., Gomez-Mestre, I. & Kam, Y. C. Salinity acclimation enhances salinity tolerance in tadpoles living in brackish water through increased Na+, K+–ATPase expression. J. Exp. Zool. A Ecol. Genet. Physiol. 321, 57–64 (2014).
35. Alexander, L. G., Lalivaux, S. P., Pechnann, J. H. & Devries, P. J. Effects of salinity on early life stages of the Gulf Coast toad, Incilius nebulifer (Anura: Bufonidae). Copeia 2012, 106–114 (2012).
36. Bernabó, I., Bonaccorsi, A., Costacuri, F., Tripepi, M. & Brunelli, E. Effects of salinity stress on Bufo bufo larvae and Bufo bufo tadpoles: Tolerance, morphological gill alterations and Na+/K+–ATPase localization. Aquat. Toxicol. 132, 119–133 (2013).
37. Kearney, B. D., Pell, R. J., Byrne, P. G. & Reina, R. D. Anuran larval developmental plasticity and survival in response to variable salinity of ecologically relevant timing and magnitude. J. Exp. Zool. A Ecol. Genet. Physiol. 321, 541–549 (2014).
38. Hsu, W. T., Wu, C. S., Hatch, K., Chang, Y. M. & Kam, Y. C. Full compensation of growth in salt-tolerant tadpoles after release from salinity stress. J. Zool. 304, 141–149 (2014).
39. Hsu, W.-T. et al. Salinity acclimation affects survival and metamorphosis of crab-eating frog tadpoles. Herpetologica 68, 14–21 (2012).
40. Lai, J.-C., Kam, Y.-C., Lin, H.-C. & Wu, C.-S. Enhanced salt tolerance of euryhaline tadpoles depends on increased Na+, K+-ATPase expression after salinity acclimation. *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* 227, 84–91 (2019).

41. Brown, M. E. & Walls, S. C. Variation in salinity tolerance among larval anurans: Implications for community composition and the spread of an invasive, non-native species. *Copeia* 2013, 543–551 (2013).

42. Balinsky, J. B. Adaptation of nitrogen metabolism to hypersaline environments in Amphibia. *J. Exp. Zool. A Ecol. Genet. Physiol.* 215, 335–350 (1981).

43. Duellman, W. & Trueb, L. *Biography of Amphibians* (John Hopkins University Press, 1994).

44. Alcala, A. C. Breeding behavior and early development of frogs of Negros, Philippine Islands. *Copeia* 1962, 679–726 (1962).

45. Gordon, M. S. & Tucker, V. A. Osmotic regulation in the tadpoles of the crab-eating frog (*Rana cancrivora*). *J. Exp. Biol.* 42, 437–445 (1965).

46. Dunson, W. A. Tolerance to high temperature and salinity by tadpoles of the Philippine frog, *Rana cancrivora*. *Copeia* 1977, 375–378 (1977).

47. Uchiyama, M., Murakami, T., Wakahusi, C. & Yoshizawa, H. Structure of the kidney in the crab-eating frog, *Rana cancrivora*. *J. Morphol.* 204, 147–156 (1990).

48. Heo, K., Kim, Y. I., Bae, Y. Jang, Y. & Borzée, A. First report of *Dryophytes japonicus* tadpoles in saline environment. *Russ. J. Herpetol.* 26, 87–90 (2019).

49. Jian, C. Y., Cheng, S. Y. & Chen, J. C. Temperature and salinity tolerances of yellowfin sea bream, *Acanthopagrus latus*, at different salinity and temperature levels. *Aquac. Res.* 34, 175–185 (2003).

50. Sardella, B. A., Sanmarti, E. & Küllü, D. The acute temperature tolerance of green sturgeon (*Acipenser medirostris*) and the effect of environmental salinity. *J. Exp. Zool. A Ecol. Genet. Physiol.* 309, 477–483 (2008).

51. Everatt, M. J., Worland, M. R., Convey, P., Bale, J. S. & Hayward, S. A. The impact of salinity exposure on survival and temperature tolerance of the Antarctic colembolan *Cryptopygus antarcticus*. *Physiol. Entomol.* 38, 202–210 (2013).

52. Kerby, J. L., Richards-Hrdlicka, K. L., Storfer, A. & Skelly, D. K. An examination of amphibian sensitivity to environmental contaminants: are amphibians poor canaries?. *Ecol. Lett.* 13, 60–67 (2010).

53. Chang, Y. M., Wu, C. S., Huang, Y. S., Sung, S. M. & Hwang, W. Occurrence and reproduction of anurans in brackish water in a coastal forest in Taiwan. *Herpetol. Notes* 9, 291–295 (2016).

54. Peng, R. T., Hsieh, Y. H. & Liu, T. S. Hydro chemical characteristics and salinization of groundwater in Yunlin area. *J. Chin. Soil Water Conserv.* 32, 173–189 (2005).

55. Gosner, K. L. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16, 183–190 (1960).

56. Phillips, S. J., Anderson, R. P., Dudík, M., Schapire, R. E. & Blair, M. E. Opening the black box: An open-source release of Maxent. *Ecography* 40, 887–893 (2017).

57. Hjumans, R. I., Cameron, S. E., Parra, J. L., Jones, P. G. & Jarvis, A. Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* 25, 1965–1978 (2005).

58. Groff, L. A., Marks, S. B. & Hayes, M. P. Using ecological niche models to direct rare amphibian surveys: A case study using the Oregon Spotted Frog (*Rana pretiosa*). *Herpetol. Conserv. Biol.* 9, 354–368 (2014).

59. Kumar, P. Assessment of impact of climate change on *Rhododendrons* in Sikkim Himalayas using Maxent modelling: limitations and challenges. *Biodivers. Conserv.* 21, 1251–1266 (2012).

60. Pineda, E. & Lobo, J. M. Assessing the accuracy of species distribution models to predict amphibian species richness patterns. *J. Anim. Ecol.* 78, 182–190 (2009).

61. Yuan, H.-S., Wei, Y.-L. & Wang, X.-G. Maxent modeling for predicting the potential distribution of Sanghuang, an important group of medicinal fungi in China. *Fungal Ecol.* 17, 140–145 (2015).

62. Chinathamby, K., Reina, R. D., Bailey, P. C. & Lees, B. K. Effects of salinity on the survival, growth and development of tadpoles of the brown tree frog, *Litoria ewingii*. *Aust. J. Zool.* 54, 97–106 (2005).

63. Metcalfe, N. B. & Monaghan, P. Compensation for a bad start: Grow now, pay later?. *Trends Ecol. Evol.* 16, 254–260 (2001).

64. Metzger, D. C., Healy, T. M. & Schulte, P. M. Conserved effects of salinity acclimation on thermal tolerance and hsp70 expression in divergent populations of threespine stickleback (*Gasterosteus aculeatus*). *J. Comp. Physiol. B.* 186, 879–889 (2016).

65. Sanabria, E. et al. Effect of salinity on locomotor performance and thermal extremes of metamorphic Andean Toads (*Rhinella spinulosa*) from Monte Desert, Argentina. *J. Therm. Biol.* 74, 195–200 (2018).

66. Sokolova, I. M. Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. *Integr. Comp. Biol.* 53, 597–608 (2013).

67. Kikawada, T. et al. Dehydration-induced expression of LEA proteins in an anhydrobiotic chironomid. *Biochem. Biophys. Res. Commun.* 348, 56–61 (2006).

68. Sanzo, D. & Hecran, S. J. Effects of road de-icing salt (NaCl) on larval wood frogs (*Rana sylvatica*). *Environ. Pollut.* 140, 247–256 (2006).

69. Wood, L. & Welch, A. M. Assessment of interactive effects of elevated salinity and three pesticides on life history and behavior of southern toad (*Anaxyrus terrestris*) tadpoles. *Environ. Toxicol. Chem.* 34, 667–676 (2015).

70. Gomez-Mestre, I., Tejedo, M., Ramayo, E. & Esteja, J. Developmental alterations and osmoregulatory physiology of a larval anuran under osmotic stress. *Physiol. Biochem. Zool.* 77, 267–274 (2004).

71. Dent, J. N. Hormonal interaction in amphibian metamorphosis 1.2. *Am. Zool.* 28, 297–308 (1988).

72. Bodensteiner, B. L. et al. Thermal adaptation revisited: How conserved are thermal traits of reptiles and amphibians?. *J. Exp. Zool. Part A Ecol. Integr. Physiol.* 335, 173–194 (2021).

73. Rezende, E. L., Tejedo, M. & Santos, M. Estimating the adaptive potential of critical thermal limits: Methodological problems and evolutionary implications. *Funct. Ecol.* 25, 111–121 (2011).

74. Mitchell, J. D., Hewitt, P. & Van Der Linde, T. D. K. Critical thermal limits and temperature tolerance in the harvester termite *Hodotermes mossambicus* (Hagen). *J. Insect Physiol.* 39, 523–528 (1993).

75. Plummer, M. V., Williams, B. K., Skiver, M. M. & Carlyle, J. C. Effects of dehydration on the critical thermal maximum of the desert box turtle (*Terrapene ornata luteola*). *J. Herpetol.* 37, 747–751 (2003).

76. Lee, S. et al. Effects of feed restriction on the upper temperature tolerance and heat shock response in juvenile green and white sturgeon. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 198, 87–95 (2019).

77. Blaustein, A. R. & Wake, D. B. Declining amphibian populations: A global phenomenon?. *Trends Ecol. Evol.* 5, 203–204 (1990).

78. Kiesecker, J. M., Blaustein, A. R. & Belden, L. K. Complex causes of amphibian population declines. *Nature* 410, 681 (2001).

79. Rohr, J. R. & Raffel, T. R. Linking global climate and temperature variability to widespread amphibian declines putatively caused by disease. *Proc. Natl. Acad. Sci. U. S. A.* 107, 8269–8274 (2010).

80. Pounds, J. A. et al. Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* 439, 161 (2006).

81. Skelly, D. & Freudenburg, L. Effects of beaver on the thermal biology of an amphibian. *Ecol. Lett.* 3, 483–486 (2000).

82. Radchuk, V. et al. Adaptive responses of animals to climate change are most likely insufficient. *Nat. Commun.* 10, 1–14 (2019).
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M.F.C., C.S.W., Y.M.C., Y.J. and Y.C.K. conceived of the study; M.F.C., Y.J.C., D.A. and Y.J.Y. collected the data; M.F.C., Y.J.C., D.A. and A.B. analyzed the data; Y.J.C. and A.B. wrote the initial manuscript; all authors edited and approved the final manuscript.

Competing interests
The authors declare no competing interests.

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