1 **Association between land use and composition of amphibian species in**
2 **temperate Brazilian forest remnants**

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19 **Abstract**

20 We evaluated the influence of landscape configuration on the diversity of anurans in
 21 Atlantic Forest remnants in southern Brazil. As natural habits provide better conditions
 22 for the survival of amphibians, we expected to find more diverse communities in areas
 23 with more forest cover. We sampled tadpoles in 28 breeding sites distributed in seven
 24 forest remnants. We recorded 22 anuran species and richness varied from 6 to 12
 25 species between sites. Most of the recorded species were not forest specialists, except
 26 for *Boana curupi* and *Crossodactylus schmidtii*. There was a significant overlap in the
 27 species composition between all remnants, and the Generalized Linear Mixed Model
 28 indicated that landscape use did not affect species richness. The PERMANOVA showed
 29 that forest and livestock farming explained the dissimilarity in the composition of the
 30 communities. One possible explanation for this is that the remnants are surrounded by a
 31 relatively well-preserved landscape, which offers favorable conditions for the
 32 maintenance of local populations and homogenizes species composition across the
 33 sampling sites. The lack of any strong association between tadpole species richness and
 34 land use suggests that anurans are primally affected by habitat characteristics that are
 35 detected only on a fine-scale analysis.

36 **Keywords** Conservation - Frog communities - Landscape - Tadpoles

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42 **Compliance with ethical standards**

43 **Conflict of interest** The authors declare that they have no conflict of interest.

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53 **Code availability** Not applicable.

54 **Authors' contributions** All authors contributed to the study conception and design,
55 material preparation, and writing of the manuscript. Data collection and species
56 identification were performed by RCS, remote sensory analysis was performed by DB
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62 **Introduction**

63 The increase of the human population generates demands such as larger areas for
64 agriculture and livestock farming, as well as more roads and buildings, which leads to
65 increased consumption of natural resources. Consequently, humans promote a variety of
66 landscape modifications (Gururaja et al. 2008; Zhou et al. 2017). When natural habitat
67 is replaced by roads, buildings or other urban facilities, many ecological interactions
68 can be affected, such as predation, competition and host/parasite relationships (Nomura
69 et al. 2011; Laufer et al. 2015; Preuss et al. 2020; Santos et al. 2020). These changes can
70 vary according to the intensity in which habitat is lost and air, soil and water are
71 polluted (Riley et al. 2005; Brand et al. 2010).

72 One of the most studied negative effects of agriculture on wildlife is its role as a
73 source of contamination by pesticides and herbicides (Koumaris and Fahrig 2016).
74 However, when a forest is replaced by agriculture or cattle pasture, besides all issues
75 listed below, there will be also a sensible reduction in both habitat complexity and
76 landscape heterogeneity (Machado et al. 2012; Saccol et al. 2017), which favors a
77 gradual reduction in species diversity (McKinney 2008; Barrows and Allen 2010). As
78 the human occupation of a landscape increases, the proximity between natural habitats
79 and urban areas reduces, causing a series of indirect (and less studied) impacts on the
80 fauna, such as light pollution (Dias et al. 2019), noise pollution (Pellet et al. 2004) and
81 road kills (Diniz and Brito 2015). In sum, the facts listed above highlight the relevance
82 of constant monitoring changes in landscape induced by human occupation.

83 Landscape evaluation is a powerful tool to access the risks of biodiversity
84 deployment and, consequently, supports environmental management. At the species
85 level, landscape configuration affects fauna displacement (Fahrig and Nettle 2005),

86 which is a baseline for testing models of metapopulation and metacommunities. In
 87 general, species with low displacement ability, such as amphibians, are more sensitive
 88 to landscape changes (Schmutzer et al. 2008; Dixo and Metzger 2010; Diniz and Brito
 89 2013; Cayuela et al. 2015). Most amphibians breed in aquatic sites, and changes in
 90 landscapes can restrain access to ponds or streams (Becker et al. 2007, 2010; Machado
 91 et al. 2012; Cayuela et al. 2015; Saccol et al. 2017; Dalmolin et al. 2019). This can
 92 promote morphological and physiological changes in tadpoles and adults (Costa et al.
 93 2017) and reduce reproductive potential, causing changes in the communities' structure
 94 and composition (Berriozabal-Islas et al. 2018; Dalmolin et al. 2020), as well as
 95 population declines or local extinctions (Marsh and Trenham 2001; Rothermel 2004;
 96 Goutte et al. 2013). Therefore, amphibians are considered good models for ecological
 97 studies on understanding the impact of landscape changes on wildlife. The monitoring
 98 of amphibian populations has been filling the gaps in our understanding of how
 99 landscape changes affect species diversity (Becker et al. 2007; Pillsbury and Miller
 100 2008; Nomura et al. 2011; Collins and Fahrig 2017) and the physiology and
 101 morphology of amphibians (Costa et al. 2017; Berriozabal-Islas et al. 2018). This
 102 highlights the importance of studies that include a greater variety of altered or natural
 103 habitats and conduct measurements in the surroundings of the sampled habitats.

104 The Brazilian Atlantic Forest is highly fragmented, with more than 97% of its
 105 fragments being smaller than 250 ha (Ribeiro et al. 2009; Zanella et al. 2012). The
 106 Atlantic Forest is a biodiversity hotspot (Myers 2000; Mittermeier et al. 2005) and
 107 harbors a high anuran richness and endemism (Haddad et al. 2013). In the southern
 108 Atlantic Forest, most of the original forest areas were replaced by agriculture, pasture,
 109 silviculture and urban areas (Ribeiro et al. 2011). Studies on amphibians in this region
 110 have associated species diversity with characteristics of habitat heterogeneity

111 (Gonçalves et al. 2015; Knauth et al. 2018; Figueiredo et al. 2019), but few have
112 analyzed wider scales that considered the effects of the landscape on species
113 composition. Landscape analyses allow the detection of changes regarding land use and
114 occupation in scales that are compatible with the species or groups in question (Hamer
115 and Parris 2011), helping define local and regional conservation strategies.

116 Many studies have focused on the role of size and connectivity of forest
117 fragments on species diversity. However, less attention is given to the role of
118 surrounding environments generated by human occupation. In this study, we evaluated
119 the influence of the surrounding landscape configuration on the diversity of anurans in
120 forest remnants in southern Brazil. Our analysis included quantification of different
121 types of land use surrounding these forest remnants. As natural habitats provide better
122 conditions for the survival, reproduction and maintenance of amphibian populations
123 (Collins and Fahrig 2017), we expected to find more diverse communities in areas with
124 more forest cover.

125 **Material and methods**

126 *Study site*

127 We conducted this study in Atlantic Forest remnants in southern Brazil (Fig. 1; Table
128 S1). Forest remnants consist of mixed ombrophilous forest and seasonal forest with a
129 variety of surrounding matrix including livestock pastures, agriculture, silviculture and
130 urban areas (SOS Mata Atlântica and INPE 2008; Ribeiro et al. 2009; Pillar and Véllez
131 2010). The climate is subtropical, with annual temperatures varying from 16 °C to 24 °C
132 and rainfall varies from 1600 to 2200 mm annually (Alvares et al. 2013). We selected
133 seven remnants based on the following criteria: a) presence of well-preserved forest
134 (public or private protected areas); b) similar climatic conditions; c) altitude between

135 300 m and 900 m above the sea level; d) similar topography. We sampled seven
136 remnants distributed across 10.500.000 ha, between the coordinates 22°30' to 33°45' S
137 (latitude) and 48°02' to 57°40' W (longitude).

138 In each remnant, we performed tadpole sampling in water bodies. We sampled a
139 total of 28 water bodies used as breeding sites (13 ponds and 15 streams). We sampled
140 all possible waterbodies including permanent and temporary ponds and streams to
141 access a greater variety of habitats and their tadpole communities (Melo et al. 2017).
142 Pond size varied from 71.20 to 2087 m² (787.27 ± 561.33). In streams, we sampled 100-
143 m-long sections with a width varying from 0.60 to 3.50 m (1.76 ± 0.79). The depth of
144 the ponds varied from 0.30 to 1.50 m (0.8 ± 0.40) and of streams from 0.15 to 0.70 m
145 (0.45 ± 0.12 ; Appendix, Table S1). We performed a time-limited sampling effort.

146 *Tadpole sampling*

147 Tadpole sampling occurred from October 2018 to March 2019, which corresponds to
148 the anuran breeding season and is the most favorable period for detection of
149 tadpoles (Both et al. 2008; Santos et al. 2008). Tadpole sampling was performed from
150 8:00 AM to 6:00 PM using a 3-mm²-mesh dip-net (Heyer 1976). Sweepings were
151 systematized and consisted of scouring the margins of the pond (Vasconcelos and
152 Rossa-Feres 2005; Santos et al. 2007; Both et al. 2009; Bolzan et al. 2016). In the
153 streams, some samples were also made using small dip nets to sample narrow spaces
154 between rocks (adapted from Jordani et al. 2017). Sampling was performed once in each
155 pond or stream. Each sample consisted of one hour of sweeping efforts through the
156 greatest possible variety of microhabitats for one hour.

157 Immediately after capture, tadpoles were euthanized by immersion in a solution
158 of 2% lidocaine, following Brazilian Regulations (CONCEA 2018), and subsequently

159 transferred to absolute ethanol. In the laboratory, tadpole species were determined with
160 the aid of a stereomicroscope and identification keys (Machado and Maltchik 2007;
161 Gonçalves 2014). The collection and manipulation of specimens were authorized by
162 ICMBio (#64962), state organs (IAP/PR #33/2018, IMA/SC #01/2019, SEMA/RS
163 #37/2018) and the Animal Ethics Committee of Universidade do Vale do Rio dos Sinos
164 (CEUA-UNISINOS # PPECEUA08.2018).

165 *Landscape assessment*

166 We assessed land use based on the analysis of satellite images (Landsat 8 multispectral
167 images, sensor Operational Land Imager from United States Geological Survey). We
168 classified the images from their vectorization in the software ArcGIS version 10.3,
169 considering a 250-m-radius buffer for each sampled water body. Buffer size was
170 defined based on the estimative of the size of habitats used by anurans (Semlitsch and
171 Bodie 2003; Smith and Green 2005; Tozetti and Toledo 2005; Canessa and Parris
172 2013). All images were captured in the current sampled year (2019) and presented the
173 minimal cloud cover available and without significant radiometric noise. We performed
174 the following stages of image preprocessing: 1) geometric corrections due to the
175 inherent geometric distortions in images collected in distinct moments, by
176 georeferencing these images; 2) atmospheric corrections aiming to reduce the
177 interference of atmospheric scattering on the images (Soares et al. 2015); and 3)
178 mosaicing and highlight of the different images in each moment aiming to reduce
179 seasonal effects on the image's visual aspect. Preprocessing was conducted with the
180 software ENVI, version 5.51. After the pre-processing stages, we defined the categories
181 of land use and occupation with adjustments based on field observations. We were able
182 to identify the following categories (landscape variables) of land use: (1) Agriculture

183 (cultivated areas, with soybean, corn or wheat); (2) Aquatic environments (streams,
184 artificial and natural ponds); (3) Forest (native forest formations); (4) Livestock pastures
185 (extensive livestock farming); (5) Urban area (buildings). The polygons regarding each
186 cover type were projected for the reference system SIRGAS 2000, Universal Transverse
187 Mercator (UTM) projection, zone 22S, and areas were calculated in km².

188 *Data analysis*

189 The comparison of species richness was based on rarefaction curves (interpolation and
190 extrapolation method) representing standardized measures of individual abundance
191 (Chao and Jost 2012). Confidence intervals (95%) associated with the curves were
192 calculated using the Bootstrap method (50 randomizations). These analyses were
193 performed using the iNEXT program (iNterpolation and EXtrapolation) (Chao and
194 Hsieh 2016).

195 We performed a Principal Components Analysis (PCA) to check which
196 variables were more associated with the landscape configuration of each remnant (and
197 its community). The PCA was performed using a water bodies matrix and a landscape
198 matrix with a 250 m buffer.

199 We tested the influence of landscape variables (Appendix, Table S2) on species
200 richness by using generalized linear mixed-effects models (GLMMs). We included
201 water bodies as a random variable. We evaluated the significance of each explanatory
202 variable by using the “ANOVA” function. The full model was analyzed using the “glm”
203 function of the lme4 package (Bates et al. 2015), in R v.3.6.0 (R Core Team 2019).

204 We assessed the differences in species composition between remnants (β -
205 diversity) and the relationship with landscape variables by using Permutational
206 Multivariate Analysis of Variance (PERMANOVA) with 999 permutations (Borcard et

al. 2011; Magurran and McGill 2011). Additionally, we used a Non-Metric Multidimensional Scaling (NMDS) to visualize and interpret these differences. PERMANOVA and NMDS were performed, respectively, by using the “adonis” and “metaMDS” functions of the vegan package in R v.3.6.0 (R Core Team 2019). For analysis using landscape variables on species diversity, only 23 water bodies were used to avoid overlapping buffers used to assess land use.

Results

We recorded 22 anuran species belonging to eight families: Bufonidae (2 species; 9.1% of the total), Hylidae (12; 54.5%), Hylodidae (1; 4.5%), Leptodactylidae (3; 13.6%), Microhylidae (1; 4.5%), Odontophrynidae (1; 4.5%), Phyllomedusidae (1; 4.5%) and Ranidae (1; 4.5%), the latter represented by the exotic species *Lithobates catesbeianus* (Table 1). Generalist species were predominant in terms of habitat use, with exception of *Boana curupi* and *Crossodactylus schimdti*, which are forest-related species. Species composition was dominated by habitat generalists (*Boana faber*, *Dendropsophus minutus*, *Lithobates catesbeianus*, *Physalaemus* cf. *carrizorum*, *P. cuvieri*, *Scinax fuscovarius*). The richness among forest remnants ranged from 6 to 12 species (8.72 ± 2.06), and abundance from 213 to 680 individuals (408.7 ± 101.6 ; Table 1). Interpolation and extrapolation curves evidenced that species richness differed between remnants (Fig. 2).

Each of the two of PCA's axes was related to different types of land use and together, explained 62% of the variation of land use surrounding the remnants (Table S3). The presence of forest, livestock pastures and aquatic environment were more associated with axis 1, while agriculture and urbanization were more associated with axis 2. Tadpole communities presented visible segregation in the biplot, with the

remnants 2, 3, 5 and 7 mainly distributed across axis 1 and remnants 1, 4 and 6, across axis 2 (Fig. 3; Table S4). However, the GLMM model showed that landscape use did not affect species richness (Table 2), with the largest amount of richness (75%) explained by a random effect ($R^2_m = 0.14$; $R^2_c = 0.89$). With exception of remnants 1 and 6, all forest remnants presented high overlap in their species composition (Fig. 4).

Satellite image analysis showed that the dominant classes of land use were forests and livestock pasture (Table S2). Together, forests and livestock farming were pointed out by PERMANOVA as the main landscape component to explain the patterns of compositional dissimilarity (beta diversity) between remnants (forest, $R^2 = 0.10$, $F = 2.35$, $p = 0.01$; livestock farming, $R^2 = 0.08$, $F = 1.93$, $p = 0.02$; see Table 3).

Discussion

We recorded 22 anuran species, which correspond approximately to two-thirds of the richness found in similar forest remnants in southern Brazil (Lucas and Fortes 2008; Iop et al. 2012; Bastiani and Lucas 2013). Hylidae represented over half of species, most of which are considered generalists and found in both forest environments and grasslands or forest edges (Lucas and Fortes 2008; Almeida-Gomes and Rocha 2014; Barbosa et al. 2014; Oliveira et al. 2017). The lack of any strong association between tadpole species richness and land use suggests that anurans are primally affected by habitat characteristics which are detected only on a fine-scale analysis. Breeding site configuration, such as pond descriptors, for example, would play a bigger role than land use over tadpole species diversity (Dalmolin et al. 2020). Although we have no detailed data to support this hypothesis, several studies have shown that microhabitat characteristics affect amphibian richness (D’Anuniação et al. 2013; Knauth et al. 2018; Figueiredo et al. 2019). Forest heterogeneity, leaf litter depth, canopy cover and

255 presence of clearings are examples of local descriptors that are not detected at a
256 landscape level but affect anuran species composition (Ferrante et al. 2017; Howell et
257 al. 2019). Most local elements of the habitats support species persistence by providing
258 fundamental resources as well as shelter (Erős et al. 2014; Landeiro et al. 2014; Datry et
259 al. 2016; Collins and Fahrig 2017). This idea is reinforced by the fact that *Boana curupi*
260 and *Crossodactylus schimdti* were registered only where local characteristics, such as
261 streams with a rocky bottom, were present (Bastiani et al. 2012; Bastiani and Lucas
262 2013; Caldart et al. 2013).

263 One possible explanation for the similarities in species composition between
264 forest remnants is the fact that they are surrounded by relatively well-preserved
265 landscapes. Although a little speculative, we believe that that they could offer favorable
266 conditions for the maintenance of local populations and homogenize species
267 composition at a regional scale. As the remnants are surrounded mainly by other forest
268 formations and pastures, they are little exposed to pollution and other anthropogenic
269 disturbs, being able to support high species diversity (Becker et al. 2010; Ferreira et al.
270 2016; Preuss et al. 2020).

271 However, we do not minimize the relevance of landscape proprieties, such as the
272 amount of available habitat, for the colonization and persistence of species (Faggioni et
273 al. 2020). Seasonal displacements, such as those related to mating and oviposition,
274 involve many risks, and landscape changes could negatively affect them, causing a
275 strong impact on reproductive cycles (Becker et al. 2010). Thus, we strongly
276 recommend new landscape-scale studies evaluating anuran diversity in a wider range.

277 Despite the predominance of forested habitats, we recorded only two forest
278 species (*Boana curupi* and *Crossodactylus schimdti*). Species composition was
279 dominated by habitat generalists (*Boana faber*, *Dendropsophus minutus*, *Lithobates*

280 *catesbeianus*, *Physalaemus* cf. *carrizorum*, *P. cuvieri*, *Scinax fuscovarius*). The
 281 predominance of habitat generalists and widely distributed species has already been
 282 pointed out in other forest remnants that were studied in this portion of the Atlantic
 283 Forest (Lucas and Fortes 2008; Lucas and Marocco 2011; Bastiani and Lucas 2013).
 284 This could be a result of the deforestation of the Atlantic forest, in which species
 285 adapted to open conditions replace specialized species that are adapted to the forest
 286 (Haddad and Prado 2005). Homogenization of biota (Ferrante et al. 2017; Nowakowski
 287 et al. 2018) could explain the lack of relationship between species composition and
 288 landscape properties that was recorded.

289 We highlight the fact that, in the whole sampled area, forest species were limited
 290 to two species (*Boana curupi* and *Crossodactylus schmidtii*). We have no information
 291 about the species composition in the studied forest remnants in past decades. Thus, we
 292 cannot affirm whether we are showing a recent or a well-established scenario about the
 293 regional anuran species composition. Forest-related species tend to be more prone to
 294 population decline due to their low ability to migrate across areas where the canopy
 295 cover is absent (Howell et al. 2019). The conversion of forest to pasture, which occurred
 296 decades ago, could have favored the local extinction of other forest-related anuran
 297 species. At the same time, these habitat changes favor the colonization by species such
 298 as *Boana faber* and *Dendropsophus minutus* (Aquino et al. 2004, 2010; Scott et al.
 299 2004; Lavilla et al. 2010; Silvano et al. 2010). These species are often found in
 300 fragmented landscapes and expanding open areas (Preuss 2018; Figueiredo et al. 2019;
 301 Menin et al. 2019). We also recorded the exotic bullfrog (*Lithobates catesbeianus*) in
 302 four of the seven remnants. This species is widely distributed and well established in
 303 altered environments of Brazil's South region (Both et al. 2011; Madalozzo et al. 2016).
 304 The process of dispersion and colonization of new localities is possibly expanding

305 (Santos-Pereira and Rocha 2015) together with anthropogenic changes. Little is still
306 known about the persistence of populations in altered environments, so this is an
307 important subject to be investigated in future studies.

308 The presence of livestock pasture (farming) and forests were the landscape
309 components that best explained the dissimilarity in species composition. The role of
310 forests and pastures over amphibian species composition is relatively well understood
311 (Haddad et al. 2013; Howell et al. 2019). At the same time, the conversion of forests
312 into areas of pasture, agriculture or urbanization may limit or prevent the permanence of
313 forest-associated species. In this sense, the composition of amphibian communities in
314 remaining natural areas may change over time as a result of the colonization or
315 permanence of generalist species (Ferrante et al. 2017). In this sense, we encourage
316 future studies that deal with past and present species composition and the role of
317 landscape changes and different surrounding matrices in past decades for current
318 species assemblages.

319

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Table 1 Tadpole abundance and observed and estimated richness (Chao 1) for the seven sampled remnants, in southern Brazil. Sites: 1 = Parque Estadual de Vila Velha; 2 = Parque Estadual Rio Guarani; 3 = Reserva Privada Enele; 4 = Parque Estadual das Araucárias; 5 = Reserva Privada Quebra Queixo; 6 = Parque Estadual Fritz Plaumann; 7 = Parque Estadual do Turvo

Family/Species	Remnants							Total
	1	2	3	4	5	6	7	
BUFONIDAE								
<i>Rhinella henseli</i> (Lutz 1934)	119	0	12	0	0	0	0	131
<i>Rhinella icterica</i> (Spix 1824)	0	401	40	0	0	0	0	441
HYLIDAE								
<i>Aplastodiscus perviridis</i> (Lutz 1950)	0	1	0	0	0	0	0	1
<i>Boana</i> cf. <i>curupi</i>	0	0	0	0	0	122	0	122
<i>Boana curupi</i> (Garcia, Faivovich and Haddad 2007)	0	0	0	148	0	90	0	238
<i>Boana faber</i> (Wied - Neuwied 1821)	0	12	54	230	48	10	46	400
<i>Boana leptolineata</i> (Braun and Braun 1977)	0	0	0	9	0	0	0	9
<i>Boana prasina</i> (Burmeister 1856)	83	0	0	0	0	0	0	83

<i>Boana pulchella</i> (Duméril and Bibron 1841)	13	0	0	0	0	0	0	13
<i>Dendropsophus microps</i> (Peters 1872)	12	0	14	0	7	40	14	87
<i>Dendropsophus minutus</i> (Peters 1872)	5	21	19	19	55	0	2	121
<i>Scinax fuscovarius</i> (Lutz 1925)	11	28	9	0	9	5	0	62
<i>Scinax granulatus</i> (Peters 1871)	3	3	14	2	0	0	0	22
<i>Scinax perereca</i> (Pombal, Haddad and Kasahara 1995)	0	1	2	0	0	9	157	169
HYLODIDAE								
<i>Crossodactylus schmidtii</i> (Gallardo 1961)	0	179	0	0	0	16	118	313
LEPTODACTYLIDAE								
<i>Leptodactylus latrans</i> (Steffen 1815)	0	0	19	72	0	0	0	91
<i>Physalaemus cuvieri</i> (Fitzinger 1826)	46	0	169	0	92	0	0	307
<i>Physalaemus</i> cf. <i>carrizorum</i> (Cardozo and Pereyra 2018)	0	0	13	0	0	8	0	21
MICROHYLIDAE								
<i>Elachistocleis bicolor</i> (Guérin-Méneville 1838)	0	2	0	0	0	0	0	2
ODONTOPHRYNIDAE								
<i>Proceratophrys avelinoi</i> (Mercadal de Barrio and Barrio 1993)	0	0	0	7	0	0	0	7

PHYLLOMEDUSIDAE

<i>Phyllomedusa tetraploidea</i> (Pombal and Haddad 1992)	88	7	0	6	0	19	56	176
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RANIDAE

<i>Lithobates catesbeianus</i> (Shaw 1802)	0	25	10	0	2	8	0	45
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Total abundance	380	680	375	493	213	327	393	2861
Observed richness	9	11	12	8	6	10	6	22
Estimated richness (Chao 1)	9	11.5	12	8	6	10	6	

635 **Table 2** Result of the Generalized Linear Mixed-Effect Models (GLMM), with the function “glm” for amphibian richness and 250-m-buffer
636 landscape variables in 23 water bodies (breeding sites) in southern Brazil, recorded from October 2018 to March 2019

Variable	numDF	denDF	F-value	P-value
Forest	1	17	55.87	0.61
Agriculture	1	17	0.25	0.60
Livestock farming	1	17	0.28	0.28
Urban area	1	17	1.22	0.28
Aquatic environment	1	17	0.59	0.44

637

638 **Table 3** Results of PERMANOVA showing the contribution of landscape variables to the dissimilarity patterns in amphibian composition in the
639 set of communities

Variable	df	Sums Sqs	Mean Sqs	F-Model	R2	P-value
Forest	1	0.91	0.91	2.35	0.09	0.01*
Agriculture	1	0.59	0.59	1.52	0.06	0.39
Livestock farming	1	0.75	0.75	1.93	0.08	0.02*
Urban area	1	0.38	0.38	0.98	0.04	0.22

Aquatic environment	1	0.28	0.28	0.74	0.03	0.34
Residuals	17	6.60	0.38		1.00	
Total	22	9.54				

640

641

642 **Figures**

643

645 **Fig. 1** Forest remnants and sampling sites of amphibian collection conducted between October 2018 and March 2019 in southern Brazil. 1 =
646 Parque Estadual de Vila Velha, Ponta Grossa/PR; 2 = Parque Estadual Rio Guarani, Três Barras/PR; 3 = Reserva Privada Enele, São Lourenço
647 do Oeste/SC; 4 = Parque Estadual das Araucárias, São Domingos/SC; 5 = Reserva Privada Quebra-Queixo, São Domingos/SC; 6 = Parque
648 Estadual Fritz Plaumann, Concórdia/SC; 7 = Parque Estadual do Turvo, Derrubadas/RS. The detail depicts sampling points of the sampling units
649 – 1 = Pond in Parque Estadual de Vila Velha; 4 = Stream in Parque Estadual das Araucárias; 5 = Pond in Reserva Privada Quebra-Queixo; 7 =
650 Stream in Parque Estadual do Turvo
651

653 **Fig. 2** Comparison of the richness of anuran species in seven Atlantic Forest remnants in southern Brazil through rarefaction
 654 (interpolation/extrapolation) based on the number of individuals. R1 (orange) = Parque Estadual de Vila Velha; R2 (navy blue) = Parque
 655 Estadual Rio Guarani; R3 (lilac) = Reserva Privada Enele; R4 (purple) = Parque Estadual das Araucárias; R5 (green) = Reserva Privada Quebra-
 656 Queixo; R6 (sky blue) = Parque Estadual Fritz Plaumann; R7 (yellow) = Parque Estadual do Turvo
 657

658

659 **Fig. 3** Ordination biplot with the two first axes of the Principal Components Analysis considering the association between 23 sampled breeding
 660 sites in seven remnants and the landscape variables in southern Brazil. Symbols ($\circ$, Δ , $+$, $\times$, $\diamond$, ∇ , $\square$) represent the remnants. $\circ$ R1 = Parque
 661 Estadual de Vila Velha; Δ R2 = Parque Estadual Rio Guarani; $+$ R3 = Reserva Privada Enele; $\times$ R4 = Parque Estadual das Araucárias; $\diamond$ R5 =
 662 Reserva Privada Quebra-Queixo; ∇ R6 = Parque Estadual Fritz Plaumann; $\square$ R7 = Parque Estadual do Turvo
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665 **Fig. 4** Non-metric multidimensional scaling (NMDS) based on Bray-Curtis distances and integrated environmental parameters depicting
666 compositional differences among forest remnants. Only two dimensions are given to facilitate interpretation. The ellipses represent the formed
667 groups, the symbols (+) represent the sampled breeding sites and the sampled forest remnants are represented as follows: + R1 = Parque Estadual
668 de Vila Velha; + R2 = Parque Estadual Rio Guarani; + R3 = Reserva Privada Enele; + R4 = Parque Estadual das Araucárias; + R5 = Reserva
669 Privada Quebra-queixo; + R6 = Parque Estadual Fritz Plaumann; + R7 = Parque Estadual do Turvo