Check List 10(5): 1014–1019, 2014

ISSN 1809-127X (available at *www.checklist.org.br*)

Check List
Journal of species lists and distribution

Lists of Species

Checklist of amphibians in a rice paddy area in the Uruguayan savanna, southern Brazil

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Abstract: With the objective of contributing to conservation strategies for areas outside conservation areas, we present an inventory of the amphibians occurring in rice fields with different types of management (conventional and organic), in Sentinela do Sul, state of Rio Grande do Sul, Brazil, a grassland area in the Uruguayan savanna ecoregion. Between August 2010 and April 2011, we collected tadpoles in rice fields and natural ponds. We recorded 14 species of six families, and only eight of these species occurred in rice fields. Species composition differed between organic and conventional fields. Habitat features (time of flooding, presence of fish, presence of vegetation) may act synergistically with agrochemical effects on anuran species composition. Since few areas of Uruguayan savanna lie within conservation units, it is paramount to develop conservation measures that help maintain biodiversity in agroecosystems.

DOI: 10.15560/10.5.1014

Introduction

Small wetlands have become one of the main priorities of conservation agendas because they support high aquatic diversity and are in decline throughout the world, due to expansion of the human population (Amezaga *et al.* 2002). Habitat loss associated with agricultural expansion is probably the principal cause of natural wetland declines (Czech and Parsons 2002). Managed by man, rice fields have been considered important wetland substitutes, and many organisms use these cultivated fields as areas for foraging and reproduction (Duré *et al.* 2008; Machado and Maltchik 2010; Piatti *et al.* 2010). Nevertheless, the use of rice fields as substitutes for natural wetlands may depend on management practices, such as use of agrochemicals or organic cultivation, irrigation water origin, and off-season use of crop area (Donald 2004; Kato *et al.* 2010). Currently, Brazil is an important area for rice production in South America, and the state of Rio Grande do Sul accounts for 67.5% of rice production in Brazil (IBGE 2012). Information about the biota associated with these agroecosystems is important because conservation units cover only 3.4% of total area of the state (SCP 2011).

Most of the currently documented amphibian species in Brazil have been discovered over the last forty years (Segalla *et al.* 2012). These new species descriptions, which have occurred at regular rates, are a strong indication that the Brazilian amphibian fauna is poorly known. Species inventories directly assess the diversity of an area over a short amount of time. This data could be critically important to conservation policies created for natural and managed areas (Silveira *et al.* 2010). Amphibian richness in Rio Grande do Sul represents approximately 10% of the species registered in Brazil, and this richness could be even higher, especially in the Uruguayan savanna ecoregion, in the southern portion of the state (Borges-Martins *et al.* 2007). Most of the wetlands and rice paddies of Rio Grande do Sul are concentrated in this region in southern Rio Grande do Sul (Maltchik *et al.* 2003).

The objective of this study was to contribute to the development of conservation strategies for areas not protected by conservation units. We surveyed amphibians in rice fields with different management regimes (conventional and organic) in the Uruguayan savanna ecoregion, and we also sampled natural ponds near the agricultural matrix to determine which species are actually using the agricultural areas. We studied tadpoles instead of adults to determine species presence, since some characteristics make these individuals good models for field inventories and aquatic monitoring studies (general abundance and longer permanence in the aquatic habitats than adults) (Andrade *et al.* 2007).

Materials and Methods

The study took place in Sentinela do Sul (Figure 1), in the central-west portion of the coastal plain of Rio Grande do Sul (30°42′00″ to 30°45′09″ S; 51°37′52″ to 51°41′58″ W), over one complete rice field cycle (2010-2011). The climate of this region is subtropical, moderately humid, and the temperature varies between 11°C in the winter and 26°C in the summer, with an average annual temperature of 18.5°C. Annual precipitation varies from 1500 to 1700 mm/year (Maluf 2000).

The rice fields were divided into two types according to management type (organic and conventional). Agrochemicals are not applied to organic fields, while in the conventionally managed fields, inorganic fertilizers, organophosphorus and carbamate insecticides, and glyphosate-based herbicides application is concentrated in the early rice growing period. The flood regime is rotated in organic fields to eliminate arthropod pests and weeds and keep the soil fertile. Cycles of flooding and dry downs last approximately 2 weeks, based on water availability in

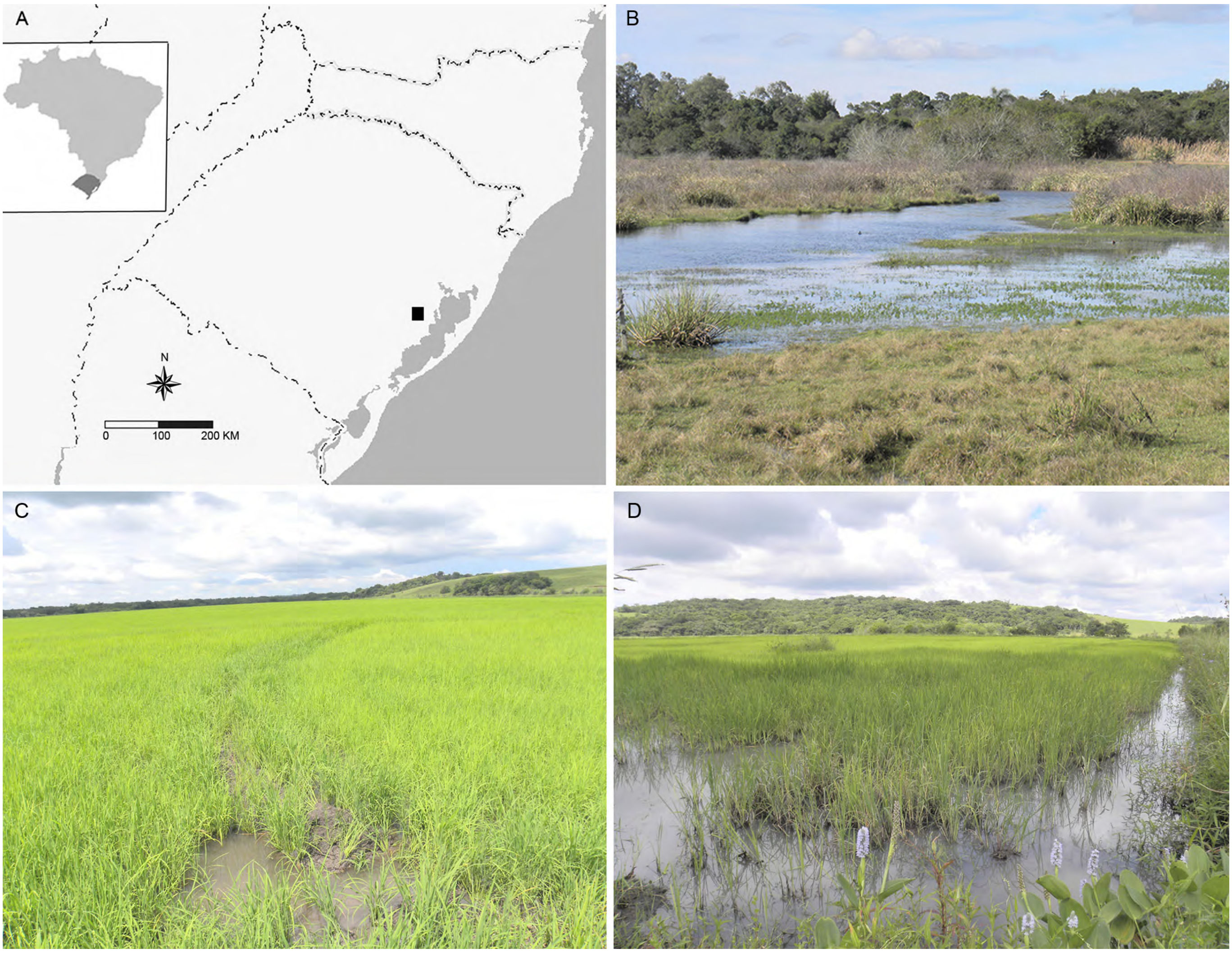

Figure 1. Study area and habitats sampled: (A) map showing the location of study area (black square) in the central-west coastal plains of Rio Grande do Sul, Brazil; (B) natural pond; (C) conventional rice field; (D): organic rice field.

the region. Organic crops also support a heterogeneous landscape around crop edges (Figure 1), which have relatively more native vegetation than conventional crops. The rice plantations in the study site were divided into various 1 ha plots that are interconnected by drainage canals and secondary roads. The canals (2–5 m wide and 0.5–1.5 m deep) are filled with water from nearby streams. Water level in the drainage canals is controlled by weirs that supply the rice plots (~10 cm water per 130 days) during the cultivation cycle.

We sampled four study plots (1 ha) in each of the two cultivation methods within a 10 km radius. We avoided using plots near other types of vegetation because we were specifically interested in the species that use rice fields. It was not possible to select fields surrounded by similar habitat in the organic fields because fields in the area are typically bordered by a strip of vegetation. We sampled four natural ponds to determine which species use agricultural areas. The mean water depth is approximately 40 cm in sampled natural ponds; however, during dry summers, the water volume of the ponds is greatly reduced. The vegetation around the ponds is composed of a mosaic of semideciduous forest and steppe (Cordeiro and Hasenack 2009). The study plots were at least 600 m from one another to minimize spatial autocorrelation.

Sampling occurred four times during the rice cultivation cycle: off-season (August 2010), soil preparation (October 2010), early growing (January 2011), and late growing (April 2011). The off-season period occurred when the land is fallow, and the fields retained water only in the irrigation canals and scattered ephemeral plots. During dry field conditions, soil preparation includes a wide range of practices, such as plowing to incorporate stubble and aerate the soil, harrowing and leveling to break and puddle clods of soil and incorporate organic matters into soil thoroughly. The early growing period is characterized by rice emergence (seedlings < 10 cm tall) and a shallow water level, while the rice was > 100 cm tall and water depth was increased to protect plants during the late growing period.

On each sampling occasion, tadpoles were collected with a dip net (0.4 × 0.3 m; 0.5 mm). The samples were collected by kicking up the substrate and then sweeping above the disturbed area. Three random sweeps of 2 m each were performed in each rice study plot (rice field or pond). Collected tadpoles were anesthetized with benzocaine and fixed in 10% formalin (collecting permit – SISBIO # 24082-2). Voucher specimens are housed in the herpetological collection of the Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul (MCP12570-12579), Brazil.

We performed an ANOVA using permutation analysis to test for differences in tadpole richness and abundance between natural ponds and rice fields. ANOVA permutation analyses were performed with the package lmPerm (Wheler 2010) in R.15.1 (R Development Core Team 2012).

Results

We found eleven anuran species of the following families: Bufonidae (1), Hylidae (4), Leptodactylidae (5), and Odontophrynidae (1) (Table 1). Tadpoles of the *Leptodactylus fuscus* group were assigned to the same taxon. Anuran richness and abundance differed between natural ponds and rice fields (Figure 2; richness $F_{2,45}$ = 4.146, p = 0.03; abundance $F_{2,45}$ = 271.08, p = 0.05). We found seven species in the rice fields, four in each management type. Hylid tadpoles were associated with organic rice fields, and Leptodactylidae species that make foam nests were found in conventional rice fields (Table 1). Natural ponds had eight species, although tadpole abundance was higher in these areas than cultivated areas. In addition, adults of six species, divided into two orders (Anura and Gymnophiona), were caught during tadpole samplings (Figure 3). Three species were found in conventional rice fields (*Elachistocleis bicolor*, *Hypsiboas pulchellus,* and *Pseudopaludicola falcipes*) and three species in natural ponds (*Chthonerpeton indistinctum*, *Leptodactylus latrans*, and *Rhinella dorbignyi*). This raised the total number of amphibians registered to 14 species of six families.

Tadpoles were registered in all samplings of natural ponds. In organic crops, we caught tadpoles in the off-season and in the early growing period of rice cultivation. We caught tadpoles only during the early growing period in conventional rice fields. With respect to temporal distribution, we caught most of species in the spring/summer samplings. Nevertheless, *Hypsiboas pulchellus* and *Physalaemus henselii* were collected only in the winter.

Discussion

In our study, the total number of species found in the rice fields and neighboring ponds represents about 60% of species richness observed in the region (Loebmann 2005; Borges-Martins *et al.* 2007; Quintela *et al.* 2011). By analyzing geographical distribution, all species are common in open areas of southern Brazil and can be found in more than one ecoregion, such as Atlantic coast restingas, Cerrado, and Uruguayan savanna (Maneyro and Carreira 2012; Haddad *et al.* 2013). Despite the low sampling effort, amphibian richness in our study is comparable with other rice paddies in South America (Table 2). Such results could be attributed to the idea that only generalist species are able to remain in areas converted to rice fields (Doody *et al.* 2006; Piatti *et al.* 2010). Amphibians that depend on ponds within or surrounded by agricultural fields may be exposed to high levels of agrochemicals, which could directly or indirectly affect biota (Peltzer *et al.* 2008;

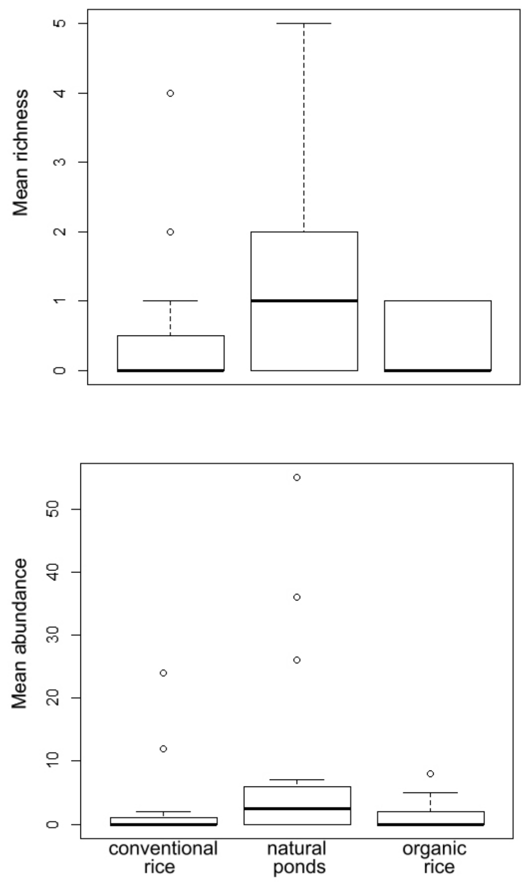

Figure 2. Mean values of tadpole richness and abundance in natural ponds and rice fields, throought the rice cultivation cycle (2010–2011), in a rice paddy area in Sentinela do Sul, RS, Brazil.

TABLE 1. List of tadpole species sampled from a rice paddy area in Sentinela do Sul, Rio Grande do Sul, Brazil, with total abundance by habitat type.

FAMILY	SPECIES	ORGANIC RICE FIELDS	CONVENTIONAL RICE FIELDS	NATURAL PONDS	TOTAL
Bufonidae	*Rhinella dorbignyi* (Duméril & Bibron, 1841)		3		3
Hylidae	*Dendropsophus minutus* (Peters, 1872)			8	8
	Hypsiboas pulchellus (Duméril & Bibron, 1841)	11		20	31
	Pseudis minuta Günther,1858			19	19
	Scinax squalirostris (A. Lutz, 1925)	2		4	6
Leptodactylidae	*Leptodactylus* gr. *fuscus* (Schneider, 1799)		30	32	62
	Physalaemus biligonigerus (Cope, 1861 “1860”)		1		1
	Physalaemus gracilis (Boulenger, 1883)			35	35
	Physalaemus henselii (Peters, 1872)			23	23
	Pseudopaludicola falcipes (Hensel, 1867)	8	6	4	18
Odontophrynidae	*Odontophrynus americanus* (Duméril & Bibron, 1841)	1			1
Total		**22**	**40**	**145**	**207**

Attademo *et al.* 2011). Agricultural intensification also affects the structure of habitat in which amphibians forage and reproduce (Peltzer *et al.* 2006; Piatti *et al.* 2010).

More generalized reproductive modes [*e.g*. mode 1 *sensu* Haddad and Prado (2005)] or modes that confer some protection against desiccation (*e.g.,* modes 11 and 30) are more favored in open areas or environments with seasonal availability of water, such as rice fields. Temporary environments are more likely to dry up and are, therefore, occupied by species with rapid development and those that build foam nests, which allows the larvae to survive until the next rain/flood (Zina 2006). Indeed, we found a higher number of tadpoles of species that deposit eggs in foam nests inside subterranean constructed chambers (mode 30). In this study, individuals of genus *Leptodactylus fuscus* species group were grouped in a single taxon. Because the tadpoles of the *L. fuscus* group have very similar morphology, external traits are not helpful for species diagnostic purposes (Langone and De Sá 2005; Prado and D'Heursel 2006; De Sá *et al.* 2007). Four species belonging to the *fuscus* group are known for the study area (*L. fuscus, L. gracilis, L. latinasus,* and *L. mystacinus*; Borges-Martins *et al.* 2007). A more detailed examination of internal oral anatomy and chondrocranium would be instructive and

Figure 3. Adult amphibians found in a rice paddy area in Sentinela do Sul, RS, Brazil, throught the rice cultivation cycle (2010–2011), during tadpole samplings. Family Bufonidae: (A) *Rhinella dorbignyi*. Hylidae: (B) *Hypsiboas pulchellus*. Leptodactylidae: (C) *Leptodactylus latrans*, (D) *Pseudopaludicola falcipes*. Microhylidae: (E) *Elachistocleis bicolor*. Caeciliidae: (F) *Chthonerpeton indistinctum*.

Table 2. Number of amphibian species found by habitat type in five rice paddy areas in three different ecoregions of South America.*: not informed.

ECOREGION	LOCALITY	RICHNESS			REFERENCE
		RICE FIELD	CHANNELS	NEIGHBOR PONDS	
Southern Cone Mesopotamian savanna	Corrientes, Argentina	10	10	7	(Duré *et al.* 2008)
Uruguayan savanna	Mostardas, Brazil	12	*	10	(Machado and Maltchik 2010)
Pantanal	Miranda, Brazil	8	9	*	(Piatti *et al.* 2010)
Uruguayan savanna	Mostardas, Brazil	*	10	*	(Maltchik *et al.* 2011)
Uruguayan savanna	Sentinela do Sul, Brazil	9	*	10	This work

help to identify these species.

The low tadpole abundance in our results were unexpected since rice fields can be potential surrogate habitats for many species that originally use natural wetlands (Machado and Maltchik 2010). Although the presence of tadpoles might be a good indicator that rice crops are effectively used for breeding activity, this must be heeded cautiously. Tadpoles might have originally occurred in other flooded areas and then ended up in the rice fields via irrigation water. This scenario seems be true for explosive breeding species (*Rhinella dorbignyi* and *Odontophrynus americanus*), because a large number of tadpoles would have been expected after a breeding event. Information about amplectant pairs and eggs are useful tools for better determining breeding sites. We were not unable to confirm breeding activity in rice quadrats, with the exception of two *Pseudopaludicola falcipes* amplectant pairs and some floating foam nests. Moreover, we observed fish species that generally prey upon tadpoles (*Hoplias* sp. and *Oligosarchus* sp.) inside rice quadrats and irrigation channels of fields under both cultivation methods. In addition to the predation effect on tadpoles and adults, the presence of fish influences breeding site selection by many amphibians (Werner *et al.* 2007; Both *et al.* 2009). These fish species were also found in natural ponds. However, additive or synergistic effects of pesticide use and habitat homogenization on tadpole predation have been demonstrated in amphibians (Mann *et al.* 2009). Thus, fish predation associated with low tadpole abundance (residents or not) could explain species absence during the late growing period.

Presence of hylid tadpoles in organic rice fields, in both off-season and growing periods, may be associated with the presence of a strip of vegetation close to the rice plots. Although the goal of our study was not to compare cultivation methods, studies indicate that the effect of organic farming might be biased by the study of species in different or multiple guilds (Fuller *et al.* 2005; Hole *et al.* 2005; Moreira and Maltchik 2014). During the off-season, the organic fields retained water in the irrigation and drainage canals, and there were ephemeral pools in the rice plots. This was not true for conventional fields which were drained and sometimes used for cattle grazing. It has been suggested that degree of permanence predicts tadpole guild composition in grasslands (Santos *et al.* 2007; Both *et al.* 2011). In this case, presence of hylid tadpoles in organic fields might simply be related to earlier flood dates (*i.e.*, longer flood period). However, this does not exclude other effects of organic management, such as no or little use of chemical pesticides/inorganic fertilizers and maintenance of a heterogeneous landscape around crop edges, of contributing to abundance in nearby areas.

Tadpole identification difficulty is a major obstacle in anuran surveys and species inventories that focus on larval forms (Rossa-Feres and Nomura 2006), even though tadpoles are easier to encounter and collect than adults. In this study, the tadpole samplings found amphibian species that are easily overlooked in active searches of adults (explosive breeders and aquatic fossorial), and they detected a good proportion of expected species in the region. Additionally, habitat features (time of flooding, presence of fish, presence of vegetation) could act synergistically with agrochemical effects on anuran species composition. In spite of the extensive coverage of the Uruguayan savanna ecoregion in the state of Rio Grande do Sul, few areas of this ecoregion are protected by conservation units. We hope that this work may stimulate others to present data on agricultural areas and encourage discussion of conservation practices outside conservation areas.

Author's Contribution Statement: LFBM carried out the design of study and performed the data collection, and drafting of the manuscript. DSK participated in the tadpole identification and drafting of the manuscript. LM helped in several aspects along this work, as well as acting in the coordination. All authors read and approved the final manuscript.

Acknowledgments: We are thankful to all private owners that authorized the use of their rice fields for our research. We also thank D. Araujo for field and laboratory assistance. We are grateful to E. Toriani-Moura and two anonymous referees for improvements on the manuscript. We thank Sistema de Autorização e Informação em Biodiversidade (SISBIO) for the collecting permit. This research was supported by funds from UNISINOS (02.00.023/00-0), Fundação de Amparo a Pesquisa no Rio Grande do Sul—FAPERGS (11/1149-7), and Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq (52370695.2).

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Received: October 2013
Accepted: August 2014
Published online: October 2014
Editorial responsibility: Camila C. Both