



# Does Organic Agriculture Benefit Anuran Diversity in Rice Fields?

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**Abstract** The debate about management practices that help maintain biodiversity in cultivated areas is an ongoing controversy in conservation biology. It has been suggested that organic agriculture supports greater levels of diversity than non-organic agriculture. This study examined anuran assemblages in natural intermittent ponds and rice fields under two types of cultivation methods (conventional and organic) in southern Brazil. We tested the differences in species richness and composition among assemblages and guilds, at different stages of rice cultivation. Overall, organic fields had a different species composition than conventional fields and natural ponds. Most of the differences observed between the natural areas and the rice fields occurred during the off-season. For semi-aquatic species, richness was higher in off-season and in earlier growing stages. We found no differences in species richness of fossorial and arboreal species across the crop cycle. The differences we observed may relate to differences in dispersal abilities among guilds. Thus, the incorporation of individual traits of each species (e.g., habitat preference and reproductive mode) is fundamental to the creation of more effective conservation strategies in agroecosystems.

**Keywords** Agroecosystems · Amphibian conservation · Community composition · Guilds · Southern Brazil

## Introduction

Agriculture occupies a larger portion of land than any other human activity (Devine and Furlong 2007), and its expansion

and intensification may be contributing to amphibian declines in some locations (Gray et al. 2004; Gallant et al. 2007). Amphibians that depend on wetlands within and surrounded by agricultural fields can be exposed to high levels of agrochemicals, which may have direct or indirect effects on the biota (Peltzer et al. 2008; Mann et al. 2009; Attademo et al. 2011). In addition, agricultural intensification affects the habitat structure for foraging and reproduction of amphibian species (Peltzer et al. 2006; Piatti et al. 2010). Overall, crop fields differ from natural wetlands in the habitat heterogeneity and hydrological cycles.

The use of organic farming techniques is currently an alternative to conventional farm management. It has been well-documented that organic agriculture supports greater levels of biodiversity than non-organic management methods (Fuller et al. 2005; Hole et al. 2005). These benefits are attributed to intrinsic, but not exclusive, practices of organic systems, such as minimal or no use of chemical pesticides and inorganic fertilizers, crop rotation and maintenance of a heterogeneous landscape around field edges. However, research suggests that response to organic agriculture varies across crops and taxonomic groups studied (Andersen and Eltun 2000; Weibull et al. 2000; Beecher et al. 2002; Fuller et al. 2005).

Rice (*Oryza* spp.) is the most common cultivated cereal in the world and occupies close to 11 % of the world's arable land (Donald 2004). In many regions, rice fields are considered important substitutes for natural wetlands and many organisms use these cultivated fields as habitat for foraging and reproduction (Wilson et al. 2007; Duré et al. 2008; Mann et al. 2009; Machado and Maltchik 2010; Piatti et al. 2010). This importance is due to a dynamic hydrological regime, with variation between aquatic and terrestrial phases. Amphibian communities are often described as being organized along a hydroperiod gradient, ranging from ephemeral ponds to large permanent wetlands (Werner et al. 2007; Both et al. 2009; Moreira et al.

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2010). So, rice production can create a mosaic of microhabitats favorable to different species, depending upon the type of management employed.

Among vertebrates, amphibians have the greatest proportion of threatened species (Beebe and Griffiths 2005; IUCN 2013), and fragmentation and habitat loss have led to these species becoming threatened (Becker et al. 2007). Various amphibian species occupy impoundments and irrigation canals since they are able to colonize these areas by terrestrial dispersal (Marsh et al. 2004; Vasconcelos and Calhoun 2004). However, one of the difficulties of managing modified landscapes is that susceptibility of amphibians to fragmentation is partially dependent on dispersal ability, reproductive mode and habitat preference of individual species (Cushman 2006; Dixo and Metzger 2010). Studies on anuran communities in agricultural environments found conflicting results among different guilds (Becker et al. 2007; Peltzer et al. 2008; Dixo and Metzger 2010). Indeed, amphibians are a group with remarkable differences in life-histories strategies. Arboreal species with aquatic larvae seem to be less tolerant of habitat alteration than species with terrestrial development. Thus, amphibian assemblages that use disparate resources may be affected differently by land use practices.

Although the relationship between amphibians and rice fields has been investigated in areas with high rice production (e.g., Fujioka and Lane 1997; Bambaradeniya et al. 2004; Kato et al. 2010), only a few recent studies of the subject have taken place in the Neotropical region (Duré et al. 2008; Machado and Maltchik 2010; Piatti et al. 2010). Information about the role of these agroecosystems in amphibian conservation is important in southern Brazil since the region contains around 10 % of the amphibian species in the country (Segalla et al. 2012), and Brazil has the greatest amphibian diversity in the world and the ninth highest rice production (FAO Stat. 2010).

We compared anuran assemblages in rice fields cultivated under different commercial cultivation methods used in southern Brazil, focusing on two aspects: 1) whether organic rice fields had a different species composition and richness than non-organic fields; 2) anuran richness and composition between rice fields and natural ponds. Based on an increased gradient of disturbance (natural ponds → organic crops → conventional crops) we predicted that anuran species richness would be highest in natural ponds, followed by organic rice cultivation, and conventional rice cultivation. We also predicted that richness of semi-aquatic and arboreal species may be lowest in rice fields (independent of cultivation method) which have homogeneous habitat and shorter hydroperiods than natural ponds. Fossorial species that are not directly associated with standing water availability and vegetative cover may be able to occupy rice fields.

## Materials and Methods

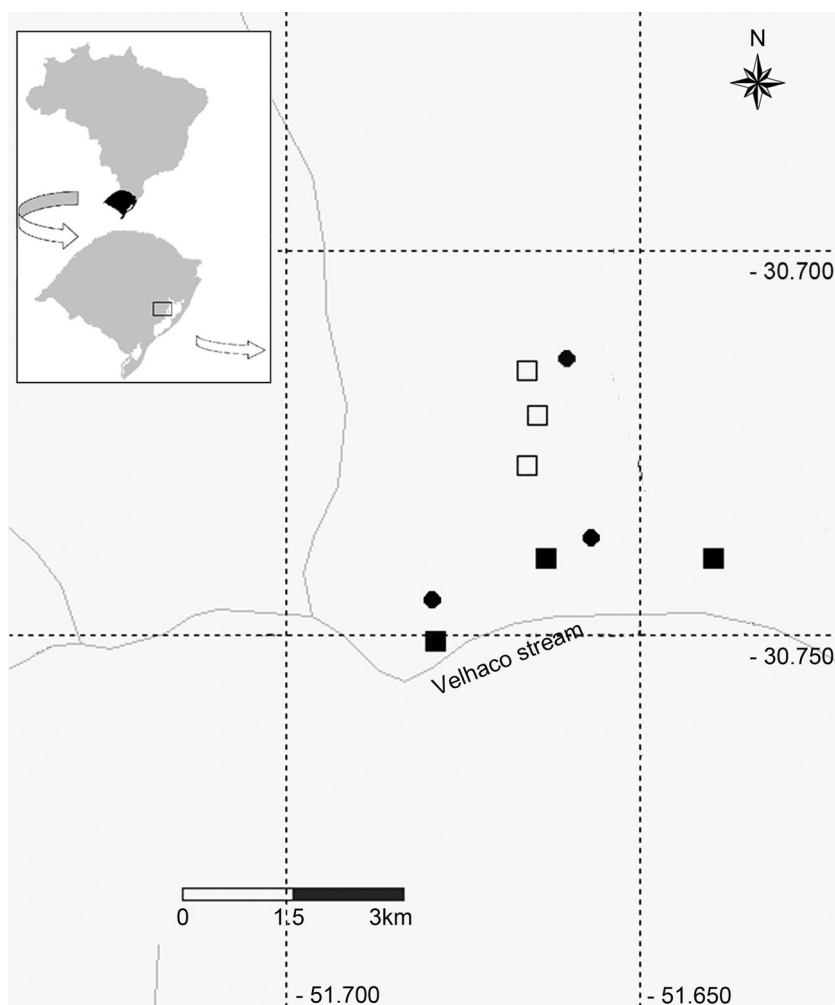
More than 64 % of the rice production in Brazil occurs in Rio Grande do Sul, and the coastal plain is an important area of rice production in South America (Azambuja et al. 2004). The study took place from August 2011 to August 2012 in an agricultural area dedicated to irrigated rice production (30.705 to 30.755 °S; 51.630 to 51.700 °W). The area is located in Sentinela do Sul, in the central–west portion of the coastal plain of Rio Grande do Sul. The climate 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 1,500 to 1,700 mm/year (Rossato 2011).

The rice plantations at Sentinela do Sul are divided into various 1 ha plots that are interconnected by secondary roads and drainage canals. The drainage canals (2–5 m wide and 0.5–1.5 m deep) are filled with water from nearby streams. Water level in the canals is controlled by weirs, which supply the rice plots (~10 cm water per 130 days) during the cultivation cycle. The rice fields were divided into two types according the management regimes used:

1. Conventional: Sowing is manual or with the aid of machinery. As soon as seedlings are established (5–10 cm tall), the fields are permanently flooded. Application of inorganic fertilizers, organophosphorus and carbamate insecticides, and glyphosate-based herbicides is concentrated in the initial growth stage (25–30 days after sowing).
2. Organic: Sowing is manual and agrochemicals are not applied to the organic fields at any stage of production. A rotation in the flooding regime is conducted 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 the region.

Three study plots (1 ha) in each of the two cultivation methods were sampled within a 10 km radius (Fig. 1). We avoided using plots near other types of vegetation because we were specifically interested in species that use rice fields. We only investigated plots surrounded by other plots of rice in the conventional rice fields. It was not possible to select fields surrounded by similar habitat in the organic rice fields because fields in the area typically are bordered by a strip of vegetation. We sampled three natural ponds to compare the anuran distribution and abundance between native areas and areas modified by rice production. We tried to select natural ponds that were similar in size to the rice fields and had temporary hydroperiods that lasted at least 5 months. The study plots were at least 600 m from one another to minimize spatial autocorrelation. The spatial independence of the nine sampling areas was tested using principal coordinates of neighbor

**Fig. 1** Map of study area in the coastal plain of Rio Grande do Sul, Brazil. *Circles*: natural ponds, *clear squares*: organic rice fields, *dark squares*: conventional rice fields



matrices (PCNM) analysis (Borcard and Legendre 2002). As the PCNM did not detect significant spatial structure ( $p=0.86$ ), it was not included in the statistical analysis.

Sampling occurred five times during the rice cultivation cycle: two samples in the off-season period (August/2011 and August/2012), one sample during early growing period (January/2012), one sample during late growing period (March/2012) and one sample during the post-harvest period (ten days after harvest) (June/2012). The off-season period occurred when the land was fallow, and the fields retained water only in the irrigation canals and scattered ephemeral pools. Early growing period was characterized by rice emergence (seedlings <10 cm tall) and shallow water level, while in the late growing phase, rice was >100 cm tall and water depth was increased to protect plants. Fields were dried completely before harvest (~15 days) and post-harvest stubble was usually plowed. Many anurans are killed during harvest, or move into terrestrial areas to avoid the disturbance.

Anuran richness and abundance was measured using visual and acoustic surveys (Crump and Scott 1994). The sampling occurred between 19:00 and 24:00 h, and the areas were

sampled in a random order. In each sample, we followed a 100 m transect perpendicular to the edge of a field or pond. Each transect was sampled for 20 min. All anurans detected visually or acoustically were recorded. The species were separated into guilds by habit (Vallan 2000; Peltzer et al. 2006): arboreal species which are generally found in herbaceous, shrubby, or low trees; fossorial which are species that excavate underground chambers for refuge or egg laying; and semi-aquatic species which are generally found in an aquatic environment or in the interface between water and land.

We compared richness of anurans among the three treatments (conventional and organic rice fields, ponds) throughout the crop cycle with a repeated measures ANOVA. When the ANOVA indicated significant differences, we conducted a Tukey test to verify which groups differed from one another (Zar 1999). The analyses were conducted separately for each guild using SYSTAT 12 program (SYSTAT 2007).

We used a permutational variance analysis (PERMANOVA) to assess differences in the anuran composition across natural ponds and rice fields, based on the Bray–Curtis dissimilarity. Differences between the treatments were

also analyzed separately between the periods of off-season, growing, and post-harvest. A non-metric multidimensional scaling (NMDS) plot was used to assist with interpretation. The analyses were conducted on the vegan package 2.0.3 for R (Oksanen et al. 2013). We also used indicator species analysis (Indval) (Dufrene and Legendre 1997) to characterize rice fields and ponds. This is a simple method that enables to identify indicator species and species assemblages characterizing groups of sites. The method combines a species' relative abundance with its relative frequency of occurrence in the various groups of sites. The matrices were constructed considering the composition of anurans in the different cultivation periods and the analyses were conducted with the package labdsv 1.5.0 for R (Roberts 2012).

## Results

We recorded a total of 17 species, representing four families (Table 1). Of the species found, six were classified as fossorial and five were classified as semi-aquatic. All five of the six species in the family Hylidae, with the exception of *Pseudis minuta*, were categorized as arboreal. *Pseudis minuta* was classified as semi-aquatic. Anuran richness among guilds differed by crop cycle. Richness in the fossorial guild did not differ across treatment or crop cycle [ANOVA repeated

measures: Treatment ( $F_{2,6}=1.870$ ;  $p=0.234$ ), Treatment X Crop cycle ( $F_{8,24}=2.078$ ;  $p=0.09$ )]. Rice fields under both cultivation methods had peaks of richness at the early growing period (Fig. 2a).

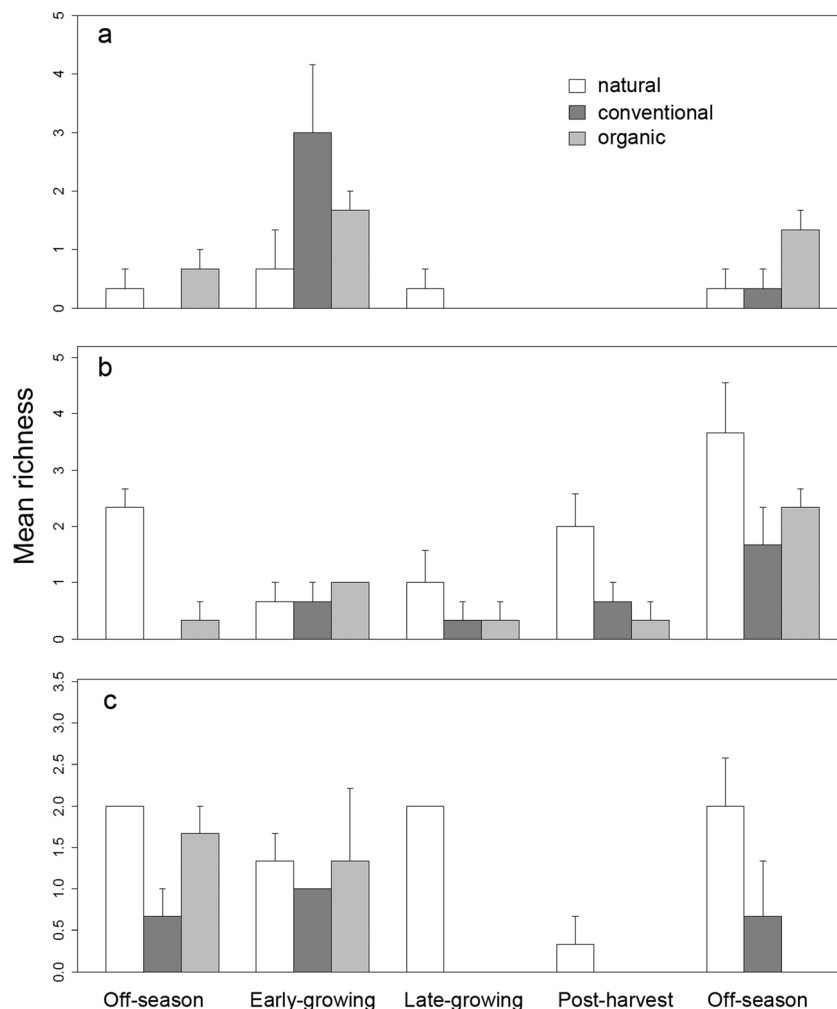
Arboreal species richness varied among treatments, although it did not differ between crop cycles [ANOVA repeated measures: Treatment ( $F_{2,6}=7.450$ ;  $p=0.024$ ), Treatment X Crop cycle ( $F_{8,24}=1.955$ ;  $p=0.119$ )]. The mean richness of arboreal species was higher in natural ponds than in organic and conventional rice fields (Fig. 2b). Richness in the semi-aquatic guild varied by crop cycle and was influenced by treatment [ANOVA repeated measures: Treatment ( $F_{2,6}=7.862$ ;  $p=0.021$ ), Treatment X Crop cycle ( $F_{8,24}=2.738$ ;  $p=0.027$ )]. Mean richness was lower in the post-harvest period than in off-season and early growing period (Tukey,  $p<0.05$ ) (Fig. 2c).

In the NMDS ordination, conventional and organic rice fields and natural ponds formed well-defined groups (Fig. 3). The anuran community composition was similar in conventional and organic crop plots. Semi-aquatic and arboreal species were abundant in ponds. Medium-sized arboreal (snout vent length >30 mm) and semi-aquatic species were associated with organic rice fields, and small arboreal (snout vent length <20 mm) and fossorial species were more common in conventional rice fields. The PERMANOVA, which included the entire cultivation cycle, showed significant differences among the amphibian communities of organic rice

**Table 1** Anuran species recorded in a rice cultivation area in Sentinela do Sul, Rio Grande do Sul, Brazil, from August 2011 to August 2012. A: Arboreal, F: Fossorial, SA: semi-aquatic

Species	Guild	Conventional cultivation			Organic cultivation			Natural ponds		
		1	2	3	1	2	3	1	2	3
Bufonidae										
<i>Rhinella dorbignyi</i>	F		x	x					x	
Hylidae										
<i>Dendropsophus sanborni</i>	A	x	x	x	x	x	x	x	x	x
<i>Dendropsophus minutus</i>	A							x	x	x
<i>Hypsiboas pulchellus</i>	A	x		x	x	x	x	x	x	x
<i>Pseudis minuta</i>	SA					x		x	x	x
<i>Scinax berthae</i>	A								x	
<i>Scinax granulatus</i>	A						x	x	x	
<i>Scinax squalirostris</i>	A	x					x	x	x	x
Leptodactylidae										
<i>Leptodactylus fuscus</i>	F						x			
<i>Leptodactylus gracilis</i>	F	x	x	x	x		x		x	
<i>Leptodactylus latinasus</i>	F		x	x	x	x				
<i>Leptodactylus latrans</i>	SA	x			x	x		x	x	x
<i>Physalaemus biligonigerus</i>	F			x	x	x				
<i>Physalaemus cuvieri</i>	SA			x		x				
<i>Physalaemus henselii</i>	SA								x	
<i>Pseudopaludicola falcipes</i>	SA	x	x	x	x	x	x	x	x	x
Microhylidae										
<i>Elachistocleis bicolor</i>	F			x			x		x	x

**Fig. 2** Mean anuran richness in a rice paddy area in Sentinela do Sul, RS, Brazil, across the rice cultivation cycle (August/2011 to August/2012). **a** fossorial species, **b** arboreal species, **c** semi-aquatic species



fields, conventional rice fields, and ponds ( $F=3.43$ ,  $df=2$ ,  $p=0.034$ ). Comparison of the assemblages between crop cycle showed that there were significant differences only during the off-season period (Table 2). We identified seven indicator species associated with ponds and conventional rice fields (Table 3), and six additional species were found in natural ponds. Indicator species during the off-season period were all hylids (arboreal and semi-aquatic guilds), whereas leptodactylid frogs (semi-aquatic and fossorial guilds) were associated with the rice growing period.

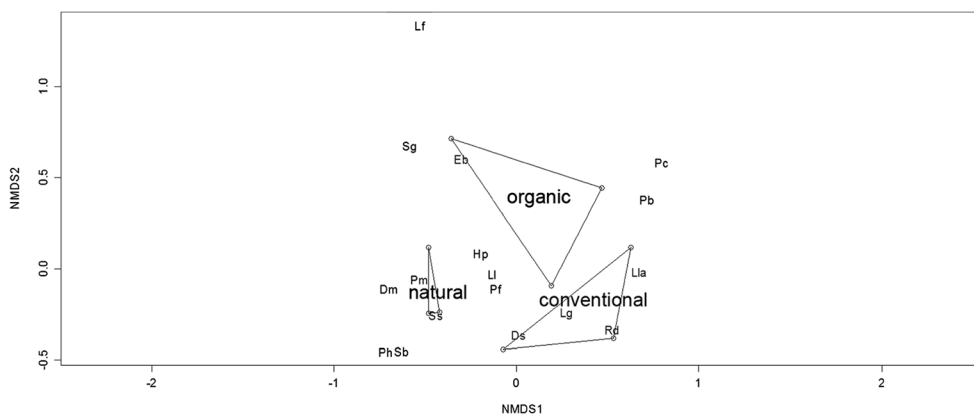
## Discussion

We found that organic rice cultivation hosted a different amphibian assemblage than non-organic rice fields. Although this pattern is consistent with previous studies in other crops (Fuller et al. 2005; Hole et al. 2005), the idea that organic agriculture favors diversity is not necessarily true. Since organic rice fields tend to be smaller than conventional fields, and have more non-cultivated edge, the effects of crop

management could be confused with the variability of the habitat. In some cases, the landscape structure seems to be more important to species diversity and composition than the type of agricultural management used (Weibull et al. 2000). In addition, the biota in agricultural landscapes may not always respond rapidly to the effect of organic farming (Jonason et al. 2011), and this time lag will depend on multiple factors such as the presence of source areas for species recolonization, vegetation succession, and biotic interactions. Although we used areas that had been under organic management for up to 25 years, amphibian guilds in organic rice fields did not differ from those of conventional fields. Thus, it is reasonable to assume that differences related to guilds, which have different life histories, and habitat heterogeneity are responsible for the differences in composition between cultivation methods. Looking at species richness among guilds rather overall species richness could be a better approach for evaluating amphibians use of different habitats (Ernst and Rödel 2008).

Species composition varied considerably between natural ponds and rice fields. In two guilds, semi-aquatic and arboreal, we noted reduced anuran richness in cultivated areas and a





**Fig. 3** Non-metric multidimensional scaling ordination for an assemblage of anurans in a rice cultivation area with different types of management (stress=0.118), in Sentinela do Sul, RS, Brazil, from August/2011 to August/2012. Rd=*Rhinella dorbignyi*, Dm=*Dendropsophus minutus*, Ds=*Dendropsophus sanborni*, Hp=*Hypsiboas pulchellus*, Pm=*Pseudis*

*minuta*, Sb=*Scinax berthae*, Sg=*Scinax granulatus*, Ss=*Scinax squalirostris*, Pb=*Physalaemus biligonigerus*, Pc=*Physalaemus cuvieri*, Ph=*Physalaemus henselii*, Pf=*Pseudopaludicola falcipes*, Lf=*Leptodactylus fuscus*, Lg=*Leptodactylus gracilis*, LI=*Leptodactylus latrans*, LLa=*Leptodactylus latinasus*, Eb=*Elachistocleis bicolor*

marked interaction with crop cycle. Changes during the crop cycle include changes in the water depth and structural diversity of the vegetation. Most of the differences detected between natural ponds and rice fields occurred during the off-season period. These results were a reflection of the greater abundance of arboreal species in natural areas that called from floating vegetation (e.g., *Dendropsophus minutus*) or herbaceous vegetation and shrubs (e.g., *Hypsiboas pulchellus*, *Scinax squalirostris*). The combination of reduced superficial water depth and the lack of vegetation cover during the off-season could turn the rice fields into a habitat that is unfavorable to these species, regardless of whether management is organic or non-organic. Similar patterns have been seen in anuran assemblages in agroecosystems in central Brazil, where low anuran richness was considered to result of habitat simplification (Piatti et al. 2010).

Comparisons among guilds showed distinct differences throughout the crop cycle in semi-aquatic species where richness was higher in off-season and the early growing periods. For other guilds, we did not find differences between crop cycles. These results could be attributed to (1) habitat

preferences and differences in the dispersal ability of the species, (2) visual surveys that may detect anuran more easily in some phases, because they are more exposed in calling aggregations, (3) size of the irrigation canals between the rice fields and the non-cultivated areas. In the study area, all of the semi-aquatic species are frequently associated with grassland and temporary ponds, and a trade-off between hydroperiod length and invertebrates predation is likely to explain anuran community structure. Since the water used for crop irrigation comes from streams or permanent ponds, predatory fish such as wolf fish (*Hoplias* spp.), catfish (*Rhamdia* spp.) and thin dogfish (*Oligosarchus* spp.) are frequently found in the rice plots and irrigation canals. In addition to the predation on tadpoles and adults, the presence of fish influences the selection of breeding sites (or habitats) by many amphibians (Resataris 2005; Werner et al. 2007; Both et al. 2009). At the study area, the rice growing period coincides with the reproductive period of most species, and we could explain the differences in richness between early and late growing periods based on anurans avoiding sites with fish. Thus, the greater water depth and homogeneous vegetation during the final cultivation period could reduce the availability of shelter or breeding sites in the rice fields. However, because rice plants occur in tall tussocks, not all strata or microhabitats within the rice fields can be sampled with equal success, particularly in the late growing period. Although sampling method may have introduced some noise in the analyses, our results showed a similar pattern with other studies focusing rice fields and anurans (Machado and Maltchik 2010; Piatti et al. 2010).

Another factor that could influence the distribution of the non-arboreal guilds is the presence of irrigation canals between the rice fields and the non-cultivated areas. Irrigation canals facilitate the movement of anurans and could also serve

**Table 2** PERMANOVA comparing anuran assemblages across natural ponds, conventional rice fields, and organic rice fields and across sampling occasions in Sentinela do Sul, Rio Grande do Sul, Brazil, from August 2011 to August 2012

Comparison	$R^2$	df*	F	p
Global comparison across all regimes	0.533	2	3.43	0.034
Off-season	0.644	2	5.42	0.005
Growing	0.337	2	1.53	0.185
Post-harvest	0.267	2	1.09	0.422

\* Degree of freedom

**Table 3** Indicator species with respective indicator value (Indval), probability for species to be listed as indicator ( $p$ ), cultivation period and sites where the species showed maximum indicator value

Species	Indval	$p$	Period	Site
<i>Dendropsophus sanborni</i>	0.518	0.04	Off-season	Natural ponds
<i>Hypsiboas pulchellus</i>	0.633	0.004	Off-season	Natural ponds
<i>Pseudis minuta</i>	0.775	0.002	Off-season	Natural ponds
<i>Scinax squalirostris</i>	0.740	0.005	Off-season	Natural ponds
<i>Pseudopaludicola falcipes</i>	0.45	0.013	Growing	Natural ponds
<i>Leptodactylus gracilis</i>	0.526	0.03	Growing	Conventional rice
<i>Leptodactylus latrans</i>	0.500	0.03	Growing	Natural ponds

as breeding sites (Mazerolle 2004; Herzon and Helenius 2008). These canals may also have lower disturbance, cool moist conditions and complex habitat relative to the crop fields. However, the suitability of canals as habitat likely varies among amphibian species. Breeding sites that are not connected with terrestrial habitats could act as population sinks due to high juvenile mortality (Rothermel 2004), and steep-sided canals could act as barriers to terrestrial anuran migration (Kato et al. 2010). The connectivity between terrestrial and aquatic habitats is a key factor for the persistence of anuran populations (Cushman 2006; Becker et al. 2007). Many studies have indicated that post-metamorphic juvenile dispersal contributes more than adult dispersal to regional persistence (Guerry and Hunter Jr 2002; Semlitsch 2008). Fossorial species can burrow into the ground when canal are dry and persist until the canals refill. However, semi-aquatic species would be more vulnerable to desiccation, relying only on soil cracks and crevices when the fields are drained.

The indicator species identified in our study are predominantly associated with open vegetation habitats (Colombo et al. 2008; Kwet et al. 2010). Indval analysis identifies indicator species as those that are well-distributed among sites within a particular treatment (i.e., cultivation method or natural ponds). Thus, the association of arboreal and semi-aquatic species with natural ponds particularly during the off-season period could be attributed to the reduced area of the ponds during the cultivation periods. Although irrigation water is not drawn directly from the sampled ponds, water level is highly affected by fluctuation in precipitation and groundwater level. There is a perception that only generalist species with high resilience are able to persist in areas converted to rice fields (Doody et al. 2006; Piatti et al. 2010). However, species may benefit from the artificial hydroperiod in rice fields. For example, anurans that lay eggs in foam nests below ground and need a flood pulse to release tadpoles from the nest could be favored in places where the flooding of rice fields coincides with breeding season. The association of *Leptodactylus gracilis* with conventional rice fields, in our Indval analysis, supports this assumption.

Our hypothesis that species richness of arboreal anurans would be higher in organic rice fields was not supported. Another important finding from our study was that the patterns of variation in anuran assemblages between natural ponds and rice fields differ among guilds. We have shown that the interpretation of the effects of organic farming on anuran diversity might be biased by the study of species in different or multiple guilds. Habitat features, such as time of flooding, and the presence of irrigation canals, which are potential barriers to dispersal or conduits for predators such as fish, could be more important in determining anuran species composition than cultivation method of these agricultural areas. However, our study was undertaken in a small section of southern Brazil, and there is a need for replicate these studies on anuran assemblages across a broader range of species and landscapes. In light the general lack of knowledge about the effects of agricultural practices on anurans, we recommend consideration of specific traits of different guilds when evaluating impacts of this land use practice on amphibians.

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