

A toad's first meal: Trophic aspects of *Rhinella jimi* (Anura: Bufonidae) froglets

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Abstract. Most anuran species present a biphasic life cycle, with an aquatic larval stage that metamorphoses into terrestrial adults. Dietary aspects of newly metamorphosed froglets differ from those of both larvae and adults. Considering the important role that recruitment of newly metamorphosed froglets has on population dynamics, it is essential to understand the trophic relations during the final stages of metamorphosis. In the present study, we investigated the diet of recently metamorphosed individuals of *Rhinella jimi* in a rainforest area located in northeastern Brazil. Sampling took place at the end of dry season. Our main objective was to answer the following questions: i) When do froglets of *R. jimi* begin feeding? ii) What is their diet composition? iii) Is prey size related with predator size? We collected 34 froglets, of which 38.2% had tail remains, all of those presenting empty stomachs. Forty-six prey items belonging to 11 categories were found in the fourteen individuals without tails. Our observations show that *R. jimi* stops feeding during the final stages of metamorphosis, and recommences once the tail has been completely absorbed. The number of prey items per stomach ranged from one to six, and was not statistically correlated with SVL, but the latter was positively correlated with maximum prey length and volume. Diptera was the most consumed prey (26.1%) and also the most frequent (53.8%). Formicidae represented the highest volumetric proportion (34.4%), followed by Diptera (30.9%). Considering the IRI, Diptera and Formicidae were the two most important prey categories, whereas prey diversity was 0.86 and the standardized niche amplitude was 0.53. According to our data and other studies, the exact stage at which feeding recommences during final stages of metamorphosis seems to be species-specific. When terrestrial prey begins to be ingested, recently metamorphosed froglets add novel items to their trophic spectrum as they grow and mouth gape enlarges. The existence of small sized prey (< 1.5 mm) is therefore crucial for maintenance of newly recruits.

Keywords. Amphibian, Development, Diet, Ecology, Metamorphosis.

Introduction

Most anurans spend part of their life as tadpoles, feeding in aquatic environments (Wells, 2007). After metamorphosis these animals move onto land where they may prey on or become prey to different species, thus evidencing their importance in energy transference between aquatic and terrestrial environments (Maneyro and Rosa, 2004; Toledo, Ribeiro and Haddad, 2007; Soave et al., 2008). As a result of the radical metamorphosis anurans undergo, these animals display a significant variation in their dietary aspects according to their life stage (Solé et al., 2002; Rodrigues, Uetanabaro and Prado, 2004; Rossa-Feres, Jim and Fonseca, 2004; Wells, 2007; Duré, Kehr and Schaefer, 2009). Therefore, in order to fully understand how

species interact with the different available resources, it is essential to understand how such resources are used during each of their life stages. Studies have shown that newly metamorphosed (hereafter froglets) and adult anurans differ in diet and foraging dynamics (Clarke, 1974; Arnold and Wassersug, 1978; Flowers and Graves, 1995; Lima, 1998; Lima and Magnusson, 1998; Blackburn and Moreau, 2006; Bull and Hayes, 2009). In some anurans, recruitment of recently metamorphosed individuals has strong influence on population growth which can occasionally be more important compared to adult survival (Richter and Seigel, 2002; Grafe et al., 2004), thus reinforcing the importance of understanding predator-prey interactions during this phase.

Natural history traits may be determined by recent ecological interactions or by phylogenetic constraints (Simon and Toft, 1991; Böhning-Gaese, Schuda and Helbig, 2003; Amat et al., 2008). The understanding of such determinants has important evolutionary consequence and may be helpful in the construction of an accurate phylogeny (Wiens, 2004; Kozak and Wiens, 2010). However, in order to distinguish between both scenarios, one must first obtain natural history data on species and latter compare how related species behave

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in different regions (Böhning-Gaese, Schuda and Helbig, 2003; Amat et al., 2008). Although the trophic relations of anurans during metamorphosis have been studied in different species (Jenssen, 1967; Hedeén, 1972; Kuzmin, 1997; Newman, 1999; Conradie et al., 2010), in the Neotropical regions these remain largely unknown.

In the present study we focus on the diet of recently metamorphosed individuals of *Rhinella jimi* (Stevaux, 2002) in a rainforest area located in northeastern Brazil. Our main objective was to answer the following questions: i) When do froglets of *R. jimi* begin feeding? ii) What is their diet composition? iii) Is prey size related with predator size?

Materials and Methods

The study took place on the margins of an artificial lake used for “fish and pay” in the municipality of Guarimiranga (04°16'S-38°56'W; altitude: 860m), State of Ceará, northeastern Brazil. Guarimiranga is located in the Maciço de Baturité, a high altitude rocky complex located within the semi-arid Caatinga domain. This blocks the clouds and allows the incidence of orographic rains, resulting in a cooler and more humid environment (details in Oliveira and Araújo, 2006). During field activity conducted in November 2009 we observed thousands of *R. jimi* froglets swarming near the edges of this lake, with many of them occupying the interface between water and land. Adults were also abundant (> 25 individuals) with males calling intensively. Other anuran species present in the lake were *Rhinella crucifer*, *Leptodactylus vastus*, and *Hypsiboas raniceps* (all were heard vocalizing). The two most abundant fish species in the lake are the Pearl cichlid (*Geophagus brasiliensis*) and the Nile tilapia (*Tilapia rendalli*). The breeding pattern observed in *R. jimi* is explosive, occurring in a punctual manner at the end of the dry season (Wells, 1977; Garda, São Pedro and Lion, 2010; Brito, pers. obs). This results in metamorphosing individuals only being available during a reduced time frame each year.

On 25 November 2010 at proximally 20:00hrs, thirty-four froglets in their final stages of metamorphosis were collected and immediately euthanized with 5% xylocain, after which they were fixed in 10% formalin and preserved in 70% isopropyl alcohol. Voucher specimens are deposited at the Coleção Herpetológica da URCA (URCAH), but have not been assigned voucher numbers at the moment of writing.

In the laboratory, specimens were measured with digital calipers and divided into two categories according to the presence or absence of tail remains (stages 44-45 and 46, respectively; Gosner, 1960). Stomachs were removed and placed on a petri dish where they were carefully analyzed for the presence of prey items. Prey items were identified (usually to order or family level) and measured using ImageJ software (Abramoff, Magelhaes and Ram, 2004). Prey volume was calculated using the ellipsoid equation (Magnusson et al., 2003). For each prey category we calculated the numerical frequency (NF; No. of prey “i” in all stomachs / all prey items), volumetric frequency (VF; Volume occupied by

prey “i” / volume occupied by all prey items), and the frequency of occurrence (FO; No. of stomachs with prey “i” / all stomachs with at least one prey item). We calculated the index of relative importance (IRI; Pinkas, Oliphant and Iverson, 1971) for each prey category, where $IRI_i = (FO_i) * (NF_i + VF_i)$.

Due to the lack of normality in the data obtained, we chose to use non-parametric analyzes. We used the Mann-Whitney U test to compare the SVL between individuals with and without tail remains. We used linear regression to investigate how froglets' SVL was related with number of prey per stomach and maximum length and volume of prey (Zar, 1996). We calculated diet diversity and trophic niche width using Simpson's and Levin's standardized indexes, respectively, as these indexes give values between 0 and 1, thus making future comparisons among studies more intuitive (Krebs, 1989). Data are shown as mean ± S.D. (range), unless noted otherwise.

Results

The mean snout-vent length of the thirty-four froglets collected was 9.45 ± 1.07 mm (range: 8.15 – 12.28mm). Among these specimens, 38.2% (N = 13) had tail remains and were statistically smaller (SVL = 8.79 ± 0.34 mm) than those without tail (SVL = 9.85 ± 1.17 mm; $t = 3.871$; $p < 0.001$). All froglets with tail remains had empty stomachs. We identified forty-six prey items belonging to 11 categories in the fourteen individuals without tail remains that had at least one prey item in their stomach (Table 1).

Snout-vent length of froglets in stage 46 with empty stomachs was 9.24 ± 0.61 mm (8.34 – 9.88mm) and did not differ significantly from those with prey items (10.15 ± 1.28 mm (8.19 – 12.28mm); $U = 32.00$; $p = 0.22$). Number of prey items per stomach ranged from one to six and was not statistically correlated with SVL ($r^2 = 0.007$; $p = 0.78$). Mean prey length and volume was 1.24 ± 0.78 mm (0.28 – 4.25mm) and 0.21 ± 0.29 mm³ (0.004 – 1.33mm³), respectively. Snout-vent length was positively correlated with maximum prey length ($r^2 = 0.398$; $p = 0.016$) and volume ($r^2 = 0.365$; $p = 0.022$). Diptera was the most consumed prey (26.1%) and also the most frequent (53.8%). Formicidae represented the highest volumetric proportion (34.4%), followed by Diptera (30.9%). Considering the IRI, Diptera and Hymenoptera (Formicidae) were the two most important prey categories, with a wide margin over the remaining prey items (Fig. 1). Prey diversity was 0.86 and the standardized niche amplitude was 0.53.

Discussion

Over the last decades there have been controversial views on whether or not anurans stop feeding during

Table 1. Prey composition of *Rhinella jimi* froglets (14 individuals) from an artificial lake in Guaramiranga, Ceará, Brazil. Legends: FO – frequency of occurrence; NF – numerical frequency; VF – volumetric frequency; UI – unidentified invertebrates; DA – Simpson's standardized index.

	FO (%)	NF (%)	VF (%)	IRI
Acari	15.38	15.22	1.45	0.02
Araneae	7.69	2.17	1.94	0.00
Coleoptera	7.69	2.17	6.95	0.01
(Curculionidae)				
Collembola	15.38	6.52	0.44	0.01
Diptera	53.84	26.09	30.89	0.31
Hymenoptera				
Formicidae	30.77	19.56	34.37	0.17
others	7.69	2.17	4.85	0.00
Lepidoptera	7.69	2.17	3.03	0.00
Larvae				
Diptera_larvae	15.38	4.35	0.47	0.01
Siphonaptera_larvae	7.69	13.04	1.42	0.01
UI	15.38	6.52	14.17	0.03
D _a	0.86			

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metamorphosis, as well as during which stages individuals stop feeding on aquatic organisms and begin to feed on terrestrial prey (Hourdry, l'Hermite and Ferrand, 1996; Kuzmin, 1997 and references therein). According to Jenssen (1967), feeding would stop after emergence of forelimbs (stage 42, Gosner, 1960) and recommence as tails are almost completely re-absorbed (stages 44-45, Gosner, 1960). Kuzmin (1997) found terrestrial invertebrates within the stomachs of stage 43 individuals of several species, with some of the bufonids studied by the author restarting to feed at stage 44-45. Although we did not analyze individuals prior to stage 44, according to our data concerning *R. jimi* froglets, individuals only began feeding on terrestrial invertebrates during stage 46 onward. These results indicate that the feeding behavior during this phase

is species-specific and may suffer from phylogenetic constraints, with closely related species displaying similar patterns (Jenssen, 1967; Flowers and Graves, 1995; Kuzmin, 1997; this study).

The energetic balance within a given population may be inferred according to the amount of individuals with empty stomachs, where low values indicate a positive balance and high values a negative balance (Huey, Pianka and Vitt, 2001). In our study we found that 33,3% of *R. jimi* individuals post stage 46 had empty stomachs. These results contrast with those found for other species in which all froglets had some content (Newman, 1999; Conradie et al., 2010). Kuzmin (1997) found rates of empty stomach varying from 0 to 50% after analyzing 18 anuran species from different families. According to the latter author, Bufonidae was the family with the

highest proportion of individuals with empty stomachs. Our results are in accordance with this trend. However, it is noteworthy to mention that the individuals analyzed in the present study were collected at night. Since Kuzmin (1997) does not mention when anurans were collected, we must consider this bias upon making any comparisons. If individuals are collected outside of their foraging period we may expect higher rates of empty stomachs, as individuals will not be feeding during this time. Only with detailed data on species' activity patterns will we better comprehend the existing results.

The positive relation between SVL and prey size such as herein detected for *R. jimi* has been observed in different anuran species and within different size ranges (Teixeira and Vrcibradic, 2003; Bull and Hayes, 2009; Quiroga, Sanabria and Acosta, 2009). This is interpreted as a consequence of morphological constraints (e.g. mouth width) imposed by predator size (Emerson, 1985). During this early phase, when froglets are feeding on whatever they can find (see below), the slightest increase in a froglet's size allows the inclusion of new prey items to its trophic spectrum. This hypothesis is supported by studies that have observed ontogenetic variation in diet composition during early post-metamorphic growth (Flowers and Graves, 1995; Newman, 1999; Blackburn and Moreau, 2006; Bull and Hayes, 2009).

Small anurans tend to consume small preys such as mites, springtail, and flies, with the tendency for mite

consumption being greater in species whose adults specialize on ants (Simon and Toft, 1991; Newman, 1999). Although the adults of *Rhinella jimi* feed mainly on ants and beetles (Brito, pers. obs. – according to stomach-flush contents of three adults), mites were the fourth most important prey with an IRI 15-fold lower than the top ranking prey. Collembolans were not very frequent, contrasting with other studies that have analyzed the diet of small sized anurans (Kuzmin, 1997; Newman, 1999; Blackburn and Moreau, 2006). Froglets have been reported to display little prey selectivity, consuming items according to their local abundance and therefore showing both temporal and spatial variation in diet composition (Flowers and Graves, 1995; Newman, 1999; Bull and Hayes, 2009). Adult bufonids are generally considered as active foraging ant specialists (Toft, 1980; Suazo-Ortuño et al., 2007; Duré, Kehr and Schaefer, 2009), although exceptions have been reported (Bonansea and Vaira, 2007; Batista et al., 2011). Future studies should aim at analyzing the diet of froglets from different water bodies, as well prey availability, in order to understand which factors are determining diet composition.

According to our results and parallel with other existing studies (see Jenssen, 1967; Kuzmin, 1997; Newman, 1999 and references therein) we conclude that the tendency to stop prey consumption during final stages of metamorphose is species specific.

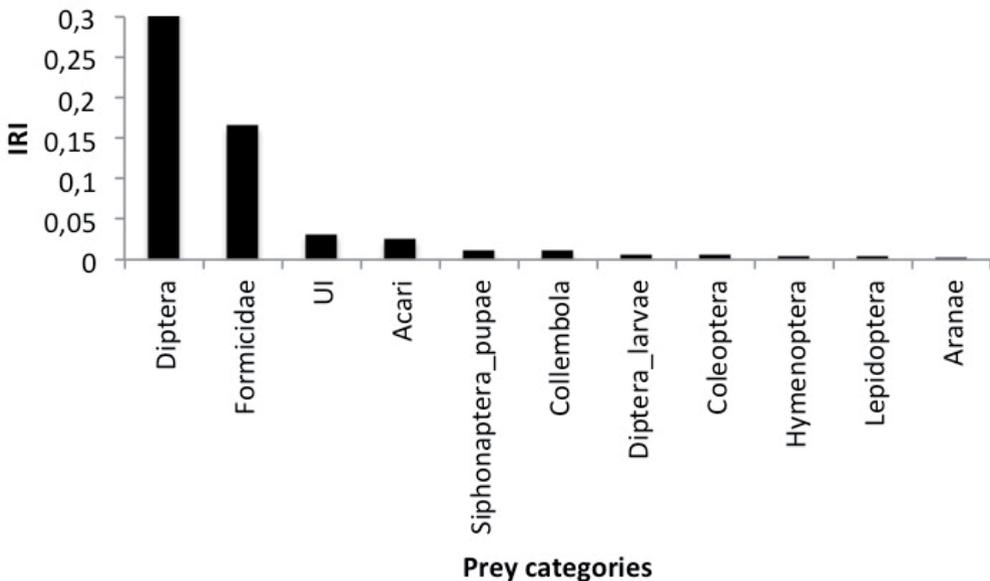


Figure 1. Index of relative importance (IRI) for prey categories found in *Rhinella jimi* froglets from an artificial lake in Guaramiranga, Ceará, Brazil. Legend: UI – unidentified invertebrates.

Studies that focus on diet during this radical phase (= metamorphosis) of life are scarce and do not allow greater general conclusions to be made. The data in Kuzmin (1997) suggests somewhat of a phylogenetic influence in the determination of when froglets begin to feed. Unfortunately, there are still too few publications, especially regarding tropical species, in order to make further comparisons. Our data show that *R. jimi* begins to feed only after complete absorption of tail (stage 46, Gosner, 1960), at which point they select available prey within adequate size for ingestion and add novel items as they grow. We encourage other researchers in the tropics to investigate the diet of different species during this intriguing phase of anuran's life.

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References

- Abramoff, M.D., Magelhaes, P.J., Ram, S.J. (2004): Image Processing with ImageJ. *Biophotonics International* **11**(7): 36-42.
- Amat, F., Pérez-Mellado, V., Hernández-Estévez, J.A., Díez, T.G. (2008): Dietary strategy of a Pyrenean lizard, *Iberolacerta aurelioi*, living in a poor resources alpine environment. *Amphibia-Reptilia* **29**: 329-226.
- Arnold, S.J., Wassersug, R.J. (1978): Differential predation on metamorphic anurans by garter snakes (Thamnophis): Social behavior as a possible defense. *Ecology* **59**(5): 1014-1022.
- Batista, R.C., De-Carvalho, C.B., Freitas, E.B., Franco, S.C., Batista, C.C., Coelho, W.A., Faria, R.G. (2011): Diet of *Rhinella schneideri* (Werner, 1894) (Anura: Bufonidae) in the Cerrado, Central Brazil. *Herpetology Notes* **4**: 17-21.
- Blackburn, D.C., Moreau, C.S. (2006): Ontogenetic diet change in the arthropod frog *Schoutedenella xenodactyloides*. *Journal of Herpetology* **40** (3): 388- 392.
- Böhning-Gaese, K., M.D. Schuda & A.J. Helbig. (2003): Weak phylogenetic effects on ecological niches of *Sylvia* warblers. *Journal of Evolutionary Biology* **16**: 956- 965.
- Bonansa, M.I., Vaira, M. (2007): Geographic Variation in the diet of *Melanophryniscus rubriventris* (Anura: Bufonidae) in Northwestern Argentina. *Journal of Herpetology* **41**(2): 231-236.
- Bull, E.L., Hayes, J. (2009): Selection of diet by metamorphic and juvenile western toads (*Bufo boreas*) in northeastern Oregon. *Herpetological Conservation and Biology* **4**(1): 85-95.
- Clarke, R.D. (1974): Food habits of toads, Genus *Bufo* (Amphibia: Bufonidae). *American Naturalist* **91**(1): 140-147.
- Conradie, W., Branch, W.R., Braack, H., Manson, M. (2010): Notes on the diet of recently metamorphosed Giant African Bullfrogs (Anura: Pyxicephalidae: *Pyxicephalus adspersus*) and growth increase during the first nine months in a semi-natural habitat. *Herpetology Notes* **3**: 215-219.
- Durá, M.I., Kehr, A.I., Schaefer, E.F. (2009): Niche overlap and resource partitioning among five sympatric bufonids (Anura, Bufonidae) from northeastern Argentina. *Phyllomedusa* **8**(1): 27-39.
- Emerson, S.B. (1985): Skull shape in frogs: Correlations with diet. *Herpetologica* **41**(2): 177-188.
- Flowers, M.A., Graves, B.M. (1995): Prey selectivity and size-specific diet changes in *Bufo cognatus* and *B. woodhousii* during early postmetamorphic ontogeny. *Journal of Herpetology* **29**(4): 608-612.
- Garda, A.A., São Pedro, V.A., Lion, M.B. (2010): The advertisement and release calls of *Rhinella jimi* (Anura, Bufonidae). *South American Journal of Herpetology* **5**(2): 151-156.
- Gosner, K.L. (1960): A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**: 183-190.
- Grafe, T.U., Kaminsky, S.K., Bitz, J.H., Lüssow, H., Linseinmair, K.E. (2004): Demographic dynamics of the afro-tropical pig-nosed frog, *Hemisus marmoratus*: effects of climate and predation on survival and recruitment. *Oecologia* **141**: 40-46.
- Hedeen, S.E. (1972): Food and Feeding Behavior of the Mink Frog, *Rana septentrionalis* Baird, in Minnesota. *The American Midland Naturalist* **88**(2): 191-200.
- Hourdry, J., l'Hermite, A., Ferrand, R. (1996): Changes in the digestive tract and feeding behavior of anuran during metamorphosis. *Physiological Zoology* **69**: 219-251.
- Huey, R.B., Pianka, E.R., Vitt, L.J. (2001): How Often do Lizards "Run on Empty"? *Ecology* **82**(1): 1-7.
- Jensen, T.A. (1967): Food Habits of the Green Frog, *Rana clamitans*, before and during Metamorphosis. *Copeia* **1967**(1): 214-218.
- Kozak, K.H., Wiens, J.J. (2010): Niche Conservatism Drives Elevational Diversity Patterns in Appalachian Salamanders. *American Naturalist* **176**(1): 40-54.
- Krebs, C. J. (1989): *Ecological methodology*. New York, Harper and Row.
- Kuzmin, S.L. (1997): Feeding of amphibians during metamorphosis. *Amphibia-Reptilia* **18**: 121-131.
- Lima, A.P. (1998): The effects of size on the diets of six sympatric species of postmetamorphic litter anurans in Central Amazonia. *Journal of Herpetology* **32**(3): 392-399.
- Lima A.P., Magnusson, W.E. (1998): Partitioning seasonal time: Interactions among size, foraging activity and diet in leaf-litter frogs. *Oecologia* **116**: 259-266.
- Magnusson, W.E., Lima, A.P., Silva, W.A., Araújo, M.C. (2003): Use of geometric forms to estimate volume of invertebrates in ecological studies of dietary overlap. *Copeia* **2003** (1): 13-19.
- Maneyro, R., Rosa, I. (2004): Temporal and spatial changes in the diet of *Hyla pulchella* (Anura, Hylidae) in southern Uruguay. *Phyllomedusa* **3** (2): 101-113.

- Newman, R.A. (1999): Body Size and Diet of Recently Metamorphosed Spadefoot Toads (*Scaphiopus couchii*). *Herpetologica* **55**: 507-515.
- Oliveira, T.S., Aratijo, F.S. (eds.). (2006): Diversidade e conservação da biota na Serra de Baturité, Ceará. Fortaleza, Companhia Energética do Ceará.
- Pinkas, L., Oliphant, M.S., Iverson, Z.L. (1971): Food habits of albacore bluefin, tuna and bonito in California waters. *California Fish Game* **152**: 1-105.
- Quiroga, L.B., Sanabria, E.A., Acosta, J.C. (2009): Size- and sex-dependent variation in diet of *Rhinella arenarum* (Anura: Bufonidae) in a wetland of San Juan, Argentina. *Journal of Herpetology* **43**(2): 311-317.
- Richter, S.C., Seigel, R.A. (2002): Annual variation in the population ecology of the endangered Gopher frog, *Rana sevosia* Goin and Netting. *Copeia* **2002**(4): 962-972.
- Rodrigues, D.J., Uetanabaro, M., Prado, C.P.A. (2004): Seasonal and ontogenetic variation in diet composition of *Leptodactylus podicipinus* (Anura, Leptodactylidae) in the southern Pantanal, Brazil. *Revista Española de Herpetología*. **18**: 19-28.
- Rossa-Feres, D.C., Jim, J., Fonseca, M.G. (2004): Diets of tadpoles from a temporary pond in southeastern Brazil (Amphibia, Anura). *Revista Brasileira de Zoologia* **21**(4): 745-754.
- Simon, M.P., Toft, C.A. (1991): Diet Specialization in Small Vertebrates: Mite-Eating in Frogs. *Oikos* **61**(2): 263-278.
- Soave, E.G., Darrieu, C.A., Aribalzaga, M.E., Camperi, A.R., Lucía, M., Williams, J., Juarez, M. (2008): Dieta del Pirincho (*Guira guira*) en el nordeste de la provincia de Buenos Aires, Argentina (Cuculiformes: Cuculidae). *Revista de Biología Tropical* **56** (4): 1883-1892.
- Solé, M., Ketterl, J., Di-Bernardo, M., Kwet, A. (2002): Ants and Termites are the diet of the microhylid frog *Elachistocleis ovalis* (Schneider, 1799) at an Araucaria forest in Rio Grande do Sul, Brazil. *Herpetological Bulletin*. **79**: 14-17.
- Suazo-Ortuño, I., Alvarado-Díaz, J., Raya-Lemus, E., Martínez-Ramos, M. (2007): Diet of the mexican marbled toad (*Bufo marmoratus*) in conserved and disturbed tropical dry forest. *Northwestern Naturalist* **52**(2): 305-309.
- Teixeira, R.L., Vrcibradic, D. (2003): Diet of *Leptodactylus ocellatus* (Anura: Leptodactylidae) from coastal lagoons of southeastern Brazil. *Cuadernos de Herpetología*. **17**(1-2): 111-118.
- Toft, C.A. (1980): Feeding ecology of thirteen syntopic species of anurans in a seasonal tropical environment. *Oecologia* **45**: 131-141.
- Toledo, L.F., Ribeiro, R.S., Haddad, C.F.B. (2007): Anurans as prey: an exploratory analysis and relationship between predators and their prey. *Journal of Zoology* **271**: 170-177.
- Wells, K.D. (1977): The social behavior of anuran amphibians. *Animal Behaviour* **25**: 666-693.
- Wells, K.D. (2007): *The Ecology and Behavior of Amphibians*, 1st edition. Chicago, The University of Chicago Press.
- Wiens, J.J. (2004): Speciation and ecology revisited: phylogenetic niche conservatism and the origin of species. *Evolution* **58**(1): 193-197.
- Zar, J.H. (1996): *Biostatistical Analysis*, 3rd edition. New Jersey, Prentice-Hall, Inc.