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Changes in land use affect anuran helminths in the South Brazilian grasslands

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Abstract

Degradation and habitat loss of natural grasslands in Southern Brazil has a negative impact on native organisms, potentially including the composition of anuran helminth communities. Here, we characterized the richness, abundance, taxonomic composition, prevalence and intensity of helminth infection in four anuran species. Host anurans were collected in 34 ponds (19 in native grasslands with livestock and 15 in agricultural cultivation) from the highland grasslands in the Brazilian states of Santa Catarina and Paraná. Our results showed a significant difference between native grasslands with livestock and agricultural cultivation regarding the structure of helminth communities for the hosts *Aplastodiscus perviridis* and *Pseudis cardosoi*. We also found a greater prevalence and intensity of infection in anurans in areas of agricultural cultivation than in native grasslands with livestock. We found that the environmental descriptors (local and landscape) seem to explain most of the differences in anuran parasitism recorded between native grasslands with livestock and agricultural areas. Thus, we emphasized that the loss of grassy habitat due to conversion to agricultural cultivation can alter helminth communities in anurans, with further work needed to understand the mechanisms involved.

Introduction

Brazil contains a greater richness of biodiversity than any other country in the world (MMA, 2003; Oliveira *et al.*, 2017), and is also where some of the greatest losses of biodiversity are occurring, mainly through the fragmentation of the natural environment by human action (MMA, 2003; Watson *et al.*, 2016; Pardini *et al.*, 2018). Among the most threatened ecosystems are the South Brazilian grasslands, whose conservation has been neglected (Overbeck *et al.*, 2007, 2015). In fact, most of these grasslands are private lands used as pasture for livestock and are under imminent threat of conversion to other uses such as silviculture and annual crops (mainly soybeans) (Pillar & Vélez, 2010; Santos *et al.*, 2014). In addition to the low level of legal protection, little is known about the biodiversity of the grasslands in Southern Brazil.

Degradation and the loss of habitat negatively impact native species, since associated processes (such as habitat fragmentation) lead to a reduction in the local abundance of species, as well as increased isolation between populations. This affects ecological processes both at the level of populations and communities (Rathcke & Jules, 1993; Brunner & Eizaguirre, 2016; Di Marco *et al.*, 2017; Powers & Jetz, 2019). Among the organisms that suffer most from environmental changes are amphibians (Miguel *et al.*, 2007), which are considered sensitive to hydrological alterations, as well as air and water contamination by chemical agents and large-scale climatic variations (Vitt *et al.*, 1990; Egea-Serrano *et al.*, 2012; Pereyra *et al.*, 2018). As a result of these pressures, amphibians are experiencing high rates of population decline and are threatened with an unprecedented risk of extinction (Verdade *et al.*, 2010; Knapp *et al.*, 2016; González-del-Pliego *et al.*, 2019).

The loss of habitat also influences the dynamics and composition of the anuran helminth communities. The structure of the helminth community depends on many factors, including host and helminth life histories (coevolution) (Janovy *et al.*, 1992; Brooks *et al.*, 2006; Brunner & Eizaguirre, 2016; Kołodziej-Sobocińska, 2019), diet and habitat (McAlpine & Burt, 1998; Poulin, 1998; Bolek & Coggins, 2003; Zelmer & Arai, 2004; Pinheiro *et al.*, 2019). It is known that helminth infections cause several negative effects on the development and fitness of anurans. For example, metacercariae of some trematodes species may interfere with the normal development of legs in anuran larvae, resulting in structurally abnormal legs, including leg duplication (Johnson *et al.*, 2007). In contrast, hosts may exhibit different defences against infection, including variations in immunity, behaviour, stress and physiological responses (Hart, 1994; Schmid-Hempel & Ebert, 2003; Kirschman & Milligan-Myhre, 2018). However, these defence responses cost the host in the form of energy expenditure, which cannot then be used for other purposes such as reproduction. Indeed, helminths can adversely affect anuran populations, so it is important to understand what may affect their prevalence and occurrence (Blaustein *et al.*, 2012; Koprivnikar *et al.*, 2012a, b; Bower *et al.*, 2018). Thus, the knowledge of the ecological processes of helminths on declining host species such as anurans is of paramount importance (McCallum & Dobson, 1995, 2002; Aguirre, 2017; Allen *et al.*, 2017).

Helminths are indicative of many biological aspects of their hosts, including diet, habitat occupation and phylogeny, and may also be good direct indicators of environmental quality (Aguiar, 2014; Dias *et al.*, 2017; Januário *et al.*, 2019). Previous studies suggested that the environment is 'healthy' when hosts are exposed to a high diversity of helminths (Marcogliese, 2004, 2005; Hudson *et al.*, 2006). Indeed, areas with anthropogenic disturbance have few helminth species; this occurs due to the fact that the life cycle of most parasitic helminths requires multiple hosts, so when a disturbance affects some of the host population, transmission may decrease or increase (Marcogliese, 2005; Hudson *et al.*, 2006). Therefore, the richness and diversity of a helminth community can indicate the richness of free-living species that live or use the ecosystem (Marcogliese, 2005; Hudson *et al.*, 2006). Thus, studies with helminths of anurans are of great importance, especially in areas of intense agricultural activity, where there is an increase in the number of infected anurans (Kiesecker *et al.*, 2004; Allen *et al.*, 2017; Guo *et al.*, 2018). In these areas, the environmental properties of ponds and the landscape matrix are a crucial factor affecting the relation between helminths and anuran hosts. Broad-scale environmental factors can make the survival, development, distribution and transmission of infective forms either difficult or easy for the host (Basualdo *et al.*, 2007). Local-scale factors (e.g. vegetation around pond edges, water temperature) and landscape factors (e.g. habitat connectivity, land-use type and habitat fragmentation in the pond's surroundings) will affect the capacity of helminths to effectively disperse between hosts and among sites (Krasnov *et al.*, 2005). Therefore, these categories of environmental descriptors have been used in other studies with free-living animals and plants for understanding patterns of biological communities related to environmental characteristics (Numa *et al.*, 2009; Mattsson *et al.*, 2013; Browne & Karubian, 2016). In fact, studies using this approach help us to better understand and predict changes in both helminth communities and helminth populations (McDevitt-Galles *et al.*, 2018).

At the local scale (i.e. pond variables), components of agricultural runoff increase helminth abundance in frogs by increasing the susceptibility to infection through immunosuppression (Kiesecker, 2002; Carey *et al.*, 2003; Christin *et al.*, 2003, 2004). In this context, any physico-chemical changes to the environment that prevent intermediate and final hosts from occupying or using a habitat may influence the transmission and establishment of helminths, especially those that depend on trophic pathways and food web structure for infection (Cone *et al.*, 1993; Marcogliese, 2003, 2004). Additionally, previous studies recorded that agricultural cultivation has been linked to an increase in infection levels of direct lifecycle nematodes and certain larval trematodes in ponds exposed to pesticides (King *et al.*, 2010).

There is evidence that the surrounding landscape matrix is a significant factor affecting trematode transmission, resulting in lower helminth species richness and diversity in areas under agricultural cultivation (King *et al.*, 2008, 2010), which fragments natural habitats, reduces definitive host activity and reduces biodiversity (McLaughlin & Mineau, 1995; Mineau & McLaughlin, 1996; Findlay & Houlahan, 1997). Landscape fragmentation can restrict the access of amphibians, birds and mammals to the area, thus preventing their trematodes from infecting other potential hosts in the ponds (King *et al.*, 2007). Several studies have evaluated how landscape factors affect anuran helminths, including the effects of land use for agricultural activities (Koprivnikar *et al.*, 2006; McKenzie, 2007; Rohr *et al.*, 2008a, b; Hartson *et al.*, 2011; Schotthoefer *et al.*, 2011; Koprivnikar & Redfern, 2012), the forest cover (King *et al.*, 2007; Hartson *et al.*, 2011; Koprivnikar & Redfern, 2012) and the road density (Urban, 2006; King *et al.*, 2007; Koprivnikar & Redfern, 2012).

Amphibians occupy a central trophic position and normally acquire helminths from invertebrates, fish and terrestrial vertebrates. Many helminths have complex life cycles and, for transmission, depend on the presence of a variety of vertebrates and invertebrates as intermediate hosts. Therefore, the low diversity of helminths in amphibians represents the absence of one or more intermediate hosts, which can represent an indicator of ecosystem stress (Marcogliese & Cone, 1997; Lafferty & Kuris, 1999; Lafferty & Holt, 2003; Marcogliese, 2005; King *et al.*, 2010). Therefore, understanding the distribution patterns across multiple spatial scales is important as a source of crucial information to describe the forces that structure and maintain biological diversity (Harte *et al.*, 2005). In this work, we compare the anuran helminth fauna in native grasslands with livestock with those in areas under agricultural cultivation in four species of anurans (*Aplastodiscus perviridis*, *Leptodactylus latrans*, *Physalaemus cuvieri* and *Pseudis cardosoi*). Thus, we made comparisons on patterns of species richness, abundance, taxonomic composition, prevalence and intensity of parasitic infection, as well as how multiple-scale descriptors change among two contrasting land uses. The extensive livestock on native grasslands is considered a less impactful land-use type in this region (Pillar & Vélez-Martin, 2010), and it was recently highlighted as more compatible with anuran conservation than crops (see Santos *et al.*, 2014; Iop *et al.*, 2020; Moreira *et al.*, 2020). Our hypotheses are: (1) anurans in native habitats with livestock and in areas under agricultural cultivation will present distinct helminth fauna; and (2) ponds in cultivated areas will present a higher prevalence and intensity of helminth infections, in congruence with changes in a set of environmental descriptors associated with land-use types.

Materials and methods

Study area

This study was carried out in the Highland Grasslands region, in the municipalities of Painel, Campo Belo do Sul and Abelardo Luz (in the state of Santa Catarina), and in the municipalities of Palmas and Tibagi (in the state of Paraná), between latitudes 24° and 30°S, 1.000–1.400 m above sea level (Hueck, 1966). This area was located in the region of the original distribution of the South Brazilian grasslands (fig. 1). Field activities were carried out in three spatial units of 5 × 5 km, characterized by native grasslands with extensive livestock (municipalities of Painel, Palmas and Tibagi), and three spatial units characterized by total replacement of the grassland matrix by soybean or maize cultivation (Campo Belo do Sul, Abelardo Luz and Tibagi). The density of livestock was spatially and temporally

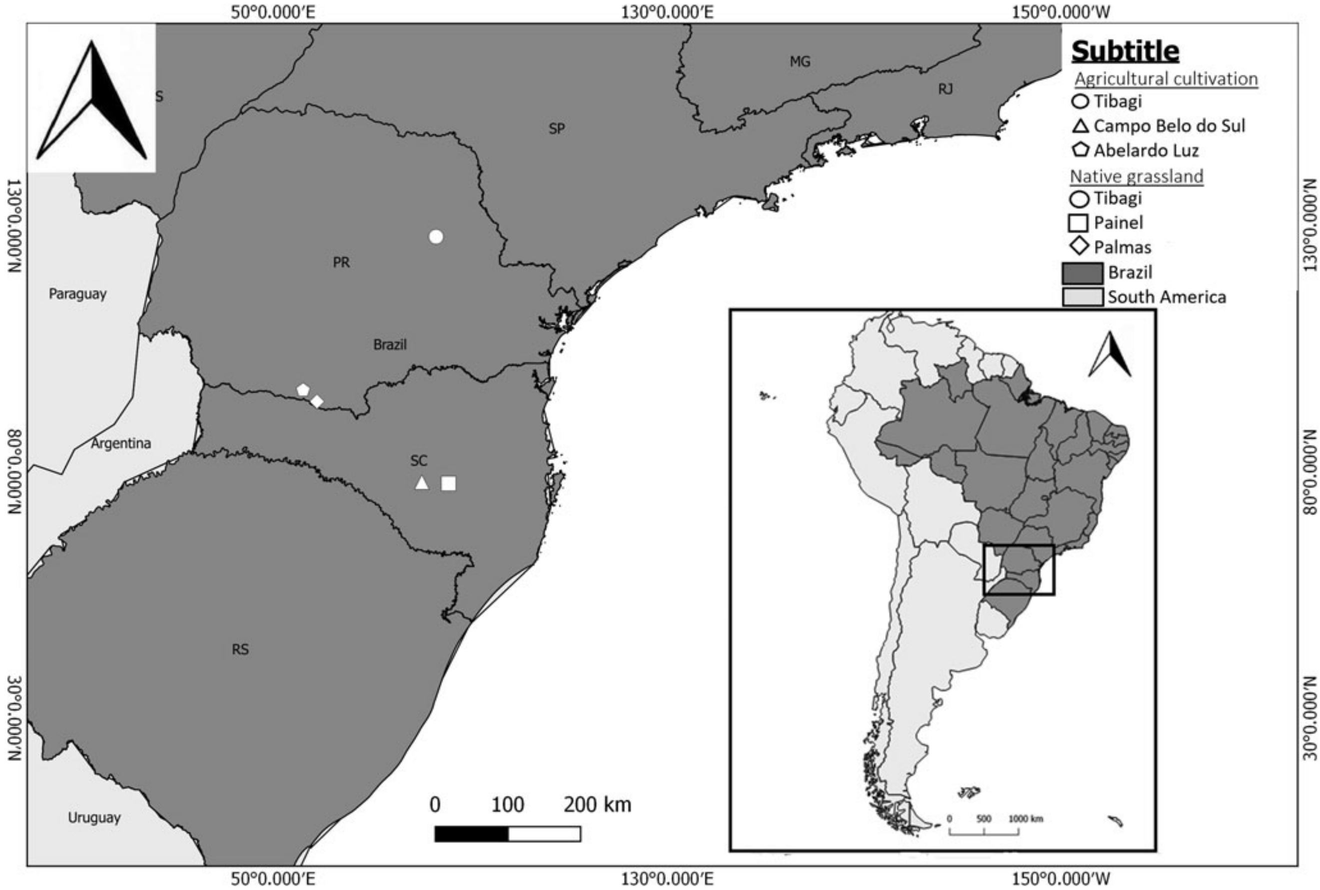

Fig. 1. Map of the location of the collection municipalities in the highland grasslands of the Brazilian states of Santa Catarina and Paraná, and ponds sampled for host anurans between January and February 2016. Municipalities with native grassland with livestock: Painel (in the state of Santa Catarina), Palmas and Tibagi (in the state of Paraná); municipalities with areas under agricultural cultivation: Campo Belo do Sul, Abelardo Luz (in the state of Santa Catarina) and Tibagi (in the state of Paraná).

variable in the grasslands spatial units. The Highland Grasslands region belongs to the Atlantic Forest biome (IBGE, 2004) and is characterized by a natural mosaic of grasslands associated with Araucaria Forest in the highlands of Southern Brazil (Oliveira-Filho & Fontes, 2000). This ecosystem contains about 2.2 thousand plant species (Boldrini, 2009) and a rich diversity of wildlife, including endemic and endangered species (Bencke, 2009). These grasslands are in a transitional region between tropical and temperate areas (Overbeck *et al*., 2007), characterized by an average annual temperature that generally varies between 12° and 18°C, with well-distributed rains throughout the year (Nimer, 1990). Cold winter nights can reach temperatures of −4° to −8°C in the highest region of Serra Geral (Nimer, 1990), where frost and snow occurrence is common.

Collection and examination of frogs

We sampled 34 ponds (19 in native grasslands with livestock and 15 in areas under agricultural cultivation), where we collected anuran hosts of two families (Hylidae: *A. perviridis*, *P. cardosoi*; and Leptodactylidae: *L. latrans*, *P. cuvieri*), between January and February 2016. Each pond was sampled once. Anurans were sampled during the twilight and night-time, using the 'survey at breeding sites' method (Scott Jr & Woodward, 1994) along the edges of selected ponds. During this survey, we aimed to collect at least three specimens of each species by each pond. Anurans were transported live to the laboratory and then euthanized with anaesthetic application to the skin (Lidocaine® 10%). Internal organs (gastrointestinal tract, lungs, kidneys, liver, gall-bladder and urinary bladder), the musculature of the anterior and posterior limbs and the coelomic cavity were examined for the presence of helminths. Anuran hosts were deposited in the Amphibian Collection of the Federal University of Santa Maria (ZUFSM), Department of Ecology and Evolution, Santa Maria, Brazil (supplementary table S1).

Nematodes were killed in warm solution (about 60°C) of 70% alcohol, fixed and kept in 70% alcohol, and cleared with Amman's lactophenol (Andrade, 2000). Trematodes, cestodes and monogenoids were killed by compression with slide and coverslip, kept moist with absolute alcohol as a fixative and preserved in 70% alcohol, after which they were stained with hydrochloric carmine (Andrade, 2000; Rey, 2001) and diaphanized with Eugenol. Helminths were mounted on temporary slides and examined in a computerized LAS V4 (Leica Application Suite) image analysis system (https://www.leica-microsystems.com/products/microscope-software/p/leica-application-suite/), adapted to the DM 2500-Leica microscopes with the interferential phase contrast system for helminth identification and collection of morphometric data and photomicrographs of helminths. The voucher species were deposited in the Helminthological Collection of the Botucatu Biosciences Institute (CHIBB), Department of Parasitology, Paulista State University, São Paulo, Brazil (supplementary table S2).

Sampling of environmental descriptors

We recorded environmental descriptors organized in two groups: (1) local scale representing pond heterogeneity, and (2) the landscape matrix. This approach was adopted because the importance of habitat descriptors can vary with spatial scale in studies on helminths and/or anuran hosts (e.g. King *et al.*, 2007, 2010; Hartson *et al.*, 2011; Schotthoefer *et al.*, 2011; Koprivnikar & Redfern, 2012; Iop *et al.*, 2012, 2020). Thus, we were interested in identifying which of these descriptor groups better explained the quantitative descriptors of parasitism. Local variables representing the environmental conditions of ponds were recorded directly at each site, by visual inspection of the entire perimeter of the pond (adapted from Vasconcelos *et al.*, 2009): vegetation structure – number of hydrophyte structural types present in the ponds (emersed, immersed and floating); the number of structural types of vegetation present on the edges (undergrowth, shrub and arboreal); percentage of each structural vegetation type on pond edge; mean height of vegetation at the pond edge; percentage of vegetation cover on the water surface; hydroperiod (permanent or temporary); origin of the water body (natural or anthropic); water physical-chemical variables (pH, dissolved oxygen, electrical conductivity, salinity and turbidity) using a Horiba® multi-parameter probe (model U-5000, Kyoto, Japan); and presence of molluscs by scanning with a long cable handle (4 mm^2 metallic mesh) along the entire perimeter of each pond only once (King *et al.*, 2007). The collected molluscs were stored in a clearly labelled 5% formalin container. To represent the landscape matrix, we recorded the shortest distance from each pond to the nearest forest fragment and human residences (King *et al.*, 2007; Hartson *et al.*, 2011; Koprivnikar & Redfern, 2012), as well as the type of land use predominant in a buffer of 500 m in the surroundings of ponds (e.g. agricultural cultivation or livestock on native grassland). This buffer zone size has been previously pointed by other studies as the zone encompassing the largest area of habitat used by amphibian species around ponds (reviews in Semlitsch & Jensen, 2001; Semlitsch & Bodie, 2003; Dodd Jr, 2010; Canessa & Parris, 2013). Land use was recorded by inspection in the field, while nearest distances were calculated using images obtained from Google Earth (https://www.google.com.br/earth/).

Statistical analyses

Quantitative descriptors of parasitism (Bush *et al.*, 1997) were calculated for all helminth species (prevalence, mean abundance and average intensity) and hosts (total helminth richness, amplitude and rarefied richness). Additionally, for each average, the respective standard error was calculated.

We tested possible differences among land uses (i.e. native grassland with livestock and land under agricultural cultivation) for helminth communities by permutational multivariate ANOVA (PERMANOVA) (Anderson, 2017), using the Bray–Curtis index and 9999 permutations. The abundance of helminths was previously transformed by dispersion weighting of species (indicated to reduce the effects of species with distinct distribution patterns), followed by fourth-root transformation (to down-weight the contributions of quantitatively dominant species to the similarities calculated between samples) (Clarke *et al.*, 2006, 2014). Anuran hosts with no helminths collected during the body examination were also incorporated into a matrix by adding a 'dummy species' with a value of 1 for all samples (frogs), before computing similarities. Land-use type (agricultural cultivation or livestock on native grassland) was included in the PERMANOVA as a fixed factor, while the natural variation associated with ponds was included as a random factor nested in the 'land-use factor'. The similarity patterns of helminth communities were contrasted for the land-use factor by bootstrap averages (150) and represented in two-dimensional space by metric-multidimensional scaling ordination (mMDS) (Clarke & Gorley, 2015), for each anuran host species. PERMANOVA, bootstrap averages and mMDS analyses were performed in Primer-E® 7.0 software (Clarke & Gorley, 2015), using anuran hosts as sampling units.

Results

A total of 171 anurans, 84 individuals in the native grassland with livestock and 87 individuals in land under agricultural cultivation were collected: *A. perviridis* ($n = 36$), *L. latrans* ($n = 60$), *P. cuvieri* (53) and *P. cardosoi* ($n = 22$). A total of 2137 helminths were found in anurans from the agricultural cultivation and 1569 from those from the native grassland with livestock, belonging to 25 taxa.

The helminth richness registered in anurans in native grassland with livestock was similar to that recorded in land under agricultural cultivation. For the general infection parameters, the helminths had a higher percentage of infection prevalence and infection intensity in the land under agricultural cultivation than in the native grasslands with livestock (table 1). Of the 84 anurans collected in the native grassland with livestock, 71 were parasitized by at least one species of helminth (a total prevalence of 84%). Of the 87 anurans collected in the land under agricultural cultivation, 82 were parasitized (total prevalence 94%). An example of this pattern was observed in *A. perviridis*, with a prevalence of infection of 88.24% in the land under agricultural cultivation and 52.63% in the native grasslands with livestock, as well as an average abundance of helminths of 26.5 in the land under agricultural cultivation and 3.0 in native grasslands with livestock (table 2). *Leptodactylus latrans* were the only host that presented a higher prevalence of infection in native grasslands with livestock (table 2).

PERMANOVA revealed that the land-use factor (native grasslands with livestock × agricultural cultivation) explained the changes in helminth communities for the hylid hosts *A. perviridis* and *P. cardosoi* (table 3). On the other hand, just the natural variation among ponds explained the changes recorded in helminth communities for the leptodactylid hosts *L. latrans* and *P. cuvieri*. These asymmetrical responses among anuran host species for the land-use factor were recovered by the bidimensional ordination (fig. 2).

Environmental descriptors data at local and landscape scales are summarized in the supplementary figs S1 and S2. Ponds in the contrasting land use (native grasslands with livestock and agriculture) were similar in most descriptors, but differed in pH, mean height of edge vegetation, shorter distance to the nearest human residence, percentage of vegetation cover on water surface, hydroperiod (if temporary or permanent) and origin (if natural or anthropic).

Table 1. Helminths collected in anurans occurring in native grasslands with livestock and in land under agricultural cultivation, in the Highland Grasslands region of the Brazilian states of Santa Catarina and Paraná.

	Livestock on native grasslands			Agriculture		
Helminths	P%	MA ± SE	MII ± SE	P%	MA ± SE	MII ± SE
Monogenea						
Polystoma cuvieri	29.2	1.0 ± 0.6	3.1 ± 1.9	0.1	0.1 ± 0.1	2.0 ± 0
Trematoda (Digenea)						
Catadiscus sp. 1	9.7	0.5 ± 0.3	4.7 ± 1.1	4.9	0.1 ± 0	1.0 ± 0
Catadiscus sp. 2	9.0	0.3 ± 0.3	3.0 ± 0.7	27.3	0.5 ± 0.3	2.0 ± 0.6
Choledocystus elegans	14.6	1.8 ± 1.3	12.6 ± 3.4	26.7	16.8 ± 8.4	63.0 ± 18.1
Choledocystus pseudium	0	0	0	36.4	1.1 ± 0.5	3.0 ± 0.4
Gorgoderina sp.	70.0	8.1 ± 1.3	11.5 ± 3.0	53.3	6.2 ± 2.0	11.7 ± 3.2
Haematoloechus ozorioi	23.3	0.8 ± 0.3	3.4 ± 0.9	16.7	2.4 ± 1.8	14.6 ± 10.4
Haematoloechus neivai	18.1	0.3 ± 0.2	1.5 ± 0	0	0	0
Rhauschiella proxima	6.6	0.1 ± 0.1	2.5 ± 0.5	10.0	0.2 ± 0.1	1.7 ± 34.0
Cestoda						
Cylindrotaenia americana	12.5	0.6 ± 0.4	5.3 ± 0	0.1	0.6 ± 0.4	5.3 ± 1.7
Plerocercoid larvae	5.3	0.3 ± 0.3	5.0 ± 0	52.9	2.5 ± 0.9	4.7 ± 1.2
Ophiotaenia sp.	26.7	1.4 ± 0.7	5.1 ± 2.0	24.4	1.3 ± 0.5	5.3 ± 1.5
Nematoda						
Cosmocercidae	16.7	1.4 ± 0.5	8.4 ± 4.6	20.4	4.8 ± 2.5	23.4 ± 10.6
Cosmocerca parva	33.3	0.7 ± 0.2	2.1 ± 0.2	29.5	0.8 ± 0.2	2.7 ± 0.4
Ochoterenella sp.	5.3	0.1 ± 0.1	1.0 ± 0	0	0	0
Falcaustra aff. *mascula*	20.0	1.8 ± 1.0	9.0 ± 3.8	6.7	0.3 ± 0.2	4.0 ± 2.0
Hedruris sp.	0	0	0	3.3	0.1 ± 0.1	3.0 ± 06.9
Pharyngodon sp.	9.1	0.1 ± 0.1	1.0 ± 0	0	0	0
Nematode larvae	10.5	4.5 ± 2.3	43.5 ± 7.0	10.5	3.8 ± 2.0	38.2 ± 14.9
Physalopteridae larvae	0	0	0	5.9	0.1 ± 0.1	2.0 ± 0
Oxyascaris oxyascaris	21.4	1.0 ± 0.3	4.5 ± 2.5	27.3	2.9 ± 1.4	10.7 ± 3.4
Rhabdias sp. 1	6.7	0.2 ± 0.2	3.5 ± 2.5	10.9	0.1 ± 0.1	1.4 ± 0.3
Rhabdias sp. 2	40.5	1.3 ± 0.3	3.1 ± 9.2	60.0	4.4 ± 1.3	7.4 ± 1.8
Rhabdias sp. 3	4.2	0.1 ± 0	1.0 ± 0	6.9	0.3 ± 0.2	4.0 ± 3.0
Unidentified cyst	13.1	0.6 ± 0.2	4.4 ± 2.0	17.0	0.8 ± 0.2	4.6 ± 1.0
Prevalence		84			94	
Mean Intensity of Infection		20.0 ± 1.4			24.0 ± 2.3	
Mean Abundance		15.0 ± 0.5			21.4 ± 2.3	
Total Richness		18			17	
Mean Richness ± SE (amplitude)		0.1 ± 0.1 (1–7)			0.1 ± 0.1 (1–7)	

P, prevalence; MA, mean abundance; SE, standard error; MII, mean intensity of infection.

Discussion

Our results showed that the helminth fauna of the anurans in the Highland Grasslands region in South Brazilian grasslands is influenced by land use. In the present study, prevalence, helminth infection intensity and helminth abundance were higher in anurans in areas under agricultural cultivation than in native grassland with livestock. Thus, our analyses suggest that the replacement of the native grassland with livestock by agricultural cultivation changes the structure and composition of the helminth community, modifying the parasitism metrics analysed.

The hypothesis that land under agricultural cultivation is associated with a higher prevalence and intensity of helminth infections in host anurans was corroborated in at least one of the two metrics in our analyses. Other studies with helminths presenting direct and indirect cycles in anurans found similar results

Table 2. Host anurans and respective helminths collected in native grasslands with livestock (N) and from land under agricultural cultivation (A), in the Highland Grasslands region of the Brazilian states of Santa Catarina and Paraná.

Host	Area	P%	MA ± SE	MII (amp)	TR	MR (amp)
Aplastodiscus perviridis (*n* = 36)	N (*n* = 19)	52.6	3.0 ± 1.7	5.0 (1–32)	5	1.1 (1–2)
	A (*n* = 17)	88.2	26.5 ± 12.2	5.8 (1–193)	4	1.5 (1–3)
Leptodactylus latrans (*n* = 60)	N (*n* = 30)	100	31.8 ± 5.0	31.8 (1–130)	10	3.1 (1–8)
	A (*n* = 30)	97.7	49.9 ± 10.2	51.6 (1–223)	11	3.1 (1–7)
Physalaemus cuvieri (*n* = 53)	N (*n* = 24)	79.2	3.9 ± 0.8	5 (1–14)	6	1.6 (1–3)
	A (*n* = 29)	93.1	4.6 ± 0.8	30.0 (1–18)	6	1.3 (1–4)
Pseudis cardosoi (*n* = 22)	N (*n* = 11)	81.8	6.3 ± 1.7	7.7 (1–17)	4	1.8 (1–3)
	A (*n* = 11)	100	4.0 ± 0.6	4.0 (1–7)	6	1.6 (1–3)

P, prevalence; MA, mean abundance; SE, standard error; MII, mean intensity of infection; amp, amplitude; TR, total richness; MR, mean richness; RR, richness rarified (95% confidence interval) of parasitic helminths.

Table 3. Permutational multivariate ANOVA based on the Bray–Curtis similarity index for helminths parasitizing anurans in native grasslands with livestock and in land under agricultural cultivation from the Highland Grasslands region of the Brazilian states of Santa Catarina and Paraná.

Host	Source of variation	df	MS	Pseudo-F	*P*
Aplastodiscus perviridis	Land-use effect	1	1771.00	2.52	0.04
	Pond variation (inside land use)	16	762.38	1.46	0.09
	Residual variation	18	522.53		
Pseudis cardosoi	Land-use effect	1	2922.70	3.12	0.03
	Pond variation (inside land use)	7	718.40	0.76	0.75
	Residual variation	13	945.57		
Leptodactylus latrans	Land-use effect	1	1691.10	1.15	0.35
	Pond variation (inside land use)	22	1710.90	2.12	<0.01
	Residual variation	36	805.08		
Physalaemus cuvieri	Land-use effect	1	138.03	0.15	0.90
	Pond variation (inside land use)	17	958.64	1.58	0.02
	Residual variation	34	605.25		

for agricultural cultivations (Hamann *et al.*, 2006; King *et al.*, 2007, 2010; Marcogliese *et al.*, 2009). Among the hosts analysed, *A. perviridis* seemed to be very sensitive to changes in the environment since it presented a higher prevalence and intensity of infection in areas under agricultural cultivation. We identified many cysts in the musculature, the body cavity and the organs of anuran hosts in the areas under agricultural cultivation. *Leptodactylus latrans* was the only host that presented a higher prevalence of parasitic infection in the native grassland with livestock, but the abundance and intensity of infection were both higher in the land under agricultural cultivation. *Leptodactylus latrans* is a terrestrial anuran, but often found in or at the water margin, giving individuals opportunities for parasitic infection in both the terrestrial and aquatic environments (Campião *et al.*, 2016). *Pseudis cardosoi*, despite the higher helminth prevalence in areas under agricultural cultivation than in the native grassland with livestock, showed higher infection intensity and higher abundance in the native grassland with livestock. This may be related to the aquatic life habit of this host, since aquatic anurans are particularly susceptible to changes in helminth communities due to environmental changes (McKenzie, 2007). The differences observed in our study can be influenced jointly by helminth characteristics, lifecycle strategy, host species, as well as limitations of ecology and phylogeny (Campião *et al.*, 2016).

In our study, some of the pond environmental descriptors differed between the two land uses, mainly at the local scale (i.e. associated to the ponds). Thus, ponds in agriculture were predominantly permanent waterbodies, nearest to human residences, presenting with higher pH, higher height of edge vegetation and lower vegetation cover on the water surface than ponds in native grasslands with livestock. Some of these differences were expected (e.g. increase of vegetation in the pond edges, increase in water pH, loss of temporary ponds) due to agricultural practices as suppression of traditional grazing (Erős *et al.*, 2020), relief softening (Iop *et al.*, 2020) and application of lime for the correction of soil acidity. It is known that environmental changes may influence the relationship between host and parasite, especially for helminths with complex life cycles that require multiple hosts for transmission, development and reproduction (King *et al.*, 2010; Koprivnikar *et al.*, 2012a, b). Agriculture helminths with complex life cycles often cannot complete their life cycle because their definitive hosts do not usually visit areas of agricultural

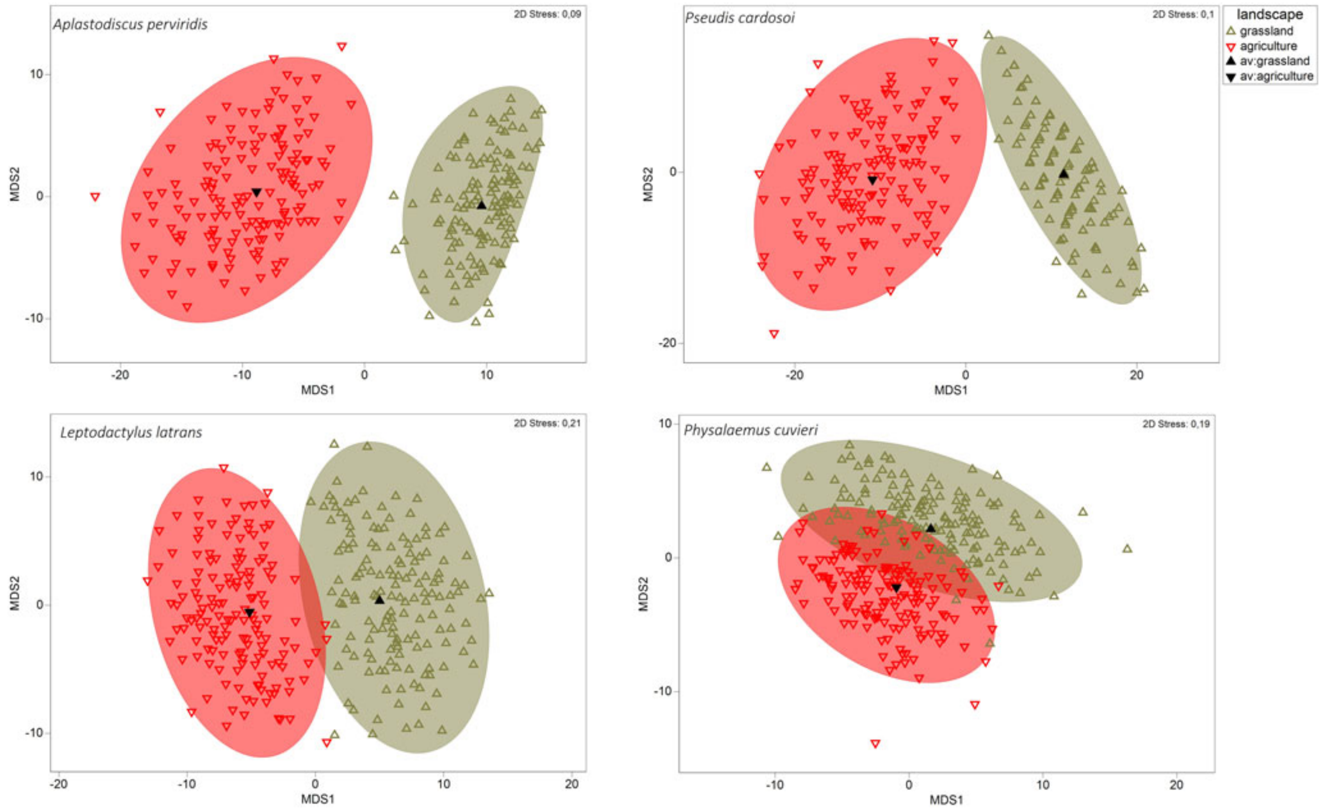

Fig. 2. mMDS ordination representing bootstrap averages (150) for comparisons of parasite helminth communities in anurans in native grasslands with livestock and in areas under agricultural cultivation, in the Highland Grasslands region of the Brazilian states of Santa Catarina and Paraná.

cultivation (Pietrock & Marcogliese, 2003; King *et al*., 2007, 2010; Koprivnikar *et al*., 2006). For instance, fewer waterbirds visit waterbodies in strongly impacted landscapes (e.g. agriculture and residences) (Bethke & Nudds, 1995; Krapu *et al*., 1997) because they prefer natural wetlands for breeding and foraging (Talent *et al*., 1982; Merendino *et al*., 1995). Another environmental descriptor that influences the helminth transmission is the marginal and aquatic vegetation, because it increases the environmental complexity, affects primary productivity, nutrient cycling and, consequently, increases the visitation of the definitive host vertebrates and intermediate hosts that use these environments (Padial *et al*., 2009; Thomaz & Cunha, 2010). The increase in water pH recorded in the ponds of agriculture can be related with components of agricultural runoff due to the traditional application of lime to correct the soil acidity. However, how this and others environmental descriptors can specifically change the infection parameters in anuran is an interesting issue for future studies. Nevertheless, we suspected that environmental descriptors may be related to the greater number of direct lifecycle helminths, as reported in similar studies (Hamann *et al*., 2006; Marcogliese *et al*., 2009; King *et al*., 2007, 2010). This occurs because helminths with direct life cycles should generally be relatively more successful in habitats with anthropogenic disturbances, and perhaps because the final hosts of helminths with complex life cycles are discouraged from visiting these sites (Hamann *et al*., 2006; King *et al*., 2007, 2010; Marcogliese *et al*., 2009).

The hypothesis that the anurans found in areas under different types of land use would present distinct helminth communities was partially corroborated by the PERMANOVA results. Indeed, helminth communities in native grasslands with livestock and in land under agricultural cultivation differed only for the hosts *A. perviridis* and *P. cardosoi*, whereas the helminths in the hosts *L. latrans* and *P. cuvieri* responded only to the random factor representing the natural variation among ponds. Interestingly, this asymmetric response of hosts seems to be related to the habitat used by the host. Land use only affected the community of helminths in hosts with more specialized habits regarding the use of the habitat. Aquatic anurans like *P. cardosoi* are particularly subject to changes in their helminth communities due to land-use changes. This is likely because water-quality changes associated with land conversion impact the composition of the helminth community (McKenzie, 2007). The community of helminths in anurans with arboreal habits, such as *A. perviridis*, was also heavily influenced by land use. Changes in land use can increase non-pathogenic helminths to high densities where they become pathogenic to hosts (McKenzie, 2007). However, *L. latrans* and *P. cuvieri* are generalists regarding their use of habitat. Natural variation among ponds is, therefore, important for the composition of their helminth communities, since each pond has different degrees of complexity in terms of vegetation cover, food availability and water quality, which can influence the success of infection and transmission of helminths of different species (Sousa & Grosholz, 1991; Wilkinson & Fenner Jr, 2007; Kruidhof *et al*., 2015).

Final remarks

In the present study, we analysed anurans from native grasslands with livestock and land under agricultural cultivation to compare the helminth communities in these two types of land uses and to assess the possible impacts of agricultural cultivation on the helminths of the anurans of these areas. Similar to the results previously reported in other studies (Kiesecker, 2002; Johnson & Chase, 2004; Koprivnikar *et al.*, 2006), we were able to verify that agricultural cultivation changes the structure and composition of helminth communities in anurans, leading to an increase in the number of infected anurans, as well as the abundance and intensity of parasitic infection. The results obtained are worrying when considering the accelerated change in land use by the conversion of native grasslands into agricultural systems (mainly for soybean cultivation) (Overbeck *et al.*, 2007; Oliveira *et al.*, 2017). Allied with government incentives to convert native grasslands to agriculture and the erroneous idea that grassy ecosystems have low biodiversity, it remains a common view that the greatest productive profitability from land can only be obtained at the expense of converting grasslands and replacing them with cultivated crops and pastures, and, as a consequence, native grasslands are poorly conserved and studied (see Overbeck *et al.*, 2007; Bond & Parr, 2010; Santos *et al.*, 2014; Pillar & Lange, 2015; Andrade *et al.*, 2019). A direct environmental consequence of the conversion of grasslands is the loss of biodiversity. The reduction of the area of remaining grasslands causes their biological impoverishment, eliminating direct ecosystem services and destabilizing or collapsing this complex network of ecological interactions (Andrade *et al.*, 2015; Pillar & Lange, 2015). Studies of this nature are extremely relevant and important for amphibian conservation, for our understanding of the ecology of wildlife diseases and environmental changes (Koprivnikar *et al.*, 2012a, b). Therefore, we emphasize the importance of understanding the processes that govern the structure of helminth communities in anurans in preserved areas, as well as in degraded and/or modified areas under agricultural cultivation. Finally, we suggest that future studies include analyses that check how each environmental descriptor influences the key metrics separately for each major helminth group (e.g. nematodes, trematodes and cestodes), as they have very different modes of transmission and life histories, and environmental variables will affect each helminth taxon differently.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0022149X20000905

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