
DIVERSITY OF AMPHIBIANS IN RICE FIELDS FROM NORTHEASTERN ARGENTINA

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SUMMARY

The anuran biodiversity and richness of a frog community inhabiting rice fields in northeastern Argentina was surveyed and analyzed. The samples were taken between October 2001 and June 2003. Twenty six species of amphibians were identified in five microhabitats: rice fields per se, ditches and paths, natural vegetation, aquatic vegetation and gullies. The natural veg-

etation was the most diverse of the microhabitats, while gullies were the poorest in species. The amphibian species of three of the microhabitats adjusted to the logarithmic range-abundance model, one to the geometric and one to the broken stick model. The data suggest that rice cropping in northeastern Argentina, if properly managed, can support a diverse anuran fauna.

DIVERSIDAD DE ANFIBIOS EN CAMPOS DE ARROZ EN EL NORESTE DE ARGENTINA

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RESUMEN

Se registró y analizó la diversidad y riqueza de una comunidad de anfibios que habita campos de arroz en el nordeste de Argentina. Las muestras fueron obtenidas en el período comprendido entre octubre 2001 y junio 2003. Se identificaron 26 especies habitando cinco microhábitats: campos de arroz, zanjas y caminos, vegetación natural, vegetación acuática y barrancas. La vegetación natural fue el microhábitat más diverso mientras

que las barrancas presentaron la menor cantidad de especies. El modelo de rango abundancia serie logarítmica fue el de mejor ajuste para tres de los microhábitats analizados, otro se ajustó al modelo geométrico y el restante al modelo de varilla rota. Los datos sugieren que los cultivos de arroz del nordeste argentino; podrían albergar una fauna muy diversa si están sujetos a un manejo apropiado.

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RESUMO

Registrou-se e analisou-se a diversidade e riqueza de uma comunidade de anfíbios que habita campos de arroz no nordeste da Argentina. As amostras foram obtidas no período compreendido entre outubro de 2001 e junho de 2003. Identificaram-se 26 espécies habitando cinco microhábitats: campos de arroz, sulcos e caminhos, vegetação natural, vegetação aquática e barrancas. A vegetação natural foi o microhábitat mais diverso en-

quanto que as barrancas apresentaram a menor quantidade de espécies. O modelo de espécie-abundância de série logarítmica foi o de melhor ajuste para três dos microhábitats analisados, outro foi ajustou ao modelo geométrico e o último ao modelo de vara quebrada. Os dados sugerem que os cultivos de arroz do nordeste argentino poderiam abrigar uma fauna muito diversa se estão sujeitos a uma manipulação apropriada.

Introduction

Rice cultivation in Argentina provides fundamental contributions to the regional economies involved. The Province of Corrientes is an important rice producer whose cultivated surface area increased considerably from

1997 to 1998 to ~86000ha (Begenesic, 1998).

Rice fields also have a fundamental role in the regulation of local water and climate cycles. The ecological features of the wetlands are a key for the preservation of wildlife in these ecosystems, since they harbor highly diverse plant

and animal species (Bambaradeniya and Amerasinghe, 2003; Bambaradeniya *et al.*, 2004; Doody *et al.*, 2004).

A field systematically set for rice cultivation possesses main and secondary channels for irrigation water supply and outlet, bounds that delimit the cultivated paddies and interior

edges that delimit the plots. The present study analyzes amphibian diversity and richness in agricultural ecosystems, particularly in rice fields. Amphibians are of interest because their special physiological (skin permeability) and ecological (compound two-phase life cycle) characteris-

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tics happen to be potentially excellent bioindicators, which in turn makes it very common for amphibian populations to be severely affected when there are serious disturbances to their natural habitats (Blaustein and Belden, 2003; Carey and Alexander, 2003; Collins and Storfer, 2003).

Given that rice fields have heterogeneous ecological conditions, organisms that live in this habitats must be well adapted to continuous changes and their survival will depend both on their physiological characteristics and their ability to migrate. Moreover, agronomic practices carried out in rice cultivation, for example when ploughing the fields, applying of herbicides, fertilizers and fungicides, and frequent adjustments of the water level, are considered limiting factors for amphibians that inhabit these ecosystems (Bambaradeniya, 2000).

The aim of this study was to determine amphibian diversity and species richness in rice fields in the Corrientes Province in relation to the different microhabitats formed inside this particular environment.

Materials and Methods

Study Site

This study was conducted in a rice field located 30km south of the city of Corrientes, in the Corrientes Province (27°47'S, 58°46'W), with a surface of ~1000ha. A reconnaissance survey was conducted prior to carrying out the field sampling, in order to select, within the study site, different habitats for the survey.

Five dominant microhabitats were selected in rice field habitats. These were a) rice fields *per se*, an area where rice was sowed and cultivated; b) ditches and paths, which included the paths and ditches on both sides of the rice fields and routes that linked the different sowed plots; c) natural vegetation, an area inside the rice-field which was not sowed, often resulting in the natural vegetation consist-

ing of shrubs and small sized trees; d) aquatic vegetation, small ponds in which floating aquatic plants had flourished, forming a layer that covered the whole surface of the water; and e) gullies, formations that could be up to 20m deep, between rice plantations and river banks.

Agricultural practices

For four years prior to this study, agricultural activities on the site being monitored were related entirely to rice cultivation. Preliminary sampling began in August, followed in October by flooding of the field with water extracted from the Parana River and, also, water distribution to the channels by means of pumping stations. The crop period lasted ~100 days. Immediately after the crop was harvested, in February and April, the plots were prepared for a new sowing. During the period between crop harvest and sowing (fallow period), the field retained some water in the channels of irrigation and drainage, as well as in small temporary pools in rice fields.

Generally, in the rice fields of the Corrientes Province, herbicides such as glyphosate or N-(fosfometil) glycine (3-6l·ha⁻¹) are applied before sowing for the control of grasses. After germination, when the plants are 7-10 days old, fertilizer is added (NPK= 5-30-15 or 5-30-20) for a final N₂ concentration of 150-180kg·ha⁻¹ and urea concentration of 100-120kg·ha⁻¹. Fertilization is generally performed in fractional applications, with half or 70% in pre-watering and the remainder during FPD (foliate primordial differentiation). During flowering, insecticides are applied for the control of ants (Fipronil) and bedbugs (Metamidofos). Other pesticides, such as deltamethrine and dimethoates, are also used. Fungicides were only used on a small scale and were generally mixed. The fumigation process was carried out by airplane.

Sampling methods

Sampling began in October 2001 and was completed in June 2003, covering two continuous rice cultivation cycles. The sampling was initiated immediately after completion of field preparation work, which coincided with spring and sowing of the rice, and continued till the fallow period. The sampling was carried out fortnightly, during day (18:00) and night (23:00), each session spanning 2.5h. A total of 41 sampling sessions were carried out during the entire survey period.

The capture method was manual, with nets, using a sampling methodology known as "visual encounters surveys" (Heyer *et al.*, 1994). Each sampling was carried out by the same three people and each microhabitat was supervised for 30-40min in order to avoid underestimation of the biodiversity. Amphibian specimens were captured inside the rice field, in channels that take the water from the river up to the plantation and connect different sowed areas, and at relictual vegetation that remains inside the area of study. The substratum on which specimens were found at the moment of capture and their behavior (i.e. singing and/or listening) were noted.

On each sampling day, the presence of anurans was recorded by direct observations or by auditive identification. The captured specimens were identified using various guides and keys (Ceí 1980, 1987; Gallardo 1987; Frost 2004; Faivovich *et al.*, 2005; Frost *et al.*, 2006).

Data analysis

Diversity was calculated by the Shannon diversity index (Shannon and Weaver, 1949), using natural logarithms. The specific diversity for each microhabitat was obtained by means of the method proposed by Zar (1996). Every calculation was made by using the Bio-Dap software (Thomas and Clay, 2000), which is based on the methods proposed by Magurran (1988).

The similarity between the specific composition of every microhabitat was estimated using the Sorensen similarity index, which uses only binary information (presence/absence) and is calculated as

$$S=2c/(a+b)$$

where a: number of taxa present in sample A, b: number present in sample B, and c: number of taxa that are present in both samples.

The Sorensen index can be also quantitative (species and their abundances in common). This is one modification of the Bray and Curtis (1957) formula and frequently named as Sorensen index (Magurran, 1988):

$$C_N= 2jN/(N_a+N_b)$$

where N_a: total number of individuals in site A, N_b: total number of individuals in site B, and 2Jn: sum of the lowest of the two abundances for species found in both sites (Magurran, 2004).

The community composition for each microhabitat was characterized by means of the range - abundance models. For every case, it was specified which of the more common three series, logarithmic, geometric and "broken stick", presented a better adjustment to the observational data. The comparisons between different variables were done by means of the Chi-square test.

To avoid underestimation of the population in some microhabitats, due to differences in sample size or to the difficulty to reach the specimens, the rarefaction model proposed by Sanders (1968) and corrected by Hurlbert (1971) and by Simberloff (1972) was used. This procedure allows to compare the species richness and diversity in different environments, regardless of the sample size. The samples were rarefied to the smallest sample size. The richness and diversity results were obtained for five classes of abundance (5, 10, 15, 20 and 25 individuals). Subsequently, the microhabitat diversity for each one of the five classes of abundance was compared with the non-parametric Kruskal-Wallis test.

Results

During the first year of sampling, the flooding of the rice field began in September 2001 and the crop was harvested in Jan-Mar 2002. The second year it began in Sep 2002 and the crop harvesting took place in Apr 2003.

A total of 1380 individuals were collected, belonging to four families: Cycloramphidae, Bufonidae, Hylidae and Leptodactylidae, and appertaining to 26 species of amphibians (Table I).

Seasonality

Significant differences were observed in species abundance in the four seasons of the year ($\chi^2= 199.90$, $df= 3$, $p<0.0001$). Autumn and spring did not witness any significant differences in relation to abun-

dance (χ^2 (with Yates correction for continuity) = 1.34, $df= 1$, $p= 0.24$). The highest abundance of different species were observed during autumn (N= 474) and spring (N= 439), which coincided with the respective emptying and flooding of the field. This number decreased slightly in summer (N= 330), during this period when the emptying of the field occurs and the labor related to rice harvest starts. In contrast, the lowest abundance of amphibians was recorded during the winter (N= 137), which coincides with low temperatures and, towards the end of winter, ploughing the field.

The Leptodactylidae family, with 10 species, was the most abundant family in the rice field and was found in every sample. Bufonidae and Hylidae were most abundant during the spring and summer and the Pseudidae

TABLE I
LIST OF AMPHIBIAN SPECIES IN A RICE FIELD
LOCATED 30km FROM CIUDAD CORRIENTES,
ARGENTINA

Family	Species
Microhylidae	<i>Elachistocleis bicolor</i> *
Cycloramphidae	<i>Odontophrynus americanus</i> (N=2)
Bufonidae	<i>Chaunus schneideri</i> (N= 57) <i>Chaunus granulatus</i> (N= 3) <i>Chaunus fernandezae</i> (N= 16) <i>Chaunus bergi</i> (N= 42)
Leptodactylidae	<i>Leptodactylus (Lithodytes) diptyx</i> (N= 7) <i>Leptodactylus podicipinus</i> * <i>Leptodactylus elenae</i> (N= 5) <i>Leptodactylus latinasus</i> (N= 70) <i>Leptodactylus chaquensis</i> (N= 317) <i>Leptodactylus ocellatus</i> (N= 9) <i>Pseudopaludicola boliviana</i> (N= 32) <i>Pseudopaludicola falcipes</i> (N= 300) <i>Physalaemus santafecinus</i> (N= 91) <i>Physalemus albonotatus</i> (N=12)
Hylidae	<i>Lysapsus limellum</i> (N= 4) <i>Hypsiboas raniceps</i> (N= 4) <i>Hypsiboas pulchellus</i> (N= 13) <i>Dendropsophus nanus</i> (N= 194) <i>Dendropsophus sanborni</i> (N= 93) <i>Scinax fuscomarginatus</i> (N= 22) <i>Scinax nasicus</i> (N= 7) <i>Scinax acuminatus</i> (N= 76) <i>Argenteohyla siemersi</i> (N=1) <i>Phyllomedusa hypochondrialis</i> (N= 3)

* Species identified only by calling or presence of tadpoles. N: number of individuals recorded,

TABLE II
RANGE - ABUNDANCE SERIES FOR EACH OF
THE MICROHABITATS CONSIDERED IN A RICE
FIELD NEAR CORRIENTES, ARGENTINA

Microhabitats	Series	X ^{2†}	df†	Significance	Diversity (Shannon index)
Ditches and paths	Logarithmic	14.13	7	14.06*	2.07
Rice-field	Logarithmic	4.31	6	12.59	2.08
Aquatic vegetation	Logarithmic	1.51	5	11.07	1.69
Natural vegetation	Broken stick	2.92	5	11.07	2.36
Gullies	Geometric	0.50	3	7.81	0.91

† χ^2 : chi square, df: degrees of freedom, significance: $p\leq 0.05$ (*).

subfamily was represented only by *Lysapsus limellum*. This species was only found on two occasions, in Feb and Mar 2002.

The Microhylidae family was represented in this area by *Elachistocleis bicolor*, although only tadpoles and vocalizations were registered in Dec 2001 and Sep 2002, and the family Cycloramphidae was represented by *Odontophrynus americanus* a species captured in the months of May and September. The most common species were *Leptodactylus chaquensis* (N= 317), *Pseudopaludicola falcipes* (N= 300) and *Dendropsophus nanus* (N= 194). All frogs were collected during the winter, despite the evident decrease in activity during these months demonstrated by the majority of species.

Microhabitat preferences

The natural vegetation was the most diverse microhabitat ($H'= 2.36$, $E= 0.82$, $N= 236$). Thereafter, in decreasing order, the rice field ($H'= 2.08$, $E= 0.77$, $N= 296$), ditches and paths ($H'= 2.07$, $E= 0.70$, $N= 723$) aquatic vegetation ($H'= 1.69$, $E= 0.74$, $N= 100$), and gullies ($H'= 0.91$, $E= 0.66$, $N= 25$). The distribution of species abundance in the microhabitats was adjusted to different series of the range - abundance model (Table II). Even though the distribution of species abundance in ditches and paths did not fit any of the series models, it came close to being within the limits of the logarithmic distribution model (Table II). Nevertheless, other microhabitats, such as the rice field and

the aquatic vegetation, fitted this series better.

The natural vegetation microhabitat presented the highest specific diversity and it was fitted to the "broken stick" model series, which represented a more suitable species abundance distribution. The lowest number of taxa was registered in the gullies, thus fitting the geometric series (Table II).

The greatest similarity, in qualitative and quantitative terms, was observed between the rice field and the ditches and paths linking the different cultivated sectors. In contrast, the gullies and the natural vegetation presented the greatest difference from a qualitative point of view, and the gullies and the ditches and paths in quantitative terms (Table III).

Significant differences were observed in the diversity of microhabitats, with the exception of the comparison between ditches and paths and the rice field (Table III). The Anuran families observed were represented in a different way in those microhabitats.

In order to compare richness and diversity amongst the five microhabitats, the calculations were rarefied to the smallest sample, e.g. gullies (N= 25; Table IV).

The majority of the species belonging to the Leptodactylidae family were observed inside the rice field and prior to the flooding phase. Once flooded, they were observed preferably in the shores of the channels and paths. The fossorial species, such as *Leptodactylus latinasus*, *L. elenae* or *L. (Lithodytes) diptyx* frequented areas with dense vegetation in those sec-

TABLE III
VALUES OF SIMILARITY (ORENSEN'S INDEX) AND COMPARATIVE ANALYSIS
OF DIVERSITY INDEX FOR EACH MICROHABITAT IDENTIFIED IN A RICE
FIELD NEAR CORRIENTES, ARGENTINA

	Ditches and paths	Rice-field	Aquatic vegetation	Natural vegetation	Gullies
Ditches and paths		Qu= 0.88† Qt= 0.55† t= 0.16 df= 598.87	Qu= 0.69 Qt= 0.23 t= 4.01 * df= 144.61	Qu= 0.70 Qt= 0.36 t= 4.12 * df= 461.39	Qu= 0.34 Qt= 0.06‡ t= 6.84 * df= 27.86
Rice-field			Qu= 0.64 Qt= 0.39 t= 3.81 * df= 190.25	Qu= 0.72 Qt= 0.47 t= 3.45 * df= 517.71	Qu= 0.42 Qt= 0.15 t= 6.72 * df= 30.97
Aquatic vegetation				Qu= 0.50 Qt= 0.53 t= 6.43 * df= 196.81	Qu= 0.43 Qt= 0.32 t= 4.20 * df= 39.64
Natural vegetation					Qt= 0.15 ‡ t= 8.28 * df= 31.65
Gullies					

Qu: qualitative, Qt: quantitative, df: degrees of freedom.

†: maximum and ‡: minimum values obtained.

* Significant differences between microhabitats (p<0.05).

tors that were kept unsown (e.g. the natural vegetation).

The two specimens found that corresponded to the Cycloramphidae *Odontophrynus americanus* were found in the ditches and paths that connect the paddy fields, always on dry land.

Concerning Bufonidae, before the flooding phase it was common to find specimens inside the rice field. However, after the flooding phase the individuals were observed on the slopes of the channels.

As soon as the rice field was flooded, and by the time that the crop had reached approximately 30cm in height, some species of Hylidae, such as *Dendropsophus nanus*, *Scinax acuminatus* and *S. fuscumarginatus*, were frequently observed inside the field. Other larger hylids, such as *Hypsiboas raniceps* or *Phyllomedusa hypo-*

chondrialis, remained in areas covered by dense vegetation bordering the sowed rice field areas near the water reservoirs. *Argenteohyla siemersi*, on the other hand, was observed very sporadically within natural vegetation on Gramineae plants exceeding 1m in height. *Lysapsus limellum* was found inside the irrigation channels, in areas inundated with water ~70cm in depth, where aquatic plants also grew alongside floating leaves.

The call of the Microhylidae *Elachistocleis bicolor* was recorded in the surrounding areas of the irrigation channels, inside natural vegetation.

Discussion

This study shows that irrigated rice fields in northeastern Argentina are more than an agricultural enterprise. Rice production currently creates a mix

of microhabitats suitable for the sustenance of a diverse anuran fauna that approximates what might be found in undisturbed wetlands, a fact previously recognized by Bambaradeniya *et al.* (2004).

The environmental impact produced by rice plantations varies depending on production methods (Donald, 2004) but there is the possibility of affecting amphibian ecology, ethology and distribution in some areas (Fujioka and Lane, 1997). Nevertheless, this environmental impact should not necessarily be classified only in negative terms, given that this type of farming practice, due to the particular characteristics of its water management, can provide an important source of microhabitats for amphibians. Anurans are important natural enemies of pest insects, and some species act as biocontrol agents of rice pest in-

sects and crabs (Bambaradeniya and Amerasinghe, 2003).

Rice production may have demonstrable positive effects inasmuch as the required creation of large flooded areas that must be maintained for extended periods represents a viable alternative to natural wetlands in providing reproductive habitats for anurans (Baker and Halliday, 1999; Knutson *et al.*, 2004). Indeed, some authors characterize rice fields as an important man-made habitat for amphibians (Bambaradeniya and Amerasinghe, 2003) and suggest that appropriately managed rice fields may somewhat ameliorate shrinking global habitats for anuran amphibians (Fujioka and Lane, 1997; Elphick, 2000).

There are many negative side effects, such as the loss of natural habitats, isolation, the harmful use of herbicides and the systematic flooding and draining of these plantations, all of which could directly affect amphibian life cycles (Fujioka and Lane, 1997; Knutson *et al.*, 2004).

The present study showed five distinctly different habitats defined by structure and plant species composition, and by the water availability. It is not surprising that, of the delineated microhabitats, natural vegetation supports the highest diversity and species richness. The rice production system studied employs an irrigation system that is intermediate between the "old-style" and "new-style" irrigation methods (Fujioka and Lane, 1997) and contains elements of both. The study site contained fields irrigated with water taken from the Parana River and raised by pumps to an earthen distribution system consisting of a network of shallow ditches. Field drainage is likewise through earthen ditches that support the growth of aquatic and terrestrial vegetation. Minimal structural changes carried out on rice fields would probably prevent a drastic decrease in frog population.

Spring is the most favorable season for studies related with the anuran communities in the rice field, because during these months the highest richness and diversity were registered.

TABLE IV
AVERAGE (\pm SD) RICHNESS AND DIVERSITY FOR EACH MICROHABITAT IDENTIFIED
IN A RICE FIELD NEAR CIUDAD CORRIENTES ARGENTINA

	Ditches and paths	Rice fields	Aquatic vegetation	Natural vegetation	Gullies
Richness	8.26 \pm 1.38	8.61 \pm 1.22	6.38 \pm 1.06	9.96 \pm 1.45	4 \pm 0
Diversity	1.79 \pm 0.17	1.83 \pm 0.17	1.55 \pm 0.14	2.04 \pm 0.14	0.91 \pm 0

Range-abundance models aided in the interpretation of the data. The most commonly used models are the geometric one, the logarithmic and the "broken stick", all of which were required to fit data from the five identified microhabitats. The geometric series was applicable to species-poor environments, and the distribution was adjusted to the least diverse microhabitat such as the gullies, in which only one species, *Dendropsophus nanus*, was present. The logarithmic series fitted better to communities characterized as being influenced by only one or a few determining factors affecting ecological interactions, with a high proportion of species found to be very uncommon (Magurran, 1988). Three of the microhabitats examined in this study, including the rice field, aquatic vegetation and ditches and paths, fitted this log distribution series, although, in the latter case, the adjustment was marginally significant.

Finally, the "broken stick" model, characterized by reflecting a more equitable condition among species and for having a more uniform distribution than the previous models (Magurran, 1988), was also best represented in the natural vegetation.

The findings underscore the critical importance of retaining patches of natural vegetation among rice paddies in order to maintain the health and diversity of anuran populations in rice cropping. This arrangement, together with the use of earthen irrigation distribution ditches, seems to assure that rice fields *per se* can serve as significant habitats for anurans, because frogs and toads and their larvae can use unimproved channels for dispersal from natural vegetation into the rice fields when conditions in the fields are favorable (i.e. flooded). Bambaradeniya *et al.* (2004) found that aquatic organisms were able to use dry rice field soil cracks and crevices for aestivation when the fields were drained. This finding is corroborated by the present study, having found *Chaunus bergi* and *Leptodactylus chaquensis* in cracks after

field drying. Moreover, *L. latinasus* foam nests were found in field soil cracks and crevices. We believe that shallow depressions, which create perennial pools and aquatic vegetation through the crop dry phase, explain the presence of these frogs in rice fields, and suggest that such features be included in the design and development of new rice growing areas, as well as enhancing them in established fields.

The existence of exclusively aquatic species, such as *Lysapsus limellum* in an environment subjected to drastic water levels variations was also of interest. The existence of pools that contain aquatic vegetation that remains immersed in water throughout the terrestrial dry phase, would explain the presence of these frogs.

Studies of this type have revealed, world wide, that rice fields have the potential to support a robust and diverse community of anurans, due to the special cultural requirements of rice production, namely the need to flood the crop. The present data suggest that natural vegetation fragments, earthen irrigation channels (as apposed to those lined with concrete) and shallow depressions that support perennial ponds and their aquatic vegetation, are landscape features that promote the use of rice fields by frogs and toads. This encouraging result invites further research designed to evaluate the impact of less obvious rice production factors on anuran communities and to optimize the benefits of management practices with negligible costs, and to evaluate the possible production benefits obtained by anuran biological control of crops.

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