Microhabitat *selection* of the poorly known lizard *Tropidurus lagunablanca* (Squamata: Tropiduridae) in the Pantanal, Brazil

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Abstract. Understanding how different environmental factors influence species occurrence is a key issue to address the study of natural populations. However, there is a lack of knowledge on how local traits influence the microhabitat use of tropical arboreal lizards. Here, we investigated the microhabitat selection of the poorly known lizard *Tropidurus lagunablanca* (Squamata: Tropiduridae) and evaluated how environmental microhabitat features influence animal's presence. We used a Resource Selection Function approach, in a case/control design where we analyzed the effect of substrate temperature and tree's diameter at breast height (DBH) in the probability of presence of lizards using mixed Conditional Logistic Regression. We found that *T. lagunablanca* uses trees with DBH from 0.40 m to 4 m and substrate temperatures ranging from 25.9°C to 42°C. Moreover, we showed that thickness of the trees and substrate temperatures significantly increased the probability of presence of *T. lagunablanca* individuals, being the probability of presence higher than 50% for trees up to 1.5 m DBH and temperature of substrate up to 37.5°C. Our study probed that *T. lagunablanca* individuals choose trees non-randomly, selecting thicker and warmer tree trunks. This information advances the knowledge of the spatial ecology of Neotropical arboreal lizards and is relevant for conservation, putting an emphasis on preserving native vegetation in the Pantanal.

Keywords. Microhabitat use; Thermal biology; Activity patterns; Substrate temperature; Thermoregulation; Lizards; Wetlands.

INTRODUCTION

Understanding how different environmental variables affect species' occurrence is of great importance for the study of natural populations and a central topic of organismal biology (Schwenk *et al.,* 2009; Ehrlén & Morris, 2015). Although other mechanisms can also influence the occurrence of species (*e.g.,* competition, coexistence, predation; Chesson, 2000; HilleRisLambers *et al.,* 2012; Cadotte & Tucker, 2017), locally, environmental filters can determine the presence of individuals. In this context, one of the most important factors for the spatial organization of population is how individuals select the resources in the environment (Pianka, 1973; Nogueira *et al.,* 2005; Rocha & Anjos, 2007; Gonçalves-Sousa *et al.,* 2019; Peixoto *et al.,* 2020). However, within their habitats, animals deal with many environmental constraints that directly influence their activity patterns and microhabitat selection (Rutherford & Gregory, 2003; Domenici *et al.,* 2007; Ariano-Sánchez *et al.,* 2020; Ortega *et al.,* 2019).

Lizards exhibit a diverse set of lifestyles and inhabit a variety of habitats, ranging from tropical forests to sandy deserts (Vitt & Pianka, 1994). In Brazil, there are high levels of species richness and endemism (Costa & Bérnils, 2018), and a high diversity of forms with striking adaptations, as psammophilous and fossorial species (*e.g., Calyptommatus, Nothobachia* and *Ophiodes*), with vestigial legs or even limbless (Rodrigues, 1996; Recoder & Rodrigues, 2020). Because lizards are ectoterms, body temperature regulation is essential for the development of all activities such as foraging, anti-predatory behaviors, and mating

strategies (Adolph, 1990; Angilletta, 2009). Microhabitat selection is, with some contribution of changes in body posture and adjustment of activity periods, the main thermoregulatory strategy of small lizards (Adolph & Porter, 1993; Bauwens *et al.,* 1996; Ortega *et al.,* 2019), including in species from deserts (*e.g., Phrynosoma platyrhinos;* Newbold & MacMahon, 2014), temperate areas (*e.g., Podarcis guadarramae;* Ortega & Pérez-Mellado, 2016), as well as in Neotropical region (*Ameivula* aff. *ocellifera* and *Tropidurus oreadicus;* Ortega *et al.,* 2019).

In general, lizards are abundant, occur in almost all types of environments, and respond strongly to local variables, thus being good models for studies focusing on habitat selection and its ecophysiological consequences in different spatial scales (Smith & Ballinger, 2001; de Andrade, 2020). A growing body of knowledge about microhabitat use and the understanding of their relations with local environmental variables has recently been produced (*e.g.,* Cosendey *et al.,* 2019; Franzini *et al.,* 2019; Xavier *et al.,* 2019). However, there is still a lack of knowledge about microhabitat selection for various lizard species and habitats, as is the case of the Pantanal (Brazil), where organisms experience complex climatic changes and flood dynamics (Alho *et al.,* 2001). Until now, and recently, only three studies evaluated the microhabitat use and/or selection of lizards in the Pantanal (*i.e.,* Terra *et al.,* 2018; Benício *et al.,* 2019; Ortega *et al.,* 2019). In this particular biome, the dynamic of organisms is primarily driven by flood and dry cycles that are quite variable in terms of recurrence and duration (Gonçalves *et al.,* 2011; Mercante *et al.,* 2011; Souza *et al.,* 2017). Thus, both flooding extension and extended droughts may limit the occurrence of species and shape local patterns of microhabitat use, reinforcing the necessity to better understand the habitat selection processes in this environment.

Here, we characterize the microhabitat use of the poorly known lizard *Tropidurus lagunablanca* (Squamata: Tropiduridae) and test hypotheses on the influence of environmental traits on microhabitat selection of this arboreal species. *Tropidurus lagunablanca* (Fig. 1) is a recently described lizard, medium-sized (SLV = 101.22 mm),

Figure 1. Adult individual of *Tropidurus lagunablanca,* Base de Estudos do Pantanal, Corumbá, Mato Grosso do Sul, Brazil.
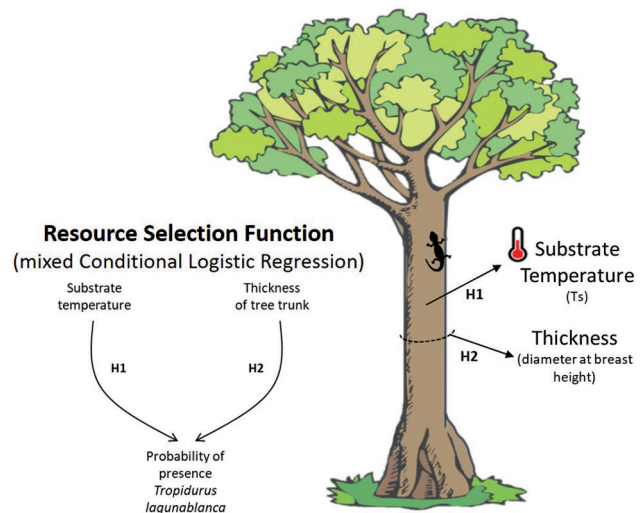

Figure 2. Hypotheses on the microhabitat selection of *Tropidurus lagunablanca* in Pantanal, Brazil. H1: *T. lagunablanca* individuals would choose trees with thermally suitable conditions for thermoregulation; H2: *T. lagunablanca* individuals would choose thicker trees, which offer more trophic and shelter resources.

diurnal, heliothermic and arboreal, sit-and-wait forager, that occurs predominantly in open formations (Carvalho, 2016). Although *T. lagunablanca* has not been yet evaluated by any red list, Carvalho (2016) suggests that, according to the rules proposed by IUCN (2001), the species could be classified as "critically endangered", mainly because of the conversion of its natural habitat into agricultural areas. Furthermore, little information is available regarding the ecology of this species (Colli *et al.,* 1992; Ávila *et al.,* 2010; Terra *et al.,* 2018).

Our specific aims were to describe microhabitat use and investigate microhabitat selection by *T. lagunablanca* in the Pantanal (Brazil), assessing the influence of environmental traits on the probability of presence of the individual lizards in the available microhabitats. Since different trees can provide different opportunities for thermoregulation, feeding, defense and mating, we formulated two major hypotheses about microhabitat selection of this species (Fig. 2): (i) *T. lagunablanca* individuals would select their microhabitats depending on their thermal properties, which would enhance thermoregulation (H1); and (ii) *T. lagunablanca* individuals would choose thicker trees, since they would offer more food and shelter, and, consequently more mating opportunities (H2).

MATERIAL AND METHODS

We conducted the study in the Pantanal region of Miranda-Abrobal, near the Base de Estudos do Pantanal (19°34'37"S, 57°00'42"W) of the Federal University of Mato Grosso do Sul (Corumbá, Mato Grosso do Sul, Brazil), in September 2017 (dry season). This article is the result of a research project of the 20th course Pantanal Ecology Course (EcoPan2017) of the Universidade Federal of Mato Grosso do Sul. The Pantanal is located in one of the most distinct macroregions formed from relief variations: the

floodplain (Prado *et al.,* 1992; Lourival *et al.,* 2000). The Pantanal occupies an area of approximately 195.000 km², which makes it one of the largest wetlands of the world (Ferreira *et al.,* 2017).

We sampled lizards along three consecutive days through active visual search (Crump & Scott Jr., 1994) from 07:00 h to 17:00 h (local time) on two transects of 1 km each, both close to Base de Estudos do Pantanal. To avoid pseudoreplicates, we divided each transect into parts, sampling each of them sequentially each day. In this way, each part of the transect was sampled only once. To avoid the effect of the observer's perception capacity, only one researcher sampled the individuals during the three days. The active visual search is effective in habitats with good visibility (such as the Pantanal), useful for presence/absence data and appropriate for monitoring populational trends, while the sampling bias is consistent over space and time (Crump & Scott Jr., 1994).

When a lizard was observed, we registered the hour (local time) and the substrate temperature (T_s, in °C) of its exact location, with a laser infrared thermometer (Benetech® Model Number GS320; Distance-to-spot ratio: 12:1; Resolution: 0.1°C) from 1 m (maintaining a constant distance in order to maximize the accuracy of the measurement). In addition, we measured the height of the point where the individual was observed and the diameter at breast height (DBH, in cm, measured always by the same observer at 1.20 m height) of the tree. We established a case/control design in order to assess the influence of these habitat traits on microhabitat selection. Thus, we simultaneously measured the environmental variables (T_s and DBH) in the four (unused) closer trees to the tree where the individual was observed. In order to understand microhabitat selection, it is important to consider unused points that could be ponentially used by the target species (keep in mind its ecology and behavior), at the temporal and spatial scale of the study. Thus, while sampling the unused trees, we excluded those trees with DBH < 7 cm, that are out of the range used by the species (see details in Terra *et al.,* 2018). Thus, for each observed lizard we gathered data of one used microhabitat VS four available (unused) ones. The study was purely observational, so we did not capture any individual, minimizing animal stress. However, many specimens of *T. lagunablanca* from the studied area are available at the Coleção Zoológica da Universidade Federal de Mato Grosso do Sul (Brazil).

To test the hypotheses of microhabitat selection, we used a Resource Selection Function (RSF) approach (Manly *et al.,* 2007). Resource Selection Functions calculate, by fitting a function on the probability of presence of the individual, the odds ratio of an individual to use a certain resource relative to its availability in the environment. We solved the RSF through a mixed Conditional Logistic Regression (mixed-CLR) analysis. The mixed-CLR was conditioned by the identity of each individual lizard, to guarantee that values of the explicative variables of each used microhabitat were paired with those available to a given individual (Duchesne *et al.,* 2010; Liedke *et al.,* 2018). In this way, the RSF allows comparing the

availability of the environmental variables for each individual simultaneously to the moment when the animal is observed in the used point. This method provides a more mechanistic and powerful approach to understand microhabitat selection than considering the general availability for all individuals of a population (Liedke *et al.,* 2018). We used Generalized Linear Mixed Models (GLMM) to fit the mixed-CLR, built with a binomial distribution, a logit link function, and the ID of individuals as a random factor (*i.e.,* random intercepts). The predictor variables were substrate temperature (T_s, in °C) and diameter at breast height (DBH, in cm), and the response variable was the presence of the individual (binomial: 0 = absence, 1 = presence). Thus, the model is as follows: Presence ~ DBH + T_s + (1|ID). We performed all analyses in the software R, version 3.5.2 (R Core Team, 2020) using the "lme4" package (Bates *et al.,* 2015).

RESULTS

We gathered data on 26 adult individuals of *T. lagunablanca*. The first active individual of *T. lagunablanca* was observed at approximately 07:30 h, and the last one was observed at approximately 17:00 h (Fig. 3). The pattern of T_s at which *T. lagunablanca* were observed varied widely throughout the day (Fig. 3), being that the average temperature of the used places was 32.06°C (ranging from 25.9°C to 42°C, SD = 4.26). Individuals of *T. lagunablanca* occurred in trees with mean DBH of 40.50 cm (ranging from 12.73 cm to 68.44 cm, SD = 15.14).

The mixed-CLR analysis showed that both T_s and DBH of the trees significantly influenced the presence of *T. lagunablanca* individuals (P = 0.002 and P < 0.001, respectively; Table 1). The probability of presence of *T. la-*

Table 1. Results of the mixed Conditional Logistic Regression analysis on substrate temperature (T_s, in °C) and diameter at breast height (DBH, in cm) of the trees on the probability of presence of *Tropidurus lagunablanca* individuals, including the individual identity as a random factor. AIC = 588.5; log likelihood = -290.3; df residual = 496.

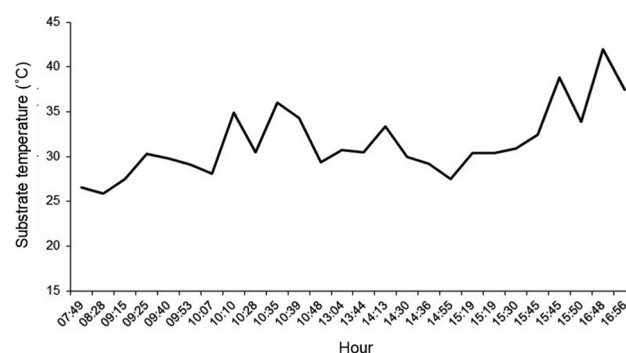|	Estimate	Std. Error	Z value	P value
T_s	0.25371	0.08163	3.108	P = 0.002
DBH	0.07239	0.01094	6.619	P < 0.001

Figure 3. Substrate temperatures (T_s) of the sites where *Tropidurus lagunablanca* were observed along daily activity period (local time) at the Base de Estudos do Pantanal, Corumbá, Mato Grosso do Sul, Brazil.
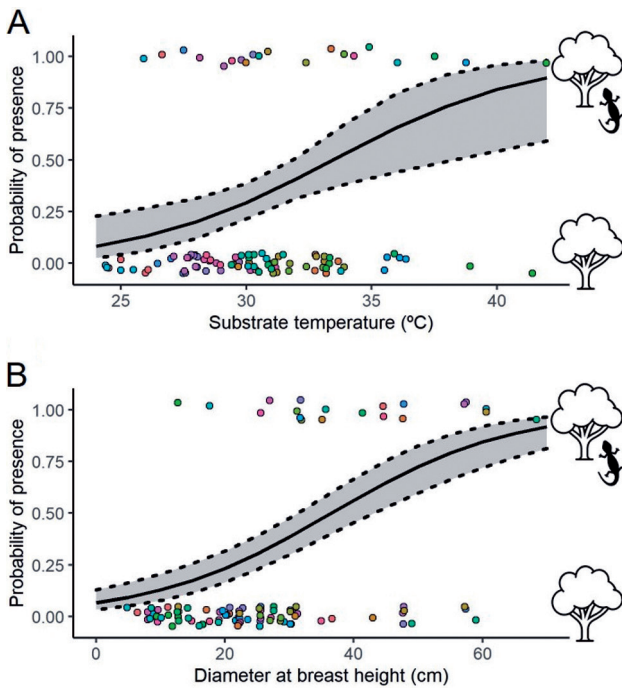
Figure 4. Result of the mixed Conditional Logistic Regression model for the presence of *Tropidurus lagunablanca* as a function of predictor variables: substrate temperature, in °C (A) and diameter at breast height (DBH, in cm) of the trees (B). The solid line represents the probability predicted by the model and the dashed lines represent the respective confidence intervals. The circles are the observed values measured in the points of presence (used microhabitats) and availability (the four respective unused microhabitats paired to each used one). As we included the ID of the observation (one ID for one used site, plus the correspondent four available microhabitats) in the model as a random factor, we colored the circles in the plot by ID, so one can have an idea of what used value corresponds to each available value. Please note that the location of the circles was vertically jittered to enhance visualization, but all points of availability correspond to the exact same probability of 0 and all points of presence correspond to the exact same probability of 1.

gunablanca is less than 30% for the mean temperature (32.06°C) (Fig. 4A) and higher than 50% for the mean DBH (40.50 m) of the trees (Fig. 4B). The probability of presence of *T. lagunablanca* is higher than the 50% for trees up to 40 cm DBH (an increase of 1 cm in DBH is associated with an increase of 0.07 units in the expected log odds of presence; that is, the probability of presence is 1.08 times higher for each cm that the DBH increases, within the studied limits). The probability of a lizard to be recorded is higher than the 50% for Ts above 34°C (an increase of 1°C implies 0.25 higher log odds of presence; that is, the probability of presence increases in ~ 1.3 times for each 1°C, within the studied limits).

DISCUSSION

We showed that the poorly known lizard *T. lagunablanca* uses trees with a mean temperature of 32.06°C and a mean thickness of 40.5 cm in the Pantanal of Brazil. By assessing microhabitat selection using a Resource Selection Function approach, we also demonstrated that lizard's presence is determined by substrate temperature

and thickness of the tree. Particularly, *T. lagunablanca* lizards are selecting thicker trees than randomly available on their habitats and, within those trees, select slightly warmer places than randomly available on their surroundings. Our results evidence that *T. lagunablanca* is a thermoregulator species, since they are choosing microhabitats non-randomly regarding substrate temperature. Besides, these findings are relevant for conservation, indicating the importance of preserving thicker trees for the conservation of this species. Finally, our results also have a practical component for field biologists, providing data on where the chances of finding these arboreal lizards are maximized, helping to save time and money in sampling efforts.

The choice for a particular warmer location in the tree in relation to the others, confirm the information suggested by Carvalho (2016) that the species is heliophilous and the frequency of specimens encountered apparently depends on environmental temperature and amount of sunlight. A recent study disentangling the effect of heat sources and structural components of the habitat found that microhabitat selection of other two Neotropical lizard species (*Tropidurus oreadicus* and *Ameivula* aff. *ocellifera*) is independent of substrate temperature, but are strongly dependent on solar radiation (Ortega *et al.*, 2019). In addition, neither substrate temperature nor sun exposure affect the microhabitat selection by *Ameiva ameiva* at the same location of this study (Benício *et al.*, 2019). However, it is important to remember that *A. ameiva* is a ground-dwelling active forager that moves constantly through space (Rocha et al., 2009), whilst *T. lagunablanca* is an arboreal ambush forager.

Our results on *T. lagunablanca* showed a clear influence of substrate temperature on microhabitat selection. This finding suggests thermoregulation, although further studies comparing the preferred thermal range of the species with operative and body temperatures can provide details on the accuracy and effectiveness of thermoregulation in *T. lagunablanca* (Hertz *et al.*, 1993; Vickers *et al.*, 2011). Despite environmental temperature is high at the Pantanal, forests act as thermal buffers (De Frenne *et al.*, 2019). Thus, thermal ecology of tropical arboreal lizards may differ from that of species inhabiting open areas. It is possible that *T. lagunablanca* lizards search for warmer substrates on their surroundings to raise their body temperatures and enhance performance. A similar pattern, of an increasing presence of lizards with increasing air temperature, has been found in another arboreal tropidurid, *Uracentron azureum* (formerly *Tropidurus azureus*) in the Amazon forest of Venezuela (Ellinger *et al.,* 2001). Thus, we suggest that microhabitat selection can be a relevant driver for thermoregulation of tropical arboreal lizards.

Adult *T. lagunablanca* actively select trees that are thicker than available. This may be due to the larger area that those trunks provide for thermoregulation, foraging and shelter. An increase in the trunk area would be related to a higher availability of arthropods (*e.g.,* Gonçalves *et al.,* 2005; Horak, 2013), resulting in better quality home ranges. This pattern of selection for thicker trees is in tune with the

typical foraging pattern observed for several other species (*e.g.*, *Christinus marmoratus, Enyalioides laticeps, Polychrus marmoratus, Uracentron flaviceps*; Garda *et al.*, 2013; Taylor *et al.*, 2016). The clear selection of trees with a higher DBH has important implications for conservation, since the removal of these trees can compromise the structure of the habitat and consequently the survival of the species.

We found a well-defined pattern of microhabitat selection by *T. lagunablanca* which may be related to its foraging, defensive, and mating strategies. Some Tropiduridae lizards (*e.g.*, *Tropidurus hispidus*), due to their ambush foraging behavior, do not need to travel large areas to find food and mates, resulting in a relative restricted home range size (Melo *et al.*, 2017). In this sense, the selection of *T. lagunablanca* for higher substrate temperature and thicker trees may also indicate, besides microhabitat selection, a possible site fidelity. However, behavioral studies on spatial and social organization are needed to better understand this issue. In any case, conservation efforts should be focused on preserving thicker trees in order to keep a suitable habitat for this species. The Pantanal is suffering from unprecedented bushfires in 2020, and the predictions estimate that bushfires will get worse in future years by climate change. Recent studies estimated a loss of more than 14.000 km^2 of native vegetation for 2,050 related to bushfires in the Pantanal (Guerra *et al.*, 2020). Therefore, this might constitute a great threat to *T. lagunablanca*, given its arboreal habits and selection for thicker trees.

In short, our results on microhabitat selection of *T. lagunablanca* showed how this species selects for microhabitats with higher substrate temperatures and thicker trees than randomly available in its habitat. This information provides knowledge on the habitat selection process of Neotropical arboreal lizards and can be used to maximize sampling efforts for the studied species. Finally, we reinforce the necessity to preserve the native vegetation in the Pantanal.

AUTHORS' CONTRIBUTIONS

R.A.B.: Conceptualization, Methodology, Formal analysis, Writing – original draft. D.C.P.: Formal analysis, Writing – review & editing. A.M.: Methodology, Formal analysis, Writing – review & editing. Z.O.: Methodology, Formal analysis, Supervision, Writing – review & editing. All the authors actively participated in the discussion of the results, they reviewed and approved the final version of the paper.

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