

Lizards in the mist: thermal niches constrained by habitat and microclimates in the Andes of southern Bolivia

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Running title: Temperature and Habitat in Andean Lizards

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Abstract

Aim: To understand how the activity budgets of ectotherms vary in mountain areas with high microclimatic and habitat heterogeneity, and how these factors together with habitat modulate the assemblage of ectotherm communities.

Location; Tajzara Basin, Cordillera de Sama (3600–4300 masl), Department of Tarija, Bolivia

Taxon: four lizard species of the genus *Liolaemus*.

Methods: After parameterizing the thermal physiology of each species and recording operative temperature time series with dataloggers, we calculated activity budgets for every species across 30 sampling sites. By multimodel inference we evaluated how

activity budgets varied across the topography. We also assessed how occurrences of each species were predicted by activity budgets, restriction time, temperature deviation, habitat covers and exposure to cold microclimate.

Results: Activity budgets were not only influenced by elevation, but also by the exposure to cold air that comes through saddles from the eastern side of the mountain divide. Although thermal physiological parameters were relatively similar among the species, their abundances were conditioned to different extents by activity budgets, restriction time, habitat and microclimates.

Main conclusions: The abundance and distribution of each species across these lizard communities reflect the microclimatic heterogeneity originated by complex topography, which uncouples activity budgets from elevation. Only one species was confined to cold microclimate refugia with higher exposure to Föhn effect. Despite having relatively similar thermal aspect of fundamental niches, lizards may differ in their realized niches by habitat selection. Habitat specialization could compromise the persistence of some species under global warming by reducing the dispersal possibilities to certain areas where population sustainability might be impaired in the long term. Understanding the heterogeneity of microclimates and habitats and the physiology of the species partially explains their distribution at local scale, and provides insights on how to best confront upcoming climate change effects.

Keywords: activity budgets, cold air drainage, ectotherm, Föhn effect, fundamental niche, mountain microclimates, realized niche, restriction time, thermoregulation

Introduction

Global climate is being altered by a general rise of temperatures, caused by the increasing amounts of greenhouse gases that human society started producing massively in the nineteenth century (Solomon et al., 2007). Scientists are increasingly reporting evidence of climate change effects on biota (Pecl et al., 2017). Nevertheless, the evaluation of the impacts of climate change on organisms is compromised by the lack of previous baseline data on biological communities. For example, there are exceptionally good distributional records of certain organisms in the past, but these are limited to few taxa in few regions (e.g. butterflies in the UK, Warren et al., 2001; lizards in Mexico, Sinervo et al., 2010). Due to the urgency of understanding the threats that species suffer from climate change, an alternative approach focusing on how niches can predict distributions has proliferated (Peterson et al., 2011).

Hutchinson (1957) defined the ecological niche of an organism as the set of multiple conditions under which it can survive, grow and reproduce. With all the ongoing environmental changes, niches of many species are being altered, leading to population declines and extinctions in the warmer margin of the distribution range, and expansions

in the colder margin, and definitively contributing to changes in biological communities across the whole planet (Pecl et al., 2017).

Since the observations of Humboldt and Bonpland (1807) across different mountains of the world, biological communities are generally assumed to be assembled along elevational belts. Despite the lack of past data, given the general decrease in temperatures with elevation, mountain ranges provide a chance to substitute space for time in the study of the variation of biological communities across microclimate heterogeneity in a relatively small extent (Jobbágy et al., 1996; Haslett, 1997; Opedal et al., 2014). Mountain ranges usually host evolutionary unique and species-rich assemblages (Körner & Spehn, 2002; Swenson et al., 2012). Dispersal to higher elevations as a response to global warming has already been documented in communities of different taxa in several regions of the world (Wilson et al., 2005; Moritz et al., 2008; Pecl et al., 2017). Moreover, because area generally decreases with elevation, range contractions are one of the expected outcomes of uphill movements. Therefore, species confined to mountain regions are considered especially threatened by climate change (La Sorte & Jetz, 2010).

Although temperature at broad scale decreases with elevation, meteorological energy flows are locally modified by topography, originating multiple microclimates at small scale (Dobrowski, 2011). These local microclimates can support the persistence of certain species although the regional climate would be unfavourable. Paleoecological data suggest that some communities survived in microclimatic refugia during glaciations, allowing rapid colonization of other areas in the interglacials (Stewart et al., 2010). Understanding mountain microclimates across space and along time can be of great value for the preservation of biodiversity (Morelli et al., 2016).

To uncouple the abiotic and biotic processes defining the fitness of a species across different conditions, it is useful to clarify the distinction between fundamental and realized niches (Kearney & Porter, 2004). In the absence of biotic interactions, all the conditions where a given species could exist define its fundamental niche. However, what we observe in nature is usually a reduced subset of possibilities, restricted by species interactions, and called realized niche (Hutchinson, 1975). Detailed studies of fundamental and realized niches within mountain biological communities are necessary to understand the ecological processes that biota are experiencing in the context of the contemporary climate change.

The effects of global warming on ectothermic animals (whose body temperatures depend on the environment) can be even more pronounced (Huey et al., 2009). Heat flows at ground level are complex, and depend on soil properties, topography, solar radiation, air movements and habitat structural elements such as stones and vegetation (Porter et al., 1973; Huey 1991). Actually, the latter may be crucial as microclimate refuges that provide optimum conditions for small organisms (Körner 1999; Molina-

Montenegro et al., 2006; Molenda et al., 2012)

Some populations of lizards have been proved to go locally extinct because the temperature increase could compromise their physiological performance (Huey et al., 2009), their activity budgets (Sinervo et al., 2010), their temperatures in retreat sites and their water balance (Walker et al., 2015).

Understanding the heterogeneity of mountain microclimates, the microclimatic refuges created by habitat structural elements and the climate change susceptibility of the species inhabiting them, are some of the current challenges that researchers and conservationists must undertake in order to know how to confront upcoming climate change effects (Morelli et al., 2016). In this manuscript, we examine how thermal fundamental niches of several species of viviparous *Liolaemus* are realized across a heterogeneous landscape with complex topography and different habitats, that challenge the simplistic generalization of elevational belts inherited from Humboldt and Bonpland (1807). We expected that the climatic heterogeneity would be reflected in the abundances of different species of ectotherms by regulating their activity budgets. Specifically, we addressed this general question by: a) measuring empirically the thermal thresholds for activity of each species; b) exploring how activity budgets based on microhabitat temperatures measured in situ are explained by topography; and c) analysing how abundance of each species is modulated by thermophysiological and habitat variables.

Materials and Methods

Area of Study

Our study was carried out in the eastern slopes of the Tajzara Basin (3600–4600 masl), situated within the Reserva Biológica Cordillera de Sama, Department of Tarija, southern Bolivia (Fig. 1). Rainfall in the region is low (usually less than 310 mm per year) and is condensed in the warmest period of the year (December–March) (Appendix S1). Monthly means of air temperature range from 2.4 °C (July) to 14.8 °C (January) (SENAMHI, <http://www.senamhi.gob.bo/sismet>). However, temperature daily variations may be pronounced all year round, from freezing to more than 30 °C. Due to its position in the eastern edge of the Andes, the Cordillera de Sama forces the air masses that bring the tropical easterlies to gain elevation, cooling them down and increasing their humidity, a phenomenon known as Föhn effect. Although most of these cold and humid air masses remain in the eastern side of the divide, part of them passes through certain mountain saddles, causing a high heterogeneity in ecosystems and microclimates of the leeward side of the Cordillera de Sama (Appendix S2). In the lower elevations (3600–3800) of the Tajzara Basin, there are endorheic lakes surrounded by a mosaic of different habitats such as *bofedales* (Andean bogs), *tholares* (formations of *tholas*, shrubs such as those in the genera *Baccharis*, *Fabiana*,

Chersodoma, *Chuquiragua*, and *Junellia*), *pajonales* (formations of Poaceae with tussock form such as *Stipa leptostachya* and *Festuca hyeronimi*), and sand dunes (Beck et al., 2001). There is a high grazing pressure, mostly by sheep (Coppus, 2002; Preston et al., 2003) that promotes abundant *k'hanllales* (formations of the thorny dwarf shrub *Tetraglochin cristatum*) and *iriales* (formations of spiniscent tussocks of *Festuca orthophylla*), especially in the vicinities of populated places (Beck et al., 2001, Preston et al., 2003). In some of the lowland habitats there is *Frankenia triandra*, a soft cushion plant. In the slopes formed on the deposits of moraines (3800–4300 masl), *pajonales* with denser and higher tussocks dominate the habitats (Beck et al., 2001). In the higher parts of glacial valleys (4000–4300 masl), where hydric conditions allow, there are patchy grasslands of *Deyeuxia violacea*, sometimes with the presence of cushions of *Pycnophyllum molle* (Beck et al., 2001).

The known lizard fauna in the Tajzara Basin consists of: *Liolaemus ornatus* (Muñoz 2002; Tarifa et al., 2007), *Liolaemus puna* (Aguayo et al., 2009), *Liolaemus orientalis* (Jiménez-Robles & De la Riva, 2017) and an undescribed species of the *Liolaemus montanus* series (De la Riva, unpublished). There are also reports of *Liolaemus chaltin* (Ocampo & Aguilar-Kiriguin, 2008), which is morphologically similar to *L. puna* and was not detected after molecular analyses of specimens from the Tajzara Basin (De la Riva, unpublished). There is no previous information on the thermal ecophysiology of *L. orientalis* and *Liolaemus* sp., and very little about that one of *L. puna* and *L. ornatus* (Marquet et al., 1989). Despite this lack of knowledge on our focal species, *Liolaemus* are known to be generally good thermoregulators, with many species living in cold environments while maintaining relatively high body temperatures (Medina et al. 2012).

Data collection

During December 2012–January 2013 and October 2013–January 2014, coinciding with the warmest months of the year, the end of the dry season and the parturition of the local lizards, we sampled an area of approximately 30 km² around the village of Arenales, in the eastern slopes of the Tajzara Basin (Fig. 1; see weather conditions and sampling schedule in Appendix S1b).

To estimate activity and abundance patterns of lizards associated with temperatures and habitat, we selected 30 sampling sites (200-m line transects) ranging 3740–4300 masl. This sampling array allowed different combinations of elevation, slope, aspect, exposition to the Föhn fogs and habitat structure (Fig. 1). In every fieldwork campaign, lizard activity was surveyed in half of the transects at least three times between 9:00 and 17:30 h (GMT-4). One of us (OJR) counted the number of active lizards seen up to 10 m each side of the progression line. Body temperature (T_b) of lizards was measured by inserting a thermometer probe in their cloaca within less than 10 seconds after capture, when possible (an average of half of the individuals observed per visit). Capture and

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handling times were subtracted from the total time spent in every survey.

Thermal characterization of sampling sites was performed by operative temperatures (T_e), by means of unanimated objects with quite similar thermal properties to those of the body of lizards. Models were built with polyvinylchloride tubing (8.8 cm length x 1.6 cm diameter x 0.2 cm thickness) sealed with cork and silicon, and covered completely with matte grey primer spray paint. Similar models have been used in other lizard thermal studies (e.g. Sinervo et al., 2010). By linear mixed-models we verified there were no significant differences in the thermal properties of polyvinylchloride models and live lizards. In the case of *Liolaemus* sp. and *L. ornatus*, the 95% confidence intervals of coefficients did not include the identity expression $y=x$, so for all calculations for these species we adjusted T_e measurements according to the fitted equation (Appendix S3).

To obtain T_e time series, lizard models were connected to dataloggers (Onset HOBO U23-003) programmed to record every six minutes. To have a representative sample of maximum and minimum basking thermal conditions per site, each datalogger was connected to two lizard models, one placed at full sun and the other in half shade. In the 15 sites surveyed per campaign, dataloggers were placed at approximately 33, 100 and 167 m along the 200-m progression lines. In the second fieldwork campaign we also put one datalogger in the centroid of every site of the first campaign. Once the dataloggers were recovered, we visualized graphically the data to check for errors. Some time series or part of them had to be discarded.

Anytime we saw a lizard we recorded its location and species identity in order to map its distribution. When possible and always respecting a 100-m buffer area around each transect, we captured lizards and brought them to a laboratory installed near Arenales. In the same way as during the transect surveys, if lizards were active we recorded their body temperatures (T_b).

Once in the laboratory, preferred temperatures were measured between one and three days after capture. We built 16 tracks of 100 cm length x 12 cm width x 45 cm height with polycarbonate board. We achieved thermal gradients from approximately 20 °C to above 50 °C by placing incandescent light bulbs (100 W) and aluminium foil covering the walls and top of the hot end of the tracks. A thin thermocouple (Omega 5SC-TT-T-40-36) with nail polish-coated tip (approximately 1 mm of final diameter), was inserted 1 cm into the cloaca and taped around the tail of each lizard with plastic paraffin film. For approximately 2.5 hours, we put the lizards in the gradients to record their body temperatures once per minute with a multichannel datalogger (Eltek Squirrel 1035). We used the highest body temperature reached by each individual as its voluntary maximum (T_{Vmax}) and the temperatures bounding the interquartile range (middle 50% of all observations) as the upper and lower set point range temperatures (T_{upset} and T_{lowset}) (Hertz et al., 1993). For these parameters, we discarded the first measures until the

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lizards could reach the first maximum in their sine-like thermoregulatory pattern, when the thermocouples got outside the cloaca or when the lizards entangled in the wire without possibility to move freely. Lizards were released back to the location of capture. Some individuals were collected as vouchers and deposited in the Colección Boliviana de Fauna, Museo Nacional de Historia Natural (CBF), La Paz, Bolivia, and Museo Nacional de Ciencias Naturales (MNCN), Madrid, Spain. Species identities were confirmed by morphological and molecular data (De la Riva, unpublished).

From the T_e time series we calculated three thermophysiological indexes using species-specific physiological thresholds. First, for each survey we calculated the average deviance of operative temperatures (d_e) (Hertz et al. 1993). Second, we calculated the daily potential activity time in every site as the cumulative sum of time in which any of the T_e was within the mean T_{Vmax} and the emergence temperature (T_{emerge}) which we defined as the 10th percentile of field body temperatures (Appendix S4). And third, in an analogous way, we calculated the daily time of heat-restricted activity (referred as restriction time from here) for each site, summing all the time in which all T_e were above T_{Vmax} . We verified that T_e recorded in each site were representative of the potential activity and restriction times, by punctually recording additional T_e (Appendix S5).

Habitat characterization was performed at nine points per site (in order to coincide with dataloggers locations; e.g.: approximately every 22 m along the 200-m progression line). We calculated the cover of different habitat elements by registering the kind of substrate of 20 points at 1-m intervals along 5-m lines radiating to the four cardinal directions (Monasterio et al., 2010).

Using the ASTER DEM (NASA and METI/Japan Space Services) of 1 arcsecond (~30 m) resolution and the R packages “raster” (Hijmans, 2017), “insol” (Corripio, 2014) and “gdistance” (van Etten, 2015) we calculated several variables: topographic position index (TPI) at sampling scales of 330, 990 and 2010 m (Guisan et al. 1999), “northness” (cosine of the slope aspect), summer solstice insolation (time that every point is insolated during the 21 December) and an index of exposure to the Föhn effect (details in Appendix S6)..

Data Analyses

By means of analyses of variance (ANOVA) we assessed the interspecific differences in T_{Vmax} . The effect of sexual condition (a three-level factor for males, non-pregnant females and pregnant females) was also tested for thermal preferences within each species. ANOVAs with statistically significant differences were followed by post-hoc Tukey HSD tests.

By means of Gaussian Generalized Linear Models (GLM), we explored the differences in potential activity and restriction times among species and how much of their

variability was explained by the values of elevation and the other topographic variables in the centroid of each transect.

We explored co-linearity in the habitat variables through redundancy analysis (RDA) using the maximum counts of every species per transect, including topographic and T_e derived variables (mean daily maximum T_e , mean daily minimum T_e , and average diurnal mean T_e). Based on our field observations and the RDA, we made species-specific habitat relationship hypotheses. We used those habitat variables as predictors of the observed lizards together with the thermophysiological characterization of transects and visits.

Because lizard counts included many hypothetical cases in which lizards were not present (or present but neither active nor detected), we ran Zero Inflated Generalized Mixed Effects Models (ZIGLMM) for the detected active individuals by means of the R package “glmmADMB” (Skaug et al., 2016). Sampling sites nested within year were included as random effects. Within the fixed elements we included the average d_e recorded during each visit, the average daily potential activity time and average restriction time in every transect, and the selected habitat variables explained above (presuming they are constant along the study). To control the effect of the duration of each survey might have on the total count for each visit, we included its logarithm as an offset in all the models.

In order to get comparable standard coefficients in the GLM and ZIGLMM, we standardized predictors such that their averages were zero and variances were one (Schiegg, 2010). We verified that none of the selected habitat variables had a variation inflation factor higher than 2 (Zuur et al., 2010).

The package “MuMIn” (Barton 2016) was used to compare different candidate nested models based in their AICc (Akaike Information Criterion corrected for small samples) and evaluate the importance of every variable for the occurrences of the four species. As we wanted to evaluate all possible effects that were significantly plausible, we selected all the models whose AICc was below that of the null models (including only random effects and offset) (Burnham & Anderson 2002). We averaged their standardized coefficients weighing by their relative AICc (Burnham & Anderson, 2002). We inspected visually if residual plots showed deviations from homocedasticity or normality.

Because pregnant females of *Liolaemus* may have different thermoregulatory behaviour (Ibargüengoytia and Cussac 2002), we also ran another set of models in which potential activity time and restriction time were calculated using thresholds obtained only from data of pregnant females. Furthermore, we also ran a similar set of models replacing the thermophysiological variables by maximum T_e during survey, mean daily maximum T_e , and average diurnal mean T_e .

Results

We carried out a total of 113 surveys in which we registered 463 lizard activity events (159 *L. puna*, 182 *L. ornatus*, 95 *Liolaemus* sp. and 27 *L. orientalis*). Apart from the data collected in the transects, we also recorded 1332 presences across the landscape (Fig. 2). *Liolaemus puna* and *L. ornatus* were the most widespread species, occurring in all the sampled elevation range. *Liolaemus* sp. was only found in some parts below 3800 masl and *L. orientalis* in valleys generally above 4000 masl.

Activity thermal thresholds

The temperature values for the activity thresholds are shown in Table 1. Voluntary maximum (T_{Vmax}) was rather similar in most comparisons between species, although values for *Liolaemus* sp. were significantly higher than those for *L. ornatus* and *L. orientalis*. Pregnant females of *L. puna* and *Liolaemus* sp. had significantly lower T_{Vmax} values than males and non-pregnant females ($F_{2, 163}=12.23$, $P<0.001$ in *L. puna*; $F_{2, 175}=1.99$, $P=0.139$ in *L. ornatus*; $F_{2, 200}=21.87$, $P<0.001$ in *Liolaemus* sp.; $F_{2, 100}=0.74$, $P=0.480$ in *L. orientalis*). When comparing T_{Vmax} of pregnant females across species, *Liolaemus* sp. had significantly lower values than *L. puna* and *L. ornatus* (Table 1).

Activity budgets

The mean daily potential activity times were 1.3–7.25 hours, mostly in the middle of the day, while restriction times were 0–1.3 hours. In the GLMs, activity budgets were explained by significant negative effects of elevation, exposure to Föhn effect and, with less extent by terrain convexity (TPI_{2010}) and a positive effect of northness. The number of plausible models (with AICc lower than the respective null model), the weighted averaged β coefficients, the sums of the AICc-based weights (W_i) of the models in which every variable appears, and the explained deviance are represented in Figure 3 (more details in Appendix S7). No model could explain the restriction times of any species.

Habitat relationships

The exploratory RDA with habitat covers yielded four eigenvectors that accounted for 36.8% of the variance (Fig. 4). This allowed elaborating different habitat-relationship hypotheses for each species. In particular, we selected covers of tussocks and stones for *L. puna*; bare ground, tussocks and *Tetraglochin* for *L. ornatus*; *Frankenia*, *Tetraglochin* and stones for *Liolaemus* sp.; and *Pycnophyllum* and exposure to Föhn effect for *L. orientalis*. We discarded other habitat variables such as terrain slope, or covers of rare plants and shrubs, because there was no clear association with the occurrence of any lizard species, or because they were highly correlated with some of the selected variables, increasing VIFs above 2 (Zuur et al., 2010).

Inference on the realized niches

In the ZIGLMs we used negative binomial distribution, except for *L. orientalis*, for which we used a Poisson distribution due to its lower overdispersion (variance similar to mean) as a consequence of the low densities of this species in our transects. Given that the three sets of models in general did not show much difference in their AICc, and the importance and effect of predictors (Appendices S8–S12), we will comment the results of multimodel inference that included the general across-sexes thermophysiological predictors. The number of plausible models (with AICc lower than the respective null model), weighted averaged β coefficients and the sums of the W_i of the models where each predictor appeared are represented in Figure 5 (detailed in Appendix S10). There was a significant positive effect of tussocks cover on *L. puna* abundances. In *L. ornatus*, the most important variable was the potential activity time, with a positive significant effect, while the a priori selected habitat features did not seem to have a significant effect. *Liolaemus* sp. counts were negatively affected by T_e during the surveys, and positively affected by the cover of *Tetraglochin* and *Frankenia*. Deviance of T_e also had an almost significant negative effect on *L. orientalis*, with restriction time and exposure to Föhn effect having negative and positive, respectively, significant effects.

Discussion

The thermal and habitat heterogeneity that exists in the Tajzara Basin causes a remarkably strong spatial structure in the abundances of each species in a relatively small extent. Beyond Humboldt's inherited view of elevational belts, mountain environments are known to be able to host many species in small areas, due to the mosaic of diverse microclimates and habitats over short distances (Scherrer & Körner 2011; Morelli et al., 2016). Such dramatic differences in species composition are mostly reported in plants (Jobbágy et al., 1996; Opedal et al., 2014) and, to a lesser extent, in arthropods (Haslett, 1997). Other studies with lizards documented variation in communities along elevation (Marquet et al., 1989, Carothers et al., 1998), but did not provide details on microclimates across the landscape. We observed how microclimates originating from local topography (such as exposure to cold air drainage, terrain concavity, northness) modify the activity budgets of each species (Appendix S12) which predicted significantly the relative abundance of two of them. As far as we know, this is the first study reporting such importance of microclimate refugia affecting the assembly of ectotherm vertebrate communities across a mountain landscape.

The effect of activity budgets on distribution and abundance was more obvious for a habitat generalist such as *L. ornatus*, whose observed counts were directly proportional to the activity budgets independently of habitat. In the opposite extreme, the relative

abundance of *L. orientalis*, was also negatively affected by the restriction time. However, we could not explain the distribution patterns of the other species without their habitat preferences. Other studies comparing the thermal physiology of several *Liolaemus* species along elevation gradients found that, despite relatively similar body temperatures, microhabitat use and elevations were different between the studied species (Marquet et al., 1989; Carothers et al., 1998). All this evidence suggests that in spite of having relatively similar thermal physiology, lizards may differentiate their realized niches by habitat selection.

Some of the lizards most threatened by climate change are mountain species, especially those that reproduce by viviparity (Sinervo et al., 2010). The reproductive specialization of high-elevation viviparous Liolaemidae might compromise their abilities to confront rising temperatures (Pincheira-Donoso et al., 2013). Two out of the three Liolaemidae possible climate-change driven extinctions reported by Sinervo et al. (2010) are viviparous Andean species. Subsequent studies also predicted future extinctions of populations of other viviparous Liolaemidae in Patagonia near their warm distribution border due to restriction of activity by temperature increase (Kubisch et al., 2016; Vicenzi et al., 2017). In our case, even when considering the thermal requirements of pregnant females, restriction hours only showed a significant negative effect on the distribution and abundance of *L. orientalis*. This finding is not surprising for these populations of *L. orientalis* as they are within the lowest latitudes of the species distribution range (Jiménez-Robles & De la Riva, 2017). At the same time, the exposure to Föhn effect has a positive effect on the abundances of *L. orientalis*, suggesting a preference for areas with high air humidity. All these facts point to the idea that *L. orientalis* has been isolated in the Cordillera de Sama and persists in relict climate refugia (saddles and areas with high exposition to Föhn effect). Therefore these populations should be monitored due their limited area and their high risk of extinction if the mountain microclimates change.

The integration of thermal ecophysiology and habitat selection in the study of biological communities can provide useful insights to predict which species may have higher risk from climate warming. Habitat generalist species such as *L. ornatus* might be able to disperse to new suitable areas. On the contrary, populations isolated in climate refugia, such as *L. orientalis*, or in patches of specific habitats, such as *Liolaemus* sp., could be compromised by the rise of temperatures (La Sorte & Jetz, 2010). Habitat selection may bring physiological consequences for the ecology and fitness of organisms, especially for ectotherms (Huey, 1991). In mountains, different plant shapes create benign microclimates where they keep important live tissues safe from the harsh environmental conditions (Körner, 1999). Some animals may benefit from these microclimate refuges created by vegetation (Porter et al., 2002). Our results suggest several vegetation preferences that could imply physiological advantages for some lizards. We repeatedly

observed lizards hiding in holes underneath *Frankenia* cushions, especially *Liolaemus* sp. and, to a lesser extent, *L. ornatus* (Appendix S14). In fact, the abundance of *Frankenia* had a positive and significant effect on the abundance of *Liolaemus* sp. Cushion plants buffer the daily temperature fluctuations and trap heat more efficiently than other plants (Körner, 1999). The microenvironment under their closed cover provides an excellent microhabitat for small ectotherms (Molina-Montenegro et al., 2006; Molenda et al., 2012). We could also observe that temperatures at mid-shade inside the *pajonales* in high elevations were sometimes higher than temperatures of sensors situated in sunny open habitat, increasing the ectotherm window of activity (Appendix S15). Grasslands create a boundary layer effect, which prevents the internal flow of cold windy air, and with the heat input of solar radiation allows a thermal environment substantially warmer than that in the open (Körner, 1999). Tussock grasses accumulate their dead leaves to increase the thermal insulation of this microenvironment (Körner, 1999). This is probably the reason why a relatively thermophilous species such as *L. puna*, specialized in a widespread habitat such as the Andean grasslands (*pajonales*), can inhabit a broad range of elevations in the Tajzara Basin, even in the less solar radiated south-facing slopes at high elevations (Figs. 2 and SXX). Therefore, some plants such as *Frankenia triandra* cushions and tussock grasses can be considered as niche constructors that provide microclimatic refuges for ectotherms in cold climate ecosystems, thus conditioning their distributions across the landscape.

In a similar way, some stones could play a key role as climate refuges (Huey et al., 1989). The most abundant populations of *Liolaemus* sp. were in hills formed by lacustrine sediments with many stones (Beck et al. 2001; Coppus 2002). Many *Liolaemus* sp. were directly under these stones, especially at midday and in the afternoon. Although they were not active on the ground surface, their body temperatures were usually within or close to their set point range (Appendix S16), and therefore were thermoregulating efficiently by thigmothermy. In some additional measures that we took, we found that when ground surface temperatures were too hot at midday or too cold in the afternoon, temperatures under the rocks could allow *Liolaemus* sp. to spend more time within its activity thresholds (Appendix S16). However, the importance of stone cover was not significant for *Liolaemus* sp., probably because stones were in many other habitats, although their size and shape were different.

Livestock grazing is one of the processes that have shaped the vegetation of the Tajzara Basin for the last centuries, increasing the abundance of certain unpalatable thorny plants (Beck et al., 2001; Preston et al., 2003). It is remarkable that *L. ornatus* and *Liolaemus* sp. are abundant in formations of *Tetraglochin* (the latter showing a positive significant effect of this plant cover on its abundances), and that *L. puna* is abundant in formations of *Festuca orthophylla*. It would be interesting to test whether different

grazing managements have an effect on the populations of different species of *Liolaemus*.

Competition with more thermophilous species has also been hypothesized as a threat for some mountain lizards (Sinervo et al., 2010). This is unlikely to impair *L. orientalis*, as their adults are the largest lizards in the area, and they are known to predate on smaller lizards (Jiménez-Robles & De la Riva 2017). Certainly, only a few specimens of *L. puna* and *L. ornatus* were found in syntopy with *L. orientalis*, suggesting that they might avoid these areas where *L. orientalis* could predate on them or their juveniles. Both *L. puna* and *L. ornatus* were also syntopic with *Liolaemus* sp., in some cases with high abundances. This, combined with the significant effects of thermal quality and habitat features on species relative abundance, make us infer that competition does not play an important role at organizing the lizard assemblages in the Tajzara Basin. Similar results were found in other vertebrate communities in mountain ranges, where temperature, habitat and competition were taken into account (e.g. Elsen et al., 2017).

Therefore, the conservation of these species rich lizard communities depends strongly on the severity of climate change effects at local scale and the preservation of the ecosystems. If clouds keep forming in the eastern side of the divide and passing through saddles, *L. orientalis* and other organisms restricted to these climate refugia could possibly persist. If habitats in the lowlands and sediment hills in the Tajzara Basin are preserved, paying special attention to refuges such as *Frankenia* cushions and stones, *Liolaemus* sp. would only have to cope with rising temperatures, which for the moment do not seem to threaten its survival. Both the habitat generalist *L. ornatus* and the widespread-habitat specialist *L. puna*, are the species with less risk of suffering any population decline due to climate change or habitat loss, as they can easily find their optimal microclimates across different parts of the landscape.

Table 1. Estimated metrics for the activity thresholds for the four species of lizards in the Tajzara Basin, Tarija, Bolivia: emergence temperatures (T_{emerge}) and voluntary maximum (T_{Vmax}). Mean \pm standard deviation, range (in parenthesis) and sample size (N). In the bottom, results of ANOVAs and post-hoc Tukey results with the groups interpreted from significant differences between species. pun refers to *L. puna*, orn to *L. ornatus*, sp to *Liolaemus* sp. and ori to *L. orientalis*.

Species	All individuals		Pregnant		
	T_{emerge} (°C)	T_{Vmax} (°C)	T_{emerge} (°C)	T_{Vmax} (°C)	
<i>L. puna</i>	26.5 N = 192	37.7 \pm 1.6 (33.3–42.4) N=166	24.5 N=13	37.9 \pm 1.6 (34.1–40.1) N=21	Con formato: Justificado
<i>L. ornatus</i>	28.1 N=286	37.5 \pm 1.5 (32–41.8) N=178	27.1 N=32	38.1 \pm 1.4 (35.3–41.5) N=19	Con formato: Justificado
<i>Liolaemus</i> sp.	25.6 N=98	37.9 \pm 1.8 (30.2–42.5) N=203	25.7 N=13	36.6 \pm 2 (30.2–40.9) N=49	Con formato: Justificado
<i>L. orientalis</i>	26.5 N=80	37.2 \pm 1.4 (33–40.9) N=103	29.3* N=5	36.7 \pm 1.3 (34.7–38.2) N=9	Con formato: Justificado
Statistics		$F_{3,646} = 4.79$		$F_{3,94} = 5.23$	Con formato: Justificado
P-value		P=0.003		P=0.002	Con formato: Justificado
Groups		sp > ori (P = 0.002)		pun>sp (P = 0.024) orn>sp (P = 0.007)	Con formato: Justificado

* Because sample size of field T_b for pregnant females of *L. orientalis* was too low, we used the same T_{emerge} as the general value for the species.

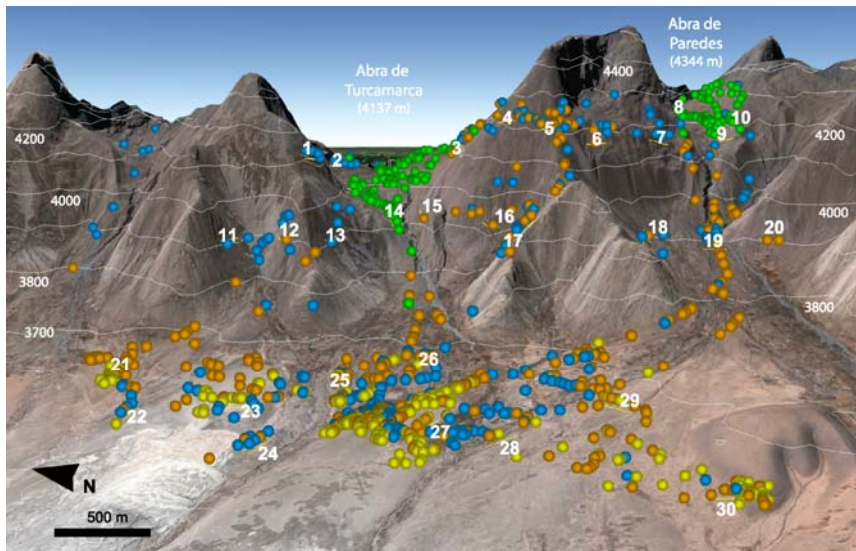
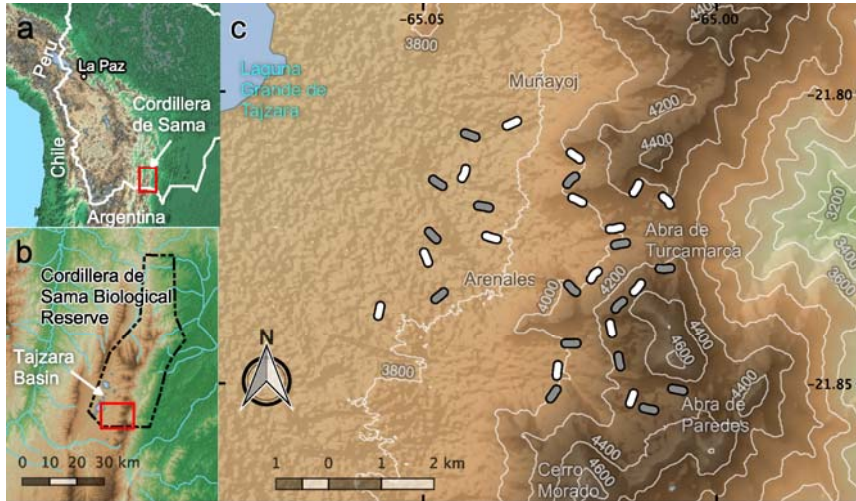
Figure 1. Maps of the study area. A) Location of Cordillera de Sama in Bolivia and the Andes. B) Limits of the Cordillera de Sama Biological Reserve and location of the Tajzara Basin. C) Line transects of 200 m where we surveyed the lizard communities repeatedly during the austral springs of 2012 (in white) and 2013 (in grey) in the vicinities of the village of Arenales.

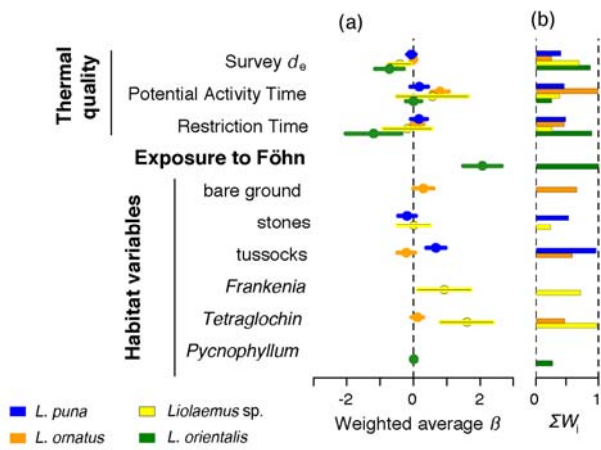
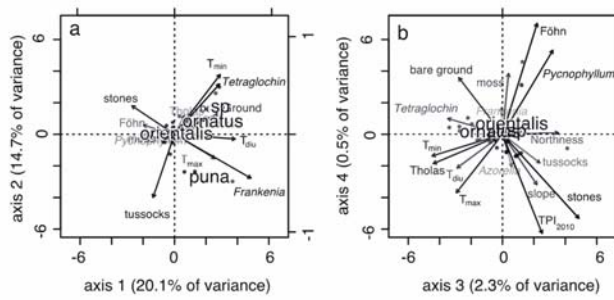
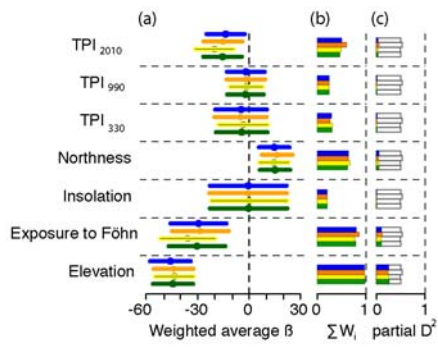
Figure 2. Distribution of four species of *Liolaemus* in the Tajzara Basin near the village of Arenales, Department of Tarija, Bolivia. Each dot represents an observation of *L. puna* (blue), *L. ornatus* (orange), *Liolaemus* sp. (yellow) and *L. orientalis* (green). Numbers 1–30 show the location of the sampling transects. Thin white lines represent 3700–4500 masl contours. Image modified from Google Earth.

Figure 3. Graphic representation of the model averages from the sets of plausible GLM models (those with AICc lower than respective null models) explaining the average daily potential activity time of the four lizard species present in the Tajzara Basin: *L. puna* (blue; averaged from 88 models), *L. ornatus* (orange; averaged from 87 models), *Liolaemus* sp. (yellow; averaged from 88 models) and *L. orientalis* (dark green; averaged from 86 models). a) Estimates of the weighted averages of standardized regression coefficients (dots) and their unconditional standard errors (lines). b) Importance of variables contributing to explain variance of each species in lizard counts, defined as the sum of the AICc-based weights of plausible models in which a particular variable appears. c) Weighted averages of the partial deviances purely explained by each predictor (the white bars represent the weighted average of total explained deviances).

Figure 4. Redundancy analysis triplots for the 30 sampled transects (represented as dots) in the Tajzara Basin, Department of Tarija, Bolivia. Lizard species are indicated in bigger black labels, while variables are in smaller labels in greyscale, being darker in proportion to their scores.

Figure 5. Graphic representation of the model averages from the sets of plausible ZIGLMM models (those with AICc lower than respective null models) explaining occurrences of the four lizard species present in the Tajzara Basin: *L. puna* (blue; averaged from 22 models), *L. ornatus* (orange; averaged from 55 models), *Liolaemus* sp. (yellow; averaged from 26 models) and *L. orientalis* (green; averaged from 27 models). a) Estimates of the weighted averages of standardized regression coefficients (dots) and their unconditional standard errors (lines). b) Importance of variables contributing to explain variance of each species in lizard counts, defined as the sum of the AICc-based weights of plausible models in which a particular variable appears.





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Biosketch

Octavio Jiménez-Robles is interested in the mechanisms that shape the spatial distribution of organisms and the assembly of biological communities. This work represents a component of his PhD at the Museo Nacional de Ciencias Naturales-CSIC on the thermal ecophysiology and distribution of lizards in mountains of Bolivia and Spain. He has mostly explored the effects of the abiotic environment and currently he is more interested in answering biogeographical questions related to biotic processes and historical effects. Ignacio De la Riva is interested on the diversity, systematics, evolution, biogeography and conservation of Neotropical herpetofauna, particularly frogs. Author contributions: OJR and IDIR conceived the ideas; OJR conducted the fieldwork and collected the data; OJR run the analyses; and OJR led the writing with assistance from IDIR.