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Amphibian diversity increases in an heterogeneous agricultural landscape

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ABSTRACT

As a group amphibians are the vertebrates most affected by anthropic activity, particularly by agriculture. The rapid advance of the agricultural frontier makes it important to identify the role of agroecosystems as habitat supply for amphibians. We analyzed the differences in amphibian assemblages and populations between habitats with different plant covers and different degrees of human intervention in northwestern Argentina. For three years we conducted 114 high frequency trap samplings to quantify abundance, specific composition and species richness of amphibian assemblages in three habitat types (lemon and sugarcane crops and secondary forest) of a piedmont agroecosystem of Tucumán province. Crops hosted more species and individuals than secondary forests, but the specific composition of forest was different from that of crops suggesting that they could be complementary. Although the assemblage abundance of every observation responded to climate, the strong effect of sampling year was not related to climatic factors suggesting that there might be long term fluctuations that were not analyzed. We also found that responses to agricultural practices were species specific, so no generalizations about these practices should be done. Our study shows that cultivated areas are not hostile environments for amphibians since they can lodge huge amphibian populations and that the occurrence of disturbances associated to agricultural practices is not necessarily reflected in a decrease in the abundance and richness of amphibians in the short term. However, our results also show that forested lands are necessary to lodge some specialist species which are very rare in the croplands. This suggests that environmental heterogeneity generated by the combination of natural and cultivated patches can increase biodiversity at landscape scale because this allows the coexistence of species related to either kind of environment.

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1. Introduction

Amphibians are the group of vertebrates most affected by anthropic activity; nearly 32% of their species being threatened or extinct (IUCN, 2014; Wake and Vredenburg, 2008). For the last decades the decline of amphibians has been recognized as a global phenomenon which was assessed quantitatively (Houlahan et al., 2000), in all the continents and in all the habitat types (Lavilla, 2001). Different processes have been mentioned as possible

causes of this decline (e.g. Collins and Storfer, 2003). For example, several studies showed that amphibians are strongly affected by environmental conditions (e.g. Prado and Rossa-Feres, 2014) and some of them describe the effects of habitat fragmentation on amphibians species richness (e.g. Peltzer et al., 2003) or show that the advance of the agricultural frontier affects amphibian assemblages through different mechanisms (e.g. Hazell et al., 2001; Johansson et al., 2005). Recurrent mechanical disturbances associated to agricultural practices alter the soil surface affecting the sites for reproduction (wetlands, ponds and pools), shelter and hibernation of amphibians (Bishop et al., 1999; Knutson et al., 2004). Pollution with agrochemicals has been regarded as a relevant factor in the decrease of the species richness and abundance of amphibians (Mann et al., 2009). Taking into account the accelerated

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advance of the agricultural frontier, it is of great importance to analyze the role of agroecosystems in biodiversity conservation and as habitat supply for amphibians (Peltzer et al., 2006). Such analyses should take into account the strong association between amphibians and environmental variables.

It is widely accepted that climatic variables, mainly temperature and rainfall are relevant abiotic factors influencing amphibians at individual and population levels (e.g. Parris, 2004; Urbina Cardona et al., 2006) due to the effects of climate on their history of life (Prado et al., 2005) and physiology (Pounds et al., 2006). In subtropical areas, the activity of amphibians and the dynamics of their populations are strongly influenced by the climatic seasonality; the reproduction of most species occurs in the warm season of the year (Conte and Machado, 2005). Thus, depending on their life histories and ecophysiological characteristics, species differ in their tolerance to climate conditions (see Wells, 2007) which determines the dynamics of amphibian populations (Sanchez et al., 2007). For example, generalist species –that use a broad spectrum of the environment– have wide habitat tolerance and are more abundant in disturbed environments than specialist species, which are restricted in their distribution (Dash and Mahanta, 1993). Moreover, it has been observed that species may respond differentially not only to climate but to the occurrence of disturbances (e.g. Pineda and Halffter, 2004). Agricultural practices can be considered as disturbances within croplands (Zhao and Neher, 2013). Amphibian populations are dynamic; population dynamics are not simple and their response to disturbance events may not be immediate, since delays are frequent in ecological processes. So it is important to evaluate the mid term effect of these disturbances on the dynamics of amphibian populations and assemblage. Since climate and disturbance may affect each species differentially (Wells, 2007), the dynamics of the properties of the assemblage would also change in response to these variables (Parris, 2004; Pineda and Halffter, 2004). These factors may affect populations and assemblage of amphibians to different extents; it is important to assess the particular contribution of every factor to the dynamics of populations of amphibians.

Our study aims at assessing the joint effect of habitat type, agricultural practices, climate and sampling year on abundance and species richness and composition of amphibians. Our three specific aims were: 1) to assess the potential of each habitat type to host amphibian populations and assemblages, and to detect potential complementary effects between them; 2) to assess differential responses of amphibian populations and assemblages to habitat type, agricultural practices and climate and to evaluate whether populations and assemblage fluctuated through the years; 3) to determine which kind of agricultural practice is more harmful to the amphibian populations and assemblages.

2. Materials and methods

2.1. Study area

We carried out samplings in the Lules Farm (26°53'20"S; 65°20'32"W), located in the Lules Department, province of Tucumán. The farm lies in the Yungas piedmont area between 450 and 750 m.a.s.l. (Sesma et al., 2010) and is characterized by a subtropical climate with an average annual temperature of 19 °C and a marked seasonality (Brown and Malizia, 2004). Rainfall regime is monzonic with dry winters and rainy summers (Grau et al., 2010). The most important hydrological system in the area is the basin of the Lules River. The water of the system has good physical and chemical characteristics, with high values of dissolved oxygen and low conductivity (Sesma et al., 2010). Some streams of this basin are channeled for irrigation and flow along the farm in a northwest to

southeast direction.

The farm is mainly covered with sugarcane and lemon plantations but in some areas, which are not apt for mechanized agriculture, remnants of natural forest are the main land cover. The resulting spatial pattern is a mosaic of croplands and secondary forests which are connected with the Yungas natural vegetation of the Sierra de San Javier. We selected six sites of Lules Farm to sample the amphibian assemblages: two within the piedmont forest, two within the sugarcane crops and two within the lemon plantation (Table 1). All the sites had similar topographies and were located at approximately 20 m from a water course, which constitutes an attractor for amphibians. To avoid spatial interaction between sampling sites they were located at least at 450 m from each other. The distance between sites with the same cover type was at least at 1200 m. Average distance between all sampling sites was 1541 m.

2.2. Sampling methods and design

We used the pitfall trapping method, modified from Corn (2001). The traps consisted of plastic cylindrical 33 cm diameter and 39 cm height buckets, with side perforations to prevent flooding. In every site, we placed 20 pitfall traps arranged into five rows separated by 40 m. In every row we placed four traps, separated by 10 m. It was not possible to install the fences suggested by Corn (2001), which increase the efficacy of the capture method, because they would have interfered with the agricultural tasks. We conducted 114 samplings from February 2007 to April 2010, which totaled 1131 days.

Traps were checked once a week during the spring and summer season and every fortnight in the autumn and winter season when the activity of amphibians decreases almost completely. Yet the interval between samplings was not homogeneous, so the interval length was taken into account in the computation of the sampling effort. As the objective of the survey was to perform a long-lasting study of the abundance of amphibian we did preliminary surveys to decide on the optimal interval between samplings taking into account the trade-off between the sampling effort and the survival of amphibians. In the survey we found few dead individuals due to dehydration, only in extremely hot days, and it was possible to identify them to the species level. Only once we found a snake in the pitfalls which could be a potential predator of amphibians. Although we acknowledge that there might be some loss of information (e.g. escaped or predated specimens), the method provides a good indicator of the abundance of amphibians and it is particularly useful when comparisons between environments are performed. In every sampling we identified the species of the captured specimens and then we freed them near the capture area. During the first sampling year, 258 captured individuals were marked by means of toe clipping but, due to the noticeably low number of recaptures (11), we discontinued the procedure in the successive sampling years.

The Famaillá Station of the National Institute of Agropecuarian Technology (INTA, 2011) provided meteorological records of daily minimum, maximum and mean temperature. Rainfall data, which is spatially variable in montane environments was provided by Lules Farm, who also provided information on the different agricultural practices performed in the croplands under study: mechanical interventions (weeding and harvesting of lemon crops and harvesting in sugarcane patches) and application of herbicides, insecticides, fungicides and fertilizers.

2.3. Data analyses

To evaluate the variation in species composition of amphibian

Table 1
Description of the six sampling sites.

| | Geographical coordinates | Cover type | Vegetation height | Plant location | Plant harvesting | Main agricultural practices | |
|--------------------|--------------------------|-----------------------|-------------------|--|----------------------------------|------------------------------|--|
| | | | | | | Mechanical interventions | Agrochemical application |
| Sugarcane crop 1 | 26°54'02"S; 65°19'44"W | Gramineous | up to 6 m | Furrow ridges in rows separated by approximately 80 cm | Annually harvested | Plant harvesting | Herbicides, insecticides and fertilizers |
| Sugarcane crop 2 | 26°52'57"S; 65°20'12"W | | | | | | |
| Lemon plantation 1 | 26°53'47"S; 65°20'39"W | Ever green fruit tree | 3 to 6 m | Furrow ridges in rows separated by approximately 8 m | Not harvested (perennial plants) | Weeding and fruit harvesting | Herbicides, insecticides, fungicides and fertilizers |
| Lemon plantation 2 | 26°53'13"S; 65°20'09"W | | | | | | |
| Piedmont forest 1 | 26°53'29"S; 65°21'26"W | Yungas forest | up to 30 m | It is the natural forest | | | |
| Piedmont forest 2 | 26°52'49"S; 65°20'40"W | | | | | | |

Sources: González Sicilia, 1960; Grau et al., 2010; Humbert, 1974; Lules Farm.

assemblages between habitat types, taking into account the sampling years and seasons of the year, we conducted a non parametric multidimensional arrangement (NMDS, Kruskal and Wish, 1978), with PC ORD (McCune and Mefford, 1999). The aim of this analysis was to assess variation between different combinations of conditions, which are considered to have significant ecological implications for amphibians. In each combination more than one single observation were clumped, thus we rearranged the raw data of observation in order to generate composites of abundance for every combination. We grouped the abundance of every species considering the habitat type, the sampling year and the season of the year, and performed a single NMDS based on their Bray Curtis' distance matrix (Legendre and Legendre, 2012). To statistically evaluate the compositional distances between the grouping variables we performed Kruskal Wallis analyses on the scores for each axis, and used Dunn test to determine which treatments differed significantly.

To evaluate the association of habitat type, sampling year, climate, and agricultural practices with the presence and abundance of amphibians we compared the fitting of a series of generalized linear models (GLM) for the whole assemblage (Gelman and Hill, 2007). In this analysis we aimed at identifying the key variables that controlled the abundance of amphibians in every sampling, so we used the raw data of abundance of each sampling. We used a zero inflated Poisson distribution to simultaneously estimate

the parameters of two components; a binomial distribution which models the amphibian presence and a Poisson distribution which models individual counts. This approach has the advantage of dealing with count data with no need of transformations at the time that it helps in the identification of possible causes of the excess of zeros (e.g. detection probability, Wenger and Freeman, 2008). The models were parameterized with the zeroinfl function from the pscl package (Jackman, 2011) of R (R Development Core Team, 2011). We compared 45 models resulting from the combination of three binomial and 15 Poisson components (Appendix 1), for the abundance and species richness of the assemblage. We also modeled the abundance of the five most abundant species, which presented at least 5 records in each habitat type (for example, we did not model the abundance of *Oreobates discoidal*, for which we had only 1 record in sugarcane and 2 records in lemon crops). For the binomial component, we evaluated one explanatory variable at a time; none, minimum temperature or rainfall. For the Poisson component, we used different combinations of four sets of explanatory variables: habitat type (forest, sugarcane crop and lemon crop), sampling year (first, second and third), climate (mean temperature and rainfall) and agricultural practices (mechanical interventions, herbicides, insecticides and fertilizers application). We did not include minimum and maximum temperature because they were highly correlated with mean temperature. Data on agricultural practices were provided by Lules Farm. Weeding and harvesting of lemon crops and harvesting in sugarcane patches were clumped within mechanical interventions. We grouped fungicides and insecticides in the same category because they were applied simultaneously in lemon crops (Appendix 2). For the analyses of the effect of these disturbances we considered one subsequent sampling to the agricultural practice. All the models included traps nights as an offset to take into account the survey effort. In previous analyses we evaluated the interactions between explanatory variables. This generated a multitude of models that did not improve the modeling. We decided to keep the simplest version to assess the individual effect of groups of factors because we were interested on their additive effects and we decided to avoid the proliferation of models. We used the Akaike information criterion (AIC) to jointly evaluate the complexity and the goodness of fit of every model to the data. As we intended to find a single model to explain the different dynamics of amphibians we used a hybrid criterion. We kept the best common model for every response variable whenever it was not significantly overcome by an alternative model, but we kept the alternative model when it significantly overcome the common one.

In order to further evaluate the short term response of amphibian populations and assemblages to the agricultural practices (application of agrochemicals and mechanical interventions), we carried out superposed epoch analyses (SEA, Haurwitz and

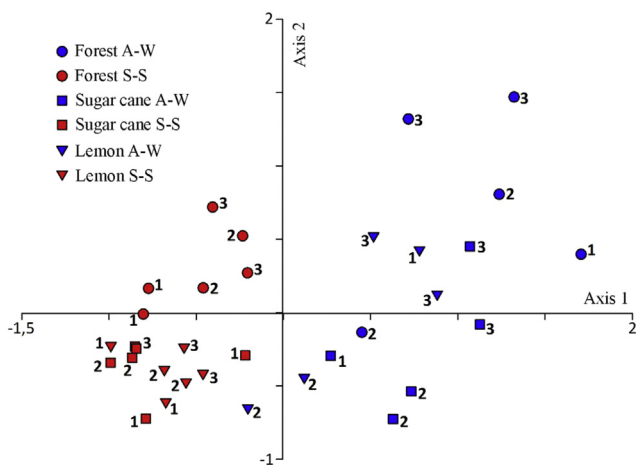


Fig. 1. Non parametric multidimensional arrangement (NMDS) of composites based on the abundance of amphibian species. Composites summarize the assemblage information of single observations based on their habitat type, sampling year and season of the year. A–W: autumn and winter; S–S: spring and summer. The numbers (1–3) correspond to three consecutive sampling years. Final stress = 10.41, $p = 0.004$.

Table 2

Results of the best generalized linear model to fit the abundance and species richness of the assemblages and the abundance of the five most abundant species: Estimate (standard error), dAIC: differential Akaike information criterion, df: degree of freedom, min. temp.: minimum temperature, mean temp.: mean temperature, mech.: mechanical interventions, herb.: herbicides application, insect.: insecticides application, fert.: fertilizers application. Bold numbers indicates no significant effect of the variable ($p > 0.05$).

| | GLM | | | Binomial distribution | | | Poisson distribution | | | | | | | | | |
|----------------------------------|------|----|--------|-----------------------|-----------------|------|-----------------------|-----------------|------------------------|-----------------|-----------------|-----------------|------------------------|-----------------|-----------------|----------------|
| | dAIC | Df | Weight | Min. temp. | Rainfall | None | Habitat type | | Sampling year | | Climatic vbles. | | Agricultural practices | | | |
| | | | | | | | Cane | Lemon | second | Third | Mean temp. | Rainfall | Mech. | Herb. | Insec. | Fert. |
| Total abundance | 0 | 13 | 0.89 | | −0.69 (0.24) | − | 0.82 (0.04) | 0.38 (0.05) | −1 (0.03) | −2.23 (0.04) | 0.52 (0.01) | 0.02 (0.01) | 0 (0.04) | −0.39 (0.04) | 0.20 (0.03) | 1.13 (0.06) |
| Total richness | 0 | 13 | 0.95 | −0.29 (0.1) | − | − | 0.26 (0.1) | −0.42 (0.13) | −0.90 (0.09) | −1.87 (0.11) | 0.44 (0.01) | 0.04 (0.01) | 0.35 (0.10) | −0.22 (0.10) | 0.33 (0.08) | 1.41 (0.16) |
| <i>Rhinella arenarum</i> | 0.5 | 13 | 0.43 | −0.2 (0.04) | − | − | 0.46 (0.05) | −0.22 (0.07) | −1.07 (0.06) | −1.93 (0.06) | 0.56 (0.01) | −0.01 (0.01) | 0.22 (0.06) | −0.56 (0.08) | 0.24 (0.05) | 0.67 (0.15) |
| <i>Pleurodema borellii</i> | 2 | 13 | 0.21 | 0 (0.04) | − | − | 0.17 (0.21) | 0.75 (0.18) | −0.79 (0.16) | −1.8 (0.17) | 0.45 (0.02) | 0.06 (0.01) | −0.01 (0.13) | −0.47 (0.21) | −0.29 (0.14) | 1.26 (0.2) |
| <i>Physalaemus biligonigerus</i> | 0 | 13 | 1 | −0.34 (0.06) | − | − | 2.63 (0.33) | 3.26 (0.34) | −0.09 (0.18) | −1.4 (0.2) | 0.19 (0.02) | 0.04 (0.01) | −0.28 (0.14) | −0.63 (0.14) | −0.41 (0.12) | 0.59 (0.2) |
| <i>Odontophrynus americanus</i> | 0 | 9 | 0.43 | −0.2 (0.09) | − | − | 2.84 (0.55) | 1.35 (0.49) | −2.31 (0.47) | −3.72 (0.47) | 0.31 (0.09) | 0.09 (0.03) | − | − | − | − |
| <i>Leptodactylus latinasus</i> | 0 | 13 | 1 | −0.18 (0.03) | − | − | 1.16 (0.08) | 1.45 (0.09) | −1.11 (0.06) | −2.11 (0.06) | 0.25 (0.01) | 0.01 (0.01) | −0.26 (0.06) | −0.52 (0.06) | −0.38 (0.05) | 0.57 (0.1) |

Brier, 1981). SEA is a re-sampling technique that permits statistically evaluating the temporal response of a variable to the occurrence of an event. It is specially designed to deal with autocorrelation effects. We matched the dates of agricultural practices with the intervals between samplings and considered, as focal time, the first sampling after the agricultural practice was carried out (time lag 0). To avoid the effect of seasonality on the practices (e.g. harvest takes place during winter) we previously built binomial GLM to account for the probability of occurrence of each type of practice in response to temperature. In the SEAs we used this modeled probability to simulate the occurrence of every practice. For the SEAs we considered a time window ranging from the sampling previous to the agricultural practice (time lag −1) to three subsequent samplings (time lag 3). In contrast with the assessment through GLM, these analyses evaluated the evolution of abundance in a longer time (up to three samplings after the application) and within a single habitat type.

3. Results

During the study, we captured 4928 individuals from 12 species: *Leptodactylus chaquensis*, *Leptodactylus latinasus*, *Leptodactylus mystacinus*, *Physalaemus biligonigerus*, *Pleurodema borellii*, *Pleurodema tucumanum* (Leptodactylidae), *Odontophrynus americanus* (Odontophrynidae), *Oreobates discoidalis* (Craugastoridae), *Rhinella arenarum* and *Rhinella schneideri* (Bufonidae), *Phyllomedusa sauvagii*, *Scinax fuscovarius* (Hylidae). *L. chaquensis*, *L. mystacinus* and *P. tucumanum* were only captured in the croplands. *Oreobates discoidalis* was captured mainly in the forest and only occasionally in the crops (Appendix 3).

The species composition strongly varied between seasons (autumn–winter and spring–summer) (Fig. 1, axis 1, Kruskal Wallis $H = 23.82$, $p < 0.001$). Although less marked, there were significant compositional differences between croplands and forests (Fig. 1, axis 2, $H = 14.68$, $p < 0.001$). The segregation between the crops and the forest was more marked during the spring and summer period than in the autumn and winter period. The segregation of forest sites between seasons was more marked than that of the crops. No segregation of sites based on species composition was observed between the sampling years (Fig. 1, Appendix 4).

The GLM that best fitted the total abundance and species richness data of amphibian assemblages was the one which combined the binomial model that included minimum temperature and the

count model (Poisson distribution) that included all the explaining variables: habitat type, sampling year, mean temperature and rainfall, and agricultural practices (Table 2).

The presence of amphibians was positively associated with the minimum temperature. Total abundance was positively related with cultivated patches, and the magnitude of the effect of sugarcane on total abundance was significant and higher than that of lemon crops. Species richness was positively associated with sugarcane and negatively with lemon crops. The second and third year showed a decrease in the total abundance and richness per sampling, being the effect of the third year bigger than that of the second year. Total abundance and species richness were positively associated with mean temperature and rainfall. When we considered one subsequent sampling from the agricultural practice, we observed positive responses of total abundance and richness to mechanical interventions and to the application of fertilizers and insecticides. By contrast, we observed a negative response of total abundance and species richness to the application of herbicides (Fig. 2A, Appendix 5 and 6).

Taking into account up to three subsequent samplings to the agricultural practice the patterns changed a little. After the application of insecticides there was a decrease in total abundance in subsequent samplings which was not detected in the GLM (SEA time lag 2 $p < 0.001$, Fig. 3A).

The GLM that best fitted the abundance data of amphibian populations of the five most abundant species was the one that combined the binomial model that included minimum temperature and the count model (Poisson distribution) that included all the proposed variables: habitat type, sampling year, mean temperature and rainfall, and agricultural practices (Table 2). The best GLM of *O. americanus*, did not include agricultural practices in the count model. The presence of the five most abundant species was positively associated with the minimum temperature. Cultivated patches presented higher abundance than forests, and the magnitude of the effect of sugarcane on the abundance of *R. arenarum* and *O. americanus* was higher than that of lemon crops. The effect of lemon crops was more important in the increase of abundance of *P. borellii*, *P. biligonigerus* and *L. latinasus*. The second and third year showed a decrease in the abundance per sampling, being the effect of the third year bigger in all the cases. The mean temperature and rainfall were positively associated with amphibian abundance. When we considered one subsequent sampling to the agricultural practice, the short term response of abundance to herbicides application was negative and the response to fertilizers was

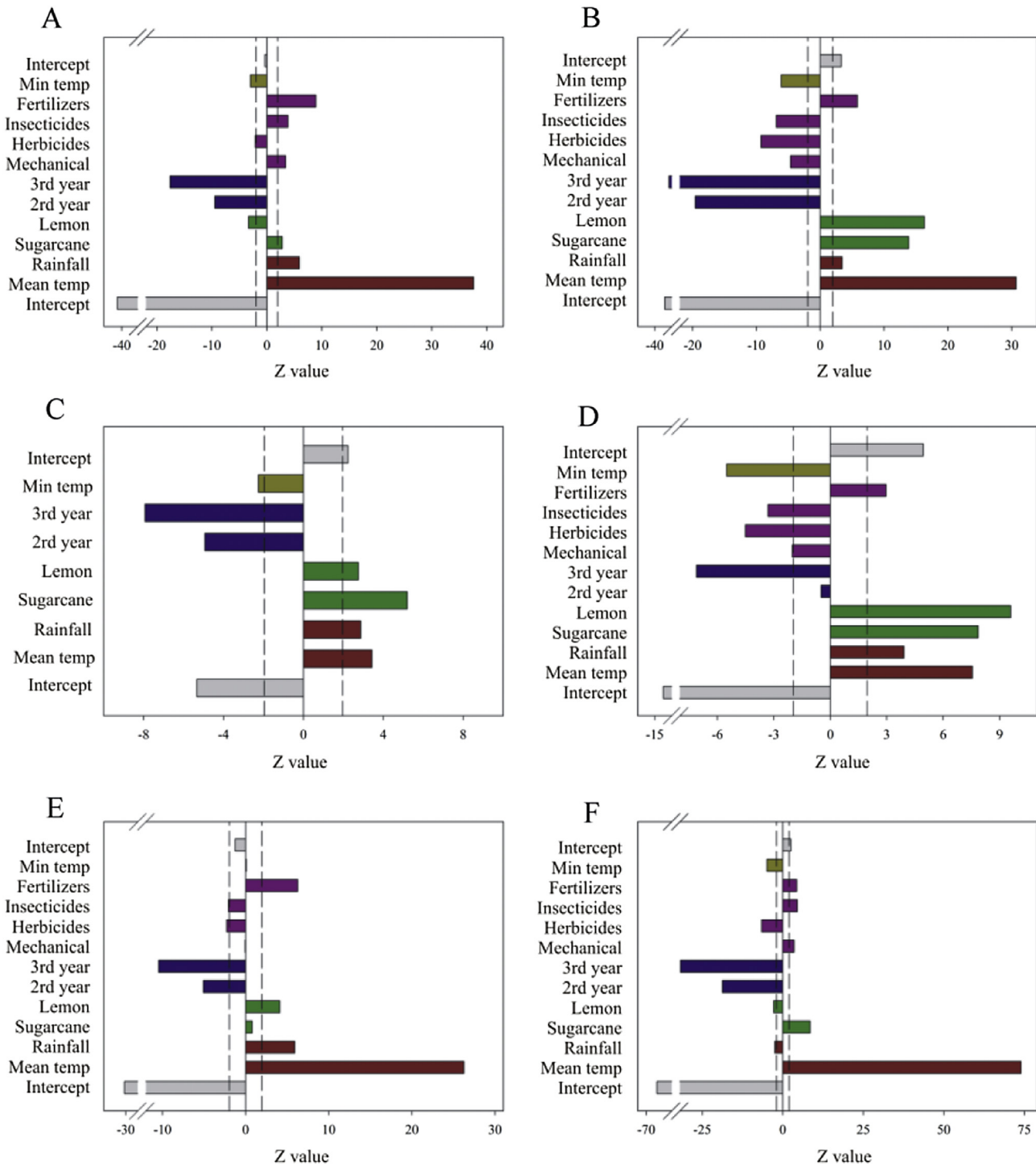


Fig. 2. The explanatory variables that best explained the presence and abundance of amphibians of a series of generalized linear models (GLM). Normalized point estimate (Z value) of every explanatory variable in the zero inflated Poisson model that best explained the presence and abundance of amphibians. Data correspond to 114 observations of three habitat types through three sampling years. The represented model obtained the lowest AIC value in a contest of 45 zero inflated Poisson models which combined three binomial and 15 count components. The two first bars correspond to Z value of the explanatory variable of the binomial component (intercept and minimum temperature or rainfall). The rest of the bars correspond to Z value of explanatory variables of the count component. A: amphibians assemblage, B: *Leptodactylus latinasus*, C: *Odontophrynus americanus*, D: *Physalaemus biligonigerus*, E: *Pleurodema borellii*, F: *Rhinella arenarum*.

positive in all the cases. The short term response of *R. arenarum* abundance to mechanical interventions and insecticides application was positive, while those of *P. borellii*, *P. biligonigerus* and *L. latinasus* were negative (Fig. 2B to F, Appendix 5 and 6).

Taking into account until three subsequent samplings to the

agricultural practice we observed different patterns. After the applications of insecticides there was a decrease in abundance of *L. latinasus* and *O. americanus* (Fig. 3B, C). After the application of herbicides there was a decrease in abundance of *O. americanus*, *P. biligonigerus* and *P. borellii* (Fig. 3D to F). In most cases, the

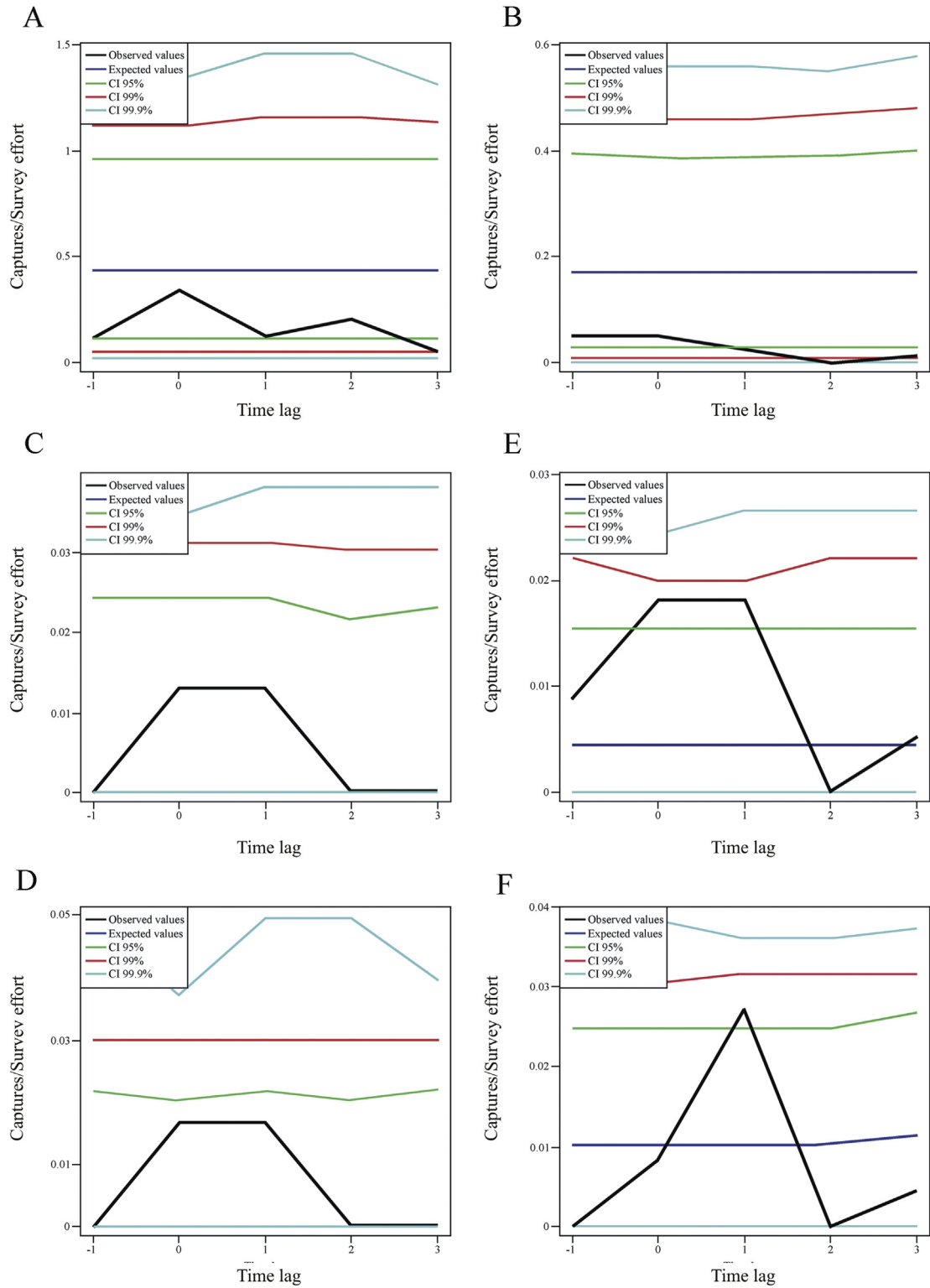


Fig. 3. The abundance of amphibians in a time window that included different agricultural practices. The short term responses of abundance were obtained through superposed epoch analyses (SEA) which average the information of samplings within the observation windows. The observation window ranges from 1 sampling before the agricultural practice (time lag -1) to three samplings (time lag 1, 2 and 3) after the agricultural practice (time lag 0). Confidence intervals are generated through 10000 re-samplings which randomize the date of the agricultural practice. A to C: Decrease in abundance of amphibians assemblage, *Leptodactylus latinasus* and *Odontophrynus americanus* after the insecticides application. D to F: Decrease in abundance of *O. americanus*, *Physalaemus biligonigerus* and *Pleurodema borellii* after the herbicides application.

response of abundance was observed two samplings after the intervention, which was not evaluated through the GLMs (SEA time lag 2, $p < 0.001$).

4. Discussion

Habitats with human intervention surveyed in this study lodged

higher species richness and abundance of amphibians than secondary forests. This pattern could be explained by the presence of depressions on the soil in the cultivated patches. In these depressions temporary ponds were formed, which were used by *L. chaquensis*, *P. biligonigerus*, *P. borellii*, *R. arenarum* and *S. fuscovarius* for their reproduction. In contrast, in forest patches there were only ephemeral streams after heavy rains, which could not be used for reproduction. Our results are supported by some studies that evidenced a positive association between diversity of amphibians and agricultural areas (e.g. Attademo et al., 2005; Kolozsvary and Swihart, 1999) because they can reproduce in wetlands, ponds and pools of disturbed habitat (Prado and Rossa-Feres, 2014). On the other hand, higher abundance of amphibians could be associated with their diet since other studies demonstrated that they are great consumers of arthropods pest in other croplands (e.g. Attademo et al., 2007a, 2007b; Peltzer et al., 2010).

At assemblage level and landscape scale, the contribution of the forest to the overall specific composition may be more significant than any of the crops. The loss of pristine areas could entail the loss of specialist taxa with the consequent homogenization of the assemblage. For example, species that thrive in mature forests such as *O. discoidalis*, are put at risk while opportunistic species such as *L. chaquensis*, *L. mystacinus* and *P. tucumanum*, selected both crops. These opportunistic species inhabit on the soil, usually near pools and flooded areas; they are common in disturbed environments (Heyer et al., 2004, 2010; Lavilla and di Tada, 2004) and they typically inhabit the Chaco region. Their invasion in the Yungas would be associated with the advance of the agricultural frontier in this region. On the other hand, *L. latinasus*, *O. americanus*, *P. biligonigerus*, *P. borellii* and *R. arenarum* were most generalist in their habitat selection. Various studies have reported a similar pattern of spatial distribution in habitat specialists and habitat generalists frogs (e.g. Dash and Mahanta, 1993; Peltzer et al., 2006; Urbina Cardona and Londoño, 2003).

Amphibian assemblages and populations responded in a similar way to the climatic variables, showing the tight relationship between temperature and rainfall with their history of life (Prado et al., 2005) and physiology (Pounds et al., 2006), independently of habitat conditions (Appendix 6). This could be due to the combination of the ectothermal condition of amphibians and the highly seasonal climate that dominates the study area. Other studies performed in other areas with seasonal climate have found similar relationships between amphibians and climatic factors (e.g. Conte and Rossa Feres, 2007; Parris, 2004). In fact, the composition of species differed between the autumn–winter and the spring–summer periods, and higher richness and abundance of amphibians in the spring–summer period than in the autumn–winter period were recorded in previous analyses which were not reported here. The relationship of amphibian abundance to the mean temperature and rainfall could be due to the influence of these variables in their reproductive activity and in the abundance and availability of their preys (Martori et al., 2005; Prado et al., 2005; Sanchez et al., 2007).

Although no compositional shift was observed between sampling years (Fig. 1), a marked decrease in the overall abundance was reported (Table 2). This trend could be explained by a long term decline or by natural interannual fluctuations which are frequent in amphibian populations (Storfer, 2003). These fluctuations have been attributed to different factors, mainly to the variation of climatic conditions due to their relationship with the amphibian reproductive activity (Prado et al., 2005). Although the mean temperatures and rainfall during the three years of study were similar, the occurrence of inter or intraannual variations in the microclimatic conditions should not be discarded. Such

fluctuations could be as diverse as variations in soil humidity, in the hydroperiod or in the availability of food and shelter places. However, we cannot discard the existence of a decline trend, which could be explained by global factors (Collins and Storfer, 2003) or local factors such as agricultural practices, whose long term effects were not assessed in this study.

The responses of abundance and richness of amphibians to agricultural practices show the existence of an effect of these disturbances on amphibians. An other evidence of the effects that habitat types can exert on amphibian assemblages was the absence of *O. discoidalis* (Craugastoridae) in the croplands. This species presents direct development and inhabits in the leaf litter from mountain forest (Lavilla et al., 2010). Their terrestrial reproduction demands high humidity to avoid the desiccation of eggs (Suazo Ortuño et al., 2008). Due to their lifestyle and reproductive mode, the habitat degradation by agricultural expansion constitutes a threat to this sensitive species (Lavilla et al., 2010). In other studies, the abundance and richness of Craugastoridae family were higher in forest than in disturbed habitats (e.g. Urbina Cardona and Londoño, 2003; Zorro Ceron, 2007). Pearman (1997) propose that some species from this family could be bioindicators of tropical humid forest quality, because they thrive under a specific combination of environmental conditions.

Our study supports the idea that environmental heterogeneity generated by the combination of natural and cultivated patches, allows the coexistence of species related to either kind of environment, increasing biodiversity at landscape level (agroecosystem). This is due to the environmental heterogeneity which implies a variety of habitats and resources that allow the coexistence of species with different environmental requirements (Benton et al., 2003), including amphibians (e.g. Rodrigues da Silva et al., 2012a, 2012b), and can give stability to the amphibian assemblage (Guerry and Hunter, 2002). On the contrary, numerous studies report lower species richness or biological diversity in monocultures or in those agroecosystems where environmental heterogeneity is scarce (e.g. Hole et al., 2005; Stoate et al., 2001). In turn, the internal regulation of natural ecosystems occurs through complex fluxes of energy and nutrient (Altieri and Nicholls, 2000). These autoregulation mechanisms could be lost with agricultural intensification and landscape simplification associated with monocultures, turning them into ecologically vulnerable environments and dependent on high agrochemicals levels (Altieri and Nicholls, 2000). Moreover, our study reinforce the importance of small forest fragments to amphibians diversity, because they provide resources that are critical for amphibians, such as habitat refuge and dispersal corridors, and preserve hydrologic regimes and water quality (Rodrigues da Silva et al., 2011).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.actao.2015.09.003>.

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