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Density, Population Size, and Habitat Use by *Ameivula nativo* (Teiidae) in a Remnant of Atlantic Forest in Brazil

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Abstract.—**We estimated the population density, size, occupancy, and detectability of the lizard *Ameivula nativo* (no English common name), an endemic and endangered species, in one of the largest remnants of the Atlantic Forest in Espirito Santo, Brazil, by using methods that consider imperfect detectability. We used linear transect surveys to estimate density and abundance. We also used transect data to estimate large and small-scale species occupancy using six covariates (sampled region, the proportion of exposed soil, the proportion of shrub cover, the proportion of herbaceous cover, air temperature, and relative humidity). The estimated overall density for *A. nativo* in the Vale Natural Reserve (VNR) was 11.03 ± 2.07 (standard error) individuals/ha with an estimated population size of 7,258 ± 1,365 individuals. At large scales, *A. nativo* occupancy probability was influenced by region, and local scale occupancy was influenced by proportion of exposed soil. Detectability was affected only by the air temperature, which may be a result of the species physiology because it is an active forager. We concluded that *A. nativo* has a relatively high density in the Natural Grasslands of VNR and occurs mainly in open areas with exposed soil and its detectability increases at high temperatures. The species density may vary according to the degree of habitat change. The high density of the species in the VNR may be indicative of good habitat quality of the species. It is of fundamental importance to protect the natural grassland areas of the VNR because the other areas where the species occurs are in increasing fragmentation and deterioration of habitat.**

Key Words.—detectability; distance sampling; lizard; occupancy modeling; reptiles

Introduction

Ameivula nativo (no English common name) is an endemic unisexual lizard common in the coastal habitats of sand dunes (restinga and campo nativo habitats) of the Atlantic Forest biome (Rocha et al. 1997), in the states of Bahia and Espírito Santo of Brazil (Rocha et al. 1997; Menezes and Rocha 2013) where some populations occur somewhat disjunctively (Colli et al. 2018). *Ameivula nativo* is a diurnal and terrestrial lizard that actively forages in open areas, the edge of bushes, and under herbaceous vegetation (Rocha et al. 2005; Peloso et al. 2008). The conservation status of this species is currently categorized as Endangered and Vulnerable in the federal and state lists of endangered fauna, respectively, due to its quite restricted distribution, reduction of populations, and intense habitat degradation within its range (Almeida et al. 2007; Colli et al. 2018).

Coastal habitats where *A. nativo* occurs are formed by beaches and sand dunes covered with herbaceous and shrubby vegetation (Araújo et al. 1998, 2008). Because restinga habitats along the coastal region are undergoing intense degradation and eradication of elements of structural habitat, including vegetation suppression due to real estate speculation and clandestine sand extraction for civil construction (Almeida et al. 2007; Rocha et al. 2007), some populations of Brazilian coastal cnemidophorine lizards (those of the genera *Ameivula* and *Glaucomastix*, previously placed in the genus *Cnemidophorus*: Rosario et al. 2019) are declining or being eradicated (e.g., Cosendey et al. 2016). Rocha et al. (2007) estimated a 50% reduction in restinga habitats in a coastal region of Brazil (Rio de Janeiro State). The low number of population studies on *A. nativo*, however, prevents consistent inferences about population declines due to habitat loss for this species, although Menezes and Rocha (2013) found evidence of decline in coastal cnemidophorine lizards due to habitat loss.

Most of the studies on *A. nativo* have focused on ecological aspects such as thermal ecology (Bergallo and Rocha 1993; Menezes and Rocha 2011), diet (Menezes et al. 2008), habitat use and activity (Peloso et al. 2008), reproductive aspects (Menezes et al. 2004a; Menezes and Rocha, 2014) and endoparasitism in the species (Menezes et al. 2004b; Menezes et al. 2018). Studies providing population size or density estimates for the species, however, are scarce (e.g., Menezes and Rocha 2013) and none of the studies regarding

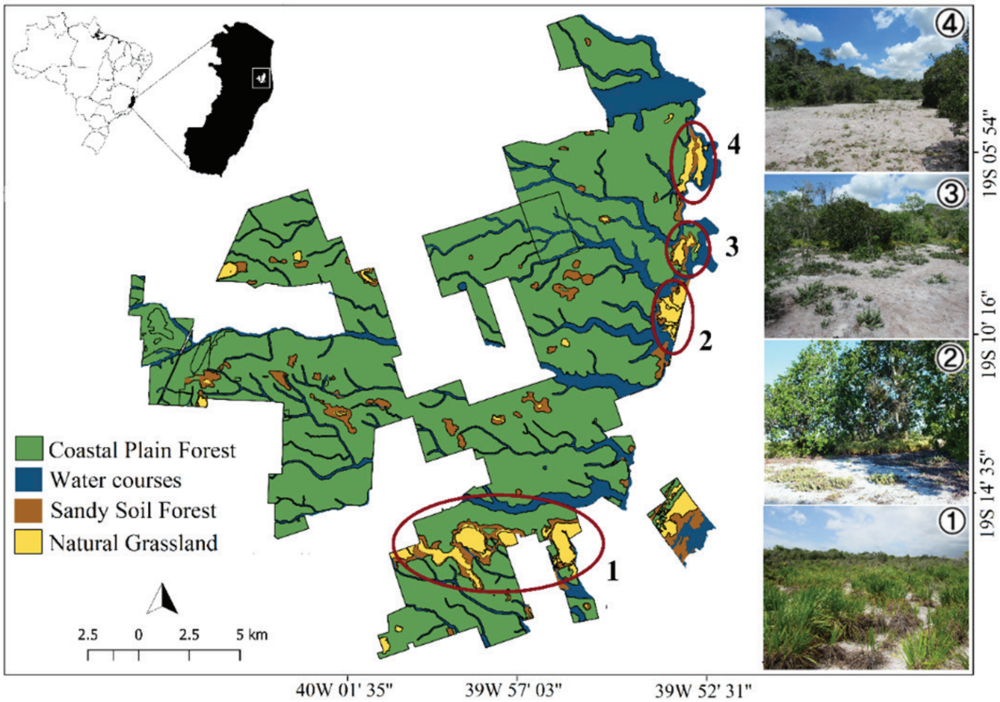

Figure 1. Location of Vale Natural Reserve, Espirito Santo, southeastern Brazil, showing the types of vegetation present in the reserve and the location and characterization of the sampled areas (1–4). (Habitats photographed by Juliane Pereira-Ribeiro).

this species used methods considering imperfect detectability. Using typical sampling methods, the likelihood of detecting reptile species may be influenced by several methodological and environmental factors (Ward et al. 2017; Ferreguetti et al. 2018a). Thus, assessing population and ecological data of a target species requires approaches that consider imperfect detection to produce more accurate data (Mazerolle et al. 2007), as these data may be critical to the application of conservation actions.

To provide data that may assist in the conservation of *A. nativo*, we estimated density, population size, occupancy, and detectability of the species in four areas of Natural Grasslands (campo nativo) in one of the largest remnants of the Atlantic Forest in Espírito Santo, Brazil, the Vale Natural Reserve (VNR). Based on prior knowledge of *A. nativo* ecology (Peloso et al. 2008; Colli et al. 2018), we modeled the occupancy and detection rate and used this to predict the response direction of six covariates: sampled region, the proportion of exposed soil, the proportion of shrub cover, the proportion of herbaceous cover, air temperature, and relative humidity. A priori, our hypothesis is that the amount of vegetation affects the occupancy of this lizard in an area. We predict that the species will have higher occupancy rates in environments with sparse vegetation (i.e., open areas, which consequently have a higher proportion of exposed soil). We also hypothesize that detectability is affected by temperature, and we predict that detection of the species will be greater at higher temperatures than at lower temperatures.

Material and Methods

Study area.—We conducted our study in the VNR (WGS84, 19°06′45″S, 40°03′03″W; mean elevation: 46 m), which is located in the northern state of Espírito Santo, southeastern Brazil (Fig.1). The reserve consists of about 23,500 ha of the Atlantic Forest central corridor, an area of great importance for the conservation of the biological diversity of the Atlantic Forest (Ministério do Meio Ambiente Brasil 2006). The region is characterized by marked seasonality, with the rainy season occurring from October to March and a dry season from April to September (Garay and Rizzini 2004). The climate in the region is tropical hot and humid, with an annual rainfall of 1,202 mm with annual temperature averaging 23.3° C (Kierulff et al. 2014).

The VNR is covered by a mosaic of habitats with four main vegetation types (adapted from Peixoto and Gentry 1990): Coastal Plain, Riparian, and Sandy Soil Forests, and Natural Grasslands. In this study, we only sampled Natural Grasslands, because it is the only type of vegetation in which *Ameivula nativo* occurs in the area (Rocha 1998; Rocha et al. 1997). Natural Grasslands are formations found in sandy, marine (up to 9 m) or river (above 28 m) soils, and occur in southern Bahia and northern Espírito Santo, where they form enclaves within the forest (Peixoto 1982; Peixoto et al. 2008). This vegetation type has a floristic composition remarkably similar to the restingas of southeastern Brazil, as well as similar edaphic conditions, such as nutrient-poor sandy substrate and shallow groundwater (Peixoto 1982; Kierulff et al. 2014). In VNR, Natural Grasslands cover about 6% of the area and the vegetation can vary from graminoid to herbaceous-shrub types (Peixoto et al. 2008).

We sampled *A. nativo* in the four main areas of Natural Grasslands in the VNR (Fig. 1). The first, Gavea Sul Natural Grassland (hereinafter NG1), is predominantly covered by a thick (i.e., 1–1.5 m) graminoid layer, with isolated shrubs throughout. The remaining three grassland areas may also have a graminoid layer but are considered open native shrub lands with clusters of shrubs interspersed by areas of exposed white sand (Peixoto et al. 2008; Kierulff et al. 2014). These three areas include Natural Grasslands of Paraju (type locality of *A. nativo*), Bomba d'agua, and Barra Seca (hereinafter NG2, NG3, and NG4, respectively).

Data collection.—To estimate the density, abundance and occupancy of *A. nativo*, we established 24 transects 500 m in length separated by at least 500 m, distributed among the four areas of Natural Grasslands, with 10 transects in NG1, seven transects in NG2, three transects in NG3, and four transects in NG4. These transects were surveyed for *A. nativo* monthly from September 2017 to January 2018 (except December 2017), totaling 48 km of surveys. We conducted field surveys between 0700 and 1300, which represents most of the activity period of the species (Peloso et al. 2008). We performed the surveys using standard distance sampling techniques (Buckland et al. 2001) and sampled each transect four times, one sample per month. The surveys were conducted by a single observer, at a walking pace of approximately 1 km/h, and we alternated the sampling order each month. We recorded the perpendicular distance of each individual observed from the transect line (with a tape measure, in centimeters) and recorded the date and time. At the beginning of each transect, we also record air temperature and humidity with a thermohygrometer.

***Data analysis.*—**We developed a hierarchical occupancy model (Nichols et al. 2008; Pavlacky et al. 2012) to estimate the detection and occupancy rates of *A. nativo* and predict habitat relationships at multiple scales for each Natural Grassland area (Nichols et al 2008). These models are useful for estimating probability of occupancy at two spatial scales and for monitoring rare species of conservation concern (Mackenzie et al. 2018). The model allowed estimation of three parameters that corresponded to each level in the nested sampling design with local records of individuals in each transect to estimate detection, and small-scale occupancy of transects in each site to estimate large-scale occupancy of each Natural Grassland area. The parameters of the model were (1) the probability of detection p_{ij} for transect j and Natural Grassland area i given the transect and Natural Grassland were occupied, (2) the probability of small-scale occupancy θ_{ij} for transect j and Natural Grassland area i given the Natural Grassland area was occupied, and (3) the probability of large-scale occupancy ψ_i for Natural Grassland area i. The assumptions of the multi-scale occupancy model were (1) no un-modeled heterogeneity in the probabilities of detection and occupancy, (2) each transect was closed to changes in occupancy over the observer occasions, (3) the detections of *A. nativo* at each transect were independent, and (4) the target species were never falsely detected (Nichols et al. 2008). We believe we meet all the assumptions of the analysis.

We fitted 20 multi-scale occupancy models using the RMark package interface (Version 2.1.13; Laake 2013; R Development Core Team 2017) for program MARK (Version 8; White and Burnham 1999). We used the region of the sampled area (i.e., the location of the four types of natural grasslands) as a covariate to model large-scale occupancy. To model small-scale occupancy (i.e., occupancy estimated within each natural grassland), we used three covariates: percentage of exposed soil (sandy substrate), percentage shrub cover, and percentage herbaceous cover. To estimate detectability, we used two occasion covariates: air temperature (°C) and percentage relative humidity. To estimate habitat covariates, we measured the proportion of exposed soil, proportion of shrub cover, and proportion of herbaceous cover in a 100-m buffer around each transect using interpreted high resolution images available in Google Earth in the Open Layer plug-in in the software QGIS 1.8.0 (http://qgis.org).

For detectability covariates, we used air temperature (°C) and percentage relative humidity data that we measured with a thermohygrometer at the beginning of each transect, on each of the four occasions. We selected the model variables that could influence the occupancy and detectability of *A. nativo* based on previous knowledge of the ecology of the species available in the literature (Peloso et al. 2008; Colli et al. 2018). We used the identity design matrix and sine link function to estimate the parameters of the model (White and Burnham 1999). Top models were selected using Akaike's Information Criterion (AIC) adjusted for small sample size (AICc). All models with a ΔAICc value < 2 were considered equivalent. We also used the weight (AICcwt) for each model, which corresponds to the amount of evidence in favor of a given model, to choose the best model that we used for testing our hypotheses.

Density and population size of *A. nativo* for each

Table 1. Distribution models used to analyze Distance sampling data of *Ameivula nativo* (no English common name), with the coefficient of variation (CV), and Akaike's Information Criterion (AIC). Parameters are ΔAIC = difference in AIC relative to the smallest value, AICw = AIC weight, and K = number of parameters.

Model	CV	AIC	ΔAIC	AICw	K
Half normal	18.41	1672.3	0	0.82	4
Uniform cosine	19.55	1695.5	23.2	0.11	4
Hazard rate	20.21	1715.2	42.9	0.07	4

site were estimated using the program DISTANCE 7.3 (Buckland et al. 2001). DISTANCE uses the perpendicular distances between the animal and survey route to estimate effective strip width (ESW) in the study area and to model the species detection. The knowledge of ESW allows the estimation of a density of individuals from the survey data (Buckland et al. 2001). This method better adjusts to the observed data in a gradient of perpendicular distances to the line transect (Laake et al. 1994; Buckland et al. 2001). We undertook exploratory analysis, examining histogram shapes using several cut points to check data behavior, distance sampling assumptions (Appendix Figure), and to select which model function to estimate density and population size (Buckland et al. 2001). There were three detection models considered (Table 1). We selected the best detection model using AIC (Akaike 1973) and we considered all models with a ΔAIC value < 2 equivalent.

Results

Occupancy models.—The best of the multi-scale occupancy models contained the variables of the location of the sampled area, soil exposure, and local temperature (Table 2). Large-scale occupancy was influenced by the sampling region. The probability of an area being occupied *by A. nativo* was highest in NG4 (Fig. 2). Small-scale occupancy increased as the proportion of exposed soil increased (Fig. 3). Detectability was affected by local temperature, with higher rates at higher temperatures (Fig. 4).

Density and population size.—We sampled 48 km of Natural Grasslands in this study (four repetitions of the 24 transects of 500 m, totaling 96 samples) and we recorded *A. nativo* 153 times. We detected *A. nativo* between 0 and 4.3 m from the transect line. The total density estimated for *A. nativo* was 11.03 ± 2.07 individuals/ha of Natural Grassland (mean ± standard error, for overall estimates) and the estimated population size was 7,258 ± 1,365 individuals for natural grassland areas within VNR, with a bandwidth (ESW) of 3.62 ± 0.40 m. The coefficient of variation (CV) for density and abundance was 18.4%. *Ameivula nativo* had the highest density in NG4, followed by NG3 (20.16 and 13.45 individuals/ha, respectively; Table 3). Regarding population size, NG1 was the area with the largest number of individuals, followed by NG4 (2,465 and 2,256 individuals, respectively; Table 3).

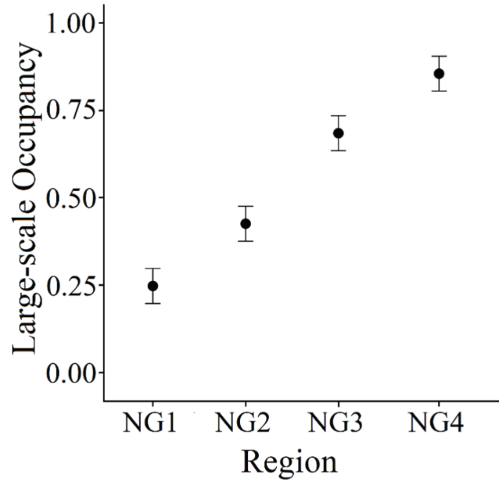

Figure 2. Relationship between the large-scale occupancy rate (mean ± standard error) of *Ameivula nativo* (no English common name) and the four areas sampled at Vale Natural Reserve, Espirito Santo, Brazil. The abbreviation NG = Natural Grassland.

Discussion

Among natural grassland areas of VNR, our estimates of occupancy probability and densities of *A. nativo* were highest in open areas (NG2, 3, and 4), characterized by the presence of sparse shrubby vegetation with portions of exposed white sand, supporting our initial hypothesis. In addition, at the local scale, the probability of occupancy of *A. nativo* was influenced by the proportion of exposed sandy substrate, with higher occupancy in areas with higher proportion of exposed sandy substrate. Although we do not have data explaining this result,

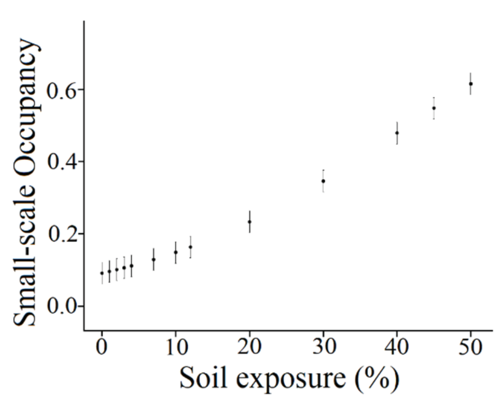

Figure 3. Relationship between the small-scale occupancy rate (mean ± standard error) of *Ameivula nativo* (no English common name) and the percentage of exposed soil in Vale Natural Reserve, Espírito Santo, Brazil.

Table 2. The 10 highest-ranking multi-scale occupancy models for the large-scale occupancy (ψ), small-scale occupancy (θ), and detectability (p) of *Ameivula nativo* (no English common name) from the data collected September 2017 to January 2018 in the Vale Natural Reserve, Espírito Santo state, Brazil. Parameters are AICc = Akaike's Information Criterion corrected for small sample size, ΔAICc = difference in AICc relative to the smallest value, AICcw = AICc weight, K = number of parameters. Covariates are Natural Grassland location (region), soil exposure (soil), shrub cover (shrub), herbaceous vegetation cover (herb), local temperature (temp), and local humidity (hum).

Model	AICc	ΔAICc	AICcw	K
Ψ(region)θ(soil)p(temp)	275.19	0	0.67	5
Ψ(region)θ(soil,herb)p(temp)	277.72	2.53	0.14	6
Ψ(region)θ(soil)p(.)	278.36	3.17	0.09	4
Ψ(region)θ(.)p(temp)	279.58	4.39	0.03	4
Ψ(region)θ(soil,herb)p(.)	281.39	6.2	0.02	5
Ψ(region)θ(herb)p(temp)	282.72	7.53	0.01	5
Ψ(region)θ(soil,herb,shrub) p(temp)	283.55	8.36	0.01	7
Ψ(region)θ(soil)p(temp,hum)	285.96	10.77	<0.01	6
Ψ(region)θ(soil,shrub)p(temp)	286.75	11.56	<0.01	6
Ψ(region)θ(herb)p(temp,hum)	288.12	12.93	<0.01	6

we believe this is probably a result of the thermal and foraging requirements of the ecology of this species. It is known that *A. nativo* prefer to move along the edge of shrubs, especially in open scrub dunes formations where it forages and thermoregulates (Bergallo and Rocha 1994; Peloso et al. 2008; Oliveira et al. 2019). Microhabitat preferences vary among coastal cnemidophorine lizards (i.e., *Ameivula*, *Glaucomastix*, and *Contomastix*) with regard to shrub density, height, and proportion of exposed sand (Dias and Rocha 2004; Ariani et al. 2011). Also, a study on the difference in the niche space between two lizard species, including one population of *A. nativo* in one of the areas of the present study (NG2), showed that *A. nativo* (formerly *Cnemidophorus ocellifer*) was found only on exposed sand and on leaf litter at the edges of shrubs (Bergallo and Rocha 1994). Similarly, a study on the activity and use of microhabitat by *A. nativo* in a sandbank in Espírito Santo showed that almost 70% of the recorded individuals were on exposed sand and vegetation edge (Peloso et al. 2008).

As we predicted from our second hypothesis, temperature positively influenced detection probability of *A. nativo* with the highest detection estimates (about 50%) between 35–38° C. *Ameivula nativo* is a highly active diurnal forager and capitalizes on warm temperatures to facilitate higher metabolic rates (Rocha et al. 1997; Menezes and Rocha 2011). Consequently, the period of activity of *A. nativo* should tend to increase

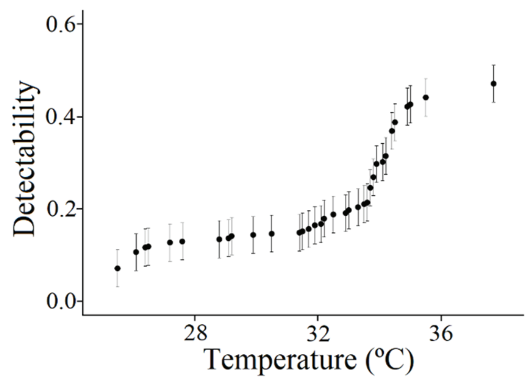

Figure 4. Relationship between *Ameivula nativo* (no English common name) detectability (mean ± standard error) and temperature (in ºC) in Vale Natural Reserve, Espirito Santo, Brazil.

with the increase of environmental air temperature, up to species-specific thermal optima, thus increasing its probability of detection. *Ameivula nativo* is a diurnal lizard that is active mainly between 0700 and 1400, with a peak activity between 0900 and 1100 (Peloso et al. 2008) and has an average body temperature of 38.4º C with range from 31°–42° C (Menezes and Rocha 2011), although the reported average body temperature of the species in the VNR is 37.6° C (Bergallo and Rocha 1993). In addition, it is known that lizards in the Teiidae are typically active at higher body temperatures than species from other families (Vitt et al. 1993; Vitt and Colli 1994; Ferreguetti et al. 2018a). Overall, several studies show that air temperature affects the activity and detection of reptiles (e.g., Ferreguetti et al. 2018a), and our results reinforce the fundamental importance of this variable in lizard detectability.

Ameivula nativo density in VNR (11 individuals/ha) was the highest estimated for the species so far and our results had a coefficient of variation that corresponded to the maximum recommended value for an accurate estimate (< 20%; Buckland et al. 2001). Menezes and Rocha (2013) estimated a density of *A. nativo* of 3.4 individuals/ha and 5.8 individuals/ha for two restinga areas near the VNR (Comboios-ES and Guriri-ES). In addition, the authors estimated a density

Table 3. The size of area (NG = Natural Grassland) sampled, density and population size (with one standard deviation) of *Ameivula nativo* (no English common name), and the coefficient of variation (CV) for areas sampled at Vale Natural Reserve, Espírito Santo, Brazil.

Area	Area size (ha)	Density (individuals/ha)	Population size (N)	CV (%)
NG1	380	6.48 ± 1.54	2,465 ± 583.49	20.34
NG2	125	10.86 ± 2.44	1,351 ± 204.63	18.41
NG3	41	13.45 ± 2.61	551 ± 102.41	16.32
NG4	112	20.16 ± 3.81	2,256 ± 426.47	18.90

of 0.5 individuals/ha in a restinga in the southern state of Espírito Santo (Setiba-ES) and a density of 1.0 individuals/ha in a restinga in the state of Bahia (Maraú-BA). The considerable difference in density values between the present study and the study by Menezes and Rocha (2013), however, may also be related to the difference in methods and sampling efforts employed by the studies. The method used by Menezes and Rocha (2013) to estimate *A. nativo* density did not consider imperfect detectability, which may bias estimates (Mackenzie et al. 2018). Therefore, we recommend that future population monitoring studies use a method that accounts for the probability of detection to produce more accurate estimates (see Mazerolle et al. 2007).

The high estimates of *A. nativo* density in VNR is important to conservation of this species because this site is the type locality (Rocha et al. 1997). In addition, the conservation status of the sampled area may also have influenced the species density. The density of *A. nativo* varies according to the degree of environmental change, usually having lower densities in areas under greater habitat degradation (Menezes and Rocha, 2013). Coastal sandy environments where *A. nativo* occurs are in an increasing and alarming rate of degradation (Rocha et al. 2007). Nevertheless, the Natural Grasslands of VNR appear to be in a comparatively better state of conservation from being surrounded by a protected reserve (pers. obs.), which would explain the local high density of the species. The fact that these natural grasslands areas are privately owned, with a systematic security system (see Ferreguetti et al. 2018b), and are considerably distant from beaches (average = 15 km) may have contributed to the conservation of the area, although we have observed impacts as the presence of domestic dog and cattle in NG1 and NG2. Thus, our results reinforce the importance of conservation of these areas of natural grasslands for the maintenance of populations of this lizard.

We conclude that *A. nativo* has a relatively high density in the VNR natural grassland areas and that it occurs mainly in open areas with exposed sandy substrate with its detectability increasing at high temperatures. The density of *A. nativo* may vary according to the degree of change in habitat conservation along the distribution of the species, and the high density of the species in the VNR may be indicative of a comparatively better habitat status for t *A. nativo*. This underscores the importance of protecting natural grassland areas of the region because *A. nativo* populations are decreasing across its range. In addition, it is worth mentioning that *A. nativo* is an endangered species and most other areas where the species currently occurs are experiencing increasing fragmentation and degradation of the habitat. Thus, we emphasize the importance of studies that estimate species density and population size in the areas of occurrence to monitor to what extent populations may be declining and if the decline is proportional to habitat loss in the area.

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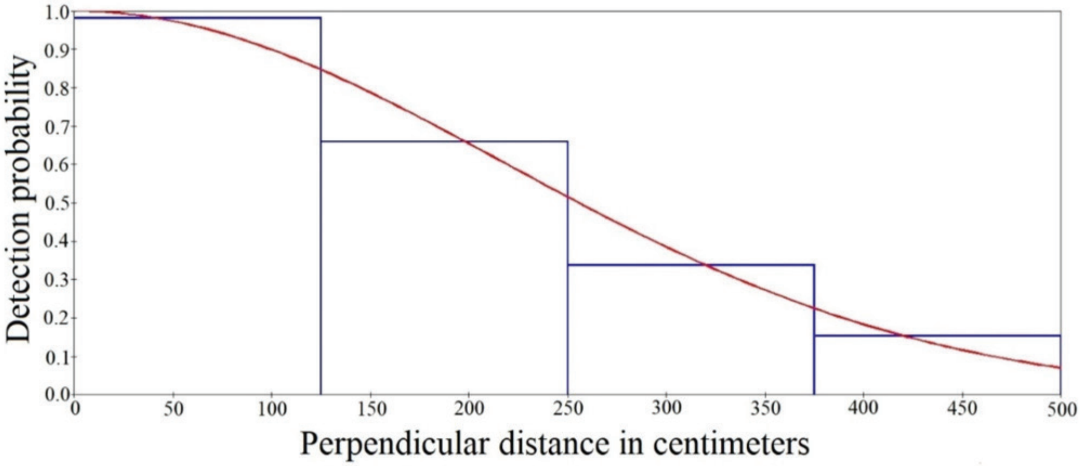

Appendix Figure. Detection model selected for *Ameivula nativo* (no English common name) at Vale Natural Reserve, Municipality of Linhares, Espírito Santo, Brazil.

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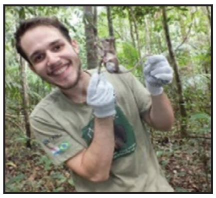

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