

# Trophic dynamics of three sympatric anuran species in a soybean agroecosystem from Santa Fe Province, Argentina

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The conversion of forests to agroecosystems presents a challenge for biodiversity conservation. In this study, the feeding habits of three species of anurans (*Rhinella fernandezae*, *Odontophrynus americanus* and *Physalaemus albonotatus*) were compared between a soybean field and a native forest in Santa Fe Province, Argentina. Our dietary assessment is based on 124 individuals (47 *R. fernandezae*, 45 *O. americanus* and 32 *P. albonotatus*). Lepidopteran larvae were the predominant item in anuran diets from the soybean field, whereas collembola, isopods and snails prevailed in the diets from forests. Generally, the three anuran species shifted their diets as prey differs in the two environments, but *R. fernandezae* and *P. albonotatus* maintained a preference for a few prey types. Mean niche overlap in the soybean field was smaller than expected by chance, suggesting that the three anuran species are competing for limited resources. Trophic studies on other native anurans in agroecosystems should be a priority in conservation efforts due to their potential use as natural control agents, as well as for assessing the consequences of the broad conversion of natural forest to agricultural use in our region and throughout the world.

*Key words:* diet, agriculture, trophic niche, *Rhinella fernandezae*, *Odontophrynus americanus*, *Physalaemus albonotatus*

## INTRODUCTION

Diets of amphibian species depend on intrinsic and extrinsic factors, such as morphological constraints related to life-history stage, size, specialization, foraging mode, diet plasticity, presence or absence of competitors and changes in resource availability due to human-mediated habitat alterations (Anderson et al., 1999; Kupfer et al., 2005; Lima et al., 2010). Although numerous studies on diet composition and feeding strategies have been conducted in native forests and areas with few anthropogenic activities (Peltzer & Lajmanovich, 1999; Duré & Kehr, 2004; Araújo et al., 2007; Crawford et al., 2009; Dietl et al., 2009), only a few have focused on agroecosystems (Hirai & Matsui, 1999; 2000; Attademo et al., 2005). In view of the gradual advance of agricultural land, a major challenge is to understand the ecological mechanisms by which a large number of species coexist and how resources are shared. Although trophic segregation is one of the main mechanisms structuring anuran assemblages (e.g. Basso, 1990; López et al., 2005), trophic niches might interact with other aspects such as structural heterogeneity (Suazo-Ortuño et al., 2007).

In Argentina, modern agriculture has led to the expansion of cultivated areas, thus exerting an increasing pressure on natural habitats (Puelo et al., 2005; Peltzer et al., 2006). In agroecosystems, native anurans may play an important role in controlling insect pests while avoiding the negative side-effects of pesticides (Attademo et al., 2005; 2007a,b).

*Rhinella fernandezae* (Bufonidae), *Odontophrynus americanus* (Cycloramphidae) and *Physalaemus albonotatus* (Leiuperidae) are widespread anurans in north-eastern and central Argentina (Frost, 2009), where they are frequently found in natural habitats (grasslands, flooded areas, savannahs, ponds) as well as in human-modified environments. Despite their well-known life histories (Aquino et al., 2004a,b; Narváes et al., 2004; Sanchez & Busch, 2008), few studies have been conducted on their feeding ecology in different environments (Lajmanovich et al., 2003; Attademo et al., 2005, 2007b). This study examines the feeding habits of sympatric populations occurring in a soybean field and a natural forest, with emphasis on prey consumption, prey importance categories and trophic interactions between species. We discuss the importance of trophic strategies and prey composition in determining the influence of human-altered environments such as agroecosystems on anuran communities, highlighting the potential use of amphibians as potential biological control agents.

## MATERIALS AND METHODS

### Study area

The study area is located in mid-eastern Argentina (Fig. 1), a part of the Espinal ecoregion (Burkart et al., 1999). Mean annual rainfall is 800 mm and mean annual temperature is 18 °C. The area is dominated by large areas of intensively managed agricultural land (cultivated mainly with soybean and rice, followed by maize, sorghum and



**Fig. 1.** Location of sampling sites in mid-eastern Argentina: soybean field (A) and native forest (B).

wheat), and interspersed with remnant wetlands and fluvial forests (Arturi, 2006; Peltzer et al., 2006).

Landsat 7 (+ ETM) satellite imagery provided by the Comisión Nacional de Actividades Espaciales (30 × 30 m pixel resolution) was used for visual selection of the sites – a soybean field (100 ha) and a native forest (40 ha, 5 km away from any agroecosystem) – based on, first, the presence of at least one pond with water during the study period, and second, the lack of a significant altitudinal gradient. Surveys were conducted during the period of soybean growth (December 2005 – March 2006).

### Sampling

Adult *R. fernandezae* (RF: Anura, Bufonidae), *O. americanus* (OA: Anura, Cycloramphidae) and *P. albonotatus* (PA: Anura, Leiuperidae) were collected from soybean (30°20'53"S; 59°58'34"W) and forest (30°18'43"S; 59°59'42"W) sites following the methodology of Greenberg et al. (1994). All specimens captured were euthanased and fixed according to ASIH et al. (2001) protocols. We obtained gastrointestinal contents using a stereomicroscope for preservation in 70% ethanol and deposition in the entomological collection of the Facultad de Bioquímica y Ciencias Biológicas of the Universidad Nacional del Litoral, Argentina. We recorded snout–vent length (SVL) of each anuran to the nearest 0.1 mm. A condition factor (CF), expressed as  $100 \times [\text{body weight (g)}] / [\text{length (cm)}]^3$  was calculated (Bagenal & Tesch, 1978). A *t*-test was used to compare CF between anuran species and sites.

### Diet analysis

Individuals containing at least one prey item were included in the overall analysis. Food items were identified to the most detailed taxonomic level possible using a binocular microscope; the number of items per digestive tract was also recorded. We measured each prey item's maximum length (L) and width (W) using digital calipers to the nearest 0.01 mm. For partially digested prey, we estimated length by measuring width and then using predetermined length–width regressions from intact prey (Hirai & Matsui, 2001).

### Intra-environment diet analysis

For each taxon, we calculated the frequency of occurrence (FO%) and numeric percentage (N%) in accordance with the methodology of Lescure (1971). Volumes (V) of each prey item were estimated using the formula for an ellipsoid (Dunham, 1983). These three parameters were considered in determining the prey importance index proposed by Biavati et al. (2004) of each prey category in the diet of each species in each environment.

*Trophic diversity.* Trophic diversity was calculated using Hurlbert's PIE (probability of interspecific encounter) index (Hurlbert, 1971). The index ranges from zero to one and represents the probability that two randomly selected individual prey will be of different taxa (Olzewski, 2004). We calculated the PIE index for each species in each environment using 1000 random permutations in the EcoSim program (Gotelli & Entsminger, 2007). We standardized the number of individuals as the lowest prey abundance in the anuran species recorded. Niche breadth was calculated using the index of Levins (1968). The individual estimations of prey were summed at random, giving the accumulated trophic diversity ( $h_k$ ) (Hurtubia, 1973), a value that was used to determine the minimum sample of digestive tracts required to evaluate sample representatives (e.g. Peltzer & Lajmanovich, 2002; López et al., 2005). One way analysis of variance (ANOVA) followed by Tukey–Kramer post hoc multiple tests was used to determine interspecific differences in prey size.

*Feeding overlap.* We calculated dietary overlaps using Pianka's index (Pianka, 1973). Overlap values of 1.0 indicate identical diets or food volume, whereas overlap values of 0 indicate total dissimilarity in food items. Overlap values were arbitrarily set at the following levels: high (>0.7), intermediate (0.4–0.7) or low (<0.4).

*Prey importance and predator–prey association.* To determine the importance of each prey category in the pooled gastrointestinal tracts of each species in each environment, we applied the formula of Biavati et al. (2004):

$$I = (FO\% + N\% + V\%) / 3$$

where F% is the occurrence percentage, N% is the numeric percentage and V% is the volumetric percentage.

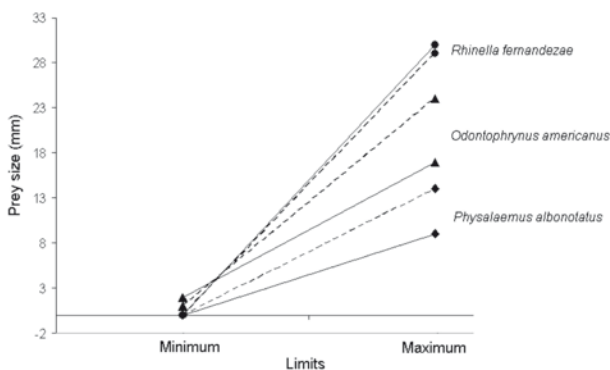
To obtain a multidimensional representation of the predator–prey associations in soybean and forest sites a correspondence analysis (CA) was performed (Legendre & Legendre, 1999). Two matrices were compiled with the

individual number of each prey category consumed by each anuran species in each study site (da Rosa et al., 2002), using MVSP software (Kovach, 2002).

### Diet comparisons and trophic structure

*Comparisons of diet composition between soybean and forest sites.* We performed a rarefaction analysis to compare prey diversities from sites that differed in prey number, involving the estimation of expected richness for a series of samples of different sizes (Gotelli & Graves, 1996). We compared intraspecific prey diversity ( $R. fernandaezeae_{\text{soybean-forest}}$ ,  $P. albonotatus_{\text{soybean-forest}}$ ,  $O. americanus_{\text{soybean-forest}}$ ) and the overall diversity (resulting from the grouping of prey within each order/class for the three species in each environment) using EcoSim (Gotelli & Entsminger, 2007), constructing rarefaction curves (Hurlbert, 1971) using Monte Carlo permutations and rarefying samples to the smallest sample. A significant difference in species diversity between sites was based on 95% confidence intervals. Wilcoxon–Mann–Whitney (U) tests were used to assess differences in Hurlbert (PIE) values between diets of each species and overall diversity in each environment. Differences in the mean size of food items between environments were tested using unpaired  $t$  tests. These analyses were performed using PAST.exe (Hammer et al., 2001).

*Niche overlap null models.* To assess whether the observed overlap values of each environment (soybean vs forest sites) occurred by chance, the observed diet composition (number of each prey category) of each anuran species was randomized by shuffling the original values among the resource states (randomization algorithms RA3; Winemiller & Pianka, 1990). This option retains the observed dietary breadth of each pair of species, but reshuffles the observed values among prey categories within species. For each pair, 1000 random Monte Carlo permutations were created, using the niche overlap module in EcoSim (Gotelli & Entsminger, 2007). The  $P$ -value for the observed mean index was estimated as the proportion of randomized index greater than observed.



**Fig. 2.** Prey size (mm) consumed by *Rhinella fernandaezeae* (●), *Odontophrynus americanus* (▲), and *Physalaemus albonotatus* (◆) in the soybean field (dotted lines) and native forest (solid lines).

## RESULTS

We analysed 47 digestive tracts of *R. fernandaezeae* ( $n=25$ , soybean field;  $n=22$ , native forest), 45 *O. americanus* ( $n=25$ , soybean field;  $n=20$ , native forest) and 32 *P. albonotatus* ( $n=16$ , soybean fields;  $n=16$ , native forest). Mean SVL of *R. fernandaezeae* was  $47.60 \pm 8.30$  ( $\pm$ SD) mm, followed by *O. americanus* ( $34.78 \pm 3.15$  mm) and *P. albonotatus* ( $21.55 \pm 4.11$  mm). The mean ( $\pm$ SD) CF values in soybean populations (*R. fernandaezeae* =  $13.9 \pm 0.48$ , *P. albonotatus* =  $14.3 \pm 0.54$ , *O. americanus* =  $27.6 \pm 0.97$ ) were similar to those from the forest (*R. fernandaezeae* =  $14.1 \pm 0.57$ , *P. albonotatus* =  $13.4 \pm 0.82$ , *O. americanus* =  $26.6 \pm 0.07$ ). No significant intraspecific differences were observed in any environment ( $t_{RF \text{ soybean-forest}} = 0.47$ ,  $P=0.79$ ;  $t_{PA \text{ soybean-forest}} = 0.88$ ,  $P=0.38$ ;  $t_{OA \text{ soybean-forest}} = 0.49$ ,  $P=0.69$ ). A total of 1460 items belonging to 42 prey types distributed into six major categories (Insecta, Arachnida, Mollusca, Myriapoda, Crustacea and plant remains) were identified.

### Intra-environment diet analysis of three anuran species

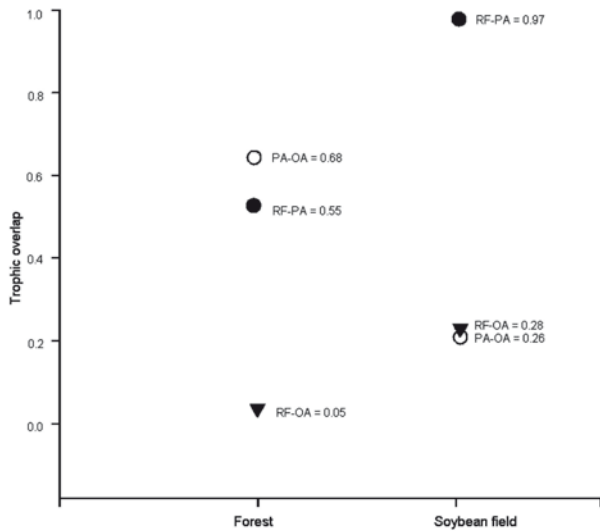
Prey abundance differed between the two study sites (Fisher's exact probability test,  $P < 0.01$ ). The diet of the three anuran species in the soybean field comprised 560 prey items belonging to 32 categories distributed into ten prey orders. The forest sample consisted of 885 prey items distributed in 28 categories from 15 prey orders. The diet composition for each species in each environment is summarized in the Electronic Appendix ([http://www.thebhs.org/pubs\\_journal\\_online\\_appendices.html](http://www.thebhs.org/pubs_journal_online_appendices.html)). Prey size varied between  $0.4 \pm 1.3$  and  $30 \pm 1.2$  mm in forest and between  $0.5 \pm 2.5$  and  $29 \pm 1.1$  mm in soybean field.

### Anuran diet composition in the soybean field

The *R. fernandaezeae* diet comprised 20 prey items ( $n=427$ ), primarily formicid ants (77%) and other hymenopterans (8%). *Physalaemus albonotatus* was represented by 14 prey categories ( $n=93$ ) and mostly fed on formicid ants (63%) and spiders (10%). The diet of *O. americanus* consisted of nine prey types ( $n=40$ ), mainly Lepidoptera (42%) and spiders (17%). *Odontophrynus americanus* had higher values of trophic diversity (PIE=0.77) and niche breadth (Nb=4.6), whereas lower values were observed in *R. fernandaezeae* (PIE=0.39; Nb=1.66, respectively). Noctuidae, crickets and formicid ants predominated volumetrically, differing in frequency of occurrence and numeric percentages (Electronic Appendix). The trophic accumulated diversity ( $h_k$ ) was stabilized between  $n=10$  (*P. albonotatus*) and  $n=13$  (*R. fernandaezeae*) digestive tracts.

Mean prey size ( $\pm 1$  SD) was significantly different in the three anuran species (Fig. 2) from the soybean field (ANOVA,  $F=15.82$ ,  $P < 0.0001$ ). The Tukey–Kramer post hoc multiple test detected significant interspecific differences ( $P < 0.01$ ) between *O. americanus* ( $12.24 \pm 6.25$  mm) and the other two species (*R. fernandaezeae* =  $7.77 \pm 6.76$  mm and *P. albonotatus* =  $3.49 \pm 2.73$  mm). The pair *R. fernandaezeae* and *P. albonotatus* also differed in prey size consumed ( $P < 0.01$ ).





**Fig. 3.** Trophic overlap among *Odontophrynus americanus* (OA)–*Rhinella fernandae* (RF)(▼), *Rhinella fernandae*–*Physalaemus albonotatus* (PA) (●), and *Odontophrynus americanus*–*Physalaemus albonotatus* (○) from soybean and forest sites.

The highest value of niche overlap in the soybean field was observed between *R. fernandae* and *P. albonotatus* ( $O_{Rf/Pa} = 0.97$ ), whereas the lowest values were recorded between *O. americanus* and the other two species ( $O_{Oa/Pa} = 0.26$ ,  $O_{Oa/Rf} = 0.28$ , respectively, Fig. 3).

### Diet composition in the forest site

The diet of *R. fernandae* comprised 19 prey items ( $n=711$ ), mostly formicid ants (88%) and isopods (5%). *Physalaemus albonotatus* consumed 18 prey categories ( $n=127$ ) dominated by formicid ants (22%) and isopods (35%). The *O. americanus* diet comprised 11 prey types ( $n=50$ ), primarily isopods (40%) and snails (30%). *Physalaemus albonotatus* had higher values of trophic diversity ( $PIE=0.80$ ) and niche breadth ( $Nb=5.35$ ), while lower values were observed for *R. fernandae* ( $PIE=0.20$ ;  $Nb=1.29$ ). Isopods were the prey representing the highest volume in the three species (Electronic Appendix). Isopods and spiders were the only shared prey items in the diet of the three species, and trophic accumulated diversity ( $h_k$ ) was stabilized between  $n=13$  (in *R. fernandae*) and  $n=15$  (in *P. albonotatus*) digestive tracts.

We found significant interspecific differences in mean prey size consumed in native forest (ANOVA,  $F=14.94$ ,  $P<0.001$ ). Prey size ranged between  $4\pm 2.55$  mm (*P. albonotatus*) and  $9.33\pm 4.71$  mm (*O. americanus*) (Fig. 2), with no significant differences between *R. fernandae* and *P. albonotatus* (Tukey–Kramer post hoc test).

The niche overlaps in the native forest were intermediate between *P. albonotatus* and *R. fernandae* ( $O_{Pa/Rf} = 0.55$ ) and *P. albonotatus* and *O. americanus* ( $O_{Pa/Oa} =$

0.68). The lowest niche overlap value was found between *O. americanus* and *R. fernandae* ( $O_{Oa/Rf} = 0.05$ , Fig. 3).

### Main prey and predator–prey associations

The main prey categories (I, both numerically and volumetrically) in pooled gastrointestinal tracts of the three species analysed in each environment are shown in the Electronic Appendix. Similar results to those obtained with the I index were observed in the CA for both environments. For soybean data (Fig. 4A), the first dimension represented 62.7% of the total variance, with a positive sign for *O. americanus* (crickets and spiders) and a negative sign for *P. albonotatus* (acarid and spiders) and *R. fernandae* (mainly formicids and noctuid lepidopterans). The second dimension (37.2% of variance) isolated *P. albonotatus* and *O. americanus* with positive loadings from *R. fernandae* with a negative loading. The CA for prey–predator association in forest sites extracted the first two dimensions that explained 99.9% of total variance (Fig. 4B). Dimensions 1 and 2 accounted for 70.2% and 29.7%, respectively. The first dimension separated *R. fernandae* (formicids and spiders) from *O. americanus* (snails and adult coleopterans) and *P. albonotatus* (isopods and collembolans). Dimension 2 identified a group composed of *R. fernandae* and *O. americanus*, as opposed to *P. albonotatus*.

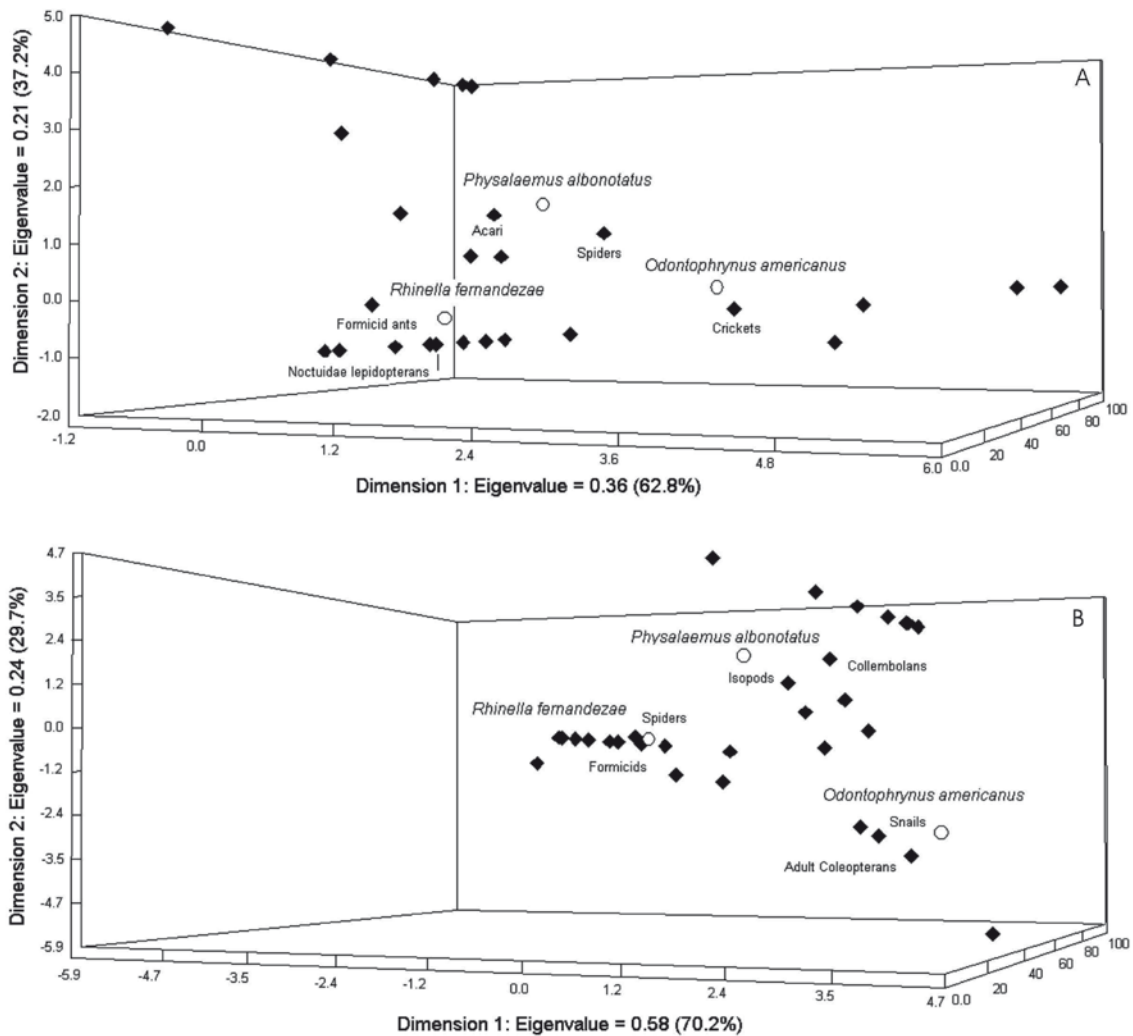
### Inter-environment diet comparison

Rarefaction analysis (based on minimum prey abundance of  $n=40$  samples; 10 intervals, 1000 permutations) showed that the prey diversities of species were different between environments (*R. fernandae*<sub>soybean-forest</sub>  $U=155$ ; *P. albonotatus*<sub>soybean-forest</sub>  $U=55$ ; and *O. americanus*<sub>soybean-forest</sub>  $U=155$ ;  $P<0.01$  in all cases). Moreover, rarefaction analysis showed that total diversity (based on minimum prey abundance of  $n=560$  samples; 19 intervals, 1000 permutations) differed significantly between prey categories (grouped in orders/classes) in both environments ( $U_{total}^{PIE\ soybean-forest} = 570$ ;  $P<0.01$ ). Mean prey size ( $\pm$ SD) was statistically different when comparing each species at both sites ( $t$ -test, *R. fernandae*<sub>soybean-forest</sub>  $t=3.05$ ,  $df=121$ ;  $P<0.01$ ; *P. albonotatus*<sub>soybean-forest</sub>  $t=2.15$ ,  $df=64$ ;  $P<0.05$ ; *O. americanus*<sub>soybean-forest</sub>  $t=2.09$ ;  $df=46$ ;  $P<0.05$ ).

Permutation tests of diet overlap null models revealed that the soybean site had  $P$ -values that were significantly smaller than expected by chance with fewer resource states ( $P[\text{mean of simulated index}] = 0.03$ ;  $P[\text{observed} \leq \text{expected}] = 0.01$ ), whereas the feeding overlap values for each species pair in the forest site were significantly greater than expected (mean of simulated index = 0.35;  $P[\text{observed} \geq \text{expected}] = 0.01$ ).

## DISCUSSION

Dietary information is crucial for understanding the impact of habitat modification on anurans (Anderson et al., 1999), and to assess whether anuran species adjust their diets to variation in prey across environments. We observed that there are differences within and between the forest and soybean sites in the feeding habits of *R. fernandae*, *P. albonotatus* and *O. americanus*.



**Fig. 4.** Plot of the first two dimensions from correspondence analysis of anuran species–prey associations in soybean (A) and forest (B) sites. For clarity, only the most related prey are named.

### Overall diet analysis

The diet of *R. fernandae*, *P. albonotatus* and *O. americanus* was mainly composed of arthropods (mostly formicids, lepidopteran larvae, isopods, orthopterans and coleopterans) and gastropods (snails). In addition, although the condition factor (CF) of each anuran species in each environment was similar, in *O. americanus* and *P. albonotatus* the values tended to be higher in the soybean field. Considering that the condition factor is a numerical relationship between body size and weight, a possible explanation is that larger (older) individuals may be better suited to survival in modified environments (soybean fields, in our case) because they are less sensitive to desiccation than smaller individuals (Mazerolle, 2005). Therefore, a diet comparison between two or more sites (natural and disturbed) in combination with skeletochronology should be considered for future studies. Another hypothesis could be that some of the insects that form plagues have high nutritional qualities (high protein content), such as caterpillars (Banjo et al. 2006) and this should be taken into account in further analysis.

### Prey variability

The diets in the soybean field mainly consisted of harmful herbivores on soybean plants (Higley & Boethel, 1994) such as lepidoptera larvae (usually represented by the velvetbean caterpillar *Anticarsia gemmatilis* and armyworms *Spodoptera* sp., among others). The life cycles of these lepidopterans coincide with seed development, and their larvae produce serious damage (Aragón, 2002). Their natural enemies are frequently reported (Saini, 2001), but few authors have considered amphibians (Hyatt & Humphrey, 1995). We suggest that the three anuran species feed on harmful herbivores and therefore are important biological control agents in soybean fields (see also Lajmanovich et al., 2003; Attademo et al., 2005, 2007a,b).

Prey availability has been demonstrated to be one of the most important factors determining the diet of amphibians (Hirai & Matsui, 1999; 2002), but resource availability is complex to measure and has not often been included in anuran diet studies (Toft, 1980). A decline in the occurrence of specialized forest prey in the diet of anurans from the soybean field (such as Cleridae beetles

whose larvae are associated with dead wood; Evans & Hogue, 2004) may generate greater food niche overlap between pairs of species such as *R. fernandezae* and *P. albonotatus*. It is also possible that they compete for food more strongly in altered and homogeneous habitats. Another possibility is that the absence of any type of prey could encourage predators to specialize in other prey (e.g. lepidopteran larvae in soybean; see also Hirai & Matsui, 1999; Maneyro & da Rosa, 2004).

### Prey-predator association

The analysis of important prey categories and prey association revealed that trophic partitioning contributes to coexistence in the forest site, but the role of microhabitat use should be taken into account in the agroecosystem to determine trophic structure. Despite the higher niche overlap in the soybean field, *R. fernandezae* displayed a stronger preference for formicids and noctuid larvae, and its pattern of occurrence was more aggregated than *P. albonotatus*, which had a broader spatial niche (marshy ponds, floating between aquatic plants, littoral zone, flooded depressions; pers. obs.). These differences in habitat use, type and size of prey taken may reduce the frequency of heterospecific encounters in the field.

### Trophic overlap

Permutation tests revealed that *P* values of the observed means decreased in the soybean field significantly more than expected by chance, suggesting that either interspecific competition or resource partitioning are occurring (Gotelli & Graves, 1996). A large mean overlap might signify common resource utilization and a lack of competition, in which some species pairs are very similar in resource use whereas others are very dissimilar (Gotelli & Graves, 1996). On the other hand, high overlap also implies strong competition that has not yet led to divergence in resource use. Both scenarios are possible and additional data on resource availability and species interactions are necessary for assessing trophic competence, considering that overlap does not equal the amount of competition (Griffiths, 1986; Connell, 1980). We found that in the soybean field, *R. fernandezae* and *P. albonotatus* had higher similarities in trophic resources than the other pairs of species, probably reflecting foraging constraints in soybean fields, where herbivores predominate (Attademo et al., 2005). That both anuran species consume similar prey types from the same areas suggests that they could compete for limited food resources (Luiselli, 2006). In forest, the niche overlap was higher than expected by chance. The mean overlap among diets was intermediate ( $O=0.4-0.7$ ) or low ( $O<0.4$ ), showing a certain degree of food partitioning in the forest. In addition, the differences in prey obtained may restrain interspecific competition, but further studies are necessary to determine if diet partitioning is a significant mechanism driving competition (Williams et al., 2006).

Our observations are consistent with published data that have shown high overlap values in human-modified environments (e.g. Luiselli, 2006). The trophic structures in both study sites seems to be a consequence of resource availability (not recorded in this study) in both soybean

and native forest, and could explain how these three anuran species can coexist at the same site by differing in trophic and spatial resource dimensions. Habitat partitioning explains high dietary overlap among competing species without exclusion (e.g. Griffiths, 1986; Duré & Kehr, 2004; López et al., 2005). In our study, *R. fernandezae* spent most of the day in burrows, while *P. albonotatus* was frequently found in the flooded peripheries of ponds, grassland or floating between submerged plants, where competing species have similar prey preferences. Therefore, data on diet composition can support ecological and behavioural field studies.

### Prey association and trophic strategies of anuran species

Trophic interactions among taxa are an important component of the regulation of populations (Wilbur, 1997). Despite the diversity of food resources exploited by *R. fernandezae*, the low individual niche breadth values and the trophic diversity in soybean and forest sites suggests a tendency toward trophic specialization. Circumstantial evidence also suggests that the presence of specialist predators at each site is based on the high abundance attained by some particular prey types (e.g. formicid ants). The importance of ants in the diet of *Rhinella* species has been repeatedly reported (e.g. Lajmanovich, 1995; Isacch & Barg, 2002). Optimal foraging theory (Pyke, 1984) proposes that dietary specialization can occur when reliable and abundant trophic resources are constantly available. It seems plausible that the reliability of ants as a food resource in our region has produced this level of dietary specialization, which is not adaptive when it exploits ephemeral resources such as in the soybean field (Muñoz-Guerrero et al., 2007; Attademo et al., 2007a). This applies, for example, to the noctuid *Anticarsia gemmatalis*, whose larvae occur in the late summer and cause damage to soybean plants (Wilkerson et al., 1986).

Formicid ants were also widely represented in the diet of *P. albonotatus* at both sites, confirming previous observations (López et al., 2005; for co-generic species see Moreira & Barreto, 1996; Attademo et al., 2007a). Ant specialists are known to have defensive toxic skin secretions (Santos et al., 2003), which might impose a cost to be detoxified, resulting in trade-offs and individual-level diet preferences (Araújo et al., 2007). Habitat quality and local arthropod diversity appear to affect access to alkaloid sources by anurans with toxic secretions (Daly et al., 2008). Long-term studies on the effect of habitat alteration on anuran diets are necessary, particularly in species that have a strong preference for one or two prey item in modified environments as a response to selective pressures or trophic plasticity (Macale et al., 2008).

### The agro-ecological context

Trophic studies on anurans in agroecosystems are still scarce and should be considered a priority in developing conservation strategies. In recent years, there has been increased attention on the impact of habitat disturbance, justifying the assessment of associated species-specific sensitivities and risks (Henle et al., 2004; Hero et al., 2005). Referring to trophic strategies, Williams et al.



(2006) pointed out that generalist species that acquire food from the available range should be less susceptible to fluctuations in any particular food type than species that forage in a more specialized way. In this context, *Odontophrynus americanus* acts as a trophic generalist in soybean, whereas *R. fernandezae* is a specialist and *P. albonotatus* an intermediate consumer. Although more studies of the effects of trophic habits on additional native anuran species need to be conducted before we can draw conclusions about the sensitivity of species to varying trophic resources in agroecosystems, the results of the present study could suggest that *O. americanus* and *P. albonotatus* are somewhat less sensitive to habitat degradation than *R. fernandezae*.

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