

# Ecophysiological variation across a forest-ecotone gradient produces divergent climate change vulnerability within species

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**ABSTRACT**

Climate change related risks and impacts on ectotherms will be mediated by habitats and their influence on local thermal environments. While many studies have documented morphological and genetic aspects of niche divergence across habitats, few have examined thermal performance across such gradients and directly linked this variation to contemporary climate change impacts. In this study, we quantified variation in thermal performance across a gradient from forest to gallery forest-savanna mosaic in Cameroon for a skink species (*Trachylepis affinis*) known to be diverging genetically and morphologically across that habitat gradient. Based on these results, we then applied a mechanistic modelling approach (NicheMapR) to project changes in potential activity, as constrained by thermal performance, in response to climate change. As a complimentary approach, we also compared mechanistic projections with climate-driven changes in habitat suitability based on species distribution models of forest and ecotone skinks. We found that ecotone skinks may benefit from warming and experience increased activity while forest skinks will likely face a drastic decrease in thermal suitability across the forest zone. Species distribution models projected that thermal suitability for forest skinks in coastal forests would decline but in other parts of the forest zone skinks are projected to experience increased thermal suitability. The results here highlight the utility of mechanistic approaches in revealing and understanding patterns of climate change vulnerability which may not be detected with species distribution models alone. This study also emphasizes the importance of intra-specific physiological variation, and habitat-specific thermal performance relationships in particular, in determining warming responses.

## INTRODUCTION

Dramatic consequences of recent environmental warming have been observed including local extinctions (Sinervo et al. 2010; Wiens 2016) and distribution shifts towards higher altitudes and latitudes (Chen et al. 2011). As climates continue to shift, the probability of such outcomes can vary depending on the characteristics of the environment occupied. The general trend for ectotherms is that species from more thermally variable environments show greater physiological tolerance to warming, hence lower vulnerability to climate change, relative to those living in more stable or homogeneous conditions (Deutsch et al. 2008; Bonebrake and Deutsch 2012; Sunday et al. 2012). Amplifying this effect is the fact that ectotherms adapted to low variability environments tend to be found in warm tropical regions, where they are already operating close to their optimal temperatures relative to those in variable environments, rendering them more likely to exceed thermal limits with warming (Deutsch et al. 2008). Similarly, and perhaps as an alternative to temperature variability as a driver of thermal performance breadth, warm-adapted species in general will tend to exhibit rapidly declining performance under warmer temperatures due to the exponential relationship between body temperature and rates of biological functions such as metabolism (Payne and Smith 2017).

Habitat plays a primary role in determining thermal environments and could also therefore influence the thermal performance of species within those habitats. For terrestrial ectotherms, species from open habitats are generally better adapted to hot environments than species from covered or forested habitats, especially in the tropics (Huey et al. 2009; Frishkoff et al. 2015; Bonebrake et al. 2016). Following this expectation, Huey et al. (2009) predicted that open habitat species might disperse towards cooler forests to cope with

climate warming, resulting in competition with the forest species which have no other refuge to seek as they approach their own upper thermal limits. Smaller scale microhabitats also play crucial roles in mediating and potentially buffering large scale climatic impacts on species (Scheffers et al. 2014).

From a palaeobiological perspective, there is evidence that ancient climate change has been an important driver in species diversification (Carstens and Knowles 2007; Pepper et al. 2011). One dominant process by which this can occur is niche divergence between populations as they adapt to new adjacent environments (Hua and Wiens 2013). In many tropical regions, habitat gradients range from rainforest to a forest-savanna transitional zone, and are important regions for studying this evolutionary phenomenon (Smith et al. 1997). Species inhabiting rainforests can also have representative populations in the gallery forests of these transitional zones, thus creating optimal conditions for niche divergence and local adaptation. In fact, there is mounting evidence that niche divergence is occurring across this ecological gradient, such as in birds and skinks in Central Africa (Smith et al. 1997; Freedman et al. 2010; Kirschel et al. 2011; Smith et al. 2011), skinks in Australia (Schneider et al. 1999) and anoles in the Caribbean (Thorpe et al. 2008).

Most studies examining adaptation across habitat gradients have focused on variation in morphological traits. While there are many abiotic and biotic factors which could be driving divergence, the thermal environment remains one of the main contrasts between the extremes of this gradient potentially limiting population ranges (Kearney and Porter 2009; Keller and Seehausen 2012). Accordingly, local adaptation may involve divergence in thermal physiology across habitats due to contrasting thermal regimes (Phillips et al. 2015). In rainforests, temperature is generally less variable, with more recurrent and consistent

precipitation than in the more open gallery forests of the rainforest-savanna transition zone (Longman and Jenik 1992). We could thus expect an intraspecific difference in thermal suitability between these habitats (Gunderson and Leal 2012).

Native to Central Africa, *Trachylepis affinis* is a widely distributed skink species for which there is known significant genetic and morphological divergence across the gradient spanning from rainforest to the forest-savanna transition zone, which we refer to as ecotone (Freedman et al. 2010). In this context, we expect there to also be divergence in thermal suitability, which could intensify or buffer the impacts of climate change. We first quantified thermal physiology, with performance as a function of body temperature (Angilletta 2009), for skinks from both habitat types. To estimate warming vulnerability we then applied a mechanistic modelling approach (Pacifi et al. 2015) to project potential activity time throughout the country and examine habitat-specific climate change vulnerability (Kearney and Porter 2004). Finally, we compared these results to species distribution models of *T. affinis* for forest and ecotone populations.

## MATERIALS AND METHODS

### *Study sites and specimen collection*

The Republic of Cameroon has an extensive latitudinal range, with an environmental gradient ranging from central rainforest in the south, to ecotone in the middle section, and eventually to savannah in the north (Supplementary material Appendix 1, Fig. A1; Smith et al. 1997; Chirio and LeBreton 2007). Across this gradient, temperature variability tends to be higher and precipitation lower towards the ecotone relative to the rainforest (Supplementary material Appendix 1, Fig. A2). Within the rainforest regions, temperatures are cooler in the Western Highlands. Temperatures also begin to cool to the northern extent

of the ecotone where there is a rise in elevation due to the Adamawa plateau. However, the country's most extreme climate lies in the dry savanna beyond this point, where temperatures are the warmest and most variable (Longman and Jenik 1992; Chiro and LeBreton 2007).

The distribution of *T. affinis* covers most of the southern forested portion of Cameroon and extends into the ecotone, yet rarely reaches beyond the Adamawa plateau (Chirio and LeBreton 2007). In the ecotone, this species is only present in the interspersed gallery forests, where the overall forest structure is similar to that of the rainforest but with a lower canopy. For this study, we had four sites: two in the ecotone and two in the rainforest (Supplementary material Appendix 1, Fig. A1). Due to the more dispersed distributions of the ecotone lizards, we encountered and collected fewer individuals during our trips to these sites relative to those in the forest. We collected live individuals by hand and ran experiments for this study on two separate field trips. From June to July of 2015, we sampled one forest (Kribi) and one ecotone site (Bazzama), and from March to April of 2016 we sampled another forest (Malimba) and ecotone site (Doume). We conducted both sampling periods during the same season, when mean temperature and precipitation were similar (Supplementary material Appendix 1, Fig. A2). Once caught, we weighed and measured the lizards, and housed them individually in an isolation room kept at a constant 25°C, where we fed them with *Hermetia illucens* larvae and water *ad libitum*.

### *Thermal physiology*

To quantify the thermal physiology of the skinks, we first let the lizards acclimate for a minimum of 72 hours. We then ran experiments to obtain raw data to model their thermal performance curves, which serve as an indication of fitness as a function of body

temperature (Angilletta 2009). For this, we needed to measure their maximum running speeds at different body temperatures, as well as their critical thermal limits to delimit the curves.

For the running speed experiments, we placed lizards in an environmental chamber (Percival® model I-36VL) at given body temperatures for at least 30 minutes each, and then coerced them to run along a 2 meter wooden track marked with 25 cm intervals. By recording the runs using a DSLR camera (D3100, Nikon, Tokyo, Japan), we analyzed each video, using the Avidemux 2.6 ([www.avidemux.org](http://www.avidemux.org), 2009) software to determine the fastest 25 cm interval. Each individual ran once at each of seven body temperatures, with two trials per day split by a break lasting at least three hours. All lizards followed the same randomized sequence (32°C, 25°C, 22°C, 12°C, 35°C, 17°C, and 29°C).

We conducted critical thermal limit experiments on the day following the last running trial, for which we measured the critical thermal minimum, the *CTmin*, and maximum, the *CTmax* (Angilletta 2009). For each lizard, we first measured the *CTmin*, followed by a four hour break before looking at the *CTmax*. We set the initial body temperature of the skinks at 10°C for the *CTmin*, and 32°C for the *CTmax*, for 30 minutes. Then, for the *CTmin*, we gradually decreased temperature at a rate of 0.7°C per minute by setting the environmental chamber to 0°C. Once the incubator reached 9.0°C, lizards were tested for a loss of righting reflex (Angilletta et al. 2002) at approximately every 60 seconds. Once this ability of righting was lost, we measured temperature with an infrared thermometer (Smart Sensor®, model AR350+), marked it as the *CTmin*, and placed the lizard in an ambient temperature water bath until it was back up and alert. The same methodology was applied for the *CTmax*, except that we gradually increased temperatures at a rate of 0.7°C per minute, and

checked for the loss of righting ability in an individual when they started to show abnormal movements such as jerking, lost control of limbs, gaping or difficulties propping the body upwards.

To model the thermal performance curves, we fit six non-linear functions (Supplementary material Appendix 1, Table A1; Landry Yuan et al. 2016) yielding the typical, unimodal left skewed shape (Angilletta 2006), and including mean  $CT_{min}$  and  $CT_{max}$  values in their equations, to the mean running speed data for each habitat group. We compared Akaike's information criterion (AICs) to determine the most suitable model overall for both groups of lizards based on the lowest score, with a difference of less than 2 being non-significant (Burnham and Anderson 2002; Angilletta 2006). Once we found an appropriate non-linear model, we applied it to the performance and critical thermal limit data for every individual in each group. We then calculated the maximum performance ( $P_{max}$ ) and thermal optima ( $T_{opt}$ ) for both ecotone and forest skinks, as well as their body temperature ranges at which 50%, 80% and 90% of  $P_{max}$  is attained, termed the  $B_{50}$ ,  $B_{80}$  and  $B_{90}$  performance breadths, respectively (Hertz et al. 1983).

We tested for significant differences in thermal physiology between habitats by applying t-tests to compare  $P_{max}$ ,  $T_{opt}$ , as well as ranges, lower and upper limits of the performance breadths. We also compared  $CT_{min}$  and  $CT_{max}$  across habitats by applying Wilcoxon's tests. All statistical analyses were implemented in R (R Core Team 2013).

#### *Estimating and comparing thermal suitability*

To gain a complete understanding of the spatial extent of thermal suitability, we incorporated our data on thermal physiology into NicheMapR, an R implementation of Niche Mapper<sup>TM</sup> (Porter et al. 1973; Kearney and Porter 2017). This is a mechanistic model



comprised of two principle functions, with the first being a “microclimate” function which defines the meteorological conditions of a specific microhabitat, as determined by its adjustable environmental parameters. To evaluate microclimates, we applied a global climate data set developed by New et al. (New et al. 1999, 2002; as described in Kearney and Porter 2017) as input for the “microclimate” function to convert this monthly global climate data to hourly estimates at a ~15 km spatial resolution (Kearney et al. 2014). The resulting output is a set of microclimatic variables over time (including wind speed, humidity, zenith angle of the sun, solar radiation and air temperature), which serves as input into the second part of the model, the “ectotherm” function, to calculate temperatures the lizard would experience over time. This is a biophysical model comprised of a set of energy balance equations and behavioral routines which consider heat transfer processes such as radiation, convection and conduction, to predict an organism’s physical relationship with its microhabitat environment. Ultimately, by parameterizing the function with data on physiology, behavior and morphology, we can employ the “ectotherm” function to estimate field body temperatures and potential activity times within thermal constraints for a given organism.

To run the model for each habitat group, we first set the “microclimate” function to run at a time interval of 365 days for one year. We set the soil type to “loam”, which is representative of the substrate in both the ecotone and forest sites. We set a maximum shade cover of 100%, and minimum shade cover of 15%, which represents a threshold for the presence of *T. affinis* at a 3 km average of 1 km pixel images (Freedman et al. 2010; unpublished data). Since this is a terrestrial species which does not typically climb trees, we

set the local height at 0.5 cm, the approximate mid-point of a typical adult individual of *T. affinis* on the ground.

We then parameterized the “ectotherm” function with the data obtained from the thermal performance curves (Supplementary material Appendix 1, Table A2; Kearney and Porter 2009), where we set the voluntary thermal minimum and maximum ( $VT_{min}$  and  $VT_{max}$ ) in the model according to the  $B_{95}$  performance breadth, which has been described by Hertz et al. (1983) to reflect the thermal optimum range. These values represent the thermal limits within which diurnal activity is possible. We used the lower  $B_{80}$  limit as the minimum basking temperature on the basis that this species is active all year round and that the values we found for the lower  $B_{80}$  were near the coolest temperatures these skinks would experience in their native habitats. There can thus be occasions where basking is possible despite inactivity. Similarly, we used the lower  $B_{50}$ , a slightly cooler measure, as the minimum temperature of emergence. Finally, we used  $T_{opt}$  as the preferred temperature during activity or inactivity, which would be maintained via shade seeking and burrowing given the adequate conditions. While  $T_{opt}$  may not precisely represent the preferred temperature (Huey and Bennett 1987; Gunderson and Leal 2016), we decided to continue with this assumption keeping in mind that we are testing for a difference in thermal constraints on activity based on thermal performance curves. For morphological traits, we set the mass according to the mean values we measured for the skinks collected and set the organism shape to the default lizard shape. In sum, we considered lizards to be active if it was daylight, if they could emerge, and if they could maintain body temperatures within their  $B_{95}$  limits by shuttling between sun and shade. We then ran the entire model for each geographic coordinate locality in Cameroon across a ~15 km grid, which is at a sufficiently

fine scale to define the rainforest to ecotone transition. The activity budget evaluation is a built-in function of the ectotherm model that produces an output for any number of days in a year as set by the user. Here, we set the model to run for all 365 days in a year, and went one step further by calculating the potential hours of diurnal activity time in hours per year. We employed this value as an estimate of thermal suitability over space and time (Kearney and Porter 2004; Kearney et al. 2008) because restriction of activity, such as that crucial for survival and reproduction, is likely to have a bigger role in species extinction rates than lethal thermal stress in reptiles (Sinervo et al. 2010; Gunderson and Leal 2016). Next, using ArcGIS, we projected this output onto a map for proper visualization of the geographic extent of thermal suitability (Kearney and Porter 2004).

#### *Climate change impacts on thermal suitability*

To estimate the shift of each group's thermal suitability throughout Cameroon with climate change by the year 2070, we applied both a mild (RCP2.6) and a severe (RCP8.5) climate change scenario according to the representative concentration pathways described by the IPCC (Pachauri et al. 2014). We obtained this data from WorldClim, from which we downloaded the current monthly averages of maximum and minimum temperatures at a 10 arc-min resolution (Hijmans et al. 2005), and then again for the future under the RCP2.6 and RCP8.5 emission scenarios (from the CCSM4 global circulation model). For each of these data sets, we first took the average of the 12 monthly averages for both the minimum and maximum temperatures. We then subtracted the current values from the future values for both RCP2.6 and RCP8.5 scenarios to obtain the projected change in average minimums and maximums throughout Cameroon. From this, we obtained a total of 4 values, an average minimum and maximum for each of the two emission scenarios. For

both the mild and severe scenarios, we modified the “microclimate” function by adding the calculated averages to the minimum and maximum temperatures to the original model inputs (Kearney et al. 2008; Mitchell et al. 2008). We then projected the new set of potential activity times spatially. For both climate change scenarios, and for both skink groups, we subtracted the current from the predicted map, yielding estimated changes in potential activity times spatially.

### *Correlative ecological niche modelling*

In order to compare our thermal suitability estimates against spatial distributions of *T. affinis* (Supplementary material Appendix 1, Fig. A1), we applied a correlative ecological niche modelling approach to predict the habitat suitability of ecotone and forest skinks throughout Cameroon in both current and future environmental conditions (Kearney and Porter 2009). This method differs from the mechanistic approach we have taken using Niche Mapper since the former does not explicitly take into account the physiology of the organisms and its resulting interactions with its environment (Kearney et al. 2010). We thus apply both strategies to obtain complementary predictions of climate change impacts on the distribution of *T. affinis* populations based on presence data and thermal physiology data.

For the ecological niche modeling, we have applied the model ensemble approach implemented in the “biomod2” R package (Thuiller et al. 2009) to presence data collected for this species across Cameroon between 2001 and 2005, which represent 18 and 91 locality points in the ecotone and forest, respectively, with duplicates removed (Supplementary material Appendix 1, Fig. A1). For both current and future distribution modeling, we used a set of 19 bioclimatic variables downloaded from WorldClim at a 30 arc-sec resolution (Hijmans et al. 2005). Before modeling current and future projections,

we formatted the presence data using the ‘random’ algorithm in “biomod2” to generate a set of pseudo-absence points matching the number of presence points. We then cross-validated a set of 8 models (Artificial Neural Network, Generalized Boosting Model, Generalized Linear Model, Flexible Discriminant Analysis, Random Forest, Classification Tree Analysis, Multiple Adaptive Regression Splines and Surface Range Envelop) over 10 runs, setting aside 50% of the data for calibration and 50% for testing, while applying the True Skill Statistic (TSS) and Relative Operating Characteristic (ROC) methods to evaluate their accuracy. We then built a total of 14 ensembles by a total consensus approach, where the ‘em.by’ argument of the “BIOMOD\_EnsembleModeling” function is set to ‘all’, from which we projected the habitat suitability of skinks from each the forest and ecotone throughout Cameroon. These projections were then combined to yield a final general ensemble projection map for each skink group. For the future distribution modelling under climate change, and for comparisons with our mechanistic estimates under a high carbon emissions scenario, we used predictions for 2070 under the RCP 8.5 emissions scenario, as generated by three global circulation models (CCSM4, MIROC5 and HadGEM2) assumed to not be co-varying (Garcia et al. 2012; Melillo et al. 2014).

## RESULTS

### *Thermal physiology*

We collected and completed experimentation on 7 individuals from the ecotone sites (4 from Bazzama and 3 from Doume), with a mean mass of  $8.57 \pm 0.92$  g (mean  $\pm$  SE) and mean snout-to-vent length of  $70.65 \pm 1.99$  mm, as well as on 29 individuals from the forest sites (14 from Kribi and 15 from Malimba), with a mean mass of  $7.08 \pm 0.52$  g and mean snout-to-vent length of  $65.45 \pm 1.75$  mm.

To shape our thermal performance curves, we found the “double exponential” (Huey and Stevenson 1979) function to best fit the data for both ecotone and forest skinks overall, for which the AIC differed by less than 2 units from the lowest score for both groups (Supplementary material Appendix 1, Table A1).

Comparing across habitats, we found a warmer-shifted curve for the ecotone skinks (Fig. 1; Supplementary material Appendix 1, Table A3). Although  $P_{max}$  values were equivalent ( $t = -1.23$ ,  $p = 0.2$ ), ecotone skinks show a  $T_{opt}$  3.1°C warmer than that for the forest ( $t = -2.8$ ,  $p = 0.01$ ). Furthermore, both  $B_{80}$  and  $B_{95}$  performance breadths were significantly warmer in the ecotone group. We found the lower  $B_{80}$  limit to be warmer by 2.7°C ( $t = -2.48$ ,  $p = 0.02$ ), the upper  $B_{80}$  by 2.5°C ( $t = -2.95$ ,  $p = 0.01$ ), the lower  $B_{95}$  by 3.1°C ( $t = -2.74$ ,  $p = 0.01$ ), the upper  $B_{95}$  by 2.9°C ( $t = -2.87$ ,  $p = 0.01$ ), and the upper  $B_{50}$  by 1.8°C ( $t = -3.04$ ,  $p = 0.01$ ). We also found performance breadth ranges to be equal for the  $B_{50}$  ( $t = -0.38$ ,  $p = 0.71$ ),  $B_{80}$  ( $t = 0.19$ ,  $p = 0.85$ ) and  $B_{95}$  ( $t = 0.44$ ,  $p = 0.67$ ).

We detected a significantly warmer  $CT_{max}$  for the ecotone skinks ( $W = 150$ ,  $p = 0.05$ ) with a mean of  $41.9 \pm 0.4^\circ\text{C}$  for the ecotone, and of  $41.0 \pm 0.2^\circ\text{C}$  for the forest. On the other hand, while the ecotone skinks show a slightly cooler  $CT_{min}$  than the forest skinks, with a mean value of  $8.9 \pm 0.6^\circ\text{C}$  for the former and  $9.6 \pm 0.3^\circ\text{C}$  for the latter, this difference was not significant ( $W = 66.5$ ,  $p = 0.17$ ).

#### *Estimating and comparing thermal suitability*

Overall, skinks from both habitats show thermal suitability ranges which greatly overlap in space, although forest skinks show more potential activity time than their ecotone counterparts in most of these areas (Fig. 2). The lower activity times shown for the ecotone skinks appear to be driven by body temperatures dropping below cold thermal constraints,

especially from June to September (Supplementary material Appendix 1, Figs. A3-A6). It is also clear that the spatial extent of thermal suitability in both groups is constrained by the northern limits of the ecotone areas, where there is a transition to the elevated savanna of the Adamawa plateau, which is too cool. To the north of this elevated region, the environment is characterized as dry savanna (Chirio and LeBreton 2007), where the climate is too hot and lacks the gallery forests typically inhabited by this species. However, the ecotone skinks do fare slightly better in this more extreme environment due to their warmer thermal limit range. To the west, there is additional cold-induced lower thermal suitability in the areas corresponding the Western Highlands and Mount Cameroon (4,095 m; Chirio and LeBreton 2007). Thermal suitability also drops, yet to a lesser extent than in the mountains, in the lower central part of the country, which is consistent with the fact that *T. affinis* is seldom known in these areas, particularly inland eastward from Mbongwana (site 2 in Freedman et al. 2010). This correlates with a change in vegetation, since the forest in this area has been described as semi-deciduous, in contrast to the wet evergreen forests along the western coast (Chirio and LeBreton 2007) where thermal suitability is high for the forest skinks and highest for the ecotone skinks.

#### *Climate change impacts on thermal suitability*

We found little change in thermal suitability distributions under the mild RCP 2.6 warming scenario (Fig. 2; average activity time increase of 2.2% for ecotone and decrease of 3.5% for forest skinks across Cameroon). However, forest skink thermal suitability decreased slightly in the north and western coast of Cameroon, yet slightly increased in Western Highlands and central semi-deciduous forests. On the other hand, ecotone skink thermal suitability appears to be slightly increasing throughout their native regions. With

the more severe RCP 8.5 warming scenario, the difference between the two skink groups is much more distinct (Fig. 2; average activity time increase of 1.5% for ecotone and decrease of 15.8% for forest skinks across Cameroon). Aside from in the Western Highlands, the Adamawa plateau, and in the central part of the semi-deciduous forest, thermal suitability drastically decreases throughout Cameroon for the forest skinks, specifically at mid-day (Supplementary material Appendix 1, Figs. A3 and A4). This contrasts the pattern shown by the ecotone skinks, where thermal suitability increases across both forest and ecotone throughout most months of the year (Supplementary material Appendix 1, Figs. A3 and A4).

#### *Correlative ecological niche modelling*

The current distributions for *T. affinis* from the ecological niche modelling show more restricted optimal ranges compared to the thermal suitability range estimates, with the ecotone and forest skinks showing high habitat suitability in only the ecotone and forest regions, respectively (Fig. 3). Here, it appears that annual precipitation strongly influenced the current forest skink projection, whereas annual precipitation and temperature seasonality moderately influenced the current ecotone skink projection (Supplementary material Appendix 1, Fig. A7). Under climate change, the ecotone skinks are predicted to see a strong decrease in habitat suitability in the ecotone regions (Fig. 3) which is contrary to the thermal suitability projections (Fig. 2), and are not predicted to see any major changes in the forest regions. For forest skinks there are slight similarities with the thermal suitability results, especially in the coastal forest regions to the west of the country, where we generally see a moderate decrease in habitat suitability (Fig. 3). However, unlike projections under the thermal suitability models, in other areas of Cameroon such as the



ecotone and the eastern forests, the ecological niche modelling shows increases in habitat suitability.

## DISCUSSION

By applying a mechanistic modelling approach to further investigate differences in thermal performance within *T. affinis*, we have detected a clear contrast in climate change implications between groups from two different habitats. We mapped the spatial extent of thermal suitability for *T. affinis* from the ecotone and forest habitats, from which we have estimated that under a high carbon emissions scenario, climate change could have a strong negative impact on forest skinks in Cameroon. Interestingly, the forest regions where there is the strongest predicted decrease in activity for the forest skinks (Fig. 2) is where we find the highest current habitat suitability, which also decreases slightly in future projections (Fig. 3). While the correlative approach offers insight on the direction of potential distribution shifts, the mechanistic approach we have applied scrutinizes the thermal physiological process by which current distributions are limited and how these range shifts are driven (Kearney et al. 2010; Mathewson et al. 2017). In this respect, this comparison demonstrates that activity loss may very well be an important challenge to be faced by these forest skinks under future warming. On the other hand, we found the opposite pattern for the ecotone skinks, for which we estimate thermal suitability to vastly increase throughout the forest and ecotone regions of Cameroon. This contrast in thermal suitability we describe is mostly driven by the 2.9°C difference in upper  $B_{95}$  limits, which we used as input for  $VT_{max}$  in NicheMapR. Therefore, our results demonstrate that forest populations could face significant thermal challenges under future warming.

In contrast to forest skinks, our results also suggest that, not only can ecotone skinks persist in their native habitat, but that they may have the potential to thrive in the rainforest zone. Accordingly, based on the fundamental niche, without taking into account any potentially restricting abiotic or biotic factors (Kearney and Porter 2009) currently limiting gene flow across the ecological gradient (Freedman et al. 2010), an invasion of rainforest habitats by ecotone skinks is possible under climate change. The underlying mechanism facilitating this invasion is changes to thermal conditions that favor the physiological adaptations of ecotone skinks, in line with more general predictions made by Huey et al. (2009).

While climate warming will likely be an important driver of changes to species' and populations' geographic ranges, the environment will also continue to change in ways not directly linked to warmer air temperatures. Since the beginning of the century, forest loss has been continuously recorded across the central forests of Cameroon, especially in the western half of the country (Hansen et al. 2013), where we predict the change in thermal suitability to be the most negative for forest skinks. This would intensify the outcome we have predicted based on thermal suitability in two ways. First, with ongoing deforestation, the forest habitats could become structurally and biologically more similar to the ecotone, hence more suitable for ecotone skinks in aspects beyond thermal physiology alone and more likely to host their future immigration (Freedman et al. 2009). Second, activity restriction imposed by loss of canopy cover and shade, rather than climate change, could be a bigger driver of extinction (Kearney 2013), meaning that forest skinks could be at a greater risk of disappearing than what is inferred from our thermal suitability-based projections alone.

Overall, the extent of an invasion of rainforest habitats by ecotone skinks, and its evolutionary consequences, will fall along a continuum of outcomes that depend upon 1) the interplay between climate change, deforestation, and currently undocumented biotic factors, 2) the heritability of thermal physiology traits, and 3) whether any reproductive isolation exists between rainforest and ecotone populations. If ecotone skinks are maladapted to rainforest habitats regardless of increasing thermal suitability, rainforest skinks will only persist if able to adapt to warming conditions. While it could be argued that rainforest skinks could persist over time via adaptive evolution of thermal physiology traits, it is a seldom documented phenomenon (Urban et al. 2014) involving genetic variation, population dynamics and multiple generations, which would require further work to better understand and apply to this case (Chown et al. 2010). Alternatively, gene flow between invading ecotone skinks and resident rainforest ones could transfer adaptive genetic variation between them: variation increasing fitness under future warming and habitat alteration into rainforest populations, and variation adaptive for current rainforest habitat into invading ecotone populations. Such cases of adaptive introgression are well known to occur across species boundaries where some degree of reproductive isolation has already evolved (Pardo-Diaz et al. 2012; Racimo et al. 2015).

Assuming that thermal tolerance in ectotherms is often thought to have a genetic basis (Angilletta 2009; Hoffmann et al. 2013), such introgression would be pronounced for genomic regions containing genes influencing thermal physiology traits. Previously, strong genetic and morphological evidence for adaptive divergence across the rainforest-savanna gradient was presented for *T. affinis* (Freedman et al. 2010), supporting a genetic basis for at least some adaptations. Given the importance of thermal regimes to ectotherm fitness,

one might expect that the differences in thermal physiology between rainforest and ecotone habitats should have a heritable component, even if the degree of that heritability is unknown. In sum, contact between these skink populations within the rainforest zone will be the outcome of complex evolutionary dynamics characteristic of hybrid zones (Harrison and Larson 2016), potentially leading to panmixia on one extreme, replacement of rainforest lineages by ecotone ones on the other, or some intermediate case where forest and ecotone lineages co-occur but remain distinct in the face of gene flow.

Plasticity could potentially play an even greater role in mitigating the consequences of warming (Chown et al. 2010; Urban et al. 2014; Llewelyn et al. 2016). For example, in this study, it is possible that the magnitude of the impact we have estimated for both habitat groups would have been less severe if we acclimated the lizards at a warmer temperature before running our experiments (Pintor et al. 2016). This is would be driven by the capacity for rapid short-term acclimation in *CT<sub>max</sub>* to warmer temperatures, termed heat-hardening, which has been observed in ectotherms in previous studies (Overgaard et al. 2011; Phillips et al. 2015). Nonetheless, there is evidence that plasticity in the upper thermal tolerance is rather limited and perhaps unable to buffer the negative effects of climate change (Hoffmann et al. 2013; Gunderson and Stillman 2015).

Despite these predictions, forest skinks are expected to benefit from climate change more than their ecotone counterparts in the Western Highlands of Cameroon (Fig. 2), a region in which they are currently known to inhabit (Chirio and Lebreton 2007). These elevated areas could serve as a refuge for forest populations. However, their potential as refugia for forest lineages is complicated by the fact that ecotone skinks are also projected to see an increase in thermal suitability in these higher elevations. Freedman et al. (2010)

did not find evidence for elevation-associated neutral population structure in *T. affinis*, instead detecting adaptive genetic divergence in a small proportion of loci. The absence of present-day population structure across the forest-montane gradient suggests that these loci alone may not be sufficient to preclude gene flow into Western Highlands populations from low elevation ones, let alone between lowland forest and ecotone populations becoming sympatric as they expand into higher elevation habitats. The degree of admixture and spatial population structure retained between these populations would likely depend upon their respective abilities to colonize higher elevation habitats, as well the degree of reproductive isolation resulting from adaptive differences in other traits such as morphology (McCormack and Smith 2008) or behavior (Ortega et al. 2016). However, considering the smaller size of Western Highlands region relative to the rest of *T. affinis*' range, our prediction that climate change will erode thermal suitability divergence across the forest-ecotone gradient overshadows the potential influence of the elevational gradient on the overall distribution of this species.

Given the nature of the projections we present here, there are ways in which such climate change vulnerability assessments can be improved or complemented. Here, we have focused on potential activity within thermal constraints, without directly assessing how far or near these limits the skinks are living. This could be adjusted by quantifying a direct measure of risk of exposure to unsuitable temperatures for both groups, such as thermal safety margins (Huey et al. 2009; Logan et al. 2013). Obtaining actual field operative temperature data from both open and shaded microhabitats at a much finer spatial scale than the New et al. (2002) data that we used could also provide stronger confidence in modeling outputs.

Furthermore, although we assume a reduction in activity time to have negative implications for mating and foraging opportunities (Sinervo et al. 2010), it is difficult to determine a threshold for the daily number of hours crucial for these behaviors. This would be made possible by first quantifying behavioral budgets in the field accounting for diel patterns, body temperature and climate. With such information, it would be possible to test for a difference in thresholds for population persistence between the forest and ecotone lizards. Another key component would be the mechanism by which potential activity time translates into behaviors actually performed. In a study on neo-tropical anoles, Gunderson and Leal (2016) developed a framework addressing this and found reproductive behavior to have a narrower thermal range than foraging. If applicable to all lizards, it would render our current thermal performance-based predictions more conservative, possibly implying more variation in thermal suitability across space, especially for the forest skinks, for which we found high current potential activity time in many regions.

There are other elements of the skinks' habitat and biology aside from temperature that may influence vulnerability to climate change, which may help explain differences in model predictions (Figs. 2 and 3). Precipitation, for example, appears to be an important driver for the current forest skink correlative model projections (Supplementary material Appendix 1, Fig. A7). While the mechanistic approach offers us insight on the specific role of thermal physiology in limiting distributions, the next step would be to develop a strategy to explore the reasons behind differences between our projections and actual distributions. One way to address this would be to isolate other potential factors which would be affected by climate change, such as prey-predator interactions, competition, resource availability or reproduction. Yet, considering the importance of behavioral thermal constraints in dictating

warming impacts (Sinervo et al. 2010; Gunderson and Leal 2016), mechanistic niche modelling remains an important first step in establishing baseline estimations of future distributions.

From this intrinsic approach, we project forest skinks will face a strong decrease in thermally suitable activity time throughout their distributions with oncoming climate change, whereas those from the ecotone would benefit, especially in the central forests. Despite these negative predictions for the forest skinks, our work highlights the importance of intra-specific variation in facilitating a species' resilience to environmental change. Accordingly, as a species, *T. affinis* does not seem to be under immediate risk of extinction due to climate change by the end of this century. These results thus hint towards the potential for the forest-ecotone gradient buffering global warming induced extinction risks, although this may come at the cost of genetic and population diversity.

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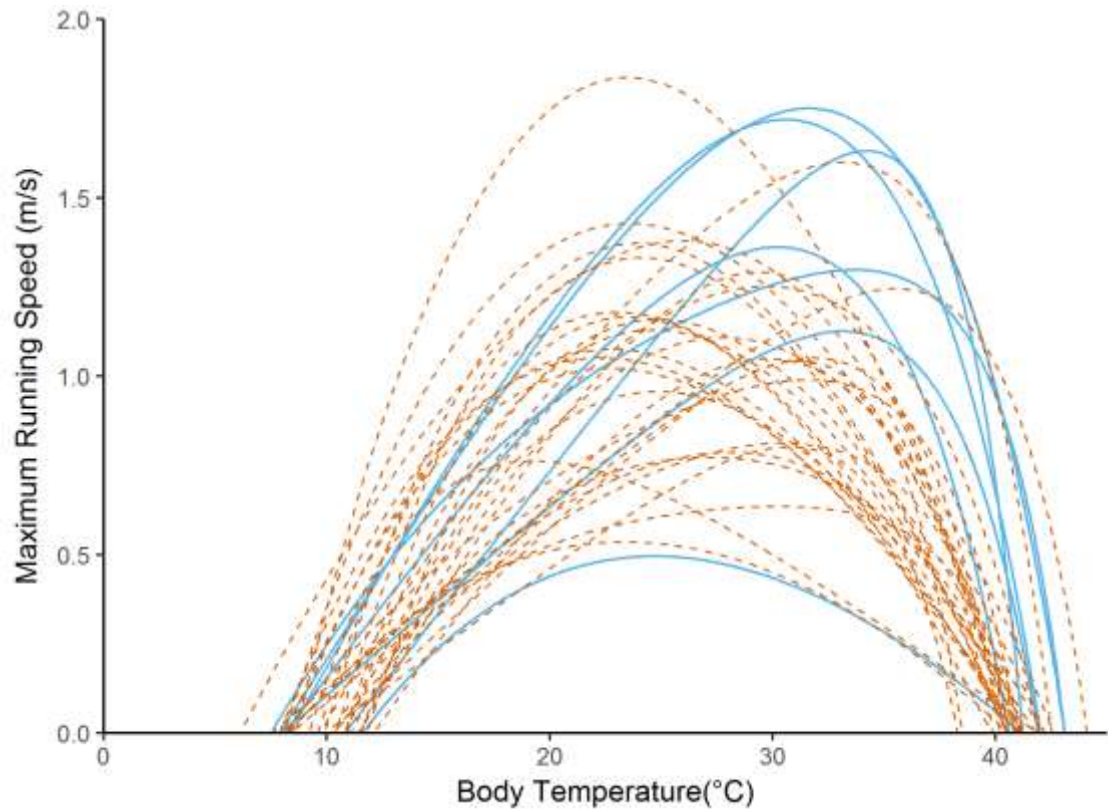
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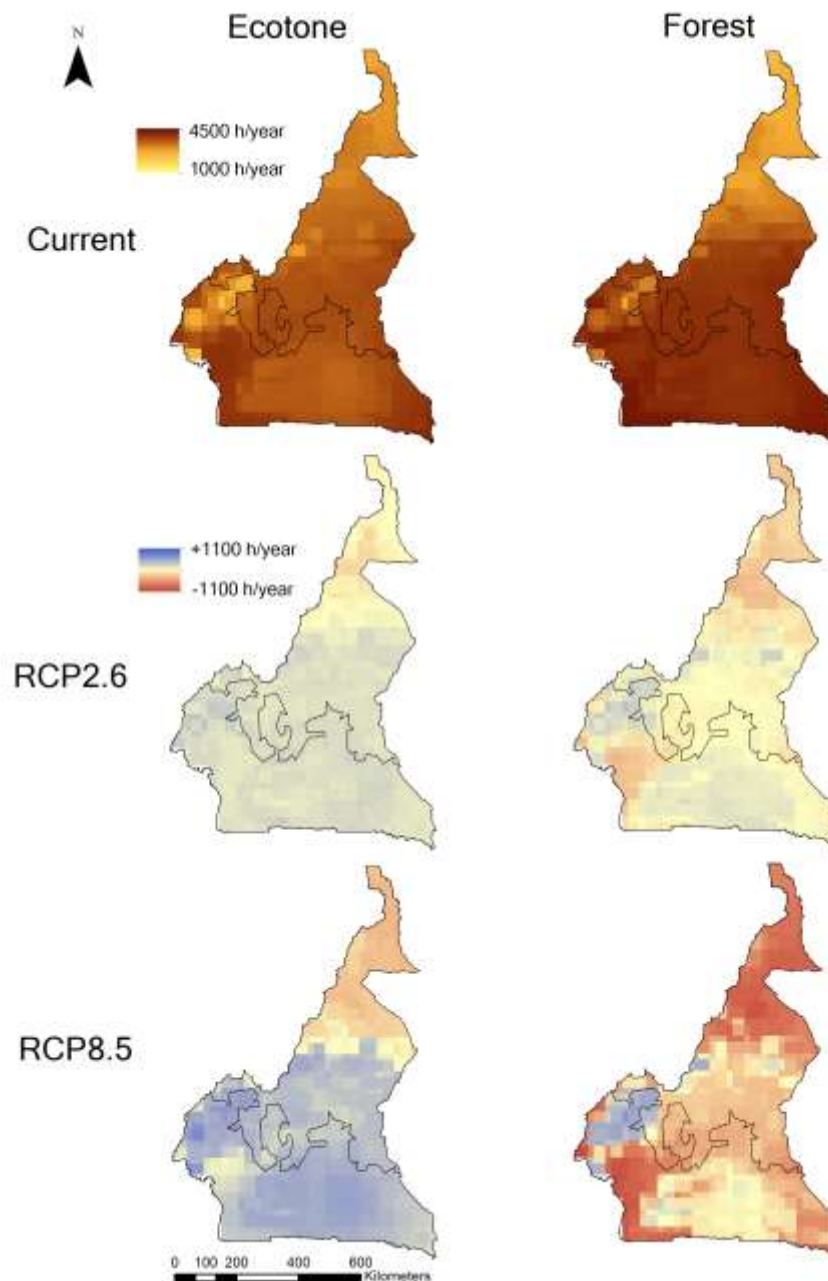
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# FIGURE LEGENDS

**Figure 1:** Thermal performance curves obtained from the chosen non-linear model applied to individual *T. affinis* skinks from the forest (red dotted lines) and ecotone (blue solid lines) habitats.



**Figure 2:** Maps in the top row depict current thermal suitability throughout Cameroon for *T. affinis* from the ecotone (left) and forest (right) populations, as estimated by potential diurnal activity time in a year (in h/year). Maps in the bottom row show projected changes in thermal suitability throughout Cameroon for *T. affinis* from the ecotone (left) and forest (right) populations under RCP2.6 (above) and RCP8.5 (below) emissions scenarios, as estimated by the change (+/-) in potential diurnal activity time in a year (in h/year). The black line marks the boundary between forest and ecotone.





**Figure 3:** Ecological niche modelling outputs depicting habitat suitability for *T. affinis* from the ecotone (top row) and from the forest (bottom row) throughout Cameroon. Current predictions are shown in the leftmost column, while future changes (+/-) for 2070 under an RCP8.5 emissions scenario are shown for three global circulation models in the other columns. The black line marks the boundary between forest and ecotone.

