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COMPOSITION OF AMPHIBIAN ASSEMBLAGES IN AGROECOSYSTEMS FROM THE CENTRAL REGION OF ARGENTINA

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The anuran diversity, abundance, richness and evenness were examined in agroecosystems from the central region of Argentina, area that was greatly altered by agricultural activities. Relationships with climatic characteristics were also analyzed. To capture anurans, pitfall trap transects were established. A total of 561 amphibians of seven species, belonging to four families (Bufonidae, Leiuperidae, Cycloramphidae, and Leptodactylidae) were recorded. Stepwise multiple linear regressions showed strong positive association between both species richness and anuran abundance with habitat variables as rainfall and rain-day. The anuran richness observed in the studied areas is similar to other sites with level of alteration comparable, but the anuran richness and abundance observed here is lower compared with the records of anuran species for the region before agricultural development. Thus, these results have suggested that amphibians may be particularly affected by habitat modification and degradation due to agricultural activities in the central region of Argentina, and it may be contributing to regression of their diversity and richness.

Keywords: agroecosystem, Argentina, anuran, soybean cropland.

INTRODUCTION

Amphibian populations are experiencing worldwide declines (Collins and Crump, 2009), which are caused by multiple factors (Burkhart et al., 2003; Blaustein et al., 2003; Henle et al., 2008). In agriculturally dominated landscapes the amphibians depend on patches of natural vegetation and ephemeral ponds for their survival and reproduction (Peltzer et al., 2003). The conversion of forest to agricultural land is occurring at rapid rates in many Neotropical areas. In this way, the pressures on uncultivated portions of the land represent an additional stress to an anuran assemblage (Peltzer et al., 2006; Romansic et al., 2006) that may contribute to reduction of their diversity and richness (e.g., Hazell et al., 2001). This presumption have spurred research to examine the role that agroecosystems play in providing habitat for Neotropical organisms (Petit and Petit, 2003; Peltzer et al., 2006).

Argentina is the third world's largest producer of soybeans (*Glycine max* Merril L). In 2008 – 2009, soybean cultivation totaled 16 million ha in the center, east and north of the country (SAGPyA, 2009) supported by a considerable use of agrochemicals (fertilizers and crop pesticides products). Particularly, the central region of Argentina has been most affected by agricultural development (INTA, 2003). Few studies, however, have been conducted on the amphibian assemblage composition and dynamics of agroecosystems in central region of Argentina. Gibbs (1998) and Gray (2002) examined the direct affects of landscape level disturbance on amphibian populations and communities and observed that landscape disturbance resulted in changes of mean morphological characteristics and in population demographics and community dynamics.

Our main objective is to determine the species richness and diversity of anuran assemblages from agroecosystems of Cordoba Province (Argentina), during one breeding season. Moreover, we asked the following questions in the same sense of Peltzer et al. (2006): (1) what habitat variables influence in the anuran assemblages in agroecosystems? (2) what is the amphibian species composition in agroecosystems? (3) agroecosystems main-

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tain abundant populations of anurans? (4) are there differences in phenology of the assemblages?

MATERIAL AND METHODS

We sampled two agroecosystems (AG) (AG 1, 9 ha: 33°05′ S 64°26′ W, AG 2, 12 ha: 33°05′ S 64°25′ W) in Rio Cuarto, Cordoba province, Argentina. The study region belongs to the Pampa Plains of central Argentina. Moderately undulating plains and a temperate climate characterize the area, with an annual mean temperature of 23°C in January and 6°C in July and with mean annual temperature of 18°C. The region is characterized by rainy and dry seasons, with rains typically starting in October and continuing through the warm months until March with a mean annual rainfall of 800 mm (Capitanelli, 1979). The agroecosystem 1 (AG 1) was devoted to soybean crop and two agroecosystems were used for cattle grazing. These study areas are sparsely vegetated by low growing plants (heights <0.5 m) with permanent and temporary ponds. The permanent and temporary ponds are used for cattle consumption and irrigation to crop during periods of drought. Average cattle density at wetlands during our study was 11.3 head (SD = 3.05) per wetland ha per month in AG 1, and 26.3 head (SD = = 3.51) per wetland ha per month in AG 2. There were fewer cattle in AG 1, but they stayed closer to wetlands than the cattle in AG 2. The level of disturbance to the aquatic and surrounding terrestrial habitats was measured on an arbitrary scale of 0 – 5 (Peltzer, 2006, adapted from Pavignano, 1988), where 0 was a natural site without alterations, and 5 was a site completely altered by floods, humans (roads, agriculture, deforestation, excavation, and intentional fire) or farm animals.

We surveyed each agroecosystems from December to April, the period of greatest breeding activity for this species (Cei, 1980). Specimens were captured using live pitfall trap transects (Corn, 1994). Two transects were arranged for each site. The average distance between transects and ponds was of 8 m. Transects were separated by 50 m. Each transect (transect length = 20 m) consisted of eight plastic traps (22 cm diameter, 40 cm tall) spaced at 2.5-m intervals. The bottom of each trap was kept wet to prevent desiccation. Sampled areas were checked at two or three day intervals. We marked individuals using a toe-clip (Waichman, 1992) to prevent resampling and we released them at their capture location. We recorded species and age class (froglet, juvenile or adult). On each visit to a site, we recorded the following habitat variables: air temperature and surface temperature (10 cm above the ground) (with standard thermometer; °C). In addition, total monthly rainfall (mm) and rain-day were provided from Cathedra of Agricultural Meteorology, National University of Rio Cuarto.

The diversity was calculated using Shannon's index (H) and evenness or equitability (E) in each area was calculated using Magurran's equation (Magurran, 1987). We used Hutchinson's t-test to evaluate the differences in amphibian diversity between the areas (Magurran, 1987). Because samples sizes were different in the two habitats, we used rarefaction methods to compare the mean diversity and the mean richness (Hamann et al., 2006; Duré et al., 2008). This method allows comparing the species richness and diversity in different environments regardless of the sample size. A Mann – Whitney U-test was used to test for differences in species richness and diversity between agroecosystems. In addition, the sample size was evaluated using rarefaction curves. Rarefaction curves were created using EstimateS software (Colwell, 1997). We used Sorensen similarity indexes to measure the similarity in the species composition (S_s) and relative abundances (C_N) between areas (Magurran, 1987). To determine the association of habitat variables with anuran abundance, richness and diversity, stepwise multiple linear regression analysis was applied used Statistica (StatSoft, 2001). We compared if the catchability of each species was similar to that of all other species (following Sanchez et al., 2007) using contingency tables. We performed the same analysis with more abundant species, to test the null hypothesis that abundance of species is similar between areas (following Martori et al., 2005).

RESULTS

A total of 561 amphibians of seven species were recorded at two agroecosystems (Table 1). The seven species collected belong to the families Leptodactylidae, Bufonidae, Cycloramphidae, and Leiuperidae. Anuran species found on AG 1 were *Rhinella arenarum*, *Leptodactylus gracilis*, *L. latinasus*, *Odontophrynus americanus*, and *Physalaemus biligonigerus* (species richness = 5). Whereas in the AG 2 *Leptodactylus latrans* and *Pleurodema tucumanum* were found in addition to the five species mentioned on AG 1 (species richness = 7). The Sorensen similarity index (S_S) between areas was 0.83. However, quantitative Sorensen index (C_N) between areas was only 0.51.

There was higher degree of alteration in AG 1 following to the scale of disturbances of Peltzer (2006), adapted from Pavignano (1988) due to modification and perturbation by crops, in addition to cattle grazing; while AG 2 was no found alteration by crops. The *max*imum anuran abundance in AG 1 was recorded in March (total 64 individuals), whereas maximum anuran abundance in

AG 2 was recorded in February (194 individuals). Species diversity in AG 1 was $H = 0.94$ and evenness value was $E = 0.58$. Diversity value in AG 2 was $H = 1.03$ and evenness value was $E = 0.53$. There was no significant difference between the diversity of two sampled areas (Hutchinson *t*-test, $p > 0.05$). Two rarefaction curves are shown in Fig. 1. The curve of both agroecosystems showed sign of reaching an asymptote. Using a rarefaction method, mean species richness (mean species richness: AG 1 = 4.26, SD = 0.96; AG 2 = = 5.15, SD = 0.86) between the two agroecosystems was statistically different (Mann – Whitney *U*-test = 183, $p < 0.0001$, $n = 31$ for both areas). Mean species diversity (mean species diversity: AG 1 = 0.81, SD = 0.12; AG 2 = 0.95, SD = 0.08) between the two areas was also statistically different (Mann – Whitney *U*-test = 90, $p < < 0.00001$, $n = 31$ for both areas). Stepwise multiple linear regression procedures revealed two significant habitat variables, rainfall and rain-day, to predict species richness (Multiple Regression, $F_{(2, 2)} = 40.11$, $p < 0.05$) and anuran abundance (Multiple Regression, $F_{(2, 2)} = = 24.67$; $p < 0.05$), AG 1 and AG 2, respectively.

There were significant differences between the catchability of each species and pooled catchability for all other species (AG 1: $X^2_{4\ R.\ arenarum} = 923.03$, $X^2_{4\ L.\ gracilis} = 625.00$, $X^2_{4\ O.\ americanus} = 193.66$, $X^2_{4\ P.\ biligonigerus} = 198.25$, $X^2_{4\ L.\ latinasus} = 122.31$; AG 2: $X^2_{4\ R.\ arenarum} = 130.87$, $X^2_{4\ L.\ gracilis} = 284.19$, $X^2_{4\ O.\ americanus} = 162.57$, $X^2_{4\ P.\ biligonigerus} = 848.48$, $X^2_{4\ L.\ latrans} = 82.88$, $X^2_{4\ L.\ latinasus} = 705.88$, $X^2_{4\ P.\ tucumanum} = = 726.00$, $p < 0.01$ in all cases). Two species, *R. arenarum* and *P. biligonigerus*, were more abundant in pitfall traps; they constituted 93 and 91% (AG 1 and AG 2, respectively) of pitfall sample (Fig. 2). Contingency tables revealed significant differences of abundance between areas for *R. arenarum* and *P. biligonigerus*($X^2_{4\ R.\ arenarum} = 420.08$ and $X^2_{4\ P.\ biligonigerus} = 283.93$, $p < 0.001$ in both cases).

Differences were found between agroecosystems with respect to the postmetamorphic recruitment. In AG 1, the largest number of individuals captured in December and March was recorded (Fig. 2), which correspond with the greatest postmetamorphic recruitment, mainly *R. arenarum* and *P. biligonigerus* individuals. The individual numbers found in December was due to froglets *R. arenarum* whereas the abundance in March was due mainly to the number of froglets and juveniles of *P. biligonigerus*. In AG 2, a gradual increase of the individual numbers was observed from December to February. The abundance in December was due mainly to froglets *R. arenarum*; in January the individual numbers was due mainly to juveniles and froglets of *P. biligonigerus* and in February, the abundance was due to the number of froglets *R. arenarum*. In AG 1 was observed larval mortality of *R. arenarum* on several temporary ponds by early drying. We collected a sample of these larvae ($n = 30$) that subsequently were examined in the laboratory and no abnormalities were detected.

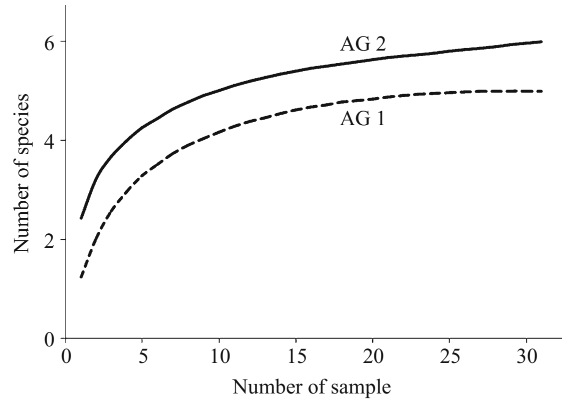

Fig. 1. Rarefaction curves to number of species vs. number of samples, for two study areas.

DISCUSSION

Disturbed habitats are less favorable to amphibians than forests (Ray et al., 2002). Habitat disturbance can lead to fewer niches, more generalists, and greater variability in physical conditions, reducing species diversity, richness and evenness (Hazell et al., 2001; Peltzer et al., 2003; Henle et al., 2008). Examining the similarity in the species richness with other amphibian assemblages in this region (Martino, 1999; Martori et al., 2005), we found approximately 60% of the total species that we would expect. In addition, our results were more similar to work conducted in agroecosystems (Martino, 1999)

TABLE 1. Presence of Species of Anurans in Both Sampling Areas During the Study Period

Species	Number of individuals	
	AG 1	AG 2
Rhinella arenarum	55	178
Leptodactylus gracilis	2	14
Leptodactylus latrans		16
Leptodactylus latinasus	2	3
Pleurodema tucumanum		1
Physalaemus biligonigerus	81	198
Odontophynus americanus	7	4

Note. AG 1, agroecosystem 1; AG 2, agroecosystem 2; bold, the most numerous species.

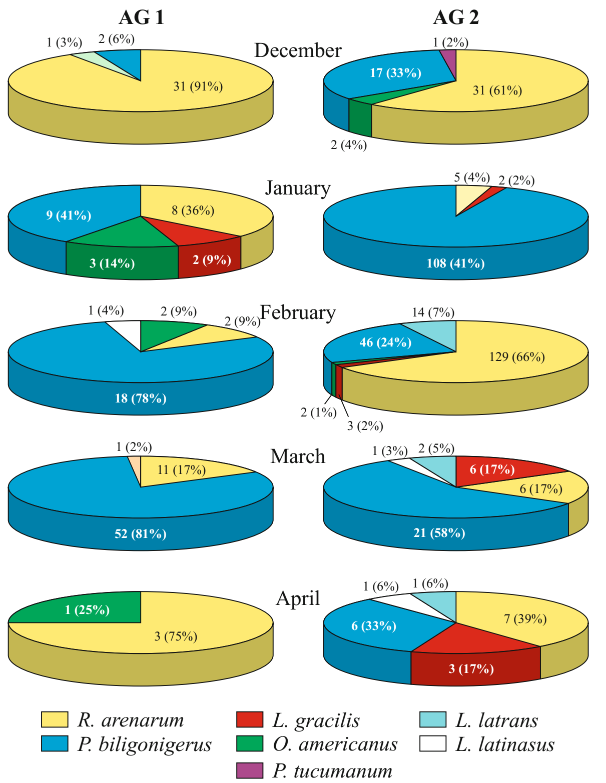

Fig. 2. Species frequencies of anurans registered by pitfall traps in AG 1 (left) and AG 2 (right). For each species the number of individuals is showed and the abundance percentage is between brackets.

than to work done in a natural site (Martori et al., 2005). In this way, the cultivated habitats reported here (our study S = 5 or 7; Martino (1999) S = 7) contain more than half of species found for a natural site (Martori et al. (2005) S = 10). Since two decades, the development of agriculture, has led to degradation in general for almost any environments, particularly in the central area of Argentina. For this reason, we could determinate the species richness of anuran before agricultural development in our region. Several studies on anuran distribution and richness (Cei, 1980; Lavilla et al., 1992; Bridarolli and di Tada 1994; di Tada, 1999) conducted prior to agricultural development, cited for the region approximately 13 anuran species. Therefore, if we consider data from these authors, the agroecosystems could not maintain a representative number of species. Although only two agroecosystems were studied, we recognized that are representative of agricultural ecosystems for the region, and considering further that, we recorded a number of species equal to that found in other agroecosystem of the region (Martino, 1999). Agricultural landscapes represent environments where unpredictable in humidity, particularly at the start of breeding season, is a factor that may limit the modes and reproductive activity in amphibians (Vasconcelos and Rossa Feres, 2005). Thus, agricultural landscapes can possibly restrict the presence of certain species. Moreover, the presence of a chemical contaminant in the breeding sites due to agricultural activities, also could contribute to regression of the assemblage anuran diversity and richness (e.g., Hazell et al., 2001; Peltzer et al., 2003). In addition, we ask whether the agroecosystems are likely to maintain large anuran populations. To address this question, we consider other studies with amphibian assemblages inhabiting natural environments in the central region of Argentina (Martori et al., 2005; Sanchez et al., 2007). Although theses studies were realized for two breeding season, the abundance of anurans in these natural environments are considerably higher (Martori et al. (2005) = 1854 individuals; Sanchez et al. (2007) = 1419 individuals) than the recorded for our study (561 individuals). Intense trampling by the cattle in and around the ponds may also contribute to increased mortality of amphibians eggs and tadpoles (Bécart et al., 2007; Burton et al., 2009), affecting the postmetamorphic abundance. In our study, cattle grazing were observed in both agroecosystems, in one area on a daily frequency. Jofré et al. (2007) provided evidence that the presence of cattle at the breeding sites reduced larval survivorship directly in Pampa de Achala toad (*Rhinella achalensis*), by trampling, and indirectly as a result of increased stream bank erosion. Also, the activities of cattle grazing may have direct or indirect negative effects by decreasing water quality through deposition of nitrogenous waste, causing eutrophication, and grazing shoreline vegetation that contributes to detrital cover and food (Schmutzer et al., 2008). Moreover, intake of water by these animals is substantial. According to Bécart et al. (2007), on average a cow can drink up to 40 liters of water per day; they also suggest that such a large intake contributes to the impact on natterjack egg and tadpole survival by early drying of ponds.

The rarefaction method indicated greater species diversity in the less disturbed agroecosystem. In AG 1, only two species mainly contributed to the diversity values, *R. arenarum* and *P. biligonigerus*; other species contributed only slightly. In the AG 2, these two species and an additional two species, *L. gracilis* and *L. latrans*, contributed significantly to the diversity values. The higher diversity in AG 2 may be due to the greatest pond area. Several ponds at AG 1 have a shorted hydroperiod because they are used as an irrigation source for crops during drought. Reduced hydroperiod decreases the availability of habitats and can have serious affects on amphibian diversity (Corn, 2005). Also, a less amount of postmetamorfic recruitment was observed at AG 1. Our data in froglets abundance for *R. arenarum* and

P. biligonigerus showed two peaks in captures in AG 1. Whereas in AG 2, three peaks in captures were observed. Thus, the proportion of recruitment was smaller in the AG 1. This may be cause for several reasons, but mainly due to the reduced hydroperiod on AG 1, larval mortality of *R. arenarum* was recorded. This in part may have been the cause of less recruitment in AG 1. Tejedo (2003) provided evidence that the duration and the hydroperiod of ponds can be a predictor causal factor of successful metamorphosis in *R. calamita* populations during a 16 years study. He found that the years without reproductive success (measured as successful metamorphosis) were those in which occurred the drying early ponds and the larvae would not be reach metamorphosis.

P. biligonigerus was most abundant species in the both agroecosystems. This is consistent with the results of Martino (1999). Similar finding were obtained by Peltzer et al. (2006) for *P. albonotatus*, recorded in 73.3% of the total ponds sampled surrounded by soybean croplands. According to Martino (1999) *P. biligonigerus* is generalist predator, r-selected population (locally abundant populations) and capable of exploiting a wide range of resources. This latter observation is particularly important because may explain an ability to persist and invade these disturbed habitats. Additionally, *P. biligonigerus* deposited their eggs in foam nest. In ephemeral ponds in which water level fluctuates, many nests may be out of water for a day or two (Duellman and Trueb, 1986). This also may represent an advantage for those species that inhabit agroecosystems, where hydrology and hydroperiod may have large effects on amphibians.

In summary, this paper described the anuran assemblages in agroecosystems of central region of Argentina and provides the first records of amphibians in disturbed agricultural areas for this region.

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