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RESEARCH ARTICLE

Density and microhabitat preference of the Southern Bristle-Tyrant (*Phylloscartes eximius*): Conservation policy implications

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ABSTRACT

Demographic and habitat preference studies are of paramount importance for conservation of birds, which is urgent in the mostly devastated Atlantic Forest. Moreover, these studies can indicate microhabitats that deserve more attention for conservation. In this article, we provide the first population density estimate and microhabitat preference assessment for the Southern Bristle-Tyrant (*Phylloscartes eximius*), a poorly known and threatened insectivorous bird endemic to the Atlantic Forest. The study was conducted at Cantareira State Park between May and December 2014. For density estimates we sampled 600 point counts using distance sampling, and for microhabitat assessment we compared 15 variables in 54 plots where birds were observed foraging with 145 random plots by adjusting generalized linear models and using hierarchical partitioning analysis. The species' density (12.7 [7.3–20.2] individuals per km²) is \sim 1/16 the density of a globally threatened congener, the Restinga Tyrannulet (*Phylloscartes kronei*), and lower than most other small insectivorous passerines in Atlantic Forest. When alone or in pairs, Southern Bristle-Tyrants preferred forests within \sim 10m of rivers and lakes; when in mixed-species flocks they preferred valleys (*grotas*). We suggest that protection of riverine forests would benefit Southern Bristle-Tyrants with positive consequences for the water supply of millions of people living in the Atlantic Forest domain.

Keywords: Atlantic Forest, riverine vegetation, Brazil's Forest Code, distance sampling, hierarchical partitioning, *Phylloscartes eximius*

Densidade e preferência de micro-habitat de *Phylloscartes eximius*: Implicações para as políticas de conservação

RESUMO

Estudos demográficos e de preferência de micro-habitat são de fundamental importância para a conservação das aves, que é urgente na devastada Mata Atlântica. Estudos desse tipo podem indicar os micro-habitats que merecem maior atenção para a conservação. Nesse artigo, fornecemos a primeira estimativa de densidade populacional e avaliação da preferência de micro-habitat pelo barbudinho (Phylloscartes eximius), um passeriforme insetívoro da Mata Atlântica, pouco conhecido e ameacado de extinção. O estudo foi realizado no Parque Estadual da Cantareira, uma unidade de conservação na Mata Atlântica, entre maio e dezembro de 2014. Para as estimativas de densidade, amostramos 600 pontos-fixos utilizando a amostragem de distâncias, e para a avaliação do uso de micro-habitat comparamos 15 variáveis em 54 parcelas onde as aves foram observadas forrageando e em 145 parcelas aleatórias ajustando modelos lineares generalizados e utilizando análises de partição hierárquica. A densidade do barbudinho (12.7 [7.3–20.2] indivíduos por km²) é considerada baixa quando comparada com outra espécie do gênero e globalmente ameaçada de extinção, a maria-da-restinga (Phylloscartes kronei; 208 [144–272] indivíduos por km²), e com outros passeriformes insetívoros da Mata Atlântica. Quando os indivíduos estavam sozinhos ou aos pares, a espécie apresentou preferência principalmente por florestas próximas (\sim 7 \pm 6.6 m) a rios e lagos; quando em bandos mistos a espécie apresentou preferência por locais situados em "vales" (grotas). Nós sugerimos que as florestas ripárias sejam protegidas para melhorar a conservação do barbudinho com consequências positivas para o abastecimento de água de milhões de pessoas que vivem no domínio da Mata Atlântica, que recentemente enfrentou a seca mais severa já registrada.

Palavras-chave: Mata Atlântica, floresta ripária, Código Florestal Brasileiro, amostragem de distâncias, partição hierárquica, Phylloscartes eximius, Pogonotriccus

INTRODUCTION

The Atlantic Forest is the second largest tropical rainforest in South America, running from 8°S to 28°S

along the coast but reaching parts of Argentina and Paraguay with its inland border. Bird diversity in the Atlantic Forest is among the highest in the world, with many species endemic and naturally rare (Goerck 1997,

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Marini and Garcia 2005). However, only 12% of its forest cover persists, and the small and isolated remaining patches still suffer from human disturbances (Ribeiro et al. 2009). Fortunately, a time lag between forest fragmentation and loss and bird extinction exists, allowing conservation efforts to be undertaken (Brooks and Balmford 1996, Brooks et al. 1999). Such measures, however, are often ill-informed because basic information on the ecology of most Neotropical birds is lacking (BirdLife International 2016). For instance, knowledge on density and population size permits the assessment of actual conservation status and the identification of areas to be protected (Buckland et al. 2001, IUCN 2016). Together with demographic information, studies on microhabitat preferences are important to indicate the environmental requirements of birds, providing clues about which habitat features should be restored in degraded areas, or which sites deserve more conservation attention (Bibby et al. 2000, Johnson 2007, Botero-Delgadillo et al. 2015).

In Neotropical forests, insectivorous birds are considered good indicators of environmental quality by being particularly sensitive to changes in microhabitats caused by selective logging (Aleixo 1999), conversion of natural areas into plantations (Greenberg et al. 1997), replacement of primary by secondary forest (Stratford and Stouffer 2013), and/or forest fragmentation (Stratford and Stouffer 2015). These birds are strongly influenced by vegetation structure and avoid altered forests if key structural elements are absent (Powell et al. 2015).

Among small Neotropical insectivorous birds, the genus Phylloscartes (Tyrannidae) comprises 23 rare and poorly known species, half of them globally threatened or nearly threatened (Fitzpatrick 2004, BirdLife International 2016). Southern Bristle-Tyrants (Phylloscartes eximius) are endemic to the Atlantic Forest, where they forage alone, in pairs, or in mixed-species flocks from the midstory to the canopy (Fitzpatrick 2004). They are distributed from southeastern to southern Brazil and part of Argentina and Paraguay, occurring mainly in mountains. Despite their wide geographic range, due to habitat degradation Southern Bristle-Tyrants have been recently recorded in only a few localities (Willis and Oniki 1993, Silveira 2009). The species is apparently suffering a sharp decline (BirdLife International 2016), which justifies its "Near Threatened" status at the global level and "Threatened" status in some Brazilian states (Silveira 2009). No information is available concerning the population size and density of Southern Bristle-Tyrants and only anecdotal knowledge is available on habitat preferences (Lowen 1996, Fitzpatrick 2004, Esquivel et al. 2007, Silveira 2009). Here we provide the first population density estimates and microhabitat preference assessment for the species. For density estimates, we used point-count distance sampling and, for microhabitat assessment, we compared plots where the birds were observed foraging with random plots.

METHODS

Study Area

Our study was conducted at Cantareira State Park (CSP; 7,900 ha), an Atlantic Forest reserve in eastern Brazil (Figure 1). The Park is located in Serra da Cantareira, one of the largest urban forests in the world at >10,000 ha (Bencke et al. 2006). CSP elevation ranges from 750 to 1,250 m above sea level and the predominant vegetation is dense mountain rain forest. Most of its vegetation results from natural regeneration that began in the late nineteenth century. Trees of the genus Vochysia, Cariniana, and Nectandra are among the most common forming the canopy, which reaches a height of ~ 15 m (Bencke et al. 2006, IF 2009). Climate is mesothermal and humid with rainy summers and dry winters (CWA Köppen), with an average temperature of 20°C and annual rainfall of 1,300 mm (IF 2009). The most preserved forests are on the urban-facing slopes of Serra da Cantareira, including CSP. Slopes facing away from urban areas are fragmented landscapes with forest patches ($\sim 10-100$ ha) embedded in agriculture and pasture matrixes including 3 recently created reserves adjacent to CSP totaling \sim 29,000 ha (IF 2009, Uezu et al. 2010). This fragmented landscape and the presence of large reserves help maintain connectivity among Serra da Cantareira and other large Atlantic Forest remnants, such as those in Serra da Mantiqueira (Uezu et al. 2010).

Serra da Cantareira avifauna is rich and diverse (326 species) and the Park harbors 80 Atlantic Forest endemic and 7 globally threatened birds, which contributed to its status as an Important Bird Area (IBA SP03; Bencke et al. 2006, Tonetti personal observation). We studied 3 areas within CSP: Pedra Grande, Engordador, and Cabuçu (Figure 1). Pedra Grande is located mainly on the top of hills with higher elevations (~1,000 m) and fewer rivers and lakes than the other 2 areas (850 m in Engordador and 800 m in Cabuçu; IF 2009). Engordador and Cabuçu have a more diversified relief, with more flat areas, "valleys," rivers, streams, and lakes than Pedra Grande (IF 2009, Tonetti personal observation).

Density

To estimate the density of Southern Bristle-Tyrants we conducted point counts using distance sampling (Buckland 2006). With ArcGIS 10.2, we established 100 fixed points at random within 100-m buffers created along all CSP available trails (one buffer for each area; Figure 1). These trails are distributed in the 3 studied areas and were ~ 6 km apart (Figure 1). Although all trails together total 30 km and cross areas with different microhabitats, we used



FIGURE 1. Cantareira State Park (CSP) with the 3 study areas (above) and the 100-m buffers (bellow). Each circle corresponds to point counts with the size proportional to the number of detections of Southern Bristle-Tyrants.

buffer areas to avoid sampling only along linear transects (the trails), which could bias the results (Marques et al. 2010). Based on a study where the size of the home range of another *Phylloscartes* species was estimated (the

Restinga Tyrannulet [*P. kronei*]; Gussoni 2014) and the territorial behavior of Southern Bristle-Tyrants (Fitzpatrick 2004, Tonetti personal observation), we established a minimum distance of 180 m between points to maintain

TABLE 1. Parameters used for density estimates of Southern Bristle-Tyrants at the Cantareira State Park and each area (Pedra Grande, Engordador, and Cabuçu): $a = area (km^2)$ of the 100-m buffers created in each area and the area of the Park as a whole; k = number of individual point counts; K = number of samples (6 for each individual point count); n = number of detected clusters; p = detection probability (%); ER = encounter rate; D = Density (individuals per km²); N = abundance; CI = confidence interval; CV = coefficient of variation.

| Area | а | k | К | п | р | ER | D (95% CI) | N (95% CI) | CV |
|-----------------------|------|-----|-----|----|------|-------|------------------|-----------------|------|
| Cantareira State Park | 79 | 100 | 600 | 46 | 6.2 | 0.076 | 12.7 (7.9–20.4) | 967 (600–1,557) | 24.3 |
| Pedra Grande | 2.83 | 34 | 204 | 1 | 0.8 | 0.004 | 0.8 (0.5–1.4) | 25 (15–41) | 24.3 |
| Engordador | 2.66 | 36 | 216 | 27 | 13.7 | 0.12 | 21.9 (13.6-35.2) | 464 (288–749) | 24.3 |
| Cabuçu | 2.33 | 30 | 180 | 18 | 8.2 | 0.1 | 17.9 (11–29.3) | 477 (291–781) | 24.3 |

independence (Buckland 2006). The 3 buffers together covered an area of 7.82 km^2 or 10% of CSP. The areas of each individual buffer and the number of random points allotted to each one are shown in Table 1.

Before sampling, we chose groups of 6 to 8 points to be visited each sampling day in a given buffer. Because the time spent visiting different points in a given area varied due to different distances between them (Figure 1), and due to relief characteristics of each buffer, the number of points sampled each day also varied (6 to 8), so that sampling was not performed after the period of high activity of the species (i.e. from dawn to 3.5 hr later; Tonetti personal observation). Although we established a minimum distance between points to avoid detecting the same individual at consecutive points, we did not sample adjacent points on the same day, as suggested by Buckland et al. (2001). The sequence of points sampled was set a priori so that the travel along trails was as short as possible. For subsequent surveys of the same group of 6 to 8 points, the sequence of visits was reversed so points were sampled at different times of the morning.

We sampled each of the 100 points (Figure 1) 6 times between May and December of 2014 for a total of 600 samples or 100 hr of sampling effort. To guarantee standardization, only the first author conducted point counts and samples were not performed on days of extreme weather conditions (rain or strong wind; Buckland et al. 2001). We spent 10 min at each point and when the species could be viewed from the point we measured the distance from the observer to the bird with a measuring tape (≤ 17 m; see Results). When we could only hear the birds, we estimated their distance to the point and walked to the place where they were singing to count the number of individuals in a flock. This was possible because Southern Bristle-Tyrants sing frequently while sallying for insects from the same branch and do not move easily with the presence of an observer (Fitzpatrick 2004, Silveira 2009, Lombardi et al. 2010, Tonetti personal observation). The low encounter rate with Southern Bristle-Tyrants (0.076 detections per point; Table 1), in addition to subsequent detections with birds distant (\sim 1.5 km) from each other, support our assumption that the time we spent

approaching birds did not interfere with us detecting more than one individual or pair of individuals foraging together at the same count point. When flock size was known, we used it directly in the analysis (see below). When Southern Bristle-Tyrants were moving and we could not estimate flock size, we used the mean group size in analyses. Mean group size was obtained from all visual records, including those made in point counts and with birds on trails while we were moving between points (Buckland et al. 2001).

Because most forest birds are detected aurally, 2 premises had to be fulfilled during bird surveys to not bias density estimates: (1) distance measures or estimates have to be exact or show minor errors, and (2) if present at a site, birds will sing with certainty (Burnham et al. 2004). Being aware of these caveats, prior to sampling, we performed a period of 4 months of training for both estimating distances to known-distance objects in the forest and to the birds detected aurally. To confirm the second premise, when an individual was not detected after a 10-min point count, we used 3 min of playback (alternating a minute of silence for each minute of song reproduction) to check if there was any response of birds not detected in spite of their presence around the point. We used playback only to test if premise (2) was met; if we had a response the distance was not used in analyses because playback attracts birds to the observer, thus biasing density estimates (Buckland et al. 2001, Burnham et al. 2004). Tailoring distance sampling methods according to particular behavior of the organism studied can increase the accuracy of results by improving data quality rather than using a pre-established protocol applied to a wide range of species (Burnham et al. 2004).

Microhabitat Preference

We assessed the microhabitat preferences of Southern Bristle-Tyrants by comparing the characteristics of plots used by birds with random plots (Manly et al. 2002; Table 2). The exact centers of plots were determined where birds were first seen sallying for insects. Random plots represented microhabitat features available for the species and did not necessarily correspond to unused sites (Manly et al. 2002). We established 5-m-radius circular plots (\sim 78

| TABLE 2. Variables measured within plots (mean [minimum-maximum]) where Southern Bristle-Tyrants were observed foraging, |
|---|
| associated or not with mixed-species flocks, as well as random plots. DBH = diameter at breast height (cm); height = estimated |
| height of trees (m); abundance = number of trees within each plot; I, II, III = classes of trees acording to DBH measurements; canopy |
| closure = estimated using a spherical densiometer; vines = quantity of these elements classified into 4 classes (0-3); slope = |
| measured with a clinometer and classified into tree classes: $1 = 0-5.45^{\circ}$, $2 = 5.45-18^{\circ}$, and $3 = >18^{\circ}$; type of habitat = classified into 3 |
| classes: 1 = valleys or grotas, 2 = hillsides, and 3 = crest or hilltops; elevation = elevation above sea level; water = presence (1) or |
| absence (0) of a river, stream, or lake within a 30-m radius of each plot center. |

| Variable | Random plots | Not associated with mixed-species flocks | Associated with mixed-species flocks | | |
|-----------------|-------------------|--|---|--|--|
| DBH I | 7.5 (5.5–10.5) | 8.0 (5.7–12.4) | 7.9 (5.9–8.9) | | |
| height I | 6.9 (2.8–12) | 6.4 (4.1–10) | 6.5 (3.8–9.3) | | |
| abundance I | 8.9 (1–25) | 7.1 (1–19) | 7.2 (1–13) | | |
| DBH II | 17.3 (12.7–24.2) | 17.8 (12.7–22) | 17.6 (13.7–24.1) | | |
| height II | 12.4 (6–22.3) | 11.0 (5–15) | 11.0 (7–15) | | |
| abundance II | 3.3 (1–9) | 2.9 (1–7) | 3.1 (1–8) | | |
| DBH III | 35.5 (25.1–89.5) | 41.3 (25.3–58.2) | 34.4 (25.3–60.5) | | |
| height III | 19.4 (6–40) | 17.5 (9–25) | 17.0 (10–25) | | |
| abundance III | 1.7 (0–4) | 1.5 (0–3) | 1.4 (0–4) | | |
| canopy closure | 6.5 (2–15) | 6.9 (4.2–13.5) | 7.5 (4–8.7) | | |
| vines | 1.5 (0–3) | 2.0 (0-3) | 1.8 (0–3) | | |
| slope | 1.6 (0–3) | 1.2 (0–3) | 1.4 (0–3) | | |
| type of habitat | 2.0 (1-3) | 1.3 (1–3) | 1.4 (1–3) | | |
| elevation | 915.0 (782–1,121) | 881.0 (810–933) | 858.0 (780–930) | | |
| water | 0.06 (0–1) | 0.6 (0–1) | 0.2 (0-1) | | |

m²) centered on locations where birds were observed foraging and at random places inside the 100-m buffers (Figure 1). Our choice of plot size was based on the literature (e.g., Stratford and Stouffer [2013] used 8-mradius plots to evaluate microhabitat preference of 9 insectivorous birds in a tropical forest), but with an adjustment based on the behavior of Southern Bristle-Tyrants (Fitzpatrick 2004, Silveira 2009, Lombardi et al. 2010, Tonetti personal observation) to finely quantify the foraging microhabitat of our focal species. To avoid sampling plots in locations where birds were only moving and not using resources, we only used observations of individuals that were foraging (Manly et al. 2002). All Southern Bristle-Tyrants observed foraging were considered for microhabitat assessment, including birds detected while we were walking on trails and moving between points as well as birds observed after aural detections in point counts. As subsequent encounters with birds occurred far from each other (~ 1.5 km), we assumed that records of foraging birds were independent of each other (Bibby et al. 2000). Random plots were selected with the aid of ArcGIS 10.2 and the number of plots in the 100-m buffers was proportional to the area covered by each buffer.

Within each plot we measured 15 microhabitat variables (Table 2), which were chosen based on literature and our experience with this group of birds (Bibby et al. 2000). We measured the diameter at breast height (DBH) of all trees with DBH>5 cm and visually estimated their heights. Since the DBH range was wide, we created 3 classes of

trees based on the quantiles of logarithmized DBH values (i.e. <25%, 25–75%, and >75%) as follows: class I (5–12.5 cm), II (12.5–25 cm), and III (>25 cm). Height estimates were made only by the first author, who practiced comparing trees with a height-measured pole. Tree species richness is high at the study area (394 species; IF 2009), and tree identification was beyond the scope of this study.

Because the presence of vines play an important role in providing foraging microhabitats for insectivorous birds (Stratford and Stouffer 2013), we estimated vine quantity in the midstory and canopy, and classified it into 4 classes: 0 for a total lack of vines; 1 when there were few vines, usually in one or two branches within the plot; 2 when vines were in more than two branches but sparse in a small number of branches (usually three to five); and 3 when a tangle of vines completely covered the trees (Schulz and Eyre 2000). To estimate canopy closure, which is directly related to light incidence and primary productive in forests, thus influencing the abundance of arthropods, we used the average of 4 readings made with a spherical densiometer facing the 4 cardinal directions from the center of each plot (Jennings et al. 1999).

Because microhabitat relief characteristics influence edaphic conditions and, consequently, vegetation structure, composition, and abundance of small insects (Bibby et al. 2000), we classified the slope and "type of habitat," and measured the elevation at each plot. The slope was measured with a clinometer and separated into 3 categories: 1 corresponding to plots situated in flat or gently inclined sites $(0-5.45^{\circ})$, 2 for plots in an interme-

TABLE 3. Estimates of concurrent models (key function + series expansion) for all Cantareira State Park and mean of models considered equally plausible (Δ AlC \leq 2). *wi* = weight; GOF = goodness-of-fit; *D* = density (individuals per km²); *N* = abundance for the Cantareira State Park; CV = coefficient of variation; CI = confidence interval.

| Model | AIC (Δ AIC) | AICwi | GOF | D (95% CV) | N (95% CI) | CV | |
|---|---------------------|-------|------|-----------------|------------------|------|--|
| Uniform + Simple polynomial | 155 92 (0) | 0 38 | 0 99 | 12 (7 9–18 1) | 911 (600–1 382) | 21 3 | |
| Uniform + Cosine | 157.11 (1.19) | 0.2 | 0.86 | 15.6 (10.1–24) | 1192 (774–1,836) | 22.1 | |
| Half-normal + Hermite polynomial | 157.14 (1.21) | 0.21 | 0.87 | 15.4 (9.1–26) | 1173 (697–1,975) | 26.8 | |
| Half-normal + Cosine | 157.14 (1.21) | 0.21 | 0.87 | 15.4 (9.1–26) | 1173 (697–1,975) | 26.8 | |
| Hazard rate + Simple polynomial | 158.64 (2.72) | - | 0.85 | 10.6 (6.5–17.4) | 808 (492–1,324) | 25.4 | |
| Hazard rate + Cosine | 158.64 (2.72) | _ | 0.85 | 10.6 (6.5–17.4) | 808 (492-1,324) | 25.4 | |
| Mean of models with $\Delta AIC \leq 2$ | - | - | - | 12.7 (7.9–20.4) | 967 (600–1,557) | 24.3 | |

diate level of declivity $(5.45-18^{\circ})]$, and 3 for the steepest sites (>18°) (Schulz and Eyre 2000). The variable "type of habitat" was categorized as 1 for valleys (i.e. between hills, also known as *grotas* in Portuguese), 2 for hillsides, and 3 for crests (or top of hills). The slope was classified within the 5-m-radius plot and it was not necessarily correlated with the type of habitat (e.g., the slope could be in the first category, flat, and the plot could be located on a hillside of a large hill, second category of "type of habitat"). We obtained elevation measures of each plot from SRTM (Shuttle Radar Topography Mission), available at the INPE website (Instituto Nacional de Pesquisas Espaciais; http:// www.inpe.br/ingles/), with the aid of ArcGIS 10.2.

Since insectivorous birds are sensitive to local variations in humidity, which affects arthropod abundance (Develey and Pongiluppi 2010, Powell et al. 2015), we also considered the presence (1) or absence (0) of water (i.e. rivers, streams, or lakes) within a 30-m radius centered at each plot. This distance (30 m) was chosen because in 20 out of 22 occasions when Southern Bristle-Tyrants were foraging near rivers or lakes they were less than 11 m from water (other distances were 22 and 29 m).

Data Analysis

We performed the analysis of distance data in the software DISTANCE 6.2 (Thomas et al. 2010). First, histograms of distances were analyzed to note possible errors during data collection (e.g., movement in response to the observer and outliers). Next, the 6 models (key function + series expansion; Table 3) deemed most appropriate according to the literature were fitted to the data truncated at 5% of the greatest distances (Buckland et al. 2001). To avoid possible errors in estimates of distances, we performed the analysis testing grouping data from 4 to 10 distance classes of equal value and, based on the visual inspection of the histograms, we selected the best grouping (Buckland et al. 2001). We used Akaike's Information Criterion (AIC) to select the best model. For models that were equally parsimonious ($\Delta AIC \leq 2$), we used the model-averaging procedure estimating the variance by bootstrap (999 randomizations; Buckland et al. 2001). Global detection functions

were adjusted using all distances collected and density was estimated for all of CSP and also separately for each of the 3 study areas.

To avoid multicollinearity in microhabitat preference analyses, we first calculated the Pearson correlation matrix and removed variables with $r \ge |0.65|$ (Zuur et al. 2010). With the remaining variables, we calculated the Variance Inflation Factor (VIF) to verify if there were any variables with values higher than 3, which were also removed from the analysis (Zuur et al. 2010). We used Generalized Linear Models (GLM) with a logit link function and binomial distribution to model the probability of a plot being used by the bird (1) or being a random plot (0). We relied upon a stepwise regression procedure using AIC and bidirectional elimination of variables as a variable selection approach (Zar 2010).

Although GLM is widely used in habitat preference studies, this method, as well as all other types of regression, is sensitive to effects of correlated data, even when data undergo some kind of treatment to reduce multicollinearity. One solution to avoid spurious results generated by GLM is to use the Hierarchical Partitioning Analyses (HP; MacNally 2000, 2002), which provides the contribution of each variable in 2 ways: independent from other variables (*I*), and combined with other variables (*J*). Variables with the largest independent contribution values in percentage (*I*%) are more likely to determine the occurrence of the species and are therefore more relevant. We evaluated the significance of the independent contribution of each variable using randomization tests and 999 randomizations (MacNally 2000, 2002).

When following mixed flocks, Southern Bristle-Tyrants foraged in places that were, at least to some extent, selected by the bird species acting as flock leaders (Munn and Terborgh 1979). Thus, we performed the analysis separately when individuals were associated with mixedspecies flocks and when they were alone or in conspecific pairs in order to test for different microhabitat preference depending on the type of association (inter- or intraspecific). We performed all microhabitat preference analyses in the software R, and used the package "hier.part" for HP analyses (Walsh and MacNally 2013).



FIGURE 2. Histograms of the distance data using 6 distance categories of equal length. Curves correspond to the detection function **(A)** and probability density **(B)** of models equally plausible ($\Delta AIC \leq 2$). The 2 models with half-normal key function showed no differences.

RESULTS

Density

We had 46 detections, all made aurally. When Southern Bristle-Tyrants were <17 m from the observer we could eventually see them and measured the distance with a measuring tape, which accounted for 8.5% of all distances used in analysis. Two detections were of birds alone, 21 detections were of pairs, and for the remaining 23 detections (i.e. when we could not see individuals after hearing their song) we used mean group size in the analysis (1.72 individuals per flock, which corresponds to the mean of all observed flocks, including flocks recorded along the trails and not during point counts; n = 87). Models (Table 3) were adjusted with 44 detections after the truncation of 5% of the greatest distance values. We decided to keep the distances grouped into 6 classes to provide the best fit of the models (Figure 2; Buckland et al. 2001). The effective radius sampled (ρ) was 61 m, and the maximum detection distance was 90 m (Buckland et al. 2001). The average of the competing models considered equally plausible indicated the density value of 12.7 (7.3-20.2) individuals per km^2 and abundance of 967 (556–1,539) individuals in the CSP. Density values for each of the 3 areas (Cabuçu,

Engordador, and Pedra Grande; Figure 1) and their coefficient of variation are in Table 1.

Microhabitat preference. We measured 199 plots, including 145 random, 8 where individuals were alone, 13 in pairs, and 33 associated with mixed-species flocks, alone or in pairs (Figure 1). DBH and height of trees in the classes II and III were correlated to each other when Southern Bristle-Tyrants were alone or in conspecific pairs (n = 21, r = 0.658 for class II and 0.81 for class III, p <0.05). In such situations, because Southern Bristle-Tyrants forage in mid- and upper canopy and are not scansorial, we hypothesized that height of trees may influence their occupancy more than DBH, thus we removed DBH from the analyses (Table 4). When individuals were in mixed-species flocks, only the DBH of trees in class III was correlated with the height and was removed from the analyses (n = 33, r = 0.82, p < 0.05). No variables showed values above 3 in the VIF test. The importance of all variables indicated by GLM was confirmed by the HP analysis (Table 4).

When not associated with mixed-species flocks, habitat features influencing bird occurrence (higher values of I%) were proximity to river or lakes (I% = 37%, positive relationship) followed by "type of habitat" (I% = 29.8%,

| TABLE 4. Values of the variables selected by the final GLM using stepwise regression and the Hierarchical Partitioning Analyses (HP). |
|--|
| All variables pointed by the GLM were confirmed by the HP; $E =$ estimate; $SE =$ standard error; $I =$ independent contribution; $J =$ joint |
| contribution; $I\%$ = independent contribution in percentage; Z = z scores in HP tests ($p < 0.05$); I, II, and III = classes of trees according |
| to DBH; DBH = diameter at breast height; n = abundance of trees; habitat = type of habitat; water = presence of river, stream, or lake |
| within a 30-m radius centered at each plot; elevation $=$ elevation above sea level. |

| | Not associated with mixed-species flocks | | | | | | Associated with mixed-species flocks | | | | | |
|-------------------|--|-----|-------------------------|------|-----------|--------|--------------------------------------|-------|------|-------------------------|-----------|------|
| | GLM | | Hierarchical partitioni | | | ng GLM | | M Hie | | erarchical partitioning | | ing |
| | E | SE | Ι | J | I% | Z | Е | SE | Ι | J | I% | Z |
| Intercept | -7.1 | 3.8 | _ | _ | - | _ | 1.8 | 4.6 | _ | _ | _ | _ |
| DBH I | 0.3 | 0.1 | 4.2 | -0.4 | 15.9 | 5 | 0.4 | 0.1 | 5.2 | -1.9 | 15.1 | 6.5 |
| height I | -0.6 | 0.3 | 2.1 | -0.7 | 8 | 2 | -0.4 | 0.2 | 1.5 | -0.3 | 4.5 | 1.5 |
| nl | - | - | - | - | - | - | -0.2 | 0.06 | 3.07 | -0.2 | 8.9 | 3.8 |
| DBH II | - | - | - | - | - | - | 0.08 | 0.03 | 2.3 | -1.2 | 6.6 | 2.5 |
| height II | 0.2 | 0.1 | 0.4 | -0.1 | 1.7 | 0.09 | - | - | - | - | - | - |
| height III | - | - | - | - | - | - | 0.09 | 0.03 | 1.04 | -0.9 | 3 | 0.7 |
| habitat | -1.4 | 0.6 | 7.9 | 4.6 | 29.8 | 8.7 | -1.6 | 0.4 | 9.3 | 1.5 | 26.9 | 11.1 |
| water | 2.4 | 0.7 | 9.9 | 4.6 | 37 | 11.5 | - | - | - | - | - | - |
| elevation | - | - | - | - | - | - | -0.01 | 0.005 | 6.3 | 0.3 | 18.6 | 7.9 |
| Null deviance | 124.4 on 159 degrees of freedom | | | | | | 168.1 on 171 degrees of freedom | | | | | |
| Residual deviance | 77.8 on 153 degrees of freedom | | | | | | 99.2 on 163 degrees of freedom | | | | | |

negative), DBH of class I trees (I% = 15.9%, positive), height of class I trees (I% = 8%, negative), and height of class II trees (I% = 1.7%, positive). We interpret these results to mean that when Southern Bristle-Tyrants are in intraspecific associations they prefer sites on the edges of rivers and lakes, in "valleys," where class I trees have higher DBH and smaller height and class II trees have higher height (Figure 3). When near a river or lake, the distance from the center of the plot to water was \sim 7 ± 6.6 m (mean \pm SD). When associated with mixed flocks, the most important variables were "type of habitat" (I% = 26.9%, with a negative relationship, thus indicating that birds also prefer valleys), elevation (I% = 18.6%, negative), DBH of class II trees (I% = 6.6%, positive), height of class I trees (I%= 4.5%, negative), and height of class III trees (I% = 3%, positive) (Figure 4).

DISCUSSION

Density and Study Area

Our results suggest a low density of Southern Bristle-Tyrants (12.7 [7.3–20.2] individuals per km²) compared to other globally threatened small insectivorous passerines in the Atlantic Forest. Using the home range of the Restinga Tyrannulet calculated with the minimum convex polygon method, Gussoni (2014) estimated a density of 208 (144–272) individuals per km², 16 times the value we found for Southern Bristle-Tyrants. In a study that used the same methodology employed by us, Cabanne et al. (2007) estimated Araucaria Tit-Spinetail (*Leptasthenura setaria*) density of 90 individuals per km². Based on anecdotal observations, we believe that, as in the Southern Bristle-Tyrant, most *Phylloscartes* species occur in low densities

when compared to other insectivorous passerines, which suggests habitat loss is one of the main threats for these birds (Goerck 1997, BirdLife International 2016; but see Gussoni 2014). Low density with forest fragmentation causes isolation of small populations that have, for example, increased inbreeding rates and vulnerability to stochastic events (Traill et al. 2010). These fragmentation effects on small populations (including inbreeding depression) may compromise the long-term conservation of several sensitive species in the Atlantic Forest (Goerck 1997).

With the exception of the Mottle-cheeked Tyrannulet (Phylloscartes ventralis), the other 8 Atlantic Forest species of the genus are considered threatened (three "Endangered") or nearly threatened at the global level (BirdLife International 2016). Their threat status may be underestimated due to the lack of knowledge (Goerck 1997, BirdLife International 2016) and we suggest that density should be estimated for the other Atlantic Forest Phylloscartes species. The territorial and slow-moving behavior of Southern Bristle-Tyrants, associated with their loud and frequent song, make the species ideal for distance sampling methods (Buckland et al. 2001, Burnham et al. 2004, Buckland et al. 2015). The coefficient of variation and goodness-of-fit values for the competing models (Table 3) indicate good accuracy of the models (Buckland et al. 2001). However, point-count distance sampling may not be adequate for all Phylloscartes species, especially those with discrete and/or fast-moving behavior (e.g., Oustalet's Tyrannulet P. oustaleti, which emits only low call notes, and the Sao Paulo Tyrannulet P. paulista, which moves constantly in the forest while foraging for insects; Fitzpatrick 2004, Tonetti personal observation). The distance sampling premises that birds will sing with



FIGURE 3. Curves of presence probability associated with variables that were significant according to the models when Southern Bristle-Tyrants were alone or in pairs. Circles on the top of each graph represent plots where the species was observed foraging and below are random plots. Circles were spaced to avoid overlap.

certainty during point counts and will not move significantly after initial detection may not be met for these 2 species, and other methods of density estimation, such as focusing on estimating home range, may be more effective. Therefore, we suggest that behavior of other *Phylloscartes* species be taken into account when choosing density estimation methods (Buckland et al. 2001, Burnham et al. 2004, Buckland et al. 2015).

CSP bird species richness (326 species; Tonetti personal observation) is similar to another Atlantic Forest reserve 70 km from our study area in Serra do Mar: the Boracéia Biological Station (323 species; Cavarzere et al. 2010). Serra do Mar, the largest Atlantic Forest patch (\sim 1,000,000 ha; Ribeiro

et al. 2009), comprises several pristine forest remmants and the similarity between CSP and the Boracéia Biological Station bird richness indicates that despite its proximity to large urban centers, the Serra da Cantareira avifauna is well preserved (Bencke et al. 2006). The area also harbors other species sensitive to human disturbance, such as the Solitary Tinamou *Tinamus solitarius* (abundant at CSP; Bencke et al. 2006, BirdLife International 2016, Tonetti personal observation). This, in addition to Southern Bristle-Tyrant tolerance to human presence (nesting was observed on a trail with intense human movement in a Brazilian Atlantic Forest reserve; Lombardi et al. 2010), suggests that the current impact of the urban area on our results is likely minimal.



FIGURE 4. Curves of presence probability associated with variables that were significant according to the models when Southern Bristle-Tyrants were associated with mixed-species flocks. Circles on the top of each graph represent plots where the species were observed foraging and below are random plots. Circles were spaced to avoid overlap.

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However, Southern Bristle-Tyrants are currently not found in several localities where they occurred decades ago, reinforcing the importance of CSP for the species' conservation (Willis and Oniki 1993, Silveira 2009). Despite its importance, this reserve alone may not guarantee the species' conservation in the long term. Although no consensus exists about the minimum number required for a population to be viable over time, this number may be on the order of thousands of individuals for most taxa (Traill et al. 2010), higher than we estimated for the Southern Bristle-Tyrant at CSP (967 [556–1,539] individuals). Moreover, the lower density of Southern Bristle-Tyrants in one area (Pedra Grande) when compared to the other 2 also suggests that even subtle environmental variation occurring in a forest continuum can cause large differences in density.

Microhabitat Preference

Regarding vegetation structure, some authors have suggested that Southern Bristle-Tyrants occupy old growth, disturbed forests, and areas near clearings and edges (Fitzpatrick 2004, Esquivel et al. 2007). Lowen (1996) suggested a possible preference for more open areas and early successional forests. On the other hand, based on anecdotal observations, Silveira (2009) reported that the species inhabits only primary or old-growth forests. Our results show a positive relationship between the presence of Southern Bristle-Tyrants and trees with 5-12.5 cm DBH, and a negative relationship with the height of trees in class I. Trees in class I are in the lower strata of forests, and their lower height associated with higher DBH indicate an altered environment, where sunlight is great (Bibby et al. 2000). Despite this, we found a positive, but weak, relationship between the species' presence and the height of class II trees when not in mixed flocks (I% = 1.7) and between class III trees when associated with mixed flocks (I% = 3; Table 4). These 2 variables are characteristics of old-growth forests (Bibby et al. 2000) and we observed Southern Bristle-Tyrants foraging both in oldgrowth forests and in intermediate stages of regeneration. In addition, the lack of preference for the remaining vegetation parameters suggests that Southern Bristle-Tyrants have some tolerance to variation in the amount of canopy closure, quantity of vines, and the size and abundance of trees. However, despite the tolerance for vegetation structure, the species showed a strong preference for habitat near rivers, lakes, and "valleys," indicating that Southern Bristle-Tyrants need more-humid forest sites. Although valleys do not necessarily have rivers or lakes, they are generally wetter than sites on hillsides or hilltops due to water accumulation caused by topography (Bibby et al. 2000).

The strong preference for forest sites close to rivers and lakes when birds were in pairs or alone was not explicitly reported in the literature (Lowen 1996, Fitzpatrick 2004, Silveira 2009); however, some records of the species in sites sampled in other Brazilian states and in Argentina were made in riverine forest (e.g., Silveira 1998, Kirwan et al. 2001, Bodrati and Cockle 2006, Bodrati et al. 2010, Lombardi et al. 2010). We hypothesize that the strong preference for riverine vegetation caused the species' rarity in Pedra Grande. Because Pedra Grande has fewer rivers and lakes (IF 2009, Tonetti personal observation), individuals may not establish their territories in such an area.

As expected, habitat use by Southern Bristle-Tyrants showed a preference for wetter areas when alone or in pairs, but not so when in mixed flocks (Table 4). We believe this pattern occurred because the species only follows mixed flocks, not acting as flock leaders (Fitzpatrick 2004, Tonetti personal observation). The leader of a mixed-species flock (usually the Red-crowned Ant-Tanager [Habia rubica] in our study area; Maldonado-Coelho and Marini 2003, Tonetti personal observation) is supposedly responsible for selecting the microhabitat exploited by the flock. We hypothesize that the benefits of associating with mixed flocks (e.g., increased foraging efficiency, decreased risk of predation, and decreased energy expenditure to defend territory; Munn and Terborgh 1979) outweigh the advantages of using preferred microhabitats (i.e. far from humid sites).

Conservation and Policy Implications

The preference for riverine forests by insectivorous passerines in the Atlantic Forest and other threatened passerines in Brazil is well known (Anjos et al. 2007, Develey and Pongiluppi 2010, Metzger 2010). These wetter microenvironments may have a higher abundance of insects (Powell et al. 2015). In addition to harboring several bird species, riparian vegetation acts as forest corridors in fragmented landscapes and facilitates dispersal of birds that do not cross open areas (Lees and Peres 2008, Metzger 2010). Given their importance, riparian forests are protected by Brazilian federal legislation, the Forest Code (FC)—the central piece of legislation regulating land use and management on private properties (Soares-Filho et al. 2014).

The characteristic that most influences quality of riparian vegetation in conserving biodiversity is width. A study performed in a fragmented landscape in Amazonia indicated that riparian vegetation less than 200 m wide was not sufficient to keep bird communities similar to continuous forest (Lees and Peres 2008). Mixed-species flocks of insectivorous birds were also negatively affected by narrow strips of riverine vegetation (Lees and Peres 2008). Although there is no similar study in the Atlantic Forest, a minimum width of 100 m is necessary for riverine forests to adequately preserve the biodiversity in this domain (Metzger 2010). Unfortunately, recent changes in the FC dramatically reduced the protection of riverine vegetation in the Atlantic Forest (Soares-Filho et al. 2014).

The new FC requires that, depending on the size of rivers or lakes, vegetation strips ranging from 30 to 500 m wide must be preserved. Moreover, a current decree (n° 7830) requires that, for small rural properties, only 5 m of riverine vegetation need be preserved, independent of the size of rivers and lakes.

Besides its direct effect on biodiversity, the reduction of forests caused by the new FC can diminish rainfall (Dobrovolski and Rattis 2015). The decrease in rainfall on the Southern Bristle-Tyrant range may be caused by both deforestation in Amazonia and the Cerrado, as well as local deforestation in the Atlantic Forest (Dobrovolski and Rattis 2015). Our study area is located in one of the largest water reservoirs in the world, the Cantareira system. Similar to several other Atlantic Forest regions (Ribeiro et al. 2009), 76.5% of the rivers comprising the Cantareira system have had their riparian forest impacted (Hirota 2014). Not surprisingly, the region recently faced the most severe drought on record, putting at risk the water supply for more than 8.8 million people (Dobrovolski and Rattis 2015). Thus, we suggest that riparian forest be restored in the Atlantic Forest to aid the conservation of Southern Bristle-Tyrants and to ensure a water supply for millions of people. To conserve the species and other forest insectivorous birds, we also suggest that Brazil's FC should be revised to enhance the protection of riverine forests.

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Authors contributions: V.R.T. and M.A.P. conceived the idea, design, and experiment. V.R.T. performed the experiments. V.R.T. and M.A.P. wrote the paper. V.R.T. developed or designed the methods. V.R.T. analyzed the data.

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