

# Niche overlap and resource partitioning among five sympatric bufonids (Anura, Bufonidae) from northeastern Argentina

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## Abstract

**Niche overlap and resource partitioning among five sympatric bufonids (Anura, Bufonidae) from northeastern Argentina.** The niche overlap and resource partitioning were analyzed for five sympatric bufonids from Northeastern Argentina: *Rhinella schneideri*, *R. bergi*, *R. fernandezae*, *R. granulosa*, and *Melanophryniscus cupreuscapularis*. The primary objectives were to analyze the diet and pattern of coexistence relative to the microhabitats among species. The bufonids, which are primarily terrestrial, exhibited a preference for small, hard prey such as formicids or coleopterans. The smallest species preferably consumed ants, while *R. schneideri* preferred beetles. Significant differences were detected for the diets of these five species. In addition, significant overlap in the trophic niche was noted for all species except between *R. granulosa* and *R. schneideri*. Studying the diet behaviors and trophic parameters of sympatric species provides important data for understanding the community and for the development of conservation guidelines.

**Keywords:** Anura, Bufonidae, diet, microhabitat use, Argentina.

## Resumo

**Sobreposição de nicho e partilha de recursos entre cinco espécies simpátricas de bufonídeos (Anura, Bufonidae) do nordeste da Argentina.** Analisamos a sobreposição de nicho e a partilha de recursos entre cinco espécies simpátricas de bufonídeos do nordeste da Argentina: *Rhinella schneideri*, *R. bergi*, *R. fernandezae*, *R. granulosa* e *Melanophryniscus cupreuscapularis*. O principal objetivo foi analisar a dieta e o padrão de coexistência entre as espécies em relação aos micro-habitats. Os bufonídeos, primariamente terrestres, mostraram preferência por presas duras e de pequeno porte, como formigas e coleópteros. As espécies menores consumiram preferencialmente formigas, enquanto *R. schneideri* preferiu besouros. Foram detectadas diferenças significativas entre as dietas das cinco espécies. Além disso, houve sobreposição significativa entre os nichos tróficos de todas as espécies, exceto entre *R. granulosa* e *R. schneideri*. O estudo do comportamento alimentar e dos parâmetros tróficos de espécies simpátricas fornece dados importantes para a compreensão da estrutura das comunidades e para o delineamento de estratégias de conservação.

**Palavras-chave:** Anura, Bufonidae, dieta, uso de micro-habitats, Argentina.

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## Introduction

Ecological communities are shaped, in part, by the manner in which similar species consume and partition food resources. Investigations of resource utilization by predators, as well as their relationship with their prey and the environment, are important for understanding the mechanisms that influence amphibian community structure. Furthermore, since two or more species often occur in sympatry, knowledge of the mechanisms that favor the co-occurrence of morphologically and ecologically similar species may be important. The degree of niche differentiation among species in the same trophic level depends on many factors, been prey availability one of the most relevant (Pianka 1969, Schoener 1974, 1989). The study of trophic relationships among sympatric species is crucial to understanding interspecific interactions (Duré and Kehr 2001, 2004).

Research on the type and composition of the diet for different species that compose a community are key components for understanding the relationships between these species and will aid the design of strategies for conservation. Understanding the overlap between the trophic niches of the species, which appear static and exhibit temporary variations, is also critical for describing the ecosystem. In addition, our findings will extend the current information on the life histories of the amphibian species that inhabit Argentina.

The Family Bufonidae is cosmopolitan and distributed across the whole world with the exception of Australia, Madagascar, and the oceanic regions. This family is composed of 33 genera and 446 species (Frost 2009).

In the present work we analyzed the diet of four species of *Rhinella* (*R. schneideri*, *R. granulosa*, *R. fernandezae* and *R. bergi*) and one species of *Melanophryniscus* (*M. cupreuscapularis*). All these species live sympatrically around Corrientes, Argentina (Duré 2004).

The main objectives of this study were: (1) to classify and quantify the prey consumed by

each species; (2) to calculate the niche width and diversity of the diet; and (3) to establish the relationship between microhabitat, diet, and foraging strategy of the five species.

## Materials and Methods

### *Study area*

All specimens were collected at the biogeographic area inside the Chaqueño Domain, Oriental District Chaqueño (Cabrera and Willink 1980, Carnevali 1994), located approximately 15 km east of Corrientes City (27° 30" S, 58° 45" W) being characterized by many temporary, semi-permanent, and permanent ponds. The mean annual temperature is 23 C and the mean annual precipitation is 1500 mm, without a pronounced dry season, however periods of rain shortages occur every four to six years (Carnevali 1994). The original plant formation at the study area was *Schinopsis balansae* "quebracho" forest, which is currently extremely degraded and largely replaced by sclerophyllous forest with prevalence of *Acacia caven*, *Celtis* spp., *Prosopis affinis*, *Prosopis nigra*, and herbaceous strata composed of grasses, numerous cacti and terrestrial bromeliads formed colonies of variable size, species that can be found in the environment are *Aechmea distichantha* and *Bromelia* spp. (Carnevali 1994).

### *Data analysis and analytical procedure*

For the diet analysis was considered the number and volume of the consumed preys. The samples were taken monthly during the period October 1994–March 2000. The capture method was manual, using visual encounters surveys (Crump and Scott Jr. 1994). Specimens were immediately fixed in 10% formalin and deposited in the Centro de Ecología Aplicada del Litoral (CECOAL-CONICET). In order to facilitate the interpretation of data, were used a minimum amount of environmental vectors

(microhabitats) grouped as: *Anthropic*: terrestrial environments that are under the man influence, for example: near homes or lighting, etc.; *Dry land*: pristine terrestrial environments with a dry substrate (soil grass or sand); *Muddy*: very wet pristine environments, the substrate is a mainly muddy. In most cases very close to water bodies; *Inundated vegetation*: grasses founded mainly along the shores of permanent, semi-permanent and temporary water bodies. This vegetation is subject to fluctuations in water level.

The variables considered for each captured specimen were: sex (detected by examination of gonads and external nuptial features), body length (mm), and maximum mouth width (mm). Only the adult specimens of each species were considered in order to avoid the ontogenetics diet variations that may cloud the interpretation of the data. The stomach content of each frog was analyzed and, for those cases in which was not prey in the stomach were analyzed the complete alimentary canal, as recommended by Schoener (1989). Prey were only included if they had at least 70% of their body undigested. For each stomach, we counted, measured (width and length, 0.01 mm, caliper) and identified prey items to order level using keys of Brewer and Arguello (1980) and Coronado Padilla and Márquez Delgado (1978).

We also calculated the individual volume of each prey item and the number of prey items per stomach for each prey category. Volume of each prey item was estimated using the formula for an ellipsoid (Dunham 1983):  $V = 4/3\pi(1/2L)(1/2W)^2$ , where  $V$  is volume,  $L$  is length, and  $W$  is width.

The diversity index used was the Shannon index (H) (Shannon and Weaver 1949):

$$H' = - \sum_{i=1}^S (p_i \ln p_i)$$

where  $p_i$  is the proportion of the resource (prey item) in the diet,  $\ln$  is the natural logarithm, and  $S$  is the total number of species (prey). In order to make a more accurate comparison of diet diversity also was used the Standardized Shannon Index ( $H_{std} = H'/\ln(s)$ ) (Krebs 1999).

For numerical data we calculated niche breadth using the Levins Index (Levins 1968):

$$Nb = (\sum P_{ij}^2)^{-1} ,$$

where  $P_{ij}$  represents the probability of finding the item  $i$  in the sample  $j$ .

We calculated dietary overlaps in two ways by considering the food proportions and the volume of each prey with the formula (Pianka 1973):

$$O_{jk} = \frac{\sum_{i=1}^n P_{ij} P_{ik}}{\sqrt{\sum_{i=1}^n P_{ij}^2 \sum_{i=1}^n P_{ik}^2}} ,$$

where  $P_{ij}$  and  $P_{ik}$  are the proportions of utilization of the  $i$ th food resource by the  $j$ th and  $k$ th species, respectively.

When comparing the composition of diet of two species, the values obtained by means of this index can fluctuate between 0 (diet or volume of ingested prey different) and 1 (diet or volume of ingested prey identical). To determine whether measured overlap values differed from what would be expected based on a random sampling of the species data, we performed a randomization analysis through the EcoSim software (Gotelli and Entsminger 2003, Duré and Kehr 2004).

Relationships between microhabitat and diet and foraging strategy for these five species were tested through a canonical correspondence analysis (CCA) (Ter Braak 1986, 1987). The CCA is a multivariate direct gradient analysis method derived from correspondence analysis, but has been modified to allow environmental data to be incorporated into the analysis. The canonical correspondence analysis let to analyse a contingency table (typically with sites as rows and species in columns) while taking into account the information provided by a set of

explanatory variables contained in a second table and measured on the same sites. The CCA test was performed using XLSTAT 2006 version 2006.3 software (Addinsoft 2006). All data were transformed to natural logarithms (Ln) before tests were run. To establish the relationship between the morphology of the predator and the volume of the prey parametric tests were used (Kehr 1994, Zar 1996).

## Results

### *Rhinella schneideri*

Twenty-eight individuals were captured, 75% (n = 21) of these were adults (males 14, females 7) with identifiable stomach or intestinal contents. The diet consisted of 13 types of prey (Table 1) and was dominated numerically (67.09%) and volumetrically (58.54%) by coleopterans. Besides, coleopterans were the most frequently represented prey in 20 individuals (95.23%). Other important preys were ants, hemipterans and miriapoda.

Prey diversity was 1.12 (Hstd = 0.43), and niche breadth was 2.04. The mean mouth width of males (n = 14) was  $50.87 \pm 13.46$  mm and for females (n = 7) was  $60.98 \pm 13.39$  mm.

A positive and significant correlation existed between body length and mouth width ( $y = 13.34 + 0.30 x$ ;  $r = 0.84$ ;  $F_{(1,19)} = 49.17$ ;  $p < 0.001$ ). Mean prey volume for each toad was not correlated with mouth width ( $y = -318.99 + 13.06 x$ ;  $r = 0.35$ ;  $F_{(1,19)} = 2.79$ ;  $p = 0.11$ ) and prey number/stomach was not correlated with Ln body length ( $y = 7.78 + 0.16 x$ ;  $r = 0.22$ ;  $F_{(1,19)} = 1.00$ ;  $p = 0.32$ ). No significant differences were observed between the diet of females and males of this species (Mann-Whitney U Test = 109.5; n = 13;  $p = 0.19$ ).

The individuals analyzed were captured mainly in anthropic areas and dry land. This species exploits environments related to the human activity, feeding of insects attracted by the houses and gardens lights.

### *Rhinella granulosa*

Thirty three individuals were captured, 81.81% (n = 27) of these were adults (males 23, females 4) with identifiable stomach or intestinal contents. The diet consisted of 7 types of prey (Table 1) and was dominated numerically (79.42%) and volumetrically (78.89%) by ants. Besides, this item was the most frequently, found in 20 individuals (74.07%). The prey diversity was 0.67 (Hstd = 0.34) and niche breadth was 1.51.

The mean mouth width of males (n = 23) was  $13.33 \pm 2.96$  mm and for females (n = 4) was  $13.4 \pm 2.37$  mm. A positive and significant correlation existed between mouth width and body length ( $y = 0.37 + 0.32 x$ ;  $r = 0.93$ ;  $F_{(1,25)} = 154.33$ ;  $p < 0.001$ ). Mean prey volume was correlated with mouth width ( $\text{Ln } y = -4.82 + 2.40 \text{ Ln } x$ ;  $r = 0.64$ ;  $F_{(1,25)} = 17.74$ ;  $p < 0.001$ ). Nevertheless, the prey number/stomach was not correlated with body length ( $y = 57.45 - 0.72 x$ ;  $r = 0.16$ ;  $F_{(1,25)} = 0.69$ ;  $p = 0.41$ ). No significant differences were observed between the diet of females and males of this species (Mann-Whitney U Test = 37.50; n = 7;  $p = 0.09$ ).

The individuals were captured mainly in dry land and this species exploits terrestrial and muddy habitats.

### *Rhinella fernandezae*

Thirty four individuals were captured, 61.76% (n = 21) of these were adults (males 12, females 9) with identifiable stomach or intestinal contents. The diet consisted of 7 types of prey (Table 1) and was dominated numerically (89.66%) and volumetrically (57.12%) by ants. Another important prey was the coleopterans, which contributed to the diet with 40.74 % of total volume. Besides, the ants were the most frequently prey, found in 19 individuals (90.47%). The diet diversity was 0.38 (Hstd = 0.19) and niche breadth was 1.23. The mean mouth width of males (n = 12) was  $15.80 \pm 3.47$  mm and for females (n = 9) was  $15.9 \pm 3.19$  mm.

**Table 1** - Types of prey in the diets of five species of bufonids nearby to Corrientes City, Argentina. F = frequency of occurrence.

Prey Category	<i>Rhinella schneideri</i> (n=21)				
	n	%	Vol. (mm <sup>3</sup> )	%	F
INSECTA					
Coleoptera	424	67.09	94310.70	58.54	20
Hemiptera	32	5.06	46911.28	29.12	11
Hymenoptera - Ants	117	18.51	1772.32	1.10	11
Hymenoptera - No Ants	6	0.95	487.47	0.30	2
Isoptera	1	0.16	7.655	0.004	1
Larvae	9	1.42	1732.89	1.07	5
Diptera	-	-	-	-	-
Homoptera	-	-	-	-	-
Collembola	-	-	-	-	-
Dictioptera	1	0.16	5311.42	3.29	1
Orthoptera	6	0.95	1660.60	1.03	4
ARACHNIDA					
Phalangida	3	0.47	200.34	0.12	1
Araneae (spiders only)	5	0.79	47.32	0.03	2
Acari (mites)	-	-	-	-	-
CRUSTACEA					
Isopoda	1	0.15	119.76	0.07	1
Miriapoda	23	3.64	4086.35	2.53	5
MOLLUSCA					
Gastropoda	4	0.63	4443.60	2.75	2
TOTAL	632	100	161091.70	100	

Prey Category	<i>Rhinella granulosa</i> (n=27)				
	n	%	Vol. (mm <sup>3</sup> )	%	F
INSECTA					
Coleoptera	11	1.44	78.42	3.54	9
Hemiptera	-	-	-	-	-
Hymenoptera - Ants	606	79.42	1747.73	78.89	27
Hymenoptera - No Ants	3	0.39	4.45	0.20	2
Isoptera	125	16.38	353.76	15.97	2
Larvae	7	0.91	29.06	1.31	2
Diptera	-	-	-	-	-
Homoptera	-	-	-	-	-
Collembola	-	-	-	-	-
Dictioptera	-	-	-	-	-
Orthoptera	-	-	-	-	-
ARACHNIDA					
Phalangida	-	-	-	-	-
Araneae (spiders only)	4	0.52	0.89	0.04	2
Acari (mites)	7	0.91	0.99	0.04	2
CRUSTACEA					
Isopoda	-	-	-	-	-
Miriapoda	-	-	-	-	-
MOLLUSCA					
Gastropoda	-	-	-	-	-
TOTAL	763	100	2215.3	100	

Table 1 - continued.

Prey Category	<i>Rhinella fernandezae</i> (n=21)				
	n	%	Vol. (mm <sup>3</sup> )	%	F
INSECTA					
Coleoptera	69	9.15	924.61	40.74	17
Hemiptera	1	0.13	18.84	0.83	1
Hymenoptera - Ants	676	89.65	1296.53	57.12	19
Hymenoptera - No Ants	2	0.26	19.51	0.86	2
Isoptera	–	–	–	–	–
Larvae	1	0.13	2.62	0.11	1
Diptera					
Homoptera	3	0.39	0.66	0.03	2
Collembola	–	–	–	–	–
Dictioptera	–	–	–	–	–
Orthoptera	–	–	–	–	–
ARACHNIDA					
Phalangida	–	–	–	–	–
Araneae (spiders only)	1	0.13	3.65	0.16	1
Acari (mites)	–	–	–	–	–
CRUSTACEA					
Isopoda	1	0.13	3.09	0.13	1
Miriapoda	–	–	–	–	–
MOLLUSCA					
Gastropoda	–	–	–	–	–
TOTAL	754	100	2269.52	100	
Prey Category	<i>Rhinella bergi</i> (n=18)				
	n	%	Vol. (mm <sup>3</sup> )	%	F
INSECTA					
Coleoptera	56	12.50	452.07	40.07	16
Hemiptera	3	0.67	11.83	1.05	3
Hymenoptera - Ants	377	84.15	602.91	53.44	16
Hymenoptera - No Ants	1	0.22	2.59	0.23	1
Isoptera	–	–	–	–	–
Larvae	9	2.01	52.28	4.63	5
Diptera	–	–	–	–	–
Homoptera	1	0.22	6.36	0.56	1
Collembola	–	–	–	–	–
Dictioptera	–	–	–	–	–
Orthoptera	–	–	–	–	–
ARACHNIDA					
Phalangida	–	–	–	–	–
Araneae (spiders only)	1	0.22	0.09	0.008	1
Acari (mites)	–	–	–	–	–
CRUSTACEA					
Isopoda	–	–	–	–	–
Miriapoda	–	–	–	–	–
MOLLUSCA					
Gastropoda	–	–	–	–	–
TOTAL	448	100	1128	100	

Table 1 - continued.

Prey Category	<i>Melanophryniscus cupreuscapularis</i> (n=22)				
	n	%	Vol. (mm <sup>3</sup> )	%	F
INSECTA					
Coleoptera	10	2.19	7.69	4.67	8
Hemiptera	2	0.44	1.03	0.62	2
Hymenoptera - Ants	349	76.53	130.95	79.42	15
Hymenoptera - No Ants	1	0.22	0.09	0.057	1
Isoptera	6	1.31	16.04	9.73	3
Larvae	2	0.43	0.27	0.16	2
Diptera	4	0.87	0.72	0.43	2
Homoptera	15	3.28	1.89	1.14	3
Collembola	26	5.70	5.41	3.28	5
Dictioptera	-	-	-	-	-
Orthoptera	-	-	-	-	-
ARACHNIDA					
Phalangida	-	-	-	-	-
Araneae (spiders only)	2	0.44	0.44	0.27	2
Acari (mites)	39	8.55	0.32	0.19	3
CRUSTACEA					
Isopoda	-	-	-	-	-
Miriapoda	-	-	-	-	-
MOLLUSCA					
Gastropoda	-	-	-	-	-
TOTAL	456	100	164.87	100	456

A positive and significant correlation existed between body length and mouth width ( $y = -0.44 + 0.36 x$ ;  $r = 0.95$ ;  $F_{(1,19)} = 199.46$ ;  $p < 0.001$ ). The Ln Mean prey volume was correlated with Ln mouth width ( $\text{Ln } y = -4.98 + 2.29 \text{ Ln } x$ ;  $r = 0.43$ ;  $F_{(1,19)} = 4.36$ ;  $p = 0.05$ ). Nevertheless, the prey number/stomach was not correlated with body length ( $y = -91.39 + 2.95 x$ ;  $r = 0.24$ ;  $F_{(1,19)} = 1.24$ ;  $p = 0.28$ ). No significant differences were observed between the diet of females and males of this species (Mann-Whitney U Test = 15.00;  $n = 8$ ;  $p = 0.065$ ).

The individuals analyzed were captured mainly in dry land. This species exploits terrestrial and muddy habitats.

#### *Rhinella bergi*

Twenty-four individuals were captured, 75.00% ( $n = 18$ ) of these were adults (males 12,

females 6) with identifiable stomach or intestinal contents. The diet consisted of 7 types of prey (Table 1) and was dominated numerically (84.15%) and volumetrically (53.44%) by ants. On the other hand, the ants were the most frequently prey, found in 16 individuals (88.88%). The diet diversity was 0.80 (Hstd = 0.41) and niche breadth was 0.36. The most important food was the Formicidae. The mean mouth width of males ( $n = 12$ ) was  $10.80 \pm 0.08$  mm and for females ( $n = 6$ ) was  $11.26 \pm 0.13$  mm.

A positive and significant correlation existed between body length and mouth width ( $y = 3.57 + 0.20 x$ ;  $r = 0.88$ ;  $F_{(1,16)} = 58.58$ ;  $p < 0.001$ ). Mean prey volume was not correlated with mouth width ( $y = -20.10 + 2.32 x$ ;  $r = 0.40$ ;  $F_{(1,16)} = 3.21$ ;  $p = 0.17$ ), and the prey number/stomach was not correlated with body length ( $y = -24.05 + 1.37 x$ ;  $r = 0.30$ ;

$F_{(1-16)} = 1.61$ ;  $p = 0.54$ ). No significant differences were observed between the diet of females and males of this species (Mann-Whitney U Test = 28.00;  $n = 7$ ;  $p = 0.65$ ).

The individuals analyzed were captured mainly in dry land. This species exploits terrestrial and muddy habitats.

#### *Melanophryniscus cupreuscapularis*

Thirty two individuals were captured, 68.75 % ( $n = 22$ ) of these were adults (males 15, females 7) with identifiable stomach or intestinal contents. The diet consisted of 11 types of prey (Table 1) and was dominated numerically (76.53%) and volumetrically (79.42%) by ants. On the other hand, the ants was the most frequently prey, found in 15 individuals (68.18%). The diet diversity was 0.95 (Hstd = 0.39) and niche breadth was 1.39.

The mean mouth width of males ( $n = 15$ ) was  $4.56 \pm 0.32$  mm and for females ( $n = 7$ ) was  $4.55 \pm 0.29$  mm.

A positive and significant correlation existed between body length and mouth width ( $y = 1.21 + 0.15 x$ ;  $r = 0.53$ ;  $F_{(1-20)} = 8.13$ ;  $p = 0.009$ ). Ln Mean prey volume was not correlated with Ln mouth width ( $\text{Ln } y = -5.71 + 2.90 \text{ Ln } x$ ;  $r = 0.21$ ;  $F_{(1,20)} = 0.93$ ;  $p = 0.34$ ), and the prey number/stomach was correlated with body length ( $y = -263.76 + 13.14 x$ ;  $r = 0.56$ ;  $F_{(1-20)} = 9.26$ ;  $p = 0.006$ ). Significant differences were observed between the diet of females and males of this species (Mann-Whitney U Test = 90.00;  $n = 11$ ;  $p = 0.049$ ).

This specie exploits vegetation inundated and muddy habitats.

#### *Comparison between diets of five species*

The trophic niche overlap (prey proportion) between the five species was high ( $O_{jk} = 0.72$ ).

Randomizations with all data produced a significant difference between measured (observed) overlaps and simulated (expected) overlaps using diet proportion ( $P$  [observed  $\leq$  expected] = 1.00, and  $P$  [observed  $>$  expected]  $< 0.001$ ). Therefore, the observed mean (0.72) was greater than the expected mean value by chance alone ( $0.089 \pm 0.067$ , iterations = 1000).

When we analyzed the trophic overlapping for couples we see that, with exception of that constituted for *R. granulosa* and *R. schneideri*, the remainder species show high values of overlapping. The highest trophic overlapping was observed between those species which diet constituted principally by ants (Table 2).

#### *Comparison of microhabitats*

The spatial niche overlap between the five species was relatively low ( $O_{jk} = 0.51$ ). Randomizations with all data produced no significant difference between measured (observed) overlaps and simulated (expected) overlaps using microhabitats ( $P$  [observed  $\leq$  expected] = 0.71, and  $P$  [observed  $>$  expected] = 0.28). Thus, the observed mean (0.51) was similar to that expected by chance ( $0.47 \pm 0.078$ , iterations = 1000).

**Table 2** - Relationships (Pianka's index) among five species of bufonids from Corrientes, Argentina. Trophic overlap values are outside brackets, habitat overlap values are between brackets. \*Low and \*\* high overlap.

	<i>R. granulosa</i>	<i>R. fernandezae</i>	<i>R. bergi</i>	<i>M. cupreuscapularis</i>
<i>R. schneideri</i>	0.276* (0.699)	0.360 (0.248)	0.403 (0.426)	0.288 (0.389)
<i>R. granulosa</i>	–	0.976 (0.186)	0.971 (0.918**)	0.974 (0.72)
<i>R. fernandezae</i>	–	–	0.998** (0.158*)	0.987 (0.648)
<i>R. bergi</i>	–	–	–	0.982 (0.789)



**Table 3** - Summary of main results of the canonical correspondence analysis (CCA) relating bufonids species to the microhabitat where they live and preys type nearby to Corrientes City, Argentina. The fix model can be observed in the Monte Carlo permutations and the pseudo F value. Note: permutations=1000; pseudo F= 0.418; p-value=0.008 and alpha 0.050.

	F1	F2	F3	F4
Eigenvalue	0.337	0.261	0.019	0.006
Constrained inertia (%)	54.108	41.833	3.118	0.941
Cumulative %	54.108	95.941	99.059	100.000
Total inertia	6.249	4.831	0.360	0.109
Cumulative %	6.249	11.080	11.440	11.548

If we analyze the microhabitat overlap between species, the biggest overlap was observed between *R. bergi* and *R. fernandezae* (Table 2). Nevertheless, the lowest overlap was observed between *R. fernandezae* with *R. bergi* and *R. granulosa*. Only a low percentage of specimens of *R. fernandezae* (14%) shared the microhabitat (land dries) with two other species before mentioned.

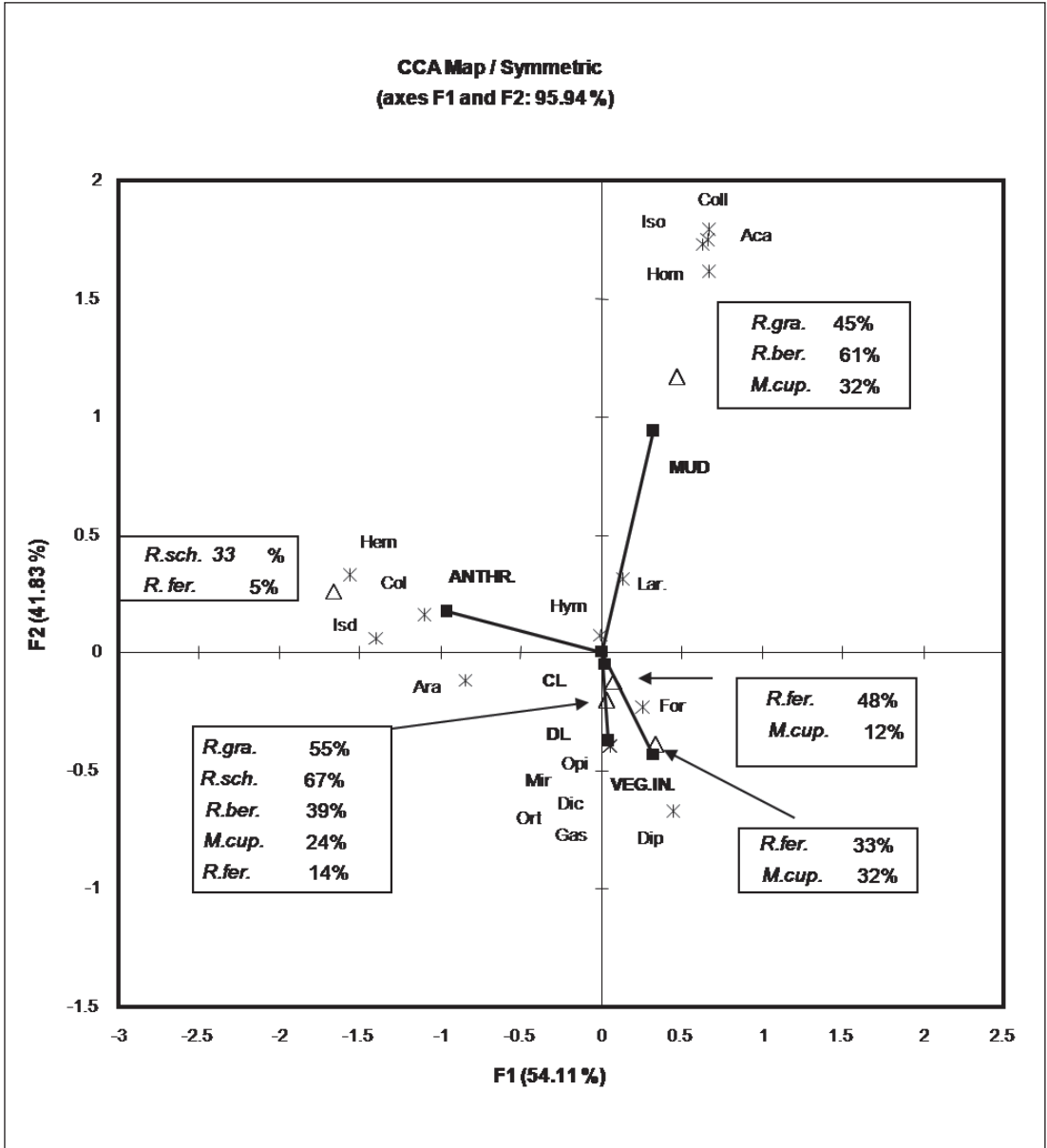
#### *Correlations between prey and microhabitats*

The CCA test indicated a very good fit to the data observed. The five species of bufonids and their preys type were linearly related to microhabitats analyzed (Monte Carlo permutation test, Pseudo F = 0.418; P = 0.008; 1000 permutations). Besides, the first 4 factors (canonical axes) account 100% of the constrained inertia explained by these variables and the sum of the first two eigenvalues adds up to 95% of the total (Table 3). A triplot of environmental variables, bufonids species and diets type on the first two ordination factors (axes) indicated that coleopterans was the prey with greater contributions on first factor (66.8%) associated with “anthropic” vector and mainly with species *R. schneideri* while in second place the formicids (13.8%) associated mainly with vectors “vegetation inundated” and

**Table 4** - Prey type contribution to each factor obtained after applications of a canonical correspondence analysis (CCA) to the data.

Preys	F1	F2
Coleoptera	0.668	0.019
Hemiptera	0.090	0.005
Formicidae	0.138	0.136
Hymenoptera	0.000	0.000
Isoptera	0.050	0.496
Diptera	0.001	0.002
Larvae	0.001	0.003
Homoptera	0.008	0.073
Orthoptera	0.000	0.001
Collembola	0.011	0.105
Araneae	0.009	0.000
Dictioptera	0.000	0.000
Acari	0.020	0.151
Opilionidae	0.000	0.001
Isopoda	0.004	0.000
Miriapoda	0.000	0.005
Gastropoda	0.000	0.001

the bufonids *R. fernandezae* and *M. cupreuscapularis* and vectors “dry land” and bufonids *R. schneideri* and *R. granulosa* (Table 4, Figure 1).



**Figure 1** - Triplot of Canonical Correspondence Analysis (CCA) results for bufonids species near to Corrientes City, Argentina. Triangles are bufonids species near to the vector extreme (in rectangles are the percentages of each one by each triangle); asterisks are the prey types, and the vectors are the microhabitats used by bufonids and their preys. *R.gra.*: *Rhinella granulosa*, *R.ber.*: *R. bergi*, *R.fer.*: *R. fernandezae*, *R.sch.*: *R. schneideri*, *M. Cup.*: *Melanophryniscus cupreuscapularis*, DL: dray land, Veg. In.: Flooded vegetation, Anthr: Anthropic. ac.: Acarina; ar.: Araneida; cl.: Collembola; co.: Coleoptera; dc.: Dictioptera; di.: Diptera; fo.: Formicidae; ga.: Gastropoda; he.: Hemiptera; ho.: Homoptera; hy.: Hymenoptera; id.: Isopoda; is.: Isoptera; la.: Larvae (Insecta); mi.: Myriapoda; op.: Phalangida; or.: Orthoptera.

The isopterans (49.6%), acari (15.1%) and collembolan (10.5%) were the preys with greater contribution on second factor associated with mud variable and the bufonids *R. bergi*, *M. cupreuscapularis* and *R. granulosa*, while formicids (13.6%) were associated mainly with vegetation inundated and *R. fernandezae* and *M. cupreuscapularis* and vectors dry land and bufonids *R. schneideri* and *R. granulosa* (Table 4, Figure 1).

In general, preferences for different microhabitat were registered for bufonids. While *R. schneideri* was associated with dry land and anthropic microhabitats, *R. granulosa* and *R. bergi* mainly with dry land and mud microhabitat respectively. *Rhinella fernandezae* preferred mainly dry land while *M. cupreuscapularis* was observed principally in mud and vegetation inundated.

## Discussion

We hypothesized that the five bufonid species studied exploit the same habitats and consume the same types of prey because they were observed feeding together in the same habitat. All species were found at different times during the year except *M. cupreuscapularis*, which exhibited a very marked seasonality. *Rhinella schneideri*, *R. granulosa*, *R. fernandezae*, and *R. bergi* were present during most of the year, while *M. cupreuscapularis* was found sporadically only after heavy rains mainly during the months of September and April, which coincide with the period of reproduction for this species.

In general, different microhabitat preferences were noted for the five bufonids. *Rhinella schneideri* preferred dry land and anthropic microhabitats while *R. granulosa* and *R. bergi* preferred dry land and muddy microhabitats. *Rhinella fernandezae* mainly preferred dry land, and *M. cupreuscapularis* preferred mud and inundated vegetation. *Rhinella schneideri*, however, has a comparatively wider trophic niche (Nb: 2.04) while *R. bergi* has a

comparatively narrow trophic niche (Nb: 0.36). The other three species (*R. granulosa*, *R. fernandezae*, and *M. cupreuscapularis*) occupied a trophic niche superior to 1.

In order to characterize the diet of these species, sampling during all the seasons of the year is important to avoid overestimation or underestimation of prey usage. Thus, samples were obtained along the year to minimize the seasonal or behavioral effects on the abundance of the preys.

The diet of these bufonid species consisted primarily of ants and coleopterans. No significant differences were observed between the numerical composition of the females and males diet for each species, with the exception of *M. cupreuscapularis* which although showed differences between sexes, the value obtained was near the limit of significance ( $p = 0.049$ ).

The smallest species (*R. granulosa*, *R. fernandezae*, *R. bergi*, and *M. cupreuscapularis*) as well as the juvenile *R. schneideri* consumed primarily ants (Duré and Kehr 1996, 2006 a,b, Duré 2004). Changes in diet due to life stage represent an important effect to the species interaction and consequently on the community. *R. schneideri*, in particular, shows a marked body size variation during its life cycle. The newly transformed and juvenile stages are characterized by consumption of a large numbers of small prey, preferably ants, similar to the rest of the bufonid species in this study. This consumption of small sized prey such as mites, collembolans, and ants was also observed in other studies on bufonids and dendrobatids (Lima and Moreira 1993, Flowers and Graves 1995, Toft 1995). The adults diet of *R. schneideri* then changes and predominantly consume coleopterans. Numerous studies have analyzed myrmecophagy in an important number of amphibian species (Toft 1981, Caldwell 1996, Vences *et al.* 1998) including bufonids. In some cases, the importance of ants in the diet of bufonids was noted (Filipello and Crespo 1994, Campeny and Montori 1995, Lajmanovich 1995, Parmelee 1999, Santana and

Juncá 2007). Interestingly, this type of prey could provide a variety of toxins that may be incorporated into the skin of these amphibians (Toft 1980, Trueb and Gans 1983, Lajmanovich 1995, Caldwell and Vitt 1999). Most animals that consume ants possess a particular morphology of heads and small mouths. (Toft 1981, Caldwell 1996, Caldwell and Vitt 1999).


These five species had a diet characteristic of both a generalist and a specialist predator. These bufonids consume a variety food items, but nevertheless, the low values of the niche amplitude indicate the preferential consumption of some type of prey (ants for *R. fernandezae*, *R. granulosa*, *R. bergi*, and *M. cupreuscapularis* and coleopterans for *R. schneideri*). In accordance with previous studies, the strategy used by specialist feeders for prey apprehension was active foraging with low metabolic activity during long periods and a preference for small, hard prey such as ants and beetles (Toft 1981, Lajmanovich 1995).

Although these five species of bufonids employed an active foraging strategy, the bufonids also alternated this strategy with apprehension to the ambush. The presence of high numbers of preferred insects in small spaces facilitated the active foraging strategy. This idea is based on field observations of these organisms feeding on ants during long periods as soon as they found a row of these insects or an anthill. *R. schneideri* applied the “sit and wait” approach to predation as these organisms were observed sitting under areas and waiting for the coleopterans to be attracted to the light of houses and gardens. This is a relevant fact because in this case a non-aggregated prey (coleopterans) is offered to the predator as an aggregated prey (like ants or termites), so the *R. schneideri* behaves like a specialist feeder.

As stated in previous works (Toft 1980, Lizana et al. 1986, Maneyro and Da Rosa 2004), the prey offer is a relevant factor that contributes to better explain the feeding behavior. Trophic behavior research that considers not only the diet aspects but also the environmental parameters and

microhabitats of the species provides valuable information regarding the functional groups and dynamics inside a community. This research provides information necessary for the implementation of strategies for conservation that involve complete groups of species that share resources and exploit them in a similar way.

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