

## Short Notes

# Population diet variation and individual specialization in the poison toad, *Melanophryniscus rubriventris* (Vellard, 1947)

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**Abstract.** Few studies have investigated the level of individual variation in diet composition of poisonous frogs and toads. We compare the diet composition of three populations of a poisonous toad, *Melanophryniscus rubriventris*, and predicted that toads within a population might be constrained to forage on particular types of alkaloid-containing prey and consequently diets among populations might not diverge from each other. Most important prey categories in the diets of the three populations consisted of the same ground-dwelling arthropods. We found evidence for individuals consuming different arrays of prey types in all populations implying that this “generalist” species is actually comprised of individuals eating different sets of the available range of prey. Formicidae, Acari, and Coleoptera were all important alkaloid-containing prey items in the diets of all populations and individuals, although there were differences in their order of importance among populations and individuals use different sets of the entire range of alkaloid-containing preys. Future research should evaluate individual diet variation in other poisonous anurans taxa given that shifts in diet composition might have important implications for understanding the consequences of alternate foraging strategies in the evolution of defensive strategies among species.

**Keywords:** anurans, chemical defense, foraging ecology, population.

The acquisition of passive chemical defenses in most poison frogs is from exogenous sources, in which the toxic/noxious substances are produced by other organism and are sequestered and stored in the skin glands for several years (Daly, 2003). Increasing evidence has shown that lipophilic alkaloids present in the skin of several poisonous frogs and toads are sequestered from their diet (Saporito et al., 2009). Studies of diet composition provide insights into the possible dietary sources of skin alkaloids, which can be tested subsequently using appropriate biochemical methods (see Daly, 2003). Several studies have examined geographic and temporal variation of the type, num-

ber, and amount of alkaloids present in poison frog and toad populations, showing that up to 26 different alkaloids can be evident within a single individual (Clark et al., 2006; Saporito et al., 2006, 2007a; Daly et al., 2007). Poisonous frogs typically had more similar alkaloid profiles within a locality than among localities, attributable to differences in the diversity of alkaloid-content in prey within the arthropod assemblages at different localities (Saporito et al., 2004, 2006, 2007a; Mebs et al., 2005; Clark et al., 2006; Daly et al., 2007, 2008). These studies also found temporal variation of alkaloid diversity within a population suggesting effects of seasonal changes, increase in habitat disturbances, and forest dynamics (Clark et al., 2006; Saporito et al., 2006). However, research to date has not evaluated variation in diet among populations of a species of poisonous frog or toad. Given that the availability of prey resources may vary in space and time (Donnelly, 1991) such studies may reveal important interactions be-

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tween feeding ecology strategies and the acquisition of chemical defensive compounds in anurans.

Individual-level variation in resource exploitation occurs when certain individuals consume only a subset of resources that are consumed by the entire population (individual specialization, *sensu* Bolnick et al., 2002). Such individual specialization were found in snails, fishes, amphibians, reptiles, and birds (Werner et al., 1987; West, 1988; Araújo et al., 2007; Bolnick et al., 2007; Costa et al., 2008). In poison frogs, functional constraints may limit the opportunities of individuals to exploit a wide range of prey types because frogs have to rely on their diet to acquire and maintain passive defenses. Alternatively, individuals might exploit a narrow range of prey types, to minimize the dietary overlap with conspecifics to avoid competition. Few studies have investigated the feeding ecology of poison anurans at the level of individual diet composition within a population (but see Donnelly, 1991; Caldwell, 1996; Darst et al., 2005; Saporito et al., 2007a).

*Melanophryniscus rubriventris* (Vellard, 1947), a diurnal and aposematic poison toad, occurs in upland montane forests of the Andean region in NW Argentina (Vaira, 2002). A rich array of alkaloids was detected in skin extracts of the species with significant geographical differences in the alkaloid profiles (Daly et al., 2007). The diet of *M. rubriventris* consists mainly of small prey such as ants, mites, aphids, burrowing bugs, and beetles. More than 40 different prey taxa may comprise the entire diet spectrum of a population (Bonansea and Vaira, 2007), suggesting that individual diets could vary considerably. Some arthropods in the diet are the source of alkaloids for *M. rubriventris* (C. Heit, M.F. Quiroga and M. Vaira, unpublished results) as has been found for other poison frogs (Saporito et al., 2009 and references therein).

In this paper, we compare the diet composition of three populations of *Melanophryniscus rubriventris* and investigate individual-

level variation in prey consumption. We predict that toads in every population will be constrained to forage on particular types of alkaloid-containing preys and consequently diet composition might not differ substantially among individuals and populations.

Diet composition data were obtained from three populations over the range of the species in Argentina: Los Toldos (22°16'S, 64°41'W; 1635 m a.s.l.), Baritú (22°29'S, 64°45'W; 1689 m a.s.l.), and Angosto de Jaire (24°01'S, 65°23'W; 1665 m a.s.l.), in 22 and 23 November 2007, and 1 December 2007 respectively. The sampled areas in all cases support a well-structured cloud forest with high vegetation species richness and vegetation density although slightly disturbed because of former clearings and the occasional presence of livestock. Numbers of toad specimens per population are indicated in table 1. We captured adult toads by hand and immediately flushed each individual's stomach following the technique of Legler and Sullivan (1979). This technique consists to slide a catheter into the stomach attached to a 30 cc syringe. Once the tube is inserted toad is oriented with its head downward and clear water is insufflated into the stomach. Flushing produced contents in a large bolus that were preserved in vials with 70% ethanol. At least three flushings per toad were performed to ensure that all stomach contents were recovered. No toads were resampled at different days. Prey items were identified to family or the lowest taxonomic level possible and counted. We considered particular prey items as potential alkaloid-source based on results of bioprospecting analysis conducted in the species and other poison frogs (Saporito et al., 2009 and references therein; C. Heit, M.F. Quiroga and M. Vaira, unpublished results). The volume of intact prey items was estimated using the formula for an ellipsoid: Prey volume =  $4/3\pi(\text{prey length}/2)(\text{prey width}/2)^2$ . To estimate the importance of each prey item in the diet, we used a dietary Importance value index for population-pooled stomach contents ( $I_p$ ) using the following formulae:  $I_p = (N\% + V\% + F\%)/3$ , where,  $N\%$  = numeric percentage,  $V\%$  = volumetric percentage, and  $F\%$  = occurrence percentage. Based on the number of preys consumed, we quantified diet population total niche width (TNW) and diet variation ( $V$ ) per population as in Bolnick et al. (2002, 2007). The values of TNW yield 0 when the entire population uses only a single prey category, increasing with both higher number of categories consumed and their evenness of use. The index of diet variation ( $V$ ) were based on the population-wide average measure of individual specialization ( $IS$ ) where  $V = 1 - IS$ , (Bolnick et al., 2007). The index ranges from 0 when all individuals use the entire range of resources consumed by the population to higher decimal values when each individual use a subset of the population resources. The  $PS$ , TNW and  $V$  indices calculations were performed with IndSpec 1.0. The program uses a non parametric Monte Carlo procedure to generate replicate null diet matrices from the population distribution from which  $P$ -values can be computed (Bolnick et al., 2002). We used 1000 replicates in

**Table 1.** Number ( $n$ ), volume ( $v$ ), occurrence percentage (%), and dietary Importance value index (Ip) of most important prey items (Ip > 25%) in the diet of three different populations of *Melanophryniscus rubriventris* (sample sizes in parentheses). Total prey numbers and volume per population are based on pooled stomachs. Mean  $\pm$  1 SD and range (in parentheses) per individual's stomach are also indicated. Values of known alkaloid-containing preys are in bold.

	Los Toldos ( $n = 41$ )				Baritú ( $n = 37$ )				Angosto de Jaire ( $n = 60$ )			
	$n$	$v$	%	Ip	$n$	$v$	%	Ip	$n$	$v$	%	Ip
<b>Acari</b>	<b>964</b>	<b>429</b>	<b>77</b>	<b>36.36</b>	<b>241</b>	<b>107</b>	<b>81</b>	<b>31.8</b>	<b>510</b>	<b>193</b>	<b>82</b>	<b>37.73</b>
	<b>24 <math>\pm</math> 46</b>	<b>10 <math>\pm</math> 20</b>			<b>6 <math>\pm</math> 7</b>	<b>3 <math>\pm</math> 3</b>			<b>8 <math>\pm</math> 10</b>	<b>3 <math>\pm</math> 4</b>		
	<b>(0-214)</b>	<b>(0-95)</b>			<b>(0-34)</b>	<b>(0-15)</b>			<b>(0-44)</b>	<b>(0-16)</b>		
Collembola	2309	313	79	48.94	1121	149	40	33.19	558	107	82	38.51
	56 $\pm$ 138	8 $\pm$ 18			30 $\pm$ 92	4 $\pm$ 12			9 $\pm$ 14	2 $\pm$ 2		
	(0-812)	(0-106)			(0-381)	(0-49)			(0-72)	(0-10)		
<b>Coleoptera</b>	<b>134</b>	<b>349</b>	<b>65</b>	<b>27.31</b>	<b>119</b>	<b>314</b>	<b>87</b>	<b>33.55</b>	<b>91</b>	<b>260</b>	<b>67</b>	<b>27.99</b>
	<b>3 <math>\pm</math> 4</b>	<b>8 <math>\pm</math> 12</b>			<b>3 <math>\pm</math> 2</b>	<b>8 <math>\pm</math> 6</b>			<b>1 <math>\pm</math> 2</b>	<b>4 <math>\pm</math> 6</b>		
	<b>(0-14)</b>	<b>(0-60)</b>			<b>(1-12)</b>	<b>(0-23)</b>			<b>(0-7)</b>	<b>(0-28)</b>		
Hymenoptera												
<b>Formicidae</b>	<b>464</b>	<b>1405</b>	<b>91</b>	<b>42.81</b>	<b>718</b>	<b>2168</b>	<b>100</b>	<b>66.62</b>	<b>533</b>	<b>1625</b>	<b>80</b>	<b>50.41</b>
	<b>11 <math>\pm</math> 12</b>	<b>34 <math>\pm</math> 37</b>			<b>19 <math>\pm</math> 18</b>	<b>58 <math>\pm</math> 56</b>			<b>8 <math>\pm</math> 14</b>	<b>27 <math>\pm</math> 45</b>		
	<b>(0-48)</b>	<b>(0-146)</b>			<b>(1-100)</b>	<b>(3-304)</b>			<b>(0-102)</b>	<b>(0-311)</b>		
Insect larvae	337	1048	65	35.17	63	196	47	18.75	126	333	62	25.7
	12 $\pm$ 26	25 $\pm$ 69			3 $\pm$ 6	5 $\pm$ 13			3 $\pm$ 4	5 $\pm$ 9		
	(1-101)	(0-314)			(1-25)	(0-77)			(1-24)	(0-63)		

Monte Carlo bootstrap simulations. For more details on the methods and indices calculated, see Bolnick et al. (2002, 2007) and the available documentation for IndSpec 1.0 at <http://www.esapubs.org/archive/ecol/E083/056/default.htm>.

We found a total of 18, 31, and 32 prey categories in the diet of *Melanophryniscus rubriventris* and a range of 2-19 taxa per individual stomach (table 1). Hymenoptera (Formicidae), Acari, Collembola, Coleoptera, and larvae were the most important prey types in each population (table 1). Total niche width population values (Los Toldos TNW = 1.81; Baritú TNW = 1.40; Angosto de Jaire TNW = 2.03) indicate important number of prey categories consumed. We found evidence of significant levels of individual specialization in each population (Los Toldos  $V = 0.46$ ; Baritú  $V = 0.51$ ; Angosto de Jaire  $V = 0.52$ ) with an observed individual diet variation higher than expected by chance (all Monte Carlo simulations  $P < 0.001$ ; null  $V$  values: 0.16, 0.15, and 0.30 respectively). Three alkaloid-containing prey categories, Formicidae, Acari and Coleoptera were always dominant prey types in the diet based on measures of pooled stomach contents with

almost no differences in their order of importance (table 1). Considering levels of individual diet variation of this three prey categories we found evidence of low levels of individual specialization in each population (Los Toldos  $V = 0.34$ ; Baritú  $V = 0.20$ ; Angosto de Jaire  $V = 0.35$ ) but an observed individual diet variation higher than expected by chance (all Monte Carlo simulations  $P < 0.001$ ; null  $V$  values: 0.16, 0.11, and 0.18 respectively) indicating that individuals use limited sets of the entire range of alkaloid-containing preys consumed by the entire population.

The diet of all three populations of *Melanophryniscus rubriventris* that we analyzed contained several prey categories as also described by Bonansea and Vaira (2007). Diets of populations consisted of several common ground-dwelling arthropods but with different alternate prey items added at low frequencies by different individuals. Overall, there were no differences in resource use of the most common items. Formicidae, Acari, Collembola, Coleoptera, and larvae were clearly dominant prey types on the diet of each population reflect-

ing interpopulational-shared prey type preferences. Previous studies have shown that various species of poison frogs consume primarily ants and mites (Toft, 1980, 1981, 1995; Donnelly, 1991; Caldwell, 1996; Darst et al., 2005). Such ground-dwelling arthropods along with coleopterans are known to be potential sources of alkaloids to this and other similar poisonous anuran species (Saporito et al., 2004, 2007b, 2009; Clark et al., 2006; C. Heit, M.F. Quiroga, and M. Vaira, unpublished results). If foraging decisions of *Melanophryniscus rubriventris* are driven for the obligate acquisition of chemical defenses through diet (Daly, 2003), such functional constraints might limit the use of a wide range of prey types and we expect a relatively specialized diet in the species. However, alkaloids persist by years in frog skin (Daly et al., 1994); thus toxicity or noxiousness may not be tied permanently to a specific alkaloid-containing prey type. Therefore, toads that sample an array of prey types enhancing diet composition could maintain and possibly even increase the diversity of alkaloids in the skin, strengthening their chemical defense systems. Also, individuals might be more efficient by foraging in places that have a high density of prey types instead of spending too much time searching a particular alkaloid-containing prey. Diet composition in *Melanophryniscus rubriventris* might be regarded as a compromise between the demands of defense acquisition and nutrition. Subtle population level changes in the importance value of dominant prey categories might also be a result of spatial and temporal changes in the relative abundance or availability of prey items. Consumption could depend upon the distribution, density, and behavior of the prey. Some patchily aggregated prey such as ants, mites, and collembolans may have different encounter rates across years and sites (Levings and Windsor, 1984; Donnelly, 1991; Bull and Hayes, 2009).

Along with population consistency in the most common items in the diet composition, we found low but significant levels of indi-

vidual specialization both in general diet and in alkaloid-containing preys consumed by all populations showing that different individuals of *Melanophryniscus rubriventris* might have diets with different arrays of prey types. This implies that a “generalist” species could be composed of individuals consuming different sets of the entire range of prey available. Other studies on diet composition have associated the existence of a generalist population of individual specialists arising under conditions of both highly diverse and available prey assemblages (Bolnick et al., 2003; Costa et al., 2008), and such individual specialization has been reported in other anuran populations (Araújo et al., 2007). Levels of individual specialization in this toad might show a compromise of competition and the need to ingest some prey items to acquire chemical defense. If there are several alkaloid-containing prey sources available to ingest, toads may probably consume alternative preys leading to greater among individuals variation. Given that shifts in diet composition might have important implications for individual skin alkaloid profiles, detailed studies of individual diet variation are needed in poison anurans to better understand the consequences of foraging strategies on the defense acquisition of the species.

**Acknowledgements.** This work was done as part of a project supported by a research fellowship from Universidad Nacional de Jujuy to M.F.Q., and was supported by a Sector-UNJu grant # D-037. Permits for sample collection of the species were provided by Delegación Técnica de Parques Nacionales, Regional Noroeste and Dirección Provincial de Políticas Ambientales y Recursos Naturales, Jujuy. We are especially grateful to M. Akmentins and D. Torrejón for the help provided during fieldwork. We greatly appreciate the comments of M. Donnelly and an anonymous reviewer in an early version of the manuscript.

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Received: September 14, 2010. Accepted: November 22, 2010.