

## Research

### Time of activity is a better predictor of the distribution of a tropical lizard than pure environmental temperatures

Gabriel H. O. Caetano, Juan C. Santos, Leandro B. Godinho, Vitor H. G. L. Cavalcante, Luisa M. Diele-Viegas, Pedro H. Campelo, Lidia F. Martins, Alan F. S. Oliveira, Júlio M. Alvarenga, Helga C. Wiederhecker, Verônica de Novaes e Silva, Fernanda P. Werneck, Donald B. Miles, Guarino R. Colli and Barry R. Sinervo

G. H. O. Caetano (<https://orcid.org/0000-0003-4472-5663>) ✉ ([gabrielhoc@gmail.com](mailto:gabrielhoc@gmail.com)) and B. R. Sinervo (<https://orcid.org/0000-0002-9241-1821>), Univ. of California Santa Cruz, 1156 High Street, Santa Cruz, CA 95064, USA. – J. C. Santos (<https://orcid.org/0000-0002-7777-8094>), St. John's Univ., Jamaica, NY, USA. – L. B. Godinho (<https://orcid.org/0000-0001-7653-5720>) and J. M. Alvarenga (<https://orcid.org/0000-0003-2276-3810>), Univ. do Estado de Mato Grosso, Campus Nova Xavantina, Nova Xavantina, MT, Brazil. – V. H. G. L. Cavalcante, Inst. Federal do Piauí, Teresina, PI, Brazil. – L. M. Diele-Viegas (<https://orcid.org/0000-0002-9225-4678>), Univ. do Estado do Rio de Janeiro, Maracanã, Rio de Janeiro, RJ, Brazil. – P. H. Campelo (<https://orcid.org/0000-0002-4048-3858>) and G. R. Colli (<https://orcid.org/0000-0002-2628-5652>), Univ. de Brasília, Campus Universitário Darcy Ribeiro, Brasília, DF, Brazil. – L. F. Martins (<https://orcid.org/0000-0002-4150-8818>), A. F. S. Oliveira (<https://orcid.org/0000-0001-6429-8545>) and F. P. Werneck (<https://orcid.org/0000-0002-8779-2607>), Inst. Nacional de Pesquisas da Amazônia, Petrópolis, Manaus, AM, Brazil. – H. C. Wiederhecker (<https://orcid.org/0000-0002-6454-0829>), Univ. Católica de Brasília, Águas Claras, Brasília, DF, Brazil. – V. de Novaes e Silva (<https://orcid.org/0000-0002-6769-361X>), Inst. Chico Mendes de Conservação da Biodiversidade, Brasília, DF, Brazil. – D. B. Miles (<https://orcid.org/0000-0001-5768-179X>), Dept of Biological Sciences, Ohio Univ., Athens, OH, USA.

#### Oikos

00: 1–11, 2020

doi: 10.1111/oik.07123

Subject Editor: Germán Orizaola

Editor-in-Chief: Dries Bonte

Accepted 5 March 2020

Environmental temperatures influence ectotherms' physiology and capacity to perform activities necessary for survival and reproduction. Time available to perform those activities is determined by thermal tolerances and environmental temperatures. Estimates of activity time might enhance our ability to predict suitable areas for species' persistence in face of climate warming, compared to the exclusive use of environmental temperatures, without considering thermal tolerances. We compare the ability of environmental temperatures and estimates of activity time to predict the geographic distribution of a tropical lizard, *Tropidurus torquatus*. We compared 105 estimates of activity time, resulting from the combination of four methodological decisions: 1) how to estimate daily environmental temperature variation (modeling a sinusoid wave ranging from monthly minimum to maximum temperature, extrapolating from operative temperatures measured in field or using biophysical projections of microclimate)? 2) In which temperature range are animals considered active? 3) Should these ranges be determined from body temperatures obtained in laboratory or in field? And 4) should thermoregulation simulations be included in estimations? We show that models using estimates of activity time made with the sinusoid and biophysical methods had higher predictive accuracy than those using environmental temperatures alone. Estimates made using the central 90% of temperatures measured in a thermal gradient as the temperature range for activity also ranked higher than environmental temperatures. Thermoregulation simulations did not improve model accuracy. Precipitation ranked higher than thermally related predictors. Activity time adds important information to distribution modeling and should be considered as a predictor in studies of the distribution of ectotherms. The distribution of *T. torquatus* is restricted by precipitation and



by the effect of lower temperatures on their time of activity and climate warming could lead to range expansion. We provide an R package ‘Maringuari’ with tools to generate spatial predictors based on the processes described herein.

Keywords: biogeography, bioinformatics, ecophysiology, mechanistic models, methodology evaluation, microclimate, species distribution modeling, thermal ecology, thermoregulatory behavior

---

## Introduction

Environmental temperatures influence ectotherms’ physiology and their capacity to perform activities necessary for survival and reproduction. Variation in ambient temperature influences foraging behavior, digestive assimilation and escape from predators; activities critical for survival, growth and reproduction (Porter et al. 1973, Adolph 1990, Adolph and Porter 1993). Because of the dependence of physiological processes on ambient temperature, most ectotherms are vulnerable to global climate change, especially in tropical areas (Deutsch et al. 2008, Huey et al. 2009, Sinervo et al. 2010). For instance, several extirpations of lizard populations due to climate change have been documented and more are predicted to occur in the next decades (Sinervo et al. 2010, Foufopoulos et al. 2011, Pontes-da-Silva et al. 2018).

Physiology modulates the effect of abiotic conditions on behavior, phenology and dispersal, which influence population dynamics and persistence (Huey 1991, Walther et al. 2002, Kearney and Porter 2009). An example of this is the interaction of climate change with thermal tolerance, changing the time available for critical activities each day (Grant and Dunham 1988, Adolph and Porter 1993, Sinervo and Adolph 1994). This might constrain the energy an individual can allocate to growth, maintenance and reproduction (Porter et al. 1973, Sinervo and Adolph 1994, Kearney and Porter 2009), determining if a species is able to inhabit a certain area (Sinervo et al. 2010).

Estimating temperature effects on the activity time of ectotherms depends on two important parameters: 1) the temperature range in which individuals may be active and 2) the environmental temperature variation experienced by individuals in nature. Numerous combinations of methodologies can be used to estimate those parameters, generating considerable variation in estimates of activity time, which could influence prediction accuracy. Therefore, researchers interested in ecophysiology need to make informed decisions before applying those methodologies.

A direct way to obtain temperature ranges for activity is to measure an animal’s body temperature in natural habitats, during an ecologically relevant period. However, the temperatures sampled may not capture the entire range of activity, since the latter may be restricted by predation risk, social interactions or lack of thermal opportunity (Hertz et al. 1983, Autumn and Denardo 1995, Ibarguengoytia 2005).

An alternative is to measure temperature ranges selected by an individual in the laboratory, using thermal gradient experiments (Licht 1965). In a lab setting, animals would not incur the aforementioned thermoregulation costs. An advantage of thermal gradient experiments is that they usually provide a greater amount of data, compared to field sampling methods, as they provide opportunity for continuous sampling once the animal is at the gradient, while field methods generally allow for the sampling of a single body temperature at the moment of capture. However, the artificial conditions in a temperature gradient may subject individuals to stresses that affect their activity patterns and selected body temperature.

Important thermal traits, such as mean preferred temperatures ( $T_{\text{mean}}$ ) or ranges of preferred temperatures ( $T_{\text{set}}$ ), can be estimated using laboratory data (Hertz et al. 1993), and we can apply the same calculations to generate similar indexes from field data, but it is important to determine which method produces the most informative estimates.  $T_{\text{mean}}$  is calculated as the mean or median temperature selected by individuals in thermal gradients, while  $T_{\text{set}}$  can be estimated as the complete range of temperatures measured or a quantile range around the median temperature (Hertz et al. 1993, Huey et al. 2009, Gutiérrez et al. 2010). The selection of  $T_{\text{mean}}$  or  $T_{\text{set}}$  to determine temperature ranges of activity, and which quantile to use for  $T_{\text{set}}$ , will influence results of subsequent analyses; thus, it is important to determine the parameter that produces the best estimates. Neurophysiological evidence suggests ectotherms regulate body temperatures between two set points (Firth and Turner 1982), but single set-points are widely used and available in the literature (Sinervo et al. 2010).

Species distribution models that include predictors based on biological processes, such as thermally constrained activity times, represent a more proximate approach than just using macroclimatic predictors (Elith et al. 2010, Dormann et al. 2012, Fordham et al. 2018). The development of biologically inspired indices premised on the process of thermoregulation can improve the output of species distribution models (Kearney and Porter 2009), especially for ectotherms. Further complexity can be added to activity time predictors by including microclimatic variation or simulations of thermoregulatory behavior. Those factors can have such importance as to override the need of physiological adaptations (i.e. the Bogert effect, Huey et al. 2003). However, the addition of complexity requires additional data, and might increase experimental and sampling errors; therefore, the benefits of increased complexity are not always evident, and these methods need to be thoroughly evaluated before they are used.

Our goal is to evaluate if thermally determined activity time estimates are better predictors of the geographic distribution of a tropical lizard than the pure environmental temperature measurements used to calculate those estimates, and which methodological decisions for the estimation of activity time generate better predictors. We assess the accuracy of different estimates of activity time in predicting the geographic distribution of the lizard *Tropidurus torquatus* (Squamata, Tropiduridae), generated under different methodological decisions. *Tropidurus torquatus* occurs throughout the

Cerrado savanna and Atlantic rainforest in South America (Rodrigues 1987). This species is locally abundant and conspicuous, making it convenient to collect and compare thermal ecology data across broad environmental gradients. Finally, we provide tools to facilitate the described estimates in a statistical package for the R programming environment, named ‘Mapinguari’.

## Material and methods

### Data collection

#### Distribution data

We used 359 distribution records from the literature and scientific collections spanning the range of *Tropidurus torquatus*. To minimize the effects of spatial autocorrelation and sampling bias, we used function ‘clean\_points’ from the R package Mapinguari to eliminate records within 40 km from each other, leaving us with 144 records. We empirically determined the size of the buffer area fitting Random Forest models under different buffers (1, 5, 10, 20, 30, 40 and 50 km) and comparing Moran’s I index (Gittleman and Kot 1990) calculated from the models’ residuals. We selected buffer distance based on the smaller distance resulting in no spatial autocorrelation. We estimated Moran’s I using the ‘Moran.I’ function from R package ‘ape’ (Paradis et al. 2004). Thirty percent of the distribution data, 44 records, was set aside for model cross-validation.

#### Physiological data

Between 2013 and 2017, we obtained physiological data from five populations of *T. torquatus* sampled during monitoring studies and field expeditions. Monitoring took place in Brasília, Distrito Federal (15°79’98”S, 47°86’45”W, 24 individuals) and Nova Xavantina, Mato Grosso (14°66’44”S, 52°35’85”W, four individuals). Short-term field sampling occurred at Gaúcha do Norte (12°96’56”S, 53°56’36”W, 13 individuals) and Alta Floresta, Mato Grosso (9°87’65”S, 56°08’55”W, three individuals); and Lagoa da Confusão, Tocantins (10°92’01”S, 50°18’33”W, eight individuals). We captured animals using pitfall traps, lassos and by hand.

We brought captured lizards to the laboratory, housed them individually and performed the thermal gradient experiments no longer than 24 h after capture. We measured the preferred temperature of each lizard using a thermal gradient, which consisted of a terrarium made of MDF plywood (100 × 15 × 30 cm – l × w × h), open at the top and with 2 cm of substrate composed of sand and vermiculite. We generated a thermal gradient approximately between 15°C and 50°C by placing a 60-W incandescent lamp at one end and an ice pack on the other (Paranjpe et al. 2013). Lizards were placed in the gradient for one hour while their body temperature was recorded every minute by a 1 mm thermocouple attached with tape to their abdomen and connected to a data logger. We allowed lizards to acclimate to the gradient for 10 min (Paranjpe et al. 2013) before recording body temperatures. We calculated for each

individual and for the whole sample: 1) the range between the 5th and 95th temperature percentile ( $T_{90}$ ), 2) range between the 25th and 75th temperature percentile ( $T_{50}$ ) and 3) average temperature ( $T_{\text{mean}}$ ).  $T_{\text{mean}}$  and  $T_{50}$  have been used in previous studies (Sinervo et al. 2010, Kubisch et al. 2016, Piantoni et al. 2016), and the broader range,  $T_{90}$ , was chosen under the hypothesis that lizards spend almost all of their time in the gradient at preferred temperatures.

We obtained field-active body temperatures from lizards at Brasília, Distrito Federal, from natural populations occurring within the city’s Zoo (15°85’12”S, 47°93’79”W, 1158 samples, 640 individuals, details in Wiederhecker et al. 2002), which was visited weekly from March 1996 to September 1998, from 08 a.m. to 06 p.m., and at Santa Terezinha, Mato Grosso (10°37’5”S, 50°51’45”W, nine samples, nine individuals) in April 1999, from 12 p.m. to 02 p.m. Active animals (i.e. those in the open, basking or moving) were captured, individually marked by toe-clipping, and had their cloacal temperature measured with a quick reading cloacal thermometer (0.2°C precision) immediately after capture. We then performed the same calculations for laboratory  $T_{90}$ ,  $T_{50}$  and  $T_{\text{mean}}$  on the aggregated field body temperatures.

The different methods of collecting body temperatures result in very different data structures. While the laboratory experiments allow extensive sampling of fewer individuals, field sampling allows the collection of many individuals, but fewer replicates per individual. In the laboratory, we sampled 52 individuals with a median of 65 samples per individual (standard deviation = 9.48), whereas in the field we sampled 649 individuals with a median of one sample per individual (standard deviation = 1.58). This presents a challenge when comparing data from the two sources, because we could calculate temperature ranges for each individual from the thermal gradient, but not from individuals in the field. Therefore, for the thermal measurements collected in the wild, we pooled the data and assumed the estimated thermal tolerances characterized the individuals from the entire sample. For laboratory data, we estimated temperature ranges as both averages of individual values or from the data aggregated from the whole sample, and then assessed which choice generated better predictors of distribution. We performed an analysis of variance with repeated measures to evaluate whether the body temperatures measured in the thermal gradient differed among individuals between populations and analysis of variance to see if there were differences between individuals in the same population.

#### Operative environmental temperatures

We recorded operative temperatures using dataloggers with sensors attached to PVC models of equivalent size and color as *Tropidurus torquatus*. This methodology has been validated by previous studies with small ectotherms (Adolph 1990, Lara-Reséndiz et al. 2015, Kubisch et al. 2016, Kirchhof et al. 2017). We placed models adjacent to pitfall trap arrays, in the locations where lizards were captured for the physiological trials, uniformly distributed in microhabitats where they were observed in activity – shaded and open spots on the ground,

on termite mounds, and at the base of trees. Data loggers were deployed during months of August 2013 and April–July 2014 in Brasília, August 2015–2016 in Nova Xavantina, August 2015 in Gaúcha do Norte, July–August 2016 in Lagoa da Confusão and July–August 2017 in Alta Floresta. Data loggers recorded temperatures every 10 min during the trapping period at each location. Variation in air temperature was also measured at the same time and locations, using another data logger without a PVC model attached to sensors, which was protected from rain and solar radiation by a PVC case suspended about 30 cm from the ground and open at the bottom to expose the sensor to the air.

## Data analysis

All analyses were performed in the R programming environment, ver. 3.5.1 (<[www.r-project.org](http://www.r-project.org)>). We used species distribution models to infer the accuracy of time of activity estimates and environmental variables in predicting the species distribution. Considering the problem at hand, all estimates are expected to be highly correlated. So, we fitted those models with two algorithms which are robust to multicollinearity (James et al. 2013): Random Forest, using R package ‘randomForest’ (Liaw and Wiener 2002) and Gradient Boosting, using R package ‘gbm’ (Ridgeway 2007).

### Pseudoabsences

We generated 100 different sets of pseudoabsences to use in those models, each with 100 pseudoabsences, the same number of presences (Barbet-Massin et al. 2012). We generated pseudoabsences by using environmental profiling with one-classification support vector machine (OCSVM), through R package ‘mopa’ (Senay et al. 2013). This methodology restricts the background sampled for pseudoabsences to a distance from presence points determined by the variation in environmental conditions and selects points representing that variation. We obtained environmental variables (maximum daily temperature, minimum daily temperature, average daily temperature, precipitation and altitude) from the WorldClim 2.0 database at 2.5 arc-min resolution for present-day (1970–2000) (Fick and Hijmans 2017). Every subsequent analysis was repeated for each pseudoabsence set and the results were averaged between sets.

To perform time of activity estimates, we developed a package for R, named ‘Mappinguari’ that provides tools for incorporating diverse biological processes in species distribution modeling. ‘Mappinguari’ is an open source program and available on a GitHub repository <[www.github.com/gabrielhoc/Mappinguari](http://www.github.com/gabrielhoc/Mappinguari)> with a tutorial on <[www.gabrielhoc.github.io/Mappinguari](http://www.gabrielhoc.github.io/Mappinguari)>. It has been submitted to the Comprehensive R Archive Network (CRAN) and will be available for installation through the R interface once it is approved. Next we detail the methods to estimate time of activity.

### Temperature variation estimation

We used three different methods to estimate daily temperature variation to which animals are subjected, and to

determine the proportion of time ambient conditions within or outside the temperature ranges for activity:

1. *Sinusoid method.* We used the approach of Sinervo and collaborators (Sinervo et al. 2010) implemented in the Mappinguari function ‘sin\_h’. This approach requires the least amount of data and assumes lizard body temperature tracks environmental temperatures. It models daily air temperature variation as a sinusoidal curve ranging between the maximum and minimum daily air temperatures at a location, extracted from WorldClim surfaces. Then we recorded how much time the environmental temperature is within  $T_{90}$ , within  $T_{50}$ , and above  $T_{\text{mean}}$ , for both field and lab temperature ranges at each occurrence and pseudoabsence point. We also did the same calculations for the lower and upper temperatures thresholds of  $T_{90}$  and  $T_{50}$  to verify if colder or hotter thresholds are more relevant than the whole range of activity. We used a time resolution of one hour to make estimates comparable with the microclim method (below), which is derived from hourly data. Hours above  $T_{\text{mean}}$  is a measure of hours of restriction to activity, so it should be interpreted on the opposite ways as the other metrics. These estimates of activity time, as well as all subsequent ones, were capped by day length at each location, calculated using Corripio’s method (Corripio 2003).
2. *Operative temperature method.* We used daily temperature variation collected with operative temperatures models ( $T_o$ ) (Bakken 1992) to estimate time of activity for each day, location and microhabitat sampled, using the same temperature ranges mentioned above. These data were regressed against maximum daily air temperatures for the same period, using Richards’ growth model (Kirchhof et al. 2017, Sinervo et al. 2018). The coefficients obtained were used to predict time of activity under present-day maximum daily air temperature data from the WorldClim 2.0 database at 2.5 arc-min resolution, for each occurrence and pseudoabsence point.
3. *Microclim method.* We used the microclim database (Kearney et al. 2014), which simulates environmental temperatures for each hour of the day in six levels of shade and different kinds of substrate for the whole world. We used air temperatures 1 cm above the soil substrate, which we regarded as the most commonly used by *T. torquatus* based on field observations. We used the function ‘summary\_microclim’ from Mappinguari to estimate time of activity, for the same temperature ranges mentioned above, at each shade level, for each occurrence or pseudoabsence point (Sinervo et al. 2018). Microclim data are available at 10 arc-min resolution, so they were re-scaled to 2.5 arc-min raster using bilinear interpolation in package ‘raster’ (Hijmans et al. 2016).

### Thermoregulation simulation

Operative temperature and microclim methods provide information on microclimatic variation at each site, so we

simulated thermoregulation, assuming lizards would choose any microhabitat with temperatures inside the preferred temperature range when available. For comparison, we generated estimates of time of activity under no thermoregulation by averaging the time of activity between all microhabitats. Names used to represent each estimate are summarized in Supplementary material Appendix 1 Table A1, hereafter the estimates will be designated by these names.

### Predictor evaluation

Estimates of activity time were used as predictors of species distribution in models constructed with different algorithms, and their quality as predictors was assessed using the protocol describe below. Each model also included average annual maximum air temperature as a comparison, since estimates were derived from maximum air temperature and should outperform it if they add any relevant information. We also include total annual precipitation, to control for variation not associated with thermal physiology.

We evaluated the performance of each algorithm by the area under the receiver operating characteristic curve (AUC), constructed with the set of presence records set aside for cross-validation and 100 sets of pseudoabsences. The agreement between algorithms variable importance indexes was used to determine predictor importance. The importance index used for Random Forest was mean decrease in accuracy (Archer and Kimes 2008) and for Gradient Boosting, relative influence (Friedman 2001). Importance measures of variables obtained from each algorithm were grouped by each methodological decision, weighted by the AUC of each algorithm and averaged to obtain a consensus of importance of each decision. Finally, activity time estimates and climate variables with greater support were used to predict the potential distribution of the animal.

## Results

Body temperatures measured in the thermal gradient did not differ significantly among populations ( $F=2.424$ ,  $df=4$ ,  $p=0.06$ ) but differed among individuals in each population (Brasília:  $F=199.31$ ,  $df=23$ ,  $p<0.0001$ ; Alta Floresta:  $F=1502.4$ ,  $df=2$ ,  $p<0.0001$ ; Gaúcha do Norte:  $F=180.82$ ,  $df=12$ ,  $p<0.0001$ ; Lagoa da Confusão:  $F=88.849$ ,  $df=7$ ,  $p<0.0001$ ; Nova Xavantina:  $F=9.51$ ,  $df=3$ ,  $p<0.0001$ ). We observed substantial variation in the distribution of body temperatures when using the pooled data or among individuals within each population (Fig. 1). However, two patterns are apparent. First, lizards in some populations have a narrow range of temperatures in the gradient, e.g. Nova Xavantina and Alta Floresta. Second, lizards in other populations exhibit a mixed pattern of either broad or narrow ranges for  $T_{\text{mean}}$ , e.g. Gaúcha do Norte and Brasília. This pattern suggests those lizards are thermal generalists and are active at a wide range of temperature and each individual is not exploring their whole thermal tolerance range in the gradient. This suggests it is

better to pool all body temperatures to characterize thermal tolerance ranges.

Median body temperature recorded in the thermal gradient was very similar to those measured on field-active lizards (laboratory =  $31.51^{\circ}\text{C}$ , field =  $32.65^{\circ}\text{C}$ ), but with higher standard deviation (laboratory =  $6.02$ , field =  $2.59$ ). This led to broader temperature ranges for activity when compared to field estimates. Physiological temperature thresholds and ranges for *T. torquatus* calculated from the gradient experiments had the following values:  $T_{\text{mean}}=31.51^{\circ}\text{C}$  (standard deviation:  $6.02$ ),  $T_{50_{\text{lwr}}}=26.68^{\circ}\text{C}$ ,  $T_{50_{\text{upr}}}=36.6^{\circ}\text{C}$ ,  $T_{90_{\text{lwr}}}=20.8^{\circ}\text{C}$ ,  $T_{90_{\text{upr}}}=38.67^{\circ}\text{C}$ , whereas estimates calculated from field temperatures had the following values  $T_{\text{mean}}=32.6^{\circ}\text{C}$  (standard deviation:  $2.59$ ),  $T_{50_{\text{lwr}}}=30.9^{\circ}\text{C}$ ,  $T_{50_{\text{upr}}}=34.6^{\circ}\text{C}$ ,  $T_{90_{\text{lwr}}}=28^{\circ}\text{C}$ ,  $T_{90_{\text{upr}}}=36.4^{\circ}\text{C}$ . Figure 2 shows the distribution of activity time estimates grouped under each methodological decision.

Random Forest had similar AUC ( $0.861 \pm 0.042$ ) to Gradient Boosting ( $0.843 \pm 0.043$ ). Predictor importance is summarized in Supplementary material Appendix 1 Table A2. Precipitation ranked highest for Gradient Boosting and third highest for Random Forest (Supplementary material Appendix 1 Table A2). For both algorithms, activity time estimates ranked higher than pure environmental temperatures, with no regard to physiology. Estimate  $\text{sin\_t90\_lwr\_lab}$  (Supplementary material Appendix 1 Table A1) ranked highest among time of activity estimates on both algorithms (Supplementary material Appendix 1 Table A2). The consensus between algorithms averaged for methodological decisions regarding the estimation of environmental temperatures experienced and thermal tolerances is summarized in Table 1 and 2, respectively. The distribution predicted by the most supported variables,  $\text{sin\_t90\_lab\_lwr}$  and precipitation, is displayed on Fig. 3.

## Discussion

Our results suggest that including ecophysiological variables, such as time available for activity determined by temperature, increases the accuracy of species distribution models. All methodological decisions evaluated here greatly affected the quality of predictors and should be carefully considered before conducting similar analysis. Estimates under many permutations of methodological decisions ranked above pure environmental temperatures, and some were as much as five times more important based on the Random Forest algorithm and 20 times more with the Gradient Boosting algorithm (Supplementary material Appendix 1 Table A2). This indicates activity time is useful to model mechanisms by which temperature restricts the distribution of a tropical lizard. Our results add to the evidence that incorporating biological mechanisms improve the accuracy of species distribution models (Buckley et al. 2010, Urban et al. 2016).

The simplest temperature variation method, sinusoid, ranked highest in both algorithms (Supplementary material

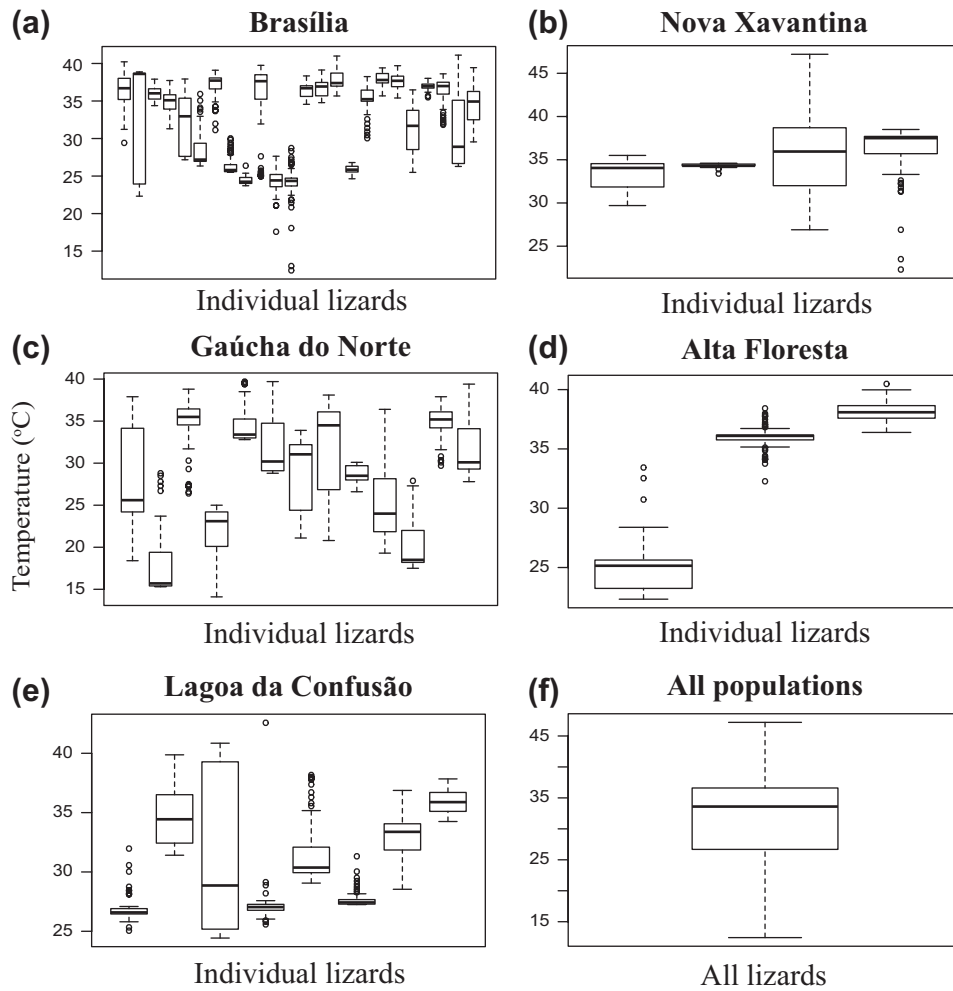


Figure 1. Distribution of preferred body temperatures of *Tropidurus torquatus* lizards from five populations in Brazil. On panels (a) through (e), each box plot represents body temperatures of a single individual collected every minute for one hour in a temperature gradient, excluding the first ten minutes and outliers, in five localities in Brazil. (a) Brasília, Distrito Federal, (b) Nova Xavantina, (c) Gaúcha do Norte and (d) Alta Floresta, state of Mato Grosso and (e) Lagoa da Confusão, state of Tocantins. Panel (f) represents the distribution of the temperatures of all individuals grouped together.

Appendix 1 Table A2), but not when averaged across all variables using this method, when it was a close second to the microclim method (Table 1). Thus, both methods are good options for estimating time of activity. Simulations based on the sinusoid model have the advantage of being simpler to estimate, lower in computational time and memory use, and simpler to extrapolate to future conditions. Simpler models have fewer sources of error and are less likely to overfit the data used to train them. The application of microclim has the advantage of including microclimatic variation in the analysis, which might be relevant to many species. Estimates made with the operative method ranked below pure environmental temperatures indicating this method needs refinement, perhaps by modeling how microclimates interact with microhabitat structure and macroclimate. Also, the operative method is likely to be very influenced by the deployment of operative models. Our uniform deployment of data loggers between microhabitats implies uniform access to each microhabitat,

so this methodology could be improved by testing different deployment designs. Access to microhabitats might also be limited by dispersal capacity and species interactions, such as competition and predation. Predators and competitors will also have their own thermal responses, which affect the strength of their interaction with *Tropidurus torquatus*. Those are complex processes and data relating to them are scarce, complicating the prospects of deriving a realistic and detailed model. Using simpler and general methods could yield more accurate predictors at lower costs.

Among time of activity estimates, the ones using temperatures above the lower threshold of  $T_{90}$  measured in laboratory as the range of activity temperatures were the best to predict distribution (Table 1). The fact the lower threshold was more important shows lower temperatures are the most limiting to *T. torquatus* distribution, unlike other species studied previously (Sinervo et al. 2010, Andrango et al. 2016, Medina et al. 2016). The upper threshold of  $T_{90}$  was rarely crossed (Fig. 2,

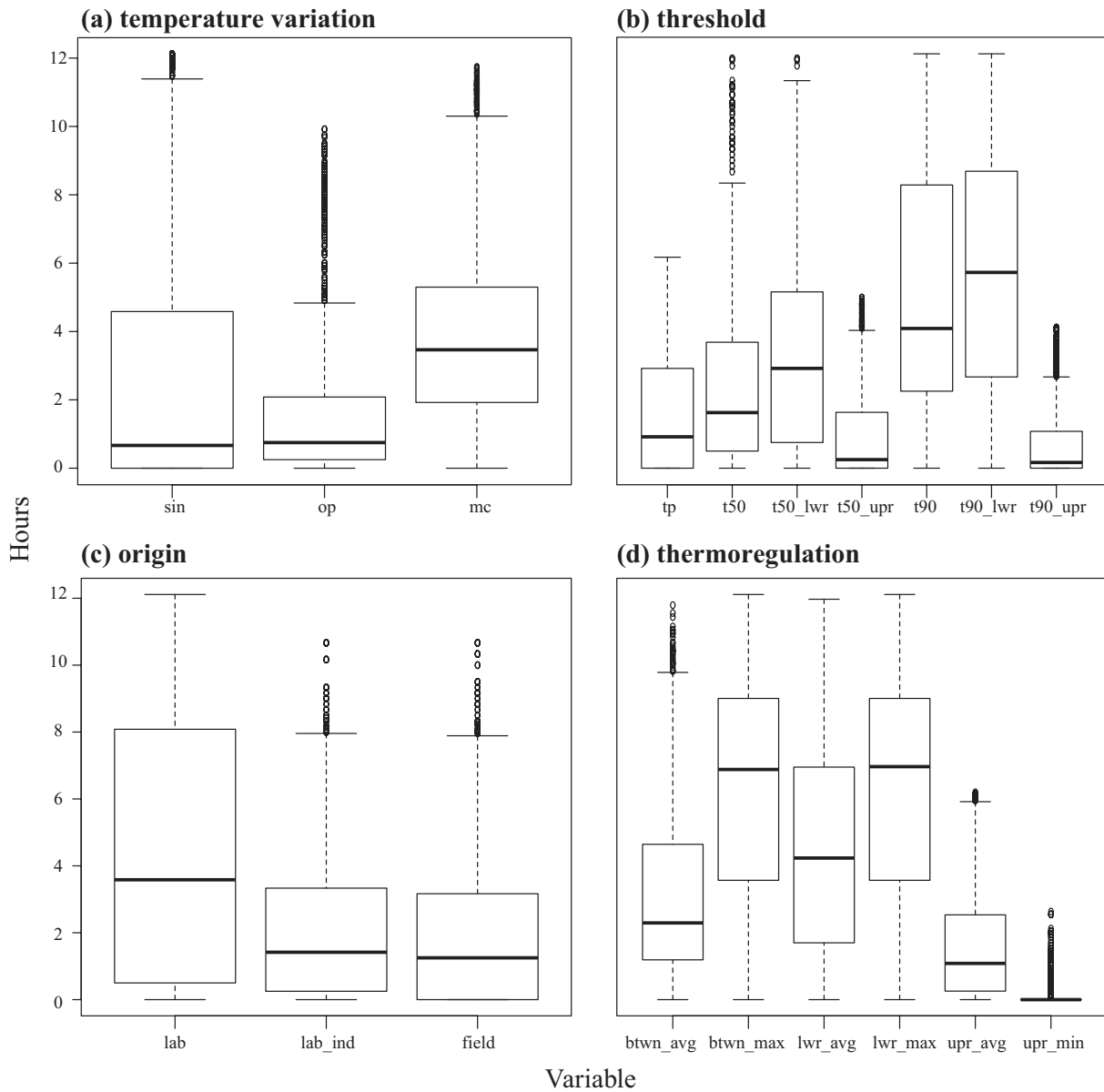


Figure 2. Distribution of activity time estimates at known occurrence sites of *Tropidurus torquatus*. (a) Grouped by temperature variation method. Sinusoid simulates temperature variation as a sine wave spanning from daily maximum to minimum air temperatures. Operative used operative temperature models to measure daily temperature variations in situ and them extrapolated time of activity measures by correlating it to air temperatures. Microclim used microclimatic surfaces (Kearney et al. 2014) containing estimates of daily temperature variation in different microhabitats. (b) Grouped by temperature range of activity.  $T_{\text{mean}}$ : above average body temperature,  $T_{50\text{-lwr}}$ : above 25th quantile,  $T_{50\text{-upr}}$ : above 75th quantile,  $T_{90\text{-lwr}}$  above 5th quantile,  $T_{90\text{-upr}}$  above 95th quantile.  $T_{50}$ : between  $T_{50\text{-lwr}}$  and  $T_{50\text{-upr}}$  and  $T_{90}$ : between  $T_{90\text{-lwr}}$  and  $T_{90\text{-upr}}$ . (c) Grouped by origin of body temperatures used to estimate temperature ranges of activity (laboratory or field), (d) grouped by use of thermoregulation simulations.

Supplementary material Appendix 1 Table A2), showing that *T. torquatus* rarely experiences temperatures above its thermal tolerance range within its current distribution, therefore those temperatures are not a significant determinant of their current distribution. Moreover, *T. torquatus* is tolerant to heat and could potentially benefit from climate warming, absent effects on important species interactions or habitat structure, expanding its distribution to areas previously too cold for it

to inhabit, where it could potentially displace native species less adapted to hotter conditions. Estimates made with interquartile ranges also ranked higher than environmental temperature profiles, so this range could also be informative and used in future studies, though our results favor the use of the 90th quantile ranges. Future studies should be done to verify if the same methodological decisions apply to more thermally restricted species.

Table 1. Median, standard deviation (SD) of activity time estimates and weighted average of their importance for predicting the distribution of the tropical lizard *Tropidurus torquatus*, grouped under different methodological decisions to estimate environmental temperatures experienced by individuals. (1) Method for estimating the temperature variation which animals experienced. Sinusoid simulates temperature variation as a sine wave spanning from daily maximum to minimum air temperatures. Operative uses operative temperature models to measure daily temperature variations in situ and them extrapolates time of activity measures by correlating it to air temperatures. Microclim uses microclimatic surfaces (Kearney et al. 2014) containing estimates of daily temperature variation in different microhabitats. (2) Use of thermoregulation simulations on operative and microclim methods of temperature variation. The average between microhabitats for each temperature range is also included for comparison. Also included are (3) climate variables: average air temperature ( $T_{avg}$ ), maximum air temperature ( $T_{max}$ ) and precipitation. Importance indexes were obtained from Random Forest (mean decrease in accuracy) and Gradient Boosting (relative influence) regressions of distribution against hours of activity and climate.

Methodological decisions	Median	SD	Importance
(1) Temperature variation			
sinusoid	0.667	3.943	0.008
operative	0.750	2.327	0.004
microclim	3.465	2.569	0.009
(2) Thermoregulation			
average - $T_{50r}$ , $T_{90}$	2.250	2.748	0.014
maximum - $T_{50r}$ , $T_{90}$	6.750	3.028	0.006
average - $T_{50_lwr}$ , $T_{90_lwr}$	4.153	3.058	0.007
maximum - $T_{50_lwr}$ , $T_{90_lwr}$	6.833	3.023	0.005
average - $T_{mean}$ , $T_{50_lwr}$	1.063	1.488	0.005
$T_{90_lwr}$			
minimum - $T_{mean}$ , $T_{50_lwr}$	0.000	0.278	0.004
$T_{90_lwr}$			
(3) Climate			
precipitation	121.333	20.909	0.124
$T_{avg}$	23.063	2.150	0.007
$T_{max}$	28.431	2.396	0.008

Laboratory measurements of body temperature produced better predictors of distribution than field measurements in general, reinforcing the importance of experiments for characterizing thermal physiology. Animals might explore wider temperature ranges in thermal gradients than in the field, avoiding constraints and costs of behavioral thermoregulation present in their natural habitats. We recommend future studies to use thermal gradients when estimating thermal tolerances. Likewise, aggregating temperatures from all individuals then calculating thermal tolerances generated better predictors of distribution than calculating thermal tolerances individually. Our interpretation of such results is that individuals might not be exploring their full thermal tolerance during thermal gradient experiments, so observing all individuals together generates a better picture of the species' thermal tolerance variability.

We could not generate informative estimates of activity time with the model of thermoregulation that we used. This may be due to the assumption that animals would have immediate access to all microhabitats available for

Table 2. Median, standard deviation (SD) of activity time estimates and weighted average of their importance for predicting the distribution of the tropical lizard *Tropidurus torquatus*, grouped under different methodological decisions to estimate their thermal tolerance. (1) Range of temperatures in which animals were considered active.  $T_{mean}$ : above average body temperature,  $T_{50_lwr}$ : above 25th quantile,  $T_{50_upr}$ : above 75th quantile,  $T_{90_lwr}$ : above 5th quantile,  $T_{90_upr}$ : above 95th quantile.  $T_{50}$ : between  $T_{50_lwr}$  and  $T_{50_upr}$  and  $T_{90}$ : between  $T_{90_lwr}$  and  $T_{90_upr}$ . (3) Origin of body temperatures used for range estimation: field or laboratory. Laboratory ranges were obtained by aggregating data from all individuals then calculating the range or by calculating the range for each individual and then averaging between them. Importance indexes were obtained from Random Forest (mean decrease in accuracy) and Gradient Boosting (relative influence) regressions of distribution against hours of activity and climate.

Methodological decisions	Median	SD	Importance
(1) Range			
$T_{mean}$	0.917	1.761	0.006
$T_{50}$	1.625	2.348	0.010
$T_{90}$	4.083	3.640	0.008
$T_{50_lwr}$	2.917	2.722	0.004
$T_{90_lwr}$	5.722	3.676	0.014
$T_{50_upr}$	0.250	1.260	0.002
$T_{90_upr}$	0.167	0.984	0.002
(2) Origin			
laboratory-pooled	3.583	4.067	0.010
laboratory-individual	1.417	2.150	0.005
field	1.250	2.133	0.005

thermoregulation. Laboratory experiments to elucidate how efficient animals are in choosing appropriate microclimates and projections of microhabitat structure and availability might help add realism to those models.

Precipitation ranked high in both algorithms, in agreement with studies that show it is a reliable predictor of lizard and other ectotherm habitat suitability (Araújo et al. 2006, Barrows 2011, Nasrabadi et al. 2018). *Tropidurus torquatus* might contract its range at more arid regions within its known distribution, such as the ecotone of the Cerrado with the semi-arid Caatinga. Modeling the processes by which rainfall affects ectotherm distribution is more complex than thermal physiology, since it might involve water-loss rates, species interactions, prey availability and egg survival, which would require data not yet widely available (but see Garcia-Porta et al. 2019). While temperature might have complex effects as well, our results suggest that direct effects on the lizard's physiology are predominant.

We found *T. torquatus* rarely experiences temperatures above its upper thermal tolerance range, suggesting high levels of warming would be required for this species to suffer significant loss of habitat suitability resulting in contraction of its distribution. Previous work has found that hours of restriction in activity computed from  $T_{mean}$  is a good predictor for persistence of desert lizard populations in Mexico (Sinervo et al. 2010), while the same estimate ranked remarkably low for *T. torquatus*. This could be due to the extreme differences in the environments and thermal physiology of



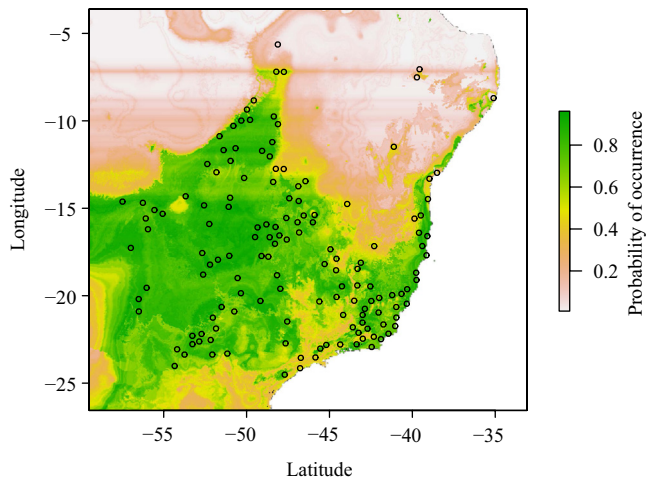


Figure 3. Probability of occurrence of the tropical lizard *Tropidurus torquatus* across central South America. Estimated using Random Forest regression of occurrence records and background points against precipitation and hours of activity. Circles represent occurrence records. Horizontal lines are artifacts generated by capping hours of activity by day length and do not have significant impacts in the figure interpretation.

both groups. For instance, Mexican desert lizards at greater risk are in montane environments, and their preferred temperatures are closer to their upper thermal tolerance limits (Sinervo et al. 2010). This indicates that the most accurate parameterization for such models is likely to be very affected by species' unique physiological traits, and thus researchers should be careful when generalizing conclusions from distribution models based on physiology to other species.

Most studies report negative effects of climate warming on Lepidosauria (Diele-Viegas and Rocha 2018), with some exceptions (Logan et al. 2015, Kubisch et al. 2016, Gilbert and Miles 2017) to which we now add *T. torquatus*. Thermal tolerant species might dominate reptile communities in the near future, given other species are expected to decline (Diele-Viegas and Rocha 2018). Other studies found lower thermal tolerance to evolve quickly in tropical lizards (Logan et al. 2014, Gilbert and Miles 2017), which might allow them to expand into colder areas if displaced from their current distribution by climate warming.

Activity time alone is not the sole factor determining the presence of an ectotherm species (Kearney 2013), but our results indicate it might be a better predictor than using pure environmental temperatures, with no regard for their interaction with species physiology. It is possible to model mechanisms by which the environment affects physiology with limited information, adding important information to distribution modelling. Package 'Maringuari' is an effort to increase accessibility to the techniques used here and facilitate their further development. We hope users of the package can generate biologically relevant information to provide policy makers with tools to mitigate the effects of climate warming on vulnerable species.

## Data availability statement

Data are available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.b2nbnzsb7>> (Caetano et al. 2020).

*Acknowledgements* – GHOC thanks L. Tourinho and A. Kilpatrick for helpful comments on the writing. We would like to thank all persons involved in the data collection effort. Instituto Brasileiro de Geografia e Estatística, Jardim Botânico de Brasília, Jardim Zoológico de Brasília, Parque Municipal do Bacaba, Universidade de Brasília and Universidade Estadual do Mato Grosso provided invaluable logistic support for data collection.

*Funding* – GRC thanks Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Apoio à Pesquisa do Distrito Federal (FAPDF) and the USAID's PEER program under cooperative agreement AID-OAA-A-11-00012 for financial support. JCS was supported by SJU start-up funds. BS and DBM thank NSF support for work in Brazil (EF-1241848). BS was supported by the Partnerships for Enhanced Engagement in Research from the U.S. National Academy of Sciences and U.S. Agency of International Development (PEER NAS/USAID; AID-OAA-A-11-00012 grant to GRC and FPW) and Pesquisador Visitante Especial (PVE) grant from CNPq awarded to BS. FPW thanks financial support from the Partnerships for Enhanced Engagement in Research-PEER from the U.S. National Academy of Sciences and U.S. Agency of International Development, CNPq, Fundação de Amparo à Pesquisas do Estado do Amazonas-FAPEAM, L'Oréal-UNESCO-ABC Para Mulheres na Ciência and L'Oréal-UNESCO For Women In Science awards. CAPES also provided a scholarship and tuition for GHOC, through the Science without Borders program (grant 99999.013716/2013-01) and for LMDV, through the Sandwich Doctorate Program (grant 88881.188452/2018-01) and Academic Excellence Program (grant 88882.182446/2018-01).

*Author contributions* – GHOC wrote the manuscript with support from JCS, GRC and BRS. DBM, BRS conceptualized the original ideas for the project. GHOC, JCS and BRS developed the statistical techniques and performed data analysis. GHOC, LBG, VHGLC, JMA, LMDV, PHC LFM, AFSSO, HCW, DBM, FPW, GRC and BRS participated in data collection and physiological experiments. HCW and VNS collected the field temperature data. FPW and GRC provided infrastructure, planning, execution and financing for data collection and experiments. All authors provided critical feedback and helped shape the research, analysis and manuscript.

*Permits* – Instituto Chico Mendes de Conservação da Biodiversidade provided the collecting license number 13324-1 to GRC. Data collection procedures were approved by the Commission of Ethics and Animal Use from University of Brasília, under process number 33786/2016.

## References

- Adolph, S. C. 1990. Influence of behavioral thermoregulation on microhabitat use by two *Sceloporus* lizards. – *Ecology* 71: 315–327.
- Adolph, S. C. and Porter, W. P. 1993. Temperature, activity and lizard life histories. – *Am. Nat.* 142: 273–295.

- Andrango, M. B. et al. 2016. Short-term predicted extinction of Andean populations of the lizard *Stenocercus guentheri* (Iguanidae: Tropidurinae). – *J. Therm. Biol.* 62: 30–36.
- Araújo, M. B. et al. 2006. Climate warming and the decline of amphibians and reptiles in Europe. – *J. Biogeogr.* 33: 1712–1728.
- Archer, K. J. and Kimes, R. V. 2008. Empirical characterization of random forest variable importance measures. – *Comput. Stat. Data Anal.* 52: 2249–2260.
- Autumn, K. and Denardo, D. F. 1995. Behavioral thermoregulation increases growth-rate in a nocturnal lizard. – *J. Herpetol.* 29: 157–162.
- Bakken, G. S. 1992. Measurement and application of operative and standard operative temperatures in ecology. – *Integr. Comp. Biol.* 32: 194–216.
- Barbet-Massin, M. et al. 2012. Selecting pseudo-absences for species distribution models: how, where and how many? – *Methods Ecol. Evol.* 3: 327–338.
- Barrows, C. W. 2011. Sensitivity to climate change for two reptiles at the Mojave–Sonoran Desert interface. – *J. Arid Environ.* 75: 629–635.
- Buckley, L. B. et al. 2010. Can mechanism inform species' distribution models? – *Ecol. Lett.* 13: 1041–1054.
- Caetano, G. H. O. et al. 2020. Data from: Time of activity is a better predictor of the distribution of a tropical lizard than pure environmental temperatures. – Dryad Digital Repository, <<http://dx.doi.org/10.5061/dryad.b2rbnzb7>>.
- Corripio, J. G. 2003. Vectorial algebra algorithms for calculating terrain parameters from dems and solar radiation modelling in mountainous terrain. – *Int. J. Geogr. Inf. Sci.* 17: 1–23.
- Deutsch, C. A. et al. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. – *Proc. Natl Acad. Sci. USA* 105: 6668–6672.
- Diele-Viegas, L. M. and Rocha, C. F. D. 2018. Unraveling the influences of climate change in *Lepidosauria* (Reptilia). – *J. Therm. Biol.* 78: 401–414.
- Dormann, C. F. et al. 2012. Correlation and process in species distribution models: bridging a dichotomy. – *J. Biogeogr.* 39: 2119–2131.
- Elith, J. et al. 2010. The art of modelling range-shifting species. – *Methods Ecol. Evol.* 1: 330–342.
- Fick, S. E. and Hijmans, R. J. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. – *Int. J. Climatol.* 37: 4302–4315.
- Firth, B. T. and Turner, S. 1982. Sensory, neural and hormonal aspects of thermoregulation. – In: Gans, C. and Pough, F. H. (eds), *Biology of the Reptilia*, vol. 12: physiology C, physiological and ecology. Academic Press, pp. 213–274.
- Fordham, D. A. et al. 2018. How complex should models be? Comparing correlative and mechanistic range dynamics models. – *Global Change Biol.* 24: 1357–1370.
- Foufopoulos, J. et al. 2011. Climate change and elevated extinction rates of reptiles from Mediterranean islands. – *Am. Nat.* 177: 119–129.
- Friedman, J. H. 2001. Greedy function approximation: a gradient boosting machine. – *Ann. Stat.* 29: 1189–1232.
- García-Porta, J. et al. 2019. Environmental temperatures shape thermal physiology as well as diversification and genome-wide substitution rates in lizards. – *Nat. Commun.* 10: 1–12.
- Gilbert, A. L. and Miles, D. B. 2017. Natural selection on thermal preference, critical thermal maxima and locomotor performance. – *Proc. R. Soc. B* 284: 20170536.
- Gittleman, J. L. and Kot, M. 1990. Adaptation: statistics and a null model for estimating phylogenetic effects. – *Syst. Zool.* 39: 227–241.
- Grant, B. W. and Dunham, A. E. 1988. Thermally imposed time constraints on the activity of the desert lizard *Sceloporus merriami*. – *Ecology* 69: 167–176.
- Gutiérrez, J. A. et al. 2010. Effect of altitude on thermal responses of *Liolaemus pictus argentinus* in Argentina. – *J. Therm. Biol.* 35: 332–337.
- Hertz, P. E. et al. 1983. Homage to Santa Anita: thermal sensitivity of sprint speed in agamid lizards. – *Evolution* 37: 1075–1084.
- Hertz, P. E. et al. 1993. Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. – *Am. Nat.* 142: 796–818.
- Hijmans, R. et al. 2016. Package 'raster.' – R package ver. 3.0-7. <<https://CRAN.R-project.org/package=raster>>.
- Huey, R. B. 1991. Physiological consequences of habitat selection. – *Am. Nat.* 137: S91–S115.
- Huey, R. B. et al. 2003. Behavioral drive versus behavioral inertia in evolution: a null model approach. – *Am. Nat.* 161: 357–366.
- Huey, R. B. et al. 2009. Why tropical forest lizards are vulnerable to climate warming. – *Proc. Biol. Sci.* 276: 1939–1948.
- Ibargüengoytia, N. R. 2005. Field, selected body temperature and thermal tolerance of the syntopic lizards *Phymaturus patagonicus* and *Liolaemus elongatus* (Iguania: Liolaemidae). – *J. Arid Environ.* 62: 435–448.
- James, G. et al. 2013. An introduction to statistical learning. – Springer.
- Kearney, M. R. 2013. Activity restriction and the mechanistic basis for extinctions under climate warming. – *Ecol. Lett.* 16: 1470–1479.
- Kearney, M. R. and Porter, W. 2009. Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. – *Ecol. Lett.* 12: 334–350.
- Kearney, M. R. et al. 2014. Microclim: global estimates of hourly microclimate based on long-term monthly climate averages. – *Sci. Data* 1: 140006.
- Kirchhof, S. et al. 2017. Thermoregulatory behavior and high thermal preference buffer impact of climate change in a Namib Desert lizard. – *Ecosphere* 8: e02033.
- Kubisch, E. L. et al. 2016. Local extinction risk of three species of lizard from Patagonia as a result of global warming. – *Can. J. Zool.* 94: 49–59.
- Lara-Reséndiz, R. A. et al. 2015. Thermoregulation of two sympatric species of horned lizards in the Chihuahuan Desert and their local extinction risk. – *J. Therm. Biol.* 48: 1–10.
- Liaw, A. and Wiener, M. 2002. Classification and regression by randomForest. – *R News* 2: 18–22.
- Licht, P. 1965. The relation between preferred body temperatures and testicular heat sensitivity in lizards. – *Copeia* 1965: 428–436.
- Logan, M. L. et al. 2014. Natural selection on thermal performance in a novel thermal environment. *Proc. Natl Acad. Sci. USA* 111: 14165–14169.
- Logan, M. L. et al. 2015. Abiotic constraints on the activity of tropical lizards. – *Funct. Ecol.* 29: 694–700.
- Medina, M. et al. 2016. Vulnerability to climate change of *Anolis allisoni* in the mangrove habitats of Banco Chinchorro Islands, Mexico. – *J. Therm. Biol.* 58: 8–14.
- Nasrabadi, R. et al. 2018. The effects of climate change on the distribution of European glass lizard *Pseudopus apodus* (PAL-LAS, 1775) in Eurasia. – *Ecol. Res.* 33: 199–204.

- Paradis, E. et al. 2004. APE: analyses of phylogenetics and evolution in R language. – *Bioinformatics* 20: 289–290.
- Paranjpe, D. A. et al. 2013. Evidence of maternal effects on temperature preference in side-blotched lizards: implications for evolutionary response to climate change. – *Ecol. Evol.* 3: 1977–1991.
- Piantoni, C. et al. 2016. Vulnerability to climate warming of four genera of New World iguanians based on their thermal ecology. – *Anim. Conserv.* 19: 391–400.
- Pontes-da-Silva, E. et al. 2018. Extinction risks forced by climatic change and intraspecific variation in the thermal physiology of a tropical lizard. – *J. Therm. Biol.* 73: 50–60.
- Porter, W. P. et al. 1973. Behavioral implications of mechanistic ecology. – *Oecologia* 13: 1–54.
- Ridgeway, G. 2007. Generalized boosted models: a guide to the gbm package. – Update 1: 2007.
- Rodrigues, M. T. 1987. Sistemática, ecologia e zoogeografia dos *Tropidurus* do grupo *Torquatus* ao sul do Rio Amazonas (Sauria, Iguanidae). – *Arq. Zool.* 31: 105.
- Senay, S. D. et al. 2013. Novel three-step pseudo-absence selection technique for improved species distribution modelling. – *PLoS One* 8: e71218.
- Sinervo, B. and Adolph, S. C. 1994. Growth plasticity and thermal opportunity in *Sceloporus* lizards. – *Ecology* 75: 776–790.
- Sinervo, B. et al. 2010. Erosion of lizard diversity by climate change and altered thermal niches. – *Science* 328: 894–899.
- Sinervo, B. et al. 2018. Climate change, thermal niches, extinction risk and maternal-effect rescue of toad-headed lizards, *Phrynocephalus*, in thermal extremes of the Arabian Peninsula to the Qinghai–Tibetan Plateau. – *Integr. Zool.* 13: 450–470.
- Urban, M. C. et al. 2016. Improving the forecast for biodiversity under climate change. – *Science* 353: aad8466.
- Walther, G.-R. et al. 2002. Ecological responses to recent climate change. – *Nature* 416: 389–395.
- Wiederhecker, H. C. et al. 2002. Reproductive ecology of *Tropidurus torquatus* (Squamata: Tropiduridae) in the Highly Seasonal Cerrado Biome of Central Brazil. – *J. Herpetol.* 36: 82–91.

Supplementary material (available online as Appendix oik-07123 at <[www.oikosjournal.org/appendix/oik-07123](http://www.oikosjournal.org/appendix/oik-07123)>).  
Appendix 1.