FEEDING HABITS OF *Physalaemus biligonigerus* (ANURA, LEPTODACTYLIDAE) FROM SOYBEAN FIELD OF CÓRDOBA PROVINCE, ARGENTINA

Andrés M. Attademo,[1,2] Paola M. Peltzer,[1] and Rafael C. Lajmanovich[1]

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Detailed investigation of feeding habits in anurans may help us to understand the ecological significance of them in a particular habitat. In this sense, diet composition of anuran living inside soybean field is scarce. We examined the diet of *Physalaemus biligonigerus* in soybean field of Córdoba Province, Argentina. A total of 27 frogs ($n = 14$ females and $n = 13$ males) were analyzed to assess gastrointestinal contents. Indeed, snout vent length, body mass and sexes were also recorded. A total of 418 prey items was found, being *Pheidole* sp. (Hymenoptera, Formicidae) and *Armadillium vulgare* (Crustacea, Isopoda) the most important preys. The diet composition of frog was compared with prey relative abundance in soybean field, estimated by pitfall traps. There were no significant correlation ($\tau = 0.48$, $p > 0.05$). Selectivity analysis showed positive values for Formicidae and Isoptera. Thus, Isopoda was consumed in the same proportion as occurrence in the environment. No significant differences were detected between females and males diet composition. Finally, we suggest that *P. biligonigerus* should be considered as potential biocontrol agents of noxious arthropods in soybean field.

Keywords: *Physalaemus biligonigerus*, Feeding habits, Prey abundance, Soybean, Biological control, Argentina.

1. INTRODUCTION

Dietary information is crucial for the understanding of anuran life history, population fluctuations, and the impact of habitat modification on those populations (Toft, 1980, 1981; Anderson et al., 1999). In this context, many of the aquatic habitats that are essential for anuran reproduction and survival in the center of Argentina have been greatly modified to the point where existing amphibian populations may be dependent of small forest remnants and altered wetland imbibed within or around agricultural areas (Peltzer et al., 2006). Particularly, in Santa Fe, Entre Ríos, Buenos Aires, and Córdoba Provinces large tracts of land were cleared for agriculture becoming the Glyphosate-tolerant GT soybean (*Glycine max*) the most important cultivation. In the last five years the cultivation of soybean increased considerably in these Provinces, comprising 10^{12} ha in 2004 – 2005.

The feeding ecology of anuran species inhabiting natural areas is well documented in Argentina (Lajmanovich, 1995, 1996; Peltzer and Lajmanovich, 1999; Duré and Kehr, 2001; Peltzer and Lajmanovich, 2002; Duré and Kehr, 2004), but documentation of diet composition of anuran living inside cultivations is insufficient (Lajmanovich et al., 2003; Attademo et al., 2005). *Physalaemus biligonigerus* is one of the commonly species usually encounter in agroecosystem (Attademo et al., 2005; Peltzer et al., 2006). This frog is a member of the family Lectodactylidae and occupies a wide geographical area, including Brazil, Argentina, Paraguay, and Uruguay (Gallardo, 1987). In spite of its relative abundance in many of these areas, virtually nothing is known of its feeding ecology.

The present study examined the feeding habits of *Physalaemus biligonigerus* in soybean field. Specifically, we asked the following questions: (1) Which is the diet composition of this frog in soybean field? (2) Which is the relationship between diet composition and prey relative abundance in soybean crops? (3) Are the frogs eating noxious prey of soybean plants? (4) Are any difference in diet composition between sexes? Answers to

[1] CONICET — High School of Health, Faculty of Biochemistry and Biological Sciences, EES-FBCB-UNL, (3001) Pje. El Pozo S/N (3000), Santa Fe, Argentina.

[2] Address correspondence to: Andrés M. Attademo, CONICET — High School of Health, Faculty of Biochemistry and Biological Sciences, EES-FBCB-UNL, (3001) Pje. El Pozo S/N (3000), Santa Fe, Argentina; Tel.: +54 (342) 474-0152; Fax: +54 (342) 475-0394; E-mail: atademo@yahoo.com.

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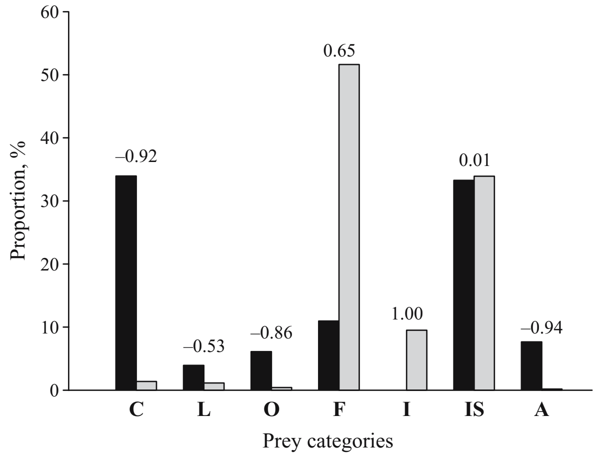

Fig. 1. Relationship between prey relative abundance in soybean field (black bars) and diet composition (gray bars) of *P. biligonigerus*. Selectivity index values (E_i) are shown at the top of each pair. **C**, Coleoptera; **L**, Lepidoptera (larvae); **O**, Orthoptera; **F**, Formicidae; **I**, Isoptera; **IS**, Isopoda (*Armadillium vulgare*); **A**, Arachnida.

these questions are not only of ecological interest but also of practical significance for the conservation management of anurans in agricultural landscape.

2. MATERIAL AND METHODS

2.1. Study Area

The study was carried out in a 10 ha soybean field of Córdoba province, Argentina (Fig. 1, 31°14′46″ S 63°33′8′ W, Argentina) during December 2002 to April 2003. The landscape is highly exploited by humans, dominated by agriculture. Only a few small wooded fragments still exist and the wooded surface is less than 25% of the landscape. Climatically, this region has an average annual rainfall is 800 mm and a mean annual temperature between 18°C.

2.2. Field Survey

To analyze diet composition, we collected 27 adults of *Physalaemus biligonigerus* ($n = 14$ females and $n = 13$ males) with sixteen pitfall traps (Corn, 1994). Each trap was a 10-liter plastic bucket with 10% formalin 10 cm deep located in the ground with the opening flush with the surface. Moreover, the sixteen pitfall traps were also used to estimate prey relative abundance (Cooper and Whitmore, 1990).

Snout vent length (SVL, with a calipers to the nearest 0.01 mm), body mass (BM, with an electronic balance to the nearest 0.1 g) and sex (detected by external nuptial features and examination of gonads) were recorded for each individual. Diets were analyzed by removing the complete gastrointestinal tracts using a binocular microscope.

Prey items (category) were determined to the lowest taxonomic categories possible and the number "per" digestive tract was recorded. We measured maximum length (L) and maximum width (W) of each item with a caliper to the nearest 0.01 mm. For partially digested prey, we estimated lengths by measuring width and then using predetermined length-width regressions from intact prey (Hirai and Matsui, 2001).

The anuran and arthropods specimens were deposited in the herpetological and entomological collections of the Faculty of Biochemistry and Biological Sciences of Santa Fe, Argentina (ESS-FBCB-UNL). In relation to the wide geographic range of the anuran specie used (IUCN, 2004), there was no indication that our modest sampling affected the population

2.3. Diet Composition

For each taxon, the frequency of occurrence (FO) (number of digestive tracts containing that particular taxon divided by the total number of digestive tracts analyzed) was calculated according to the formula of Lescure (1971). Volumes (V) of each prey item was calculated using the formula for an ellipsoid (Dunham, 1983):

$$V = \frac{4\pi}{3}\frac{L}{2}\left(\frac{W}{2}\right)^2,$$

where L represents the length and W the width. To determine the trophic diversity Shannon's index (Shannon and Weaver, 1949) was followed.

2.4. Prey Selectivity

Prey selection indices for each arthropod taxa were calculated using Ivlev's E_i formula (Ivlev, 1961):

$$E_i = (n_i - r_i)/(n_i + r_i),$$

where n_i represents the relative abundance of prey taxa i in stomach contents and r_i represents the abundance in the environment (soybean field). This index scale symmetrically from –1 (negative selectivity) to 1 (positive selectivity), the 0 (nonselection) indicating that prey is taken in the same proportion as occurrence. In this analysis, we used only taxa that were commonly found in both potential prey sample and gastrointestinal contents (Hirai and Matsui, 2000). We also examined relationships between prey relative abundance and diet composition, by Kendall's rank correlation coefficients (τ) (Seigel, 1956).

2.5. Comparison between Sexes

Differences between sexes in SVL and BM measurements were tested using the Mann–Whitney *U*-test. We test sexual differences in diet by comparing the presence or absence of each prey taxon by Fisher's exact probability test.

3. RESULTS

3.1. Diet Composition

Table 1 summarized the diet composition of *P. biligonigerus* from a soybean field of Córdoba Province. The diet of *P. biligonigerus* based on the identification of 418 prey items (Table 1), was composed of 18 prey categories (1 vegetal and 17 animals). The most frequently taken prey items were *Pheidole* sp. (35.17%, Hymenoptera, Formicidae) and *Armadillium vulgare* (33.97%, Crustacea, Isopoda). Moreover, *Armadillium*

TABLE 1. Diet Composition in *P. biligonigerus* (27 individuals) in Soybean Field

Prey taxa	*N*	%	Freq.	FO	V
Insecta					
Coleoptera					
Elateridae					
Agriotes sp.*	3	0.72	3	11.11	0.34
Scaraboidae					
Anomala sp.*	3	0.72	3	11.11	0.94
Lepidoptera (larvae)					
Noctuidae					
Spodoptera sp.*	1	0.24	1	3.70	3.78
*Anticarsia gemmatalis**	3	0.72	2	7.40	11.24
*Rachiplusia nu**	1	0.24	1	3.70	0.29
Orthoptera					
Gryllidae					
*Anurogryllus muticus**	1	0.24	1	3.70	4.11
Gryllotalpidae					
*Scapteriscus borelli**	1	0.24	1	3.70	3.73
Homoptera					
Delphacidae					
Adult (n.i.)	1	0.24	1	3.70	0.03
Hemiptera					
Pentatomidae					
Nezara sp.*	3	0.72	2	7.41	11.81
Hymenoptera					
Formicidae					
Pheidole sp.	147	35.17	12	44.44	8.81
Acromyrmex sp.*	22	5.26	3	11.11	2.10
Solenopsis sp.	6	1.43	1	3.70	0.07
Adult (n.i.)	41	9.81	9	33.33	3.73
Isoptera*	40	9.57	3	11.11	2.07
Diptera					
Culicidae	1	0.24	1	3.70	0.02
Crustacea					
Isopoda					
*Armadillium vulgare**	142	33.97	18	66.67	46.53
Arachnida	1	0.24	1	3.70	0.10
Diplopoda	1	0.24	1	3.70	0.29
Animal parts (n.i.)	×	×	27	100	×
Vegetal remnants (n.i.)	×	×	3	11.11	×
Diversity	**1.54**				
Total prey	**418**				

Note. N, total numbers of preys; Freq., absolute frequency in the gastrointestinal tracs; FO, frequency of occurrence; V, volumetric proportion; ×, not numerical value; (n.i.), not identified. *Herbivore species.

vulgare predominated in volume (46.53%), followed by *Nezara* sp. (11.81%) and *Anticarsia gemmatalis* (11.24%).

Eleven prey items are hurtful to soybean plant (*Anticarsia gemmatalis*, *Spodoptera* sp., *Rachiplusia nu*, *Agriotes* sp., *Anomala* sp., *Anurogryllus muticus*, *Scapteriscus borelli*, *Nezara* sp., Isoptera, *Armadillium vulgare*, and *Acromyrmex* sp.) and represented 64.70% of the total animal prey categories consumed. Thus, the trophic diversity of frogs was $H = 1.54$

3.2. Prey Selectivity

Only seven prey categories shown in Fig. 1 these constituted 95.89% ($n = 8544$) of pitfall sample and 98.53% ($n = 418$) of gastrointestinal contents. Formicidae and Isoptera were more frequently in frog's diet than in the soybean field, whereas Coleoptera, Lepidoptera (larvae), Orthoptera, and Arachnida were underrepresented in the diet. Indeed, the selectivity index showed that frogs selected positively Formicidae and Isoptera ($E_i = 0.65$ and 1, respectively), and negatively Coleoptera, Lepidoptera (larvae), Orthoptera, and Arachnida ($E_i = -0.92$, -0.53, -0.86, and -0.94, respectively). Isopoda (*Armadilliun vulgare*) was abundant to frog in the environment and was consumed in higher proportion. Also, the frog diet composition and prey relative abundance in soybean field did not showed significant correlation ($\tau = 0.48$, $p > 0.05$).

3.3. Comparisons between Sexes

Adult females were significantly larger (SVL: 36.25 ± 1.94 and 34.34 ± 0.89 mm in females and males, respectively; U-test = 128, $p < 0.05$) and higher weight (BM: 9.85 ± 2.2 and 8.05 ± 0.83 g in females and males, respectively; U-test = 214, $p < 0.05$) than adult males. Moreover, frequency of occurrence of all prey taxa did not differed significantly between the sexes (Fisher's exact probability test, $p > 0.05$) (Table 2).

4. DISCUSSION

The knowledge of diet composition of anuran living inside cultivations is one important applied aspect to understand their role in controlling pest insects; a few field studies have evidence the relationship between hurtful arthropods of field agroecosystem and feeding habits of the wild amphibians (Premo and Atmowidjojo, 1987; Lajmanovich et al., 2003; Attademo et al., 2005).

The diet of *Physalaemus biligonigerus* in soybean of Córdoba Province consisted on a much higher quantity of mobile arthropods. The majority of arthropods we found were associated with soybean field and represent important noxious herbivores. The diet composition of males and females did not differed in the frequency of occurrence for all taxa consumed. These of evidence

TABLE 2. Dietary Comparison of Males and Females of *P. biligonigerus*

Prey taxa	Males ($N = 13$)				Females ($N = 14$)			
	N	%	Frequency	FO	N	%	Frequency	FO
Insecta								
Coleoptera	2	0.97	2	15.38	4	1.87	3	21.42
Lepidoptera								
Noctuidae (larvae)	3	1.46	3	23.07	2	0.94	2	14.28
Orthoptera	1	0.49	1	7.69	1	0.47	1	7.14
Homoptera	—	—	—	—	1	0.47	1	7.14
Hemiptera	—	—	—	—	3	1.40	2	14.28
Hymenoptera								
Formicidae	162	79.02	10	76.92	54	25.35	7	50
Isoptera	—	—	—	—	40	18.78	3	21.42
Diptera	1	0.49	1	7.69	—	—	—	—
Crustacea Isopoda	36	17.56	9	69.23	106	49.76	9	64.28
Arachnida	—	—	—	—	1	0.47	1	7.14
Diplopoda	—	—	—	—	1	0.47	1	7.14
Animal parts (n.i.)	×	×	13	100	×	×	14	100
Vegetal remnants (n.i.)	×	×	1	7.69	×	×	2	14.28
Total prey	**205**				**213**			

Note. N, total number of organisms found in the digestive tracts; %, percentage of each category in the total numbers of preys; Freq., absolute frequency in the gastrointestinal tracs; FO, frequency of occurrence, ×, not numerical value; (n.i.), not identified.

may indicate that food resource were not partitioned of males and females of *P. biligonigerus*, as well as for other frog species (Hirai and Matsui, 2000), despite their morphological differences. Moreover, the relationship between prey relative abundance of arthropod in the soybean field and their abundance in the gastrointestinal contents of *P. biligonigerus*, showed no significant correlation. In this sense, this frog took prey taxa in different proportions from these relative abundance in the environment. Accordingly to the selectivity index, frogs showed positive selectivity to Formicidae and Isoptera, and negative values for Coleoptera, Lepidoptera larvae, Orthoptera, and Arachnida. Overrepresentation of ants and isopterans in the diet suggest that this frog is a generalist predator with high selectivity to these invertebrates. Moreover, Isopoda (*Armadillium vulgare*) was taken in the same proportion as occurrence in the environment indicating that frog no selected this prey category. In this context, is important to note that technical of direct seeding that implies the non removal of the floor and rest of previous crops has provided suitable habitats (e.g., humid) to noxious organisms. Some of the organisms that have been benefited with this seeding type are the *Armadillium vulgare* and ants. This environmental condition increased their populations considerably, causing damages in the seeds or different part of the plant, and consequently the plant's death (Aragón, 2002). It is important to know that these damages are usually found in soybean plants through different locations. (INTA, 2005). To solve this problem, insecticides are massive used to control these arthropods (Lajmanovich et al., 2002, 2004). Thus, it has become increasingly evident that biological methods might be economically advantageous (Hilje and Hanson, 1998). Saini (2001) review and discuss the published information available on natural enemies in soybean suggested invertebrates predators species, parasitoid species and fungi infestation. Few authors postulated amphibians as potential natural enemies of herbivores in cultivation (Wood, 1976; Hyatt and Humphrey, 1995)

In this context, anurans may be contributed to the control of noxious species (Hirai and Matsui, 1999; Peltzer and Lajmanovich, 2002; Lajmanovich et al., 2003; Attademo et al., 2005). Our study reveled that *P. biligonigerus* consumes noxious arthropods of soybean field such as: *Armadillium vulgare*, *Agriotes* sp., *Anomala* sp., *Anurogryllus muticus*, *Scapteriscus borelli*, *Nezara* sp., Isoptera, *Spodoptera* sp., *Anticarsia gemmatalis*, and *Acromyrmex* sp. (Brewer and Arguello, 1980; Morrone and Coscarón, 1998; PIF, 1999; Saini, 2001).

Finally, the results of our study provide the first data of the diet composition of *P. biligonigerus* in agroecosystems of center Argentina. In conclusion, we suggest that *P. biligonigerus* should be considered as potential biocontrol agents of noxious arthropods in soybean field. We finally suggest that future studies are necessary to determine the role of this vertebrate in agroecosystem.

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