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Diet of the Andean Frog *Ranitomeya virolinensis* (Athesphatanura: Dendrobatidae)

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ABSTRACT.—**The effects of sex, ontogeny, and season on the diet of *Ranitomeya virolinensis* were studied over one year. The diet of this Andean species is composed mainly of small prey; the most important prey categories according to index of relative importance (IRI) were Acari, Formicidae, Holometabolous larvae, and Collembola. There were no differences in total prey gut content over time assessed in number and volume; however, the diet composition of this Santander Poison Frog changed between dry and wet seasons, with Formicidae, holometabolous larvae and Collembola as the prey categories that contributed most to the difference. Males had fewer prey in their guts than females, but there were no compositional differences between sexes. There was a shift in the importance of prey caused by ontogeny. Mean prey volume increased with body size, whereas the occurrence of Acari and Formicidae varied inversely with SVL. Formicidae was the second most important prey category according to IRI. Therefore it is an important category as has been described previously for other Dendrobatid species. However, its importance fluctuated with the season. Acari was the most significant prey type in *R. virolinensis* as is the case for other small related species of the genus, although its importance changed with the ontogeny. The diet of this frog had a substantial phylogenetic component because closely related species have similar diets; nevertheless, its diet is clearly affected by other intrinsic and extrinsic factors.**

Dendrobatidae is considered a monophyletic taxon known as Dart-Poison Frogs (Vences et al., 2000; Haas, 2003; Grant et al., 2006), all of which are included in the superfamily Dendrobatoidea (Grant et al., 2006). Certain dendrobatid frogs are characterized by territorial behavior (Roithmair, 1994; Lima and Keller, 2003; Pröhl, 2005), parental care (Wijngaarden and Bolaños, 1992; Caldwell and Oliveira, 1999), and the presence of alkaloid skin secretions that are used as a chemical defense against predation (Daly, 1998; Mortari et al., 2004; Daly et al., 2005). Alkaloids in Dart-Poison Frogs are accumulated directly from their diet (Daly et al., 1994a,b; Daly, 1998). Recent studies have hypothesized an evolutionary correlation between aposematism and toxicity at the species level (Summers and Clough, 2001; Santos et al., 2003). Although there are interpopulational differences in frog toxicity and coloration, there is no apparent correlation between toxicity and the color of the frogs among populations (Daly and Myers, 1967). It has been further proposed that a specialized diet on ants may be linked to these two features (Caldwell, 1996; Santos et al., 2003; Darst et al., 2005). However, there are many ecological factors influencing the relationship between frog diet and toxicity, including the locality of the studied species and availability of alkaloid-containing arthropods at these locations (Toft, 1980b; Biavati et al., 2004; Saporito et al., 2006, 2007b), the effects of long-term bioacummulation of alkaloids, and spatial and temporal variation in alkaloids among arthropod prey (Smith and Jones, 2004; Saporito et al., 2004, 2007a,b). Ants are an important dietary source of alkaloids in Dendrobatid (Jones et al., 1999; Saporito et al., 2004); however, mites have recently been shown to be the largest source of alkaloids at least in one Dendrobatidae, *Oophaga pumilio* (Takada et al., 2005; Saporito et al., 2006, 2007a). It is crucial to gain a further understanding of the importance of certain prey categories in the diet of dendrobatids and their relationship with intrinsic factors such as phylogeny, morphology, sex, and ontogeny and extrinsic factors such as season and locality before inferring relationships between diets and toxicity. Therefore, additional studies are needed on the diets of dendrobatid species.

Anuran diet can be modified by shifts in dietary resources, such as geographic and seasonal shifts in arthropod availability (Toft, 1980a,b; Biavati et al., 2004). Ants are the main prey category identified in the diet of the dendrobatid frogs studied in Amazonian and Central American wet forests (Toft, 1980a,b;

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Caldwell, 1996). However, ants may not be an important dietary component in dendrobatid frogs in the Cerrado biome. For example, *Ameerega flavopicta*, a toxic species that inhabits this biome, is not an ant specialist as are their Amazonian and Central America relatives (Biavati et al., 2004). The dietary importance of ants or other prey categories in Andean amphibian species is poorly known (Piñero-Bonilla and La Marca, 1996).

Ant consumption is strongly influenced by phylogeny, which leads to similar diets in sister species. However, ant consumption is not solely linked to phylogeny, and in many Neotropical anuran species, it changes over time, linked to seasonal fluctuations in prey resources (Toft, 1980a,b; Donnelly, 1991). In addition, behavioral activity, directly associated with reproduction, may lead to temporal changes in the energetic investments of frogs (Girish and Saidapur, 2000). If this is true, then other factors that are restricted by morphology and behavior may be important in understanding the trophic ecology of anurans, such as sex (Donnelly, 1991), ontogeny (Lima and Moreira, 1993), and reproduction (Biavati et al., 2004).

Ranitomeya virolinensis (Ruiz-Carranza and Ramírez-Pinilla, 1992), commonly known as Santander Poison Frog, is a small (up to 18.9 mm SVL) dendrobatid frog distributed throughout the northern montane wet forests of the western slope of the Cordillera Oriental of Colombia (Ruiz-Carranza and Ramírez-Pinilla, 1992). Its distribution ranges throughout the Departaments of Boyacá, Cundinamarca, and Santander. The diet of *R. virolinensis* is completely unknown; therefore, it is of great interest to ask the following questions: (1) Is *R. virolinensis* an ant specialist, as has been suggested for most of the studied lowland dendrobatids? (2) What is the annual feeding pattern of this Andean species? (3) Are there any changes in the diet of *R. virolinensis* associated with differences in sex, ontogeny, and reproductive condition? To address these questions, we investigated the diet of a population of this species.

MATERIALS AND METHODS

This study was conducted in a secondary wet forest located on a mountain at approximately 6°6′N, 73°13′W, at an elevation of 1,803 m; this area is known as Costilla del Fara, Corregimiento de Virolín, Santander, Colombia. This zone has an annual mean temperature of 18.4°C and a mean annual rainfall of 2,916 mm. Historical rainfall levels show that rainfall follows a bimodal seasonal pattern (Fig. 1; Hijmans et al., 2005). The vegetation of the site is characteristic of low montane forest (Holdridge, 2000). The site is located on a steep hill with a small stream where oaks (*Quercus humboltii* and *Colombobalanus excelsa*) and arums are very common, and there are many epiphytes located on the ground and on trees. The roots of trees extend along the ground, making a net of roots and rocks that are covered by leaf litter. Males of *R. virolinensis* use these areas as territories for calling.

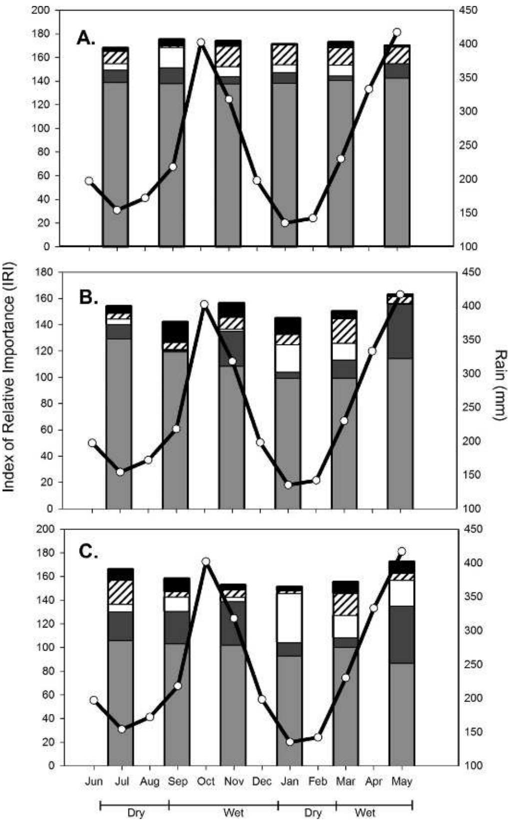

FIG. 1. Relative Importance Index (IRI divided by 100) expressing annual seasonal pattern and monthly diet (from July 2005 to May 2006) of five major prey categories in *Ranitomeya virolinensis*. (A) Juveniles. (B) Males. (C) Females. ▒, Acari; ▓, Formicidae; ☐, larvae; ▨, Collembola; ■, other taxa. Line graph describes rain pattern, according to data compiled by Hijmans et al., 2005.

Frog sampling was conducted once every two months from July 2005 to May 2006, to collect data from all different seasons of the year. Within the boundaries of a pre-established 2,300-m^2 area (see Gil, 2005), three collectors walked up and down the hill collecting frogs from 0800–1600 h for three days during each sampling period. A minimum of 20 postmetamorphic individuals was collected per month by searching the leaf-litter, old vegetation on the ground, and inside rocks and root caves where calling males and courting couples were found (Gil, 2005). Given the small size of these frogs (mean SVL = 16.01 mm, range 9.05–18.44 mm), we could not stomach flush individuals to obtain dietary items. Therefore, specimens were euthanized with 10% ethanol and immediately fixed in 10% formalin to preserve gut contents with minimum digestion. In the laboratory, specimens were transferred to 70% ethanol for storage. All of the collected specimens are housed in the Colección Herpetológica, Museo de Historia Natural UIS (UIS-A-3584 to UIS-A-3750).

Snout–vent length (SVL) and mouth width (MW) were recorded to the nearest 0.02 mm in the laboratory with a Vernier caliper. Specimens were dissected, and the sex and size of gonads were recorded for each individual. The complete reproductive and digestive tracts were also dissected and stored separately in 70% ethanol. Sex and sexual maturity of specimens were determined by examination of gonads and sexual ducts and by histological analyses of the gonadal tissues (M. Valderrama-Vernaza, V. H. Serrano-Cardozo, and M. P. Ramírez-Pinilla, unpubl. data).

We analyzed 166 specimens of *R. virolinensis* (61 adult males, 66 adult females and 39 juveniles). Adult females (SVL mean ± SD = 17.38 ± 0.53 mm) were significantly larger than adult males (16.72 ± 0.54 mm; $t_{124} = 6.91$, $P < 0.001$, $N = 127$). Only four of 66 adult females were nongravid, with only previtellogenic follicles and slightly convoluted oviducts. Therefore, we did not have a representative sample of nonreproductive females to compare its diet with reproductive females. For all gut samples, prey items were sorted, counted, and identified to the lowest possible taxonomic category, and measured for length and width (to the nearest 0.01 mm) with a digital caliper under a dissecting stereomicroscope. The volume of intact prey was estimated with the formula for a prolate spheroid.

$$\text{Prey Volume} = \frac{4\pi}{3}\left(\frac{\text{length}}{2}\right)\left(\frac{\text{width}}{2}\right)^2$$

To describe the importance of each prey category consumed (t), the index of relative importance (Pinkas et al., 1971) was calculated as $\mathrm{IRI}_t = \%O_t (\%N_t + \%V_t)$, where $\%O_t$ is the occurrence percentage (i.e., the number of guts containing each t category), $\%N_t$ is the percentage of the number of t items in all guts, and $\%V_t$ is the percentage of the volume of t items in all guts. Niche breadth was calculated for females, males, and juveniles separately to compare the use of prey categories using Levins standardized formula (Hurlbert, 1978):

$$B_A = \frac{\left(1/\sum p_i^2\right) - 1}{n - 1}$$

where p_i = the proportion in number or volume of each prey category with respect to the total number or volume of prey in each age-sex category of *R. virolinensis* diet; n = the number of prey categories in the diet of frogs. B_A ranges from 0 to 1, and a value of 1 for B_A means that all prey categories were used in equal proportions, whereas a value near zero for B_A means that only one or a few categories were used in high proportions and that most prey categories were used in low proportions.

Correlations between frog SVL and mean prey volume and total prey content (in volume and number) of ingested prey were tested using Spearman Rank Correlation to see if prey size depends on frog body size. The variation of consumption (both in number and volume) of the four most important prey categories according to IRI was also checked employing a Spearman Rank Correlation. The effects of ontogeny on diet were also investigated using a non metric multidimensional scaling (NMDS) technique were the diet of juveniles class was compared with the data of diet of adult females and males. Because the categories of prey do not represent independent data because the amount of food that can be eaten is constrained by stomach capacity, it is not appropriate to apply independent tests to each prey category (Lima et al., 2000). An increase in the consumption in number and volume of one prey type will automatically cause a decrease in the consumption of other taken prey. Therefore, a multidimensional scaling technique was used to summarize the differences in the diet among sex-age stages and among seasons. Gross differences between sex and age stages were compared using a NMDS. NMDS is the result of iteratively fitting nonparametric regressions of the distances between sex-age categories and seasons in a two-dimensional space on the similarity values. The final stress coefficient provides a measure of goodness-of-fit of the regression, stress values larger than 0.20 cannot be interpreted reliably. A one-way analysis of similarities (ANOSIM; Clarke and Warwick, 1994) was used to discern significantly among the diet of sex-age categories and seasons groups. Both the NMDS and the ANOSIM based on a Bray Curtis similarity matrix (Clarke and Warwick, 1994). The index of the similarity matrix was calculated on the basis of the IRI instead of numeric or volumetric percentages because, as it combines %*N*, %*V* and %*F*, it provides more information about the diet of the

TABLE 1. Diet of *Ranitomeya virolinensis* from a montane rainforest at the Cordillera Oriental of Colombia. N = Number of prey of each item, $\%N$ = Percentage of N, V = Volume of prey of each prey item, $\%V$ = Percentage of V, F = Frequency of each prey item, $\%F$ = Percentage of F, IRI = Index of Relative Importance (Pinkas et al., 1971).

Prey type	*N*	%*N*	*V*	%*V*	*F*	%*F*	IRI
Acari	7766	84.79	72.83	25.18	165	100	10997.44
Hymenoptera							
Non-Formicidae	68	0.74	3.65	1.26	45	27.27	54.62
Formicidae	328	3.58	74.48	25.75	103	62.42	1,831.14
Total Holometabolous larvae	264	2.88	67.15	23.22	80	48.48	1,265.43
Coleoptera larvae	210	2.29	29.25	10.11	64	38.78	481.23
Lepidoptera larvae	9	0.10	33.70	11.65	8	4.84	56.97
Nonidentified larvae	18	0.20	5.47	1.89	8	4.84	10.12
Collembola	488	5.33	18.79	6.50	118	71.52	845.74
Diptera	34	0.37	3.52	1.22	18	10.91	17.33
Coleoptera	91	0.99	32.09	11.10	61	36.97	446.98
Pseudoescorpiones	23	0.25	0.91	0.32	9	5.45	3.09
Tisanoptera	44	0.48	2.00	0.69	23	13.94	16.34
Gastropoda	18	0.20	7.82	2.70	11	6.67	19.34
Myriapoda	15	0.16	3.07	0.01	12	7.27	1.27
Aranae	20	0.22	2.90	0.01	14	8.48	1.94

frogs (López et al., 2007). These analyses compare mean rank of dissimilarities of samples within and among groups and do not make the assumption that data are normally distributed or that variances or covariances are equal (Anderson and Underwood, 1994). The ANOSIM test statistic, R ranges from -1 to 1, the closer to its maximum value the greater the dissimilarities between groups than within groups. Statistical significance is achieved by comparing the observed R with those produced by a random permutation procedure (Serrano-Cardozo et al., 2008; K. R. Clarke and R. N. Gorley, PRIMER v5, 2001).

The variation of the total volume and number of prey was analyzed for sexes and seasons employing an ANCOVA. Seasonal differences were also assessed using a NMDS and one-way ANOSIM as in the sex-age comparison (Clarke and Warwick, 1994). Where significant differences occurred between seasons, the prey types that contribute most to the difference were determined using similarity percentages (SIMPER; Clarke and Warwick, 1994). Both wet periods and both dry periods of the annual raining pattern were combined on a single group called wet season and dry season respectively. To check whether rainfall levels were related to changes in diet, we employed a Spearman Rank Correlation between the monthly rain level and the mean monthly volume of total content and four major prey categories. The multidimensional scaling ordination was constructed employing PAST Software package (Hammer et al., 2001). Both the ANOSIM and SIMPER procedures were conducted using the PRIMER Software package (Clarke and Warwick, 1994).

RESULTS

All of the examined frogs of *R. virolinensis* contained at least one complete prey item in their guts. A total of 8,993 prey items was analyzed, representing 13 prey types (Table 1). Mean number of prey items found in guts of *R. virolinensis* was 56 ± 37.64 (N = 165). The Relative Importance Index showed that the diet of this frog in both sexes and in all developmental stages was dominated by the following prey types ranked in order of significance: Acari, Formicidae, Collembola, and Holometabolous larvae (Coleoptera, Lepidoptera, and nonidentified larvae; Fig. 1 and Table 1). Niche breadth was near to zero for numeric data (juveniles $B_A = 0.0102$, females $B_A = 0.0386$, and males $B_A = 0.0224$) and volumetric data (juveniles $B_A = 0.2129$, females $B_A = 0.2814$, and males $B_A = 0.3128$), suggesting that *R. virolinensis* tended to concentrate on a select group of prey types.

There was an ontogenetic shift in composition and number of prey (Fig. 2). There was a negative relationship between number of total prey and SVL (Spearman Rank Correlation, $r_s = -0.3129$, $P < 0.001$, $N = 165$; Fig. 2A) which is expected given that one of the smallest prey types, Acari was significantly correlated with SVL in number (Spearman Rank Correlation; $r_s = -0.3310$, $P < 0.001$, $N = 165$) and volume

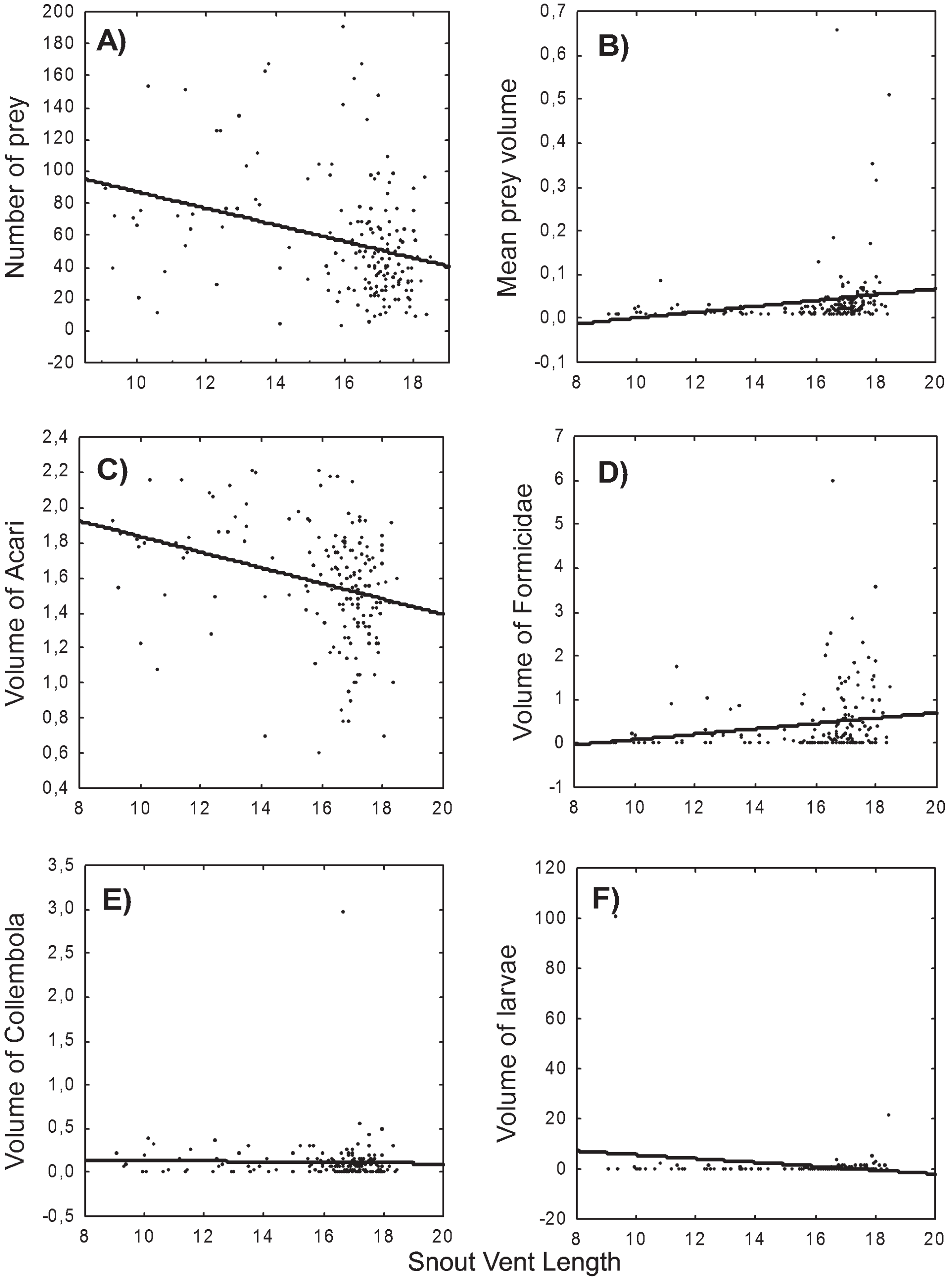

FIG. 2. Volume of stomach contents for each prey category in relation to the SVL of the frogs. (A) Total number of prey. (B) Mean prey volume. (C) Acari. (D) Formicidae. (E) Collembola. (F) Holometabolous larvae.

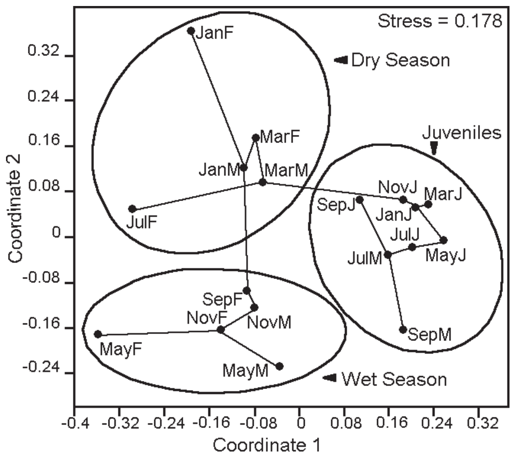

FIG. 3. Multidimensional scaling ordination showing seasonal and sex-age variation of the diet of *Ranitomeya virolinensis* based on Bray-Curtis similarity matrix constructed on the IRI (Importance Relative Index) of diet. ''F'' denotes females from corresponding month; ''M'' denotes males from corresponding month; and ''J'' denotes juveniles from corresponding month.

(Spearman Rank Correlation; $r_s = -0.3307$, $P <$ 0.001, $N = 165$; Fig. 2c). The mean prey volume increased with frog body size (Spearman Rank Correlation, $r_s = 0.4674$, $P < 0.001$, $N = 165$; Fig. 2B), which also explains the increase in volume consumption of larger prey such as Formicidae (Spearman Rank Correlation; $r_s =$ 0.2100, $P = 0.006$, $N = 165$; Fig. 2D). No other prey types were significantly correlated with body size either for volume or number (Spearman Rank Correlation, $P > 0.05$, $N = 165$; Collembola and Holometabolous larva, Fig. 2E, F).

The ontogenetic variation in diet was also shown in the NMDS analysis in which data from juveniles from all months tended to cluster in one single group (Fig. 3). NMDS of sex-age and season data yielded a stress coefficient of 0.178, indicating that the resulting ordination plot may be interpreted reliably. A global one-way ANOSIM showed this variation was significant (global $R = 0.317$, $P = 0.001$). Pairwise comparisons using ANOSIM showed significant differences between juveniles and adults (both females and males; Table 2). SIMPER results showed that Acari, Formicidae, holometabolous larvae, and Collembola are the prey types that contributed most to the difference between juveniles and adults (Table 2).

Although females had a significantly higher number (ANCOVA, $F_{1,127} = 12.51$, $P = 0.0005$, $N = 127$) and volume (ANCOVA, $F_{1,127} = 6.70$, $P = 0.0109$, $N = 127$) of total prey (Fig. 4), pairwise comparisons using ANOSIM revealed no significant differences between the diets of females and males (Table 2). Although the diets of adult females and males was different from juveniles, there were no sex differences in the composition of prey consumed (Fig. 3).

The relative importance of prey types in the diet showed visible fluctuations over time with apparent increase of Formicidae when rains were high (Fig. 1). In spite of the lack of significant differences in total number (ANCOVA, $F_{5,127} = 1.19$, $P = 0.3197$) and volume (ANCOVA, $F_{5,127} = 0.93$, $P = 0.4633$) of total prey among months, this trend of seasonal variation in diet was confirmed by the multidimensional scaling analysis (Fig. 3). The diet of *R. virolinensis* from months with relative high rainfall (November and May) tended to group together, whereas months with a relative dry period (January and July) tended to form another group (Fig. 3); the dissimilarity between these two groups was confirmed with an ANOSIM (Global $R = 0.779$, $P = 0.001$). The diet of the frogs collected (both males and females) in March are within the dry period, given that this is just the beginning of the wet season, whereas in September, which is the beginning of the other wet season, the diet tends to be more similar to the wet period. This seasonal trend was observed in the diet of adults only; the diet of juveniles from all months did not follow any seasonal pattern (Fig. 3). Pairwise comparisons using ANOSIM showed significant differences between the

TABLE 2. Pairwise comparisons showing significant differences among sex-age classes in the diet of *Ranitomeya virolinensis* and contributing prey categories to the difference.

			Contribution (%)				Cumulative contribution (%)			
Comparison	R	P	Formicidae	Acari	Larvae	Collembola	Formicidae	Acari	Larvae	Collembola
Adult females and adult males	0.172	0.082	24.59	30.38	20.53	—	54.97	30.38	75.50	—
Adults females and juveniles	0.631	0.002	21.92	40.02	16.28	—	61.94	40.02	78.22	—
Adult males and juveniles	0.219	0.028	23.65	30.00	15.65	14.28	—	53.64	69.28	83.58

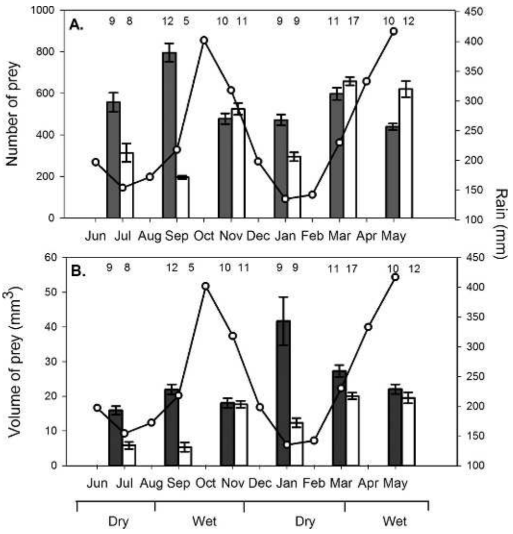

FIG. 4. Frequency of total preys in guts of *Ranitomeya virolinensis* frogs from July 2005 to May 2006. Line rainfall (mm) data compiled by Hijmans et al., 2005 showing historical annual seasonal pattern. ■, Females; □, Males. Numbers over the bars indicate sample size. The whiskers show the mean and standard deviation.

adults of wet and dry period (Table 3). In pairwise comparison, SIMPER showed that differences in the IRI of Formicidae, holometabolous larvae, and Collembola were the main contributing factor to the dissimilarity between seasons (Table 3). None of the prey categories or the total volume of prey was significantly correlated with the amount of rain in each month (Spearman Rank Correlation, $P > 0.1$; $N = 6$).

DISCUSSION

This study illustrated that *Ranitomeya virolinensis* is a species whose diet was composed of small sized prey. Niche breadth and IRI values showed that *R. virolinensis* had a narrow diet, with Acari as the most important prey in adults and juveniles. The high consumption (both in number and volume) of mites could correspond to different interacting issues. One explanation is that the diet of *R. virolinensis* was similar to that of other small anurans from different regions, including a moist forest from Malawi, Africa (Blackburn and Moreau, 2006), a montane forests of the Andean Region of Argentina (Bonansea and Vaira, 2007), a montane moss forest of Papua New Guinea, Oceania (Simon and Toft, 1991), Central America (Donnelly, 1991), and Amazonian forests (Simon and Toft, 1991). Although information that describes the ecology of mites in South America is poor, mites occur in bromeliads, leaf litter, and soil (Santos and Albuquerque, 2006). *Ranitomeya virolinensis* is found in these same microhabitats, which may explain in part why mites are a common prey category in their diet according to IRI. The other reason that may also favor the consumption of mites is the association between mites and alkaloids (Takada et al., 2005; Saporito et al., 2007a). Nevertheless mites remarked importance in relation to other prey categories according to IRI values in guts of *R. virolinensis* could be caused by the high percentage of chitin that negatively affect the gut passage time of food and the long-term assimilation rate of energy (Díaz and Carrascal, 1993) caused by the surface to volume ratio (Simon and Toft, 1991).

There was a shift in diet of *R. virolinensis* with ontogeny. The consumption in volume and number of Acari decreased and the consumption in volume of Formicidae increased, likely as a consequence of the relationship between frog body size and prey size (Lima and Moreira, 1993). Furthermore, the number of prey items decreased, whereas the mean size of prey items increased with body size in *R. virolinensis*. Lima and Magnusson (1998) also found a reduction in the proportion of total mass of mites and an increase in the proportion of total mass of ants at larger body size classes of frogs assessed with an NMDS in an assemblage of diurnal leaf litter frogs in the Amazonian wet forest resulting

TABLE 3. Pairwise comparisons showing significant differences and prey categories contributing to the dissimilarity between the diet of *Ranitomeya virolinensis* in the wet and dry season, based upon NMDS plot (Fig. 3).

			Contribution (%)				Cumulative contribution (%)			
Comparison	R	P	Formicidae	Acari	Larvae	Collembola	Formicidae	Acari	Larvae	Collembola
Adults of wet season and dry season	0.556	0.008	32.87	—	22.92	17.48	32.87	—	55.78	73.27
Adults of dry season and juveniles	0.766	0.001	—	41.61	23.04	15.24	—	41.61	64..65	79.89
Adults of wet season and juveniles	0.888	0.001	36.57	36.03	—	—	36.57	72.60	—	—

from mean prey size differences among arthropods taxa. The diet of *O. pumilio* also changes ontogenetically, because mites are more important for juveniles than for adults and because there is a positive correlation between body size and number of ants consumed (Donnelly, 1991). These ontogenetic shifts of prey size are expected in dendrobatids and nondendrobatids (Lima and Moreira, 1993; Hirai and Matsui, 2002). *Ranitomeya virolinensis* did not omit small prey categories such as mites and collembolans when they became adult; nonetheless, based on the NMDS comparison the importance of ants and these prey categories was dissimilar among juveniles and adults.

Sex influenced the total number of prey items consumed but not the type of prey in the diet of *R. virolinensis*. In *O. pumilio*, females fed more frequently than males to obtain the energetic requirements associated with vitellogenesis and rearing of tadpoles (Donnelly, 1991). Males of *R. virolinensis* transport tadpoles to pools of water in bromeliads (Ruiz-Carranza and Ramírez-Pinilla, 1992); therefore, the main energy requirements of the females of this Andean species should be vitellogenesis. Besides the transport of offspring, males invest time and energy in calling activity, courtship behavior, and defending territories. Thus, there are differences in mobility between males and females. Females have more time to forage than the males as has been stated by Donnelly (1991) in *O. pumilio*. Furthermore, males of *R. virolinensis* call from underground roots and rock cavities which constitute their territory; this could restrict the activity of males to those sites. If the foraging site of males is limited to its territory and its surroundings, males may not be able to search for an aggregate resource such as ants or mites, which corresponds to the main prey categories of *R. virolinensis*. As in *Ameerega flavopicta* (Biavati et al., 2004) and *Aromobates alboguttatus* (Piñero-Bonilla and La Marca, 1996), there were no differences in the composition of diet between sexes.

The total volume or number of prey items did not change with season; however, there was a trend toward a shift in the composition of diet associated with season. The diet of *R. virolinensis* tended to be more similar among months with similar rainfall levels and more dissimilar between months of the dry and the wet seasons. Arthropods are positively associated with moisture content in transitions between seasons and sites (Janzen and Schoener, 1968; Leving, 1983; Leving and Windsor, 1984; Pearson and Derr, 1986). Although there are no studies on seasonal arthropod patterns for the site examined in this study, the behavior of arthropods, in particular ants in other sites in response to desiccation stress between seasons and among sites within a season (Kaspari and Weiser, 2000; Hahn and Wheeler, 2002) presumably explains the high contribution of Formicidae to the dissimilarity between the wet and the dry season. Although none of the prey categories was significantly correlated with rainfall level, other factors than rain may be causing differences in diet among different periods. The feeding pattern of *R. virolinensis* contrasts with the diet of other anurans and reptiles from other Neotropical regions (Toft, 1980a; Donnelly, 1991; Whitfield and Donnelly, 2006), where ants were less common in the guts of frogs during the wet season or did not change at all the consumption of certain prey types (Magnusson and Da Silva, 1993; Biavati et al., 2004). The differences in the seasonal diet trends among studies could be resulting from differences in ambient conditions influencing arthropod prey of the different sites studied. Therefore, the overgeneralization that food is more limiting during the wet season resulting from deterioration of leaf litter (Toft, 1980a) should be viewed with caution because diet temporal trends change from site to site and arthropod taxa are independent from each other (Biavati et al., 2004; Whitfield and Donnelly, 2006).

The most important prey type of *R. virolinensis* according to IRI was Acari as in its closely related congeners that inhabit Central America (*Ranitomeya minuta* and *Ranitomeya fulgurita* studied by Simon and Toft, 1991). The consumption of mites (in volume) was inversely correlated with that of ants. Although ant and mite importance according to IRI is high in *R. virolinensis*, as in other dendrobatids (Toft, 1980a; Simon and Toft, 1991; Donnelly, 1991; Caldwell, 1996), it varied with season, size, and sex. Thus, diet is strongly influenced by evolutionary relatedness among species; however, it is also related to ecological and other intrinsic issues (Simon and Toft, 1991; Donnelly, 1991; Biavati et al., 2004).

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