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Diet of critically endangered Valcheta frog, *Pleurodema somuncurense* (Anura: Leptodactylidae), in the Somuncura Plateau, Patagonia, Argentina

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Abstract. In this work, we studied the diet of *Pleurodema somuncurense* and assessed selection patterns based on resource availability of terrestrial and aquatic prey items. We discuss potential implications for the current conservation status of this species and for management actions conducted with this species. *Pleurodema somuncurense* is endemic to the Valcheta Stream, Somuncura Plateau, Patagonia, Argentina. The conservation status of this frog is Critically Endangered, according to the IUCN red list. Our analysis framework was based on assessing the diet composition in frogs through stomach flushing technique versus prey availability in the habitat. We also estimated the relative importance for each consumed prey. Of the 55 sampled individuals, we found identifiable contents in 47 stomachs. Our results showed that diet sample is composed of 179 prey items, most of them arthropods, including aquatic and terrestrial preys. The highest IRI was for Diptera (mostly from Family Tipulidae), Lepidoptera (Family Tineidae), Amphipoda (Family Hyalellidae), Araneae (mostly from Family Linyphiidae) and Isopoda (Family Armadillidiidae). The highest volumetric percentage was for Lepidoptera and Diptera. The use *versus* availability test showed active prey selection of *P. somuncurense* for Armadillidium isopods, and rejection of several prey items, particularly ants that were the most abundant terrestrial item. This dependence on terrestrial preys makes *P. somuncurense* susceptible to the indirect effect on land use that affect prey availability, at least on aestival months when diet samples were taken.

Key words: allochthonous items, coverage-based rarefaction, diet, prey selection, trophic niche.

Introduction

The Valcheta frog, *Pleurodema somuncurense* (Cei 1969), is endemic to the headwaters of the Valcheta Stream, one of the main watercourses of the Somuncura Plateau, Patagonia, Argentina. The conservation status of the Valcheta frog is of great concern since its spatial occupation along the stream and its shores is restricted to an extent of occurrence of 32 km^2, an area of occupancy of just 1.8 km^2, and the declining wild populations are isolated and facing several human-related threats (IUCN 2016). The main threats are degradation and loss of aquatic habitats due to current water operations (irrigation and dams), poor livestock management (bovine, ovine, equine and caprine), chytrid infection (Arellano et al. 2017), and the introduction of exotic predatory fishes (Menni 2004, Lavilla 2009). For this reason, specialists listed the Valcheta frog as "Endangered" at the national red list (Vaira et al. 2012) and as "Critically Endangered" at the IUCN red list (IUCN 2016). Despite its threat status, current knowledge on its natural history is still scarce and further knowledge is of key importance for the development of a Conservation Action Plan.

Feeding habits are one of the most relevant features of the natural history of a species, as it affects survival and regulates future recruitment, providing elements to take conservation and management decisions regarding an endangered species (Sanabria et al. 2005; Cuello et al. 2006, Lobos et al. 2016, Watson et al. 2017). Although there are some scattered observations related to the natural history of the Valcheta frog (Cei 1969, Diminich 2006), its feeding habits and prey preferences remain unknown.

Pleurodema somuncurense is an aquatic frog (Cei 1969a, 1969b, 1970), clearly differentiated from other species within the *Pleurodema thaul* clade, which are mostly terrestrial or semi-aquatic (Faivovich et al. 2012). Thermal waters of the Valcheta Stream allow these frogs to remain active throughout the year, even in the extremely cold winter of the Patagonian steppe (Cei 1980). Its aquatic life habit and the arid conditions of the surrounding environment led us to expect that aquatic invertebrates would be the basis of the diet of *P. somuncurense*, and that allochthonous preys (either terrestrial or flying) would be a secondary and opportunistic food item.

In this work, we analyzed the diet of *P. somuncurense* and assessed selection patterns based on resource availability of terrestrial and aquatic prey items. Finally, we discuss potential implications for its current conservation status and for management actions conducted with this species.

Material and Methods

We performed this study in the Northern part of the Somuncura Plateau, Rio Negro province, Argentina. The area belongs to the floors of the ravines or streams filtration described in Cei (1969a), with altitudes ranging from 500 to 800 m.a.s.l. The habitat is characterized by low and xerophytic vegetation, corresponding to the ecotone between the Monte and the Patagonia regions (Betinelli & Chebez 1986), which was called "Ecotono Rionegrino" by León et al. (1998). One of the main streams in this region is the Valcheta Stream, an endorreic watercourse that supports several endemic species (e.g. the Valcheta frog, the naked characin *Gymnocharacinus bergii* and two mollusks: *Heleobia rionegrina* and *Potamolithus valchetensi*). The Valcheta Stream, which has thermal waters, is 80 km long and is formed by the confluence of four major branches. The western pair is locally known as the Cold Branches (18-23 °C), while the eastern pair is called Hot Branches (20-26 °C). We developed Visual Encounter Survey (VES; Crump & Scott 1994) and surveys to account for prey availability (see below) at two parallel and narrow creeks (no more than 1,5 m width) of first order, separated by 500 m and located within the type locality of the species "Estancia El Rincón" (40° 59'26.89"S, 66° 40'37.07"W) which encompasses almost one-third of the known range of this species in the Cold Branch (Velasco et al. 2016).

We conducted a Visual Encounter Survey (VES; Crump & Scott 1994) by walking downstream from the thermal springs, covering a total of 400 m along the stream banks, between 21:00 and 24:00 hours during February and March 2015. We captured frogs manually and obtained the stomach contents by applying the technique of stomach flushing (Legler & Sullivan 1979, Solé et al. 2005). Before being released at the same capture site, we recorded sex (only recognizable in adults) and snout-vent length (SVL, with an accuracy of 1 mm) and we marked each individual with a unique code by using visible implanted elastomers, which allowed us sampling each frog just once. In this study we only considered adult frogs to avoid harming individuals during the stomach flushing process. We determined adults as those individuals with SVL higher than 28 mm that was the lower measure where secondary sexual traits were recognizable in males (nuptial pads, Cei 1980). We determined those individuals higher than 28 mm but without nuptial pads, as females. In some cases, we confirmed the sex of females by observing abdominal eggs against the light.

To account for prey availability, we synchronically applied complementary sampling methods to trophic ecology. For terrestrial items, we distributed a total of 25 pitfall traps, consisting of plastic dishes (20 cm diameter and 5 cm deep), with water and unscented detergent (1570 cm^3), located at 15 cm from the stream banks along the sampling transects. We cleared the traps once per day during fieldwork. For aquatic items, we made kick sampling, washing one square meter of substrate of stream at the same sites where terrestrial items were sampled. We fixed both sources of samples (stomach contents and preys' availability) in the field with 70 % ethanol and stored in independent plastic containers.

We analyzed the stomach contents under the stereomicroscope. We identified preys under level of Phyllum, in the case of Platyhelminthes and Nematomorpha; under the level of Order, in the case of Mollusca and Arthropoda (with exception of Acari, for which we considered a higher level) but larvae and worms were pooled into the category Larvae, under the assumption of no taxonomically selection of this organisms by frogs (Bonansea & Vaira 2007). We then evaluated the representativeness of the diet sample by constructing a coverage-based (species richness) rarefaction curve for incidence data (Chao & Jost 2012), using iNEXT package version 2.0.5 (Chao et al. 2016) in the program R (R Core Team 2015).

We measured length (L) and width (W) of each item to estimate the prey volume based on an ellipsoid (Dunham 1983). Then, the relationship between frogs' SVL and mean volume of preys were assessed with a linear regression and tested for significance using a Monte Carlo test. For estimation of the relative contribution of each prey item to the diet, we used the Index of Relative Importance (IRI) proposed by Pinka et al. (1971).

In order to assess the patterns of diet selection in the Valcheta frog, we used the models of use versus availability developed in Pledger et al. (2007). These models allow testing the preference for a specific resource based on a higher use than expected according to its availability, while rejection was considered as the opposite. Non-significant values mean that the target species uses a specific resource according to its availability. We developed null models to assess preferences, considering the alfa index of Manly as an indicator of use. For comparing observed *versus* expected values, we compared them with the dissimilarity index of Bray-Curtis. For the general test, we used a null model with a random distribution. Because significant differences between use and availability were observed, we applied *a posteriori* test to determine whether each prey item was used more or less than expected. This *a posteriori* test was based on the D index (Jacobs 1974), which measures the deviation between the alfa index of Manly and the random expected value. The selection of a particular prey item is considered as "preference" when $D > 0$, while as "rejection" when $D < 0$. Because we performed 21 consecutive tests (one for each prey item), we applied a sequential Bonferroni adjustment for p values (Holm 1979). For a detailed description of the methods used in this work, see Pledger et al. (2007).

To compare the diet between males and females we used: a) the Jaccard's coefficient to compare similarity between stomach contents, and; b) the zeros' randomization algorithm (RA3) to assess niche overlap (Winemiller & Pianka 1990, Gotelli & Graves 1996). We then compared SVL between males and females using a Monte Carlo simulation based on Student´s t distribution to analyze if different patterns could be attribute of sex or size. We also modeled the relationship between frogs' SVL and mean volume of preys with a linear regression and tested for significance using a Monte Carlo approach.

We performed most statistical tests and analyses using null models with the software EcoSim and Past 3. Uses versus availability models were also based on null models and we developed it manually in Excel (Microsoft® Office 2011). We run randomizations on a basis of 1,000 permutations and the significance level used for all statistics was 0.05. We presented all results as mean and standard deviation (SD).

Results

Of the 55 sampled individuals, we found identifiable contents in 47 stomachs. The mean of SVL in mm of all studied individuals (21 females, 24 males and 2 indeterminate individuals) was of 39.6 (1SD: 6.8). Diet samples were composed by 179 prey items, most of them arthropods, including aquatic and terrestrial preys (Table 1). The coverage-based rarefaction curve showed that the sampling effort was appropriate and reached a high degree of completeness for prey diversity items (Fig. 1). Frogs consumed 17 from the 22 available preys. Stomach contents also had mineral granules, ashes, plant debris (mainly leaves, stems and seeds) and mucous strings.

The Index of Relative Importance (IRI) evidenced the contribution of each prey to the diet. The highest IRI were for Diptera (mostly Family Tipulidae), Lepidoptera (Family Tineidae), Amphipoda (Family Hyalellidae), Araneae (mostly Family Linyphiidae) and Isopoda (Family Armadillidiidae). The highest volumetric percentage was for Lepidoptera and Diptera (Table 1).

The mean of preys by stomach was of 3.7 (1SD: 1.2), and the volumetric percentage of preys in mm^3 was of 6.2 (1SD: 12.3). We found a significant but moderate correlation between the SVL of frogs and the mean volume of preys ($rho_{45} = 0.33$, $p = 0.03$) with the following relationship SVL = 1.03 * prey volume + (-11.48) (Fig. 2).

The use versus availability test was significant, indicating a pattern of selectiveness that showed active prey selection by *P. somuncurense*, which preferred Armadillidium isopods, and rejected several prey items, particularly ants that were the most abundant terrestrial item (Fig. 3, Table 2).

Although males SVL (mean 34.6, 1SD: 4.2 mm) were significantly smaller than females SVL (mean 41.2, 1SD: 4.9 mm) ($t_{45} = 7.74$; $p < 0.05$), we did not find differences between their diets. The similarity index of Jaccard indicated a very similar diet between males and females (71%).

Table 1. Prey consumed (n = 176) by *Pleurodema somuncurense* N% = percentage of each item from all stomachs; F% = percentage of occurrence of each item from all stomachs; V% = volumetric percentage per item; IRI = Index of Relative Importance.

Prey items	N%	F0%	V%	IRI	IRI%
Terrestrial Items					
Insecta					
Coleoptera	3.35	4.40	0.40	31.94	0.67
Colembolla	1.12	2.20	0.01	4.76	0.10
Diptera	27.93	24.18	13.43	1936.35	40.39
Hemiptera	1.12	2.20	2.07	13.58	0.28
Hymenoptera (Formicidae)	3.35	6.59	0.92	54.53	1.14
Lepidoptera	7.82	10.99	49.35	1216.31	25.37
Odonata	0.56	1.099	0.01	1.21	0.03
Tricoptera	1.68	1.10	2.39	8.65	0.18
Chelicerata					
Acari	1.12	2.20	0.01	4.76	0.10
Araneae	10.06	13.19	4.49	371.48	7.75
Crustacea					
Isopoda	9.50	7.69	13.13	337.05	7.03
Mollusca					
Pulmonata	1.12	2.20	1.38	10.61	0.22
Aquatic Items					
Insecta					
Coleoptera	0.56	1.10	0.02	1.23	0.03
Larvae	8.94	6.59	0.44	119.75	2.50
Hemiptera	1.12	2.20	4.52	24.01	0.50
Crustacea					
Amphipoda	20.67	12.09	7.44	657.81	13.72

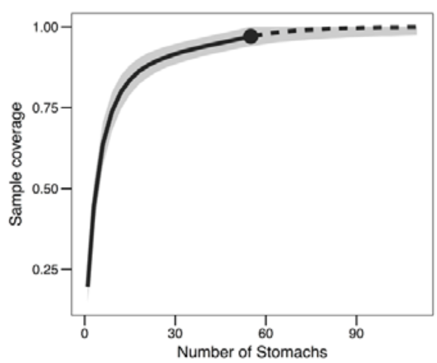

Figure 1. Coverage-based rarefaction (solid lines) and extrapolation (dashed lines) curves for prey diversity completeness (Hill numbers of order q = 0) of the analyzed stomachs of *Pleurodema somuncurense*. The 95% confidence intervals (gray area) were calculated based on 200 bootstrap replicates.

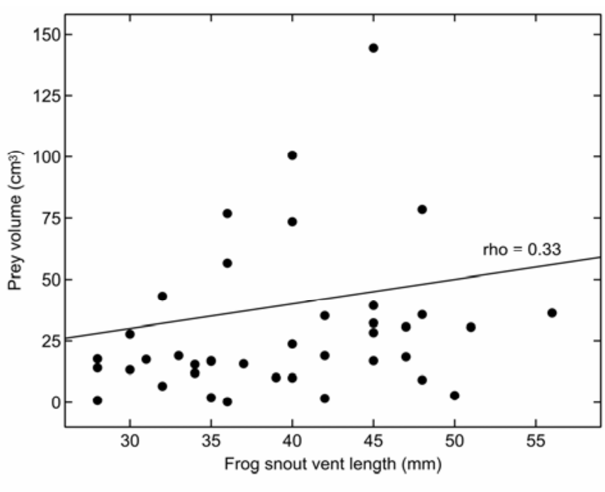

Figure 2. Regression curve between frogs' sizes and volumetric percentage of prey.

Table 2. Values of use (fr), availability (gr) and D index for each prey item (negative values indicate rejection and positive one selection). Significant values of p are remarked in bold.

Available items	fr	gr	D	p
Terrestrial Items				
Coleoptera	6	14	-0.03	0.13
Colembolla	2	81	-0.04	0.00
Diptera	50	238	-0.04	0.00
Hemiptera	2	12	-0.04	0.12
Hymenoptera (Formicidae)	6	513	-0.05	0.00
Hymenoptera (No Formicidae)	0	4	-0.05	0.00
Lepidoptera	14	18	-0.02	0.17
Odonata	1	1	-0.01	0.36
Orthoptera	0	6	-0.05	0.00
Tricoptera	3	1	0.07	0.05
Acari	2	1	0.03	0.21
Araneae	18	34	-0.03	0.04
Pseudoescorpionida	0	1	-0.05	0.00
Isopoda	17	3	0.61	0.00
Pulmonata	2	4	-0.03	0.38
Aquatic Items				
Coleoptera	1	23	-0.04	0.04
Larvae	16	110	-0.04	0.00
Hemiptera	2	25	-0.04	0.03
Amphipoda	37	253	-0.04	0.00
Pulmonata	0	1	-0.05	0.00
Nematomorpha	0	3	-0.05	0.00
Tricladida	0	20	-0.05	0.00

Indeed, the RA3 algorithm indicated a high overlap of trophic niches (87%), with no difference between the diet of males and females ($p > 0.05$).

Discussion

The present work represents the first study on feeding habits of the Valcheta frog. Overall, our results showed that *P. somuncurense* has a broad trophic niche, consuming nearly 80% of the potential prey items registered. Most preys were consumed according to their availability, although a clear pattern of selectivity was observed for some items, with a preference for woodlice and avoidance of ants. Although *P. somuncurense* has aquatic life habits, aquatic preys represented only a quarter of its diet, exhibiting a high dependence on allochthonous food items, in contrast to our predictions.

Plant debris represented a small percentage of the frog´s stomach contents (24%), and we consider that this item is eaten at random while they prey on animals that are settled on plants (Díaz-Páez & Ortiz 2003). The presence of ashes in some of the stomachs is a human consequence of deliberate fires on grasslands, aimed at promoting regrowth of pastures for livestock management. These fires have a

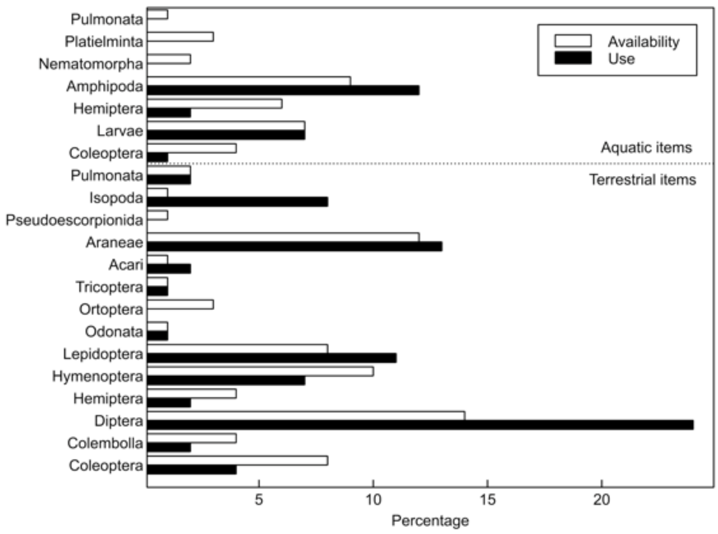

Figure 3. Percentage of available items and items consumed by frogs.

negative impact on frog´s habitats, reducing the availability of shelters, reproductive sites, and terrestrial preys.

We were able to observe some of the frogs' foraging behavior: most of the individuals were observed ambushing the preys semi-submerged in the emergent vegetation, and only a few individuals were seen out of the water, although close to the stream banks. Based on these observations and on the consumption of preys according to their availability, we inferred that individuals mostly exhibit a non-restricted sit-and-wait behavior, which can turn into an active search for gregarious and aquatics preys like amphipod shrimps. This hypothesis is supported by observations of individuals in captivity, which were sometimes observed actively searching for their preys, and eating completely submerged while gathering the prey into their mouth with their forearms (Calvo, pers. comm. 2016).

The dependence of the Valcheta frog on allochthonous preys contrasts with the diet of other species. For example, the aquatic form of *Atelognathus patagonicus* preys exclusively on aquatic items and has a strong specialization on amphipods shrimps of the genus *Hyalella* (Cuello et al. 2006). Conversely, *Pleurodema somuncurense* showed a similar diet pattern to the semi-aquatic *P. thaul*, evidencing a phylogenetic trend (Díaz-Páez & Ortiz 2003). However, its sister taxon *P. bufoninum* show a high preference of ants, whilst *P. somuncurense* showed a clear avoidance, suggesting a strong difference in trophic ecology between the two species (Pincheira-Donoso 2002, Bello et al. 2005). The predominance pattern of aquatic or allochthonous (either terrestrial or flying) preys in the diet vary among Neotropical aquatic frogs. Pipid and telmatobid frogs feed primarily on aquatic preys and have a specialized suction mechanism to capture preys underwater (Carreño & Nishikawa 2010, Barrionuevo 2016). On the contrary, hylid frogs of the genera *Lysapsus* and *Pseudis* prey mostly on terrestrial invertebrates (Duré & Kehr 2001, Brandão et al. 2003, Teixeira et al. 2004, Garda et al. 2007, Downie et al. 2010). Likewise, *P. somuncurense* is mainly a sit-and-wait predator without a specialized mechanism for feeding underwater. This dependence on terrestrial preys makes *P. somuncurense* susceptible to land use practices that affect prey availability, at least on aestival months, when the diet samples were taken.

Since anuran assemblages from the Patagonia steppe are composed of only a few species, it will be interesting to determine the degree of trophic niche overlap between *P. somuncurense* and other two species that are present in the study area, i.e. *Rhinella arenarum* and *Odontophrynus occidentalis*. In the case of *R. arenarum* (argentine common toad), which is the most abundant species in the stream, it has been observed mostly preying on isopods (Attademo et al. 2007, Cossovich et al. 2011), therefore, some overlap with *P. somuncurense* is expected, at least in relation with this item.

Studies on trophic ecology are of key relevance for the preservation of endangered species (Lobos et al. 2016, Watson et. al. 2017) and have a direct application in conservation actions. In the case of *P. somuncurense*, this information has served as a basis for improving the conditions of an *ex-situ* colony of the species for conservation purposes. At the same time, our results will help warning about the negative effects of the current land use practices over native species in the study area. For example, overgrazing and intentional fires for promoting pasture renewal might have direct effects on the availability of preys. Currently, as part of the *in situ* conservation actions for *P. somuncurense*, experimental management actions are being carried out in Estancia El Rincón. These actions consist in the exclusion of livestock and the removal of the invasive exotic rosehip plant (*Rosa* sp.), in order to restore and avoid further degradation of the habitat where the frogs live. Indirect effects such as a potential competition for trophic resource between Valcheta frog and the exotic fishes *Onchorhynchus mikys* and *Cheirodon interruptus* should be evaluated in further studies.

Finally, to get a more comprehensive picture of the possible overlap of trophic niches, it will be interesting to repeat the diet analysis during winter months. In this sense, we will be able to determine if there is a switch in the predominance of aquatic items when there is a decline in the abundance of terrestrial preys during the cold winter of the Patagonian steppe.

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