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On the feeding ecology of *Pelophylax saharicus* (Boulenger 1913) from Morocco

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Abstract. The Sahara frog is the most common amphibian found in North Africa. However, the knowledge of its natural history is rather fragmentary. In the present work we studied the trophic ecology of *Pelophylax saharicus* at some areas of Morocco through the analysis of 130 gastric contents. We did not find any significant sexual dimorphism in body size of adult individuals. Consumed prey show similar sizes in both sexes, while bigger frogs normally eat larger prey. As in other Palearctic frogs, the diet is basically insectivorous, including terrestrial and aquatic prey. We found some differences in the diet of juveniles, with a higher proportion of flying prey, probably indicating a foraging strategy closer to ambush hunting. In the Atlas region, the high consumption of slow-moving terrestrial prey, as Gastropoda, stands out. Only in the Atlas region, the diet was similar to that described from other areas of North Africa, as Tunisia.

Keywords. Trophic ecology, Ranidae, Green frogs, Morocco, *Pelophylax saharicus*.

The Sahara frog, *Pelophylax saharicus,* inhabits a large portion of North Africa, from South Sahara to the Mediterranean coast, through the Atlas Mountains (Pasteur and Bons, 1959; Amor et al., 2010), living at altitudes of more than 2600 m.a.s.l. Its distribution ranges from Morocco to Egypt, being the most common green frog of North Africa (Salvador, 1996; Amor et al., 2010). The species is strictly aquatic and is found both in natural and artificial permanent ponds, even when these are slightly eutrophized (Salvador, 1996).

Pelophylax saharicus is currently considered a full species, following Bons and Géniez (1996) that summarized the discussion about the controversial status of Moroccan green frogs. Molecular studies support the specific status of *P. saharicus* (Plötner, 1998; Frost et al., 2006; Lymberakis et al., 2007; Lansari et al., 2015; Nicolas et al., 2015), ranging it as the sister group of *Pelophylax perezi*, apart from the other species of the genus. Both males and females reach the sexual maturity in the second year of life, and are able to live as much as six years (e.g. Oromi et al., 2011). Previous studies concluded that *P. saharicus* does not show sexual dimorphism in the size of adult animals (Esteban et al., 1999).

In a preliminary study of feeding ecology of Palearctic frogs, Smith (1951) described the diet of *Rana ridibunda ridibunda.* Then, Lizana et al. (1989) compared the feeding ecology of *P. perezi* with other Iberian amphibians and with trophic availability at an area of the central Iberian Peninsula. Subsequent studies have addressed the feeding ecology of other *Pelophylax* species (Çiçek et al., 2006; Sas et al., 2009; Mollov et al., 2010; Paunović et al., 2010; Bogdan et al., 2012, 2013; Plitsi et al., in press). A recent study assesses the effect of temperature, density and food in the growth and metamorphosis of *P. saharicus* tadpoles (Bellakhal et al., 2014). Regarding its trophic ecology, some data have been published about

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P. saharicus in the oases of Kettana, in Tunisia (Hassine and Nouira, 2009). Here we present the first data about trophic ecology of the Sahara frog in Morocco.

All samples used in this study came from Moroccan areas within the semi-arid Mediterranean zone of North Africa (Le Houéron, 1989). Frogs were captured during 1996 in three different areas of Morocco: (1) The Western Plateau, an area of subhumid to semiarid climate and two localities were sampled: El Borj and Zwiat Cheikh, (2) Rif Mountains and adjacent areas (this is the most humid area of Morocco with more than 600 mm of annual rainfall), and (3) The Middle Atlas, with a climate of strong continental characteristics. Sample sizes were 9 males, 4 females and 5 juveniles for the Western Plateau, 26 males, 52 females and 6 juveniles for the Rif Mountains, and 11 males, 16 females and 1 juvenile for the Middle Atlas.

Frogs were euthanized during the field work because they were captured to study helminthic parasites in the framework of a parasitological research (see Navarro and Lluch, 2006). Maturity and sex of the individuals were determined by direct examination of the gonads after dissection. The analysis included 130 gastric contents. Prey items were identified to Family or Order level. Prey size was measured from intact items with a micrometric ocular. Afterwards, absolute frequencies of each prey type and its percentage in the diet were calculated for each region, as well as the number of gastric contents in which such prey was present.

We used Spearmann rank correlation and ANCOVA on prey size, with SVL (snout-vent length) as a covariate, to study the relation between body length of frogs and the size of consumed prey for each category (adult males, adult females, and juveniles). Then, we estimated and compared diet diversities using the approach proposed by Pallmann et al. (2012). Instead describing diet diversity through a given index as, for example, Simpson or Shannon indices, we converted these "raw" indices into "true" diversities. That is, regarding different measures as special cases of Hill's general definition of diversity measures (Hill, 1973). To study differences in diversity between males, females and juveniles, we performed two-tailed tests for integral Hill numbers of orders $-1 \leq q \leq 3$. This selection includes the transformed versions of the three following indices: the species richness index, H_{sr} ($q = 0$), the Shannon entropy index, H_{sh} ($q \to 1$) and the Simpson concentration index, H_{si} ($q = 2$). All comparisons among diversities of groups were made with Tukey-like contrasts employing a resampling procedure. We did 5000 bootstrap replications so as to obtain reliable p-values (Westfall and Young, 1993). Methods described here are implemented in R package "simboot" (Scherer and Pallmann, 2014) and are fully described in Pallmann et al. (2012). All calculations were done in R version 3.0.3 (R Core Team, 2014). Finally, in order to visualize differences in the composition of the diet of adults of both sexes and juveniles, we conducted a discriminant function analysis. Box's M test of equality of covariance matrix was not significant, so data were suitable for discriminant analysis. Only two variables (Dictyoptera larvae and Dermaptera larvae) failed the tolerance test, so were excluded from the analysis, the rest of the variables (Table 3) were suitable for analysis (tolerance test with $P > 0.05$).

The diet of *P. saharicus* was mainly insectivorous and more varied in females than in males or juveniles. But, we did not find significant differences in the diversity values of males, females and juveniles ($P > 0.05$ in all pairwise comparisons, Table 2). Diptera were the most important prey item. The diet of juvenile individuals, principally dominated by Formicidae and other small Hymenoptera, was less diverse than that of adult males and females.

We observed a high proportion of Hymenoptera in the diet of Western Plateau frogs, much higher than for Tunisian populations (Hassine and Nouira, 2009). This is principally due to the massive presence of this prey in five juvenile individuals, in which we found 95.58% (65 of 68 prey items) of all sampled Hymenoptera. In addition, all adult individuals of *P. saharicus* from the Plateau ate proportionally more Hymenoptera than those from the Atlas or Rif regions, suggesting a greater availability of such prey at the Plateau. Alternatively, these differences can be due to a different foraging behaviour in different areas. According to our results, there is no sexual dimorphism in adult individuals of *P. saharicus* (see also Esteban et al., 1996; Meddeb et al., 2007).

SVL of juveniles was 51.00 ± 2.00 mm (mean ± SE, n = 8). We did not find significant differences in body size of adult males and females of *P. saharicus* (one-way ANOVA of log-transformed data, $F = 0.304$, $P = 0.583$, homogeneous variances, Levene test, $P = 0.90$; SVL of adult males, mean = 88.43 ± 4.96 mm, range = 48-97 mm, n = 44; adult females, mean = 92.51 ± 4.40 mm, range = 48-223, n = 74), even if females were slightly larger than males. We measured 803 prey items (mean = 5.26 ± 0.18 mm, range = 0.5-70 mm). We found a significant correlation between frog body size (SVL) and prey size (Spearmann Rank correlation, $R_s = 0.510$, $P < 0.001$, n = 803). This correlation was also maintained within adult individuals ($R_s = 0.399$, $P < 0.001$, n = 695; mean = 5.68 ± 0.20 mm, range = 0.5-70 mm). According to this result, we analysed the prey size in both genders employing the SVL as the covariate. Adult females ate prey of significantly larger size than adult males (ANCOVA analysis, $F = 4.816$, $P = 0.029$, with no significant differences in regression slopes, $F = 1.055$, $P = 0.305$; mean = 5.04

Table 1. Data from the analysis of 130 stomach contents of *Pelophylax saharicus*. F_i is the absolute frequency of each type of prey item group in the sample, % F_i the relative frequency of the group in the sample, P is the presence of each group (i.e. the number of stomach contents in which the group appears), and % P the percentage of the presence of the item in the sample.

Group	Total				Juveniles				Adult males				Adult females			
	F_i	% F_i	P	% P	F_i	% F_i	P	% P	F_i	% F_i	P	% P	F_i	% F_i	P	% P
Gastropoda	48	5	21	16.2	2	1.74	2	18.18	9	2.90	6	13.64	37	6.93	13	17.57
Araneae	21	2.18	17	13.1	0	0	0	0	13	4.19	11	25	8	1.50	6	8.11
Acarina	2	0.21	1	0.8	2	1.74	1	9.09	0	0	0	0	0	0	0	0
Ostracoda	5	0.52	2	1.5	0	0	0	0	0	0	0	0	5	0.94	2	2.70
Isopoda	7	0.73	6	4.6	0	0	0	0	1	0.32	1	2.27	6	1.12	5	6.76
Crustacea	1	0.1	1	0.8	0	0	0	0	1	0.32	1	2.27	0	0	0	0
Diplopoda	1	0.1	1	0.8	0	0	0	0	1	0.32	1	2.27	0	0	0	0
Chilopoda	6	0.62	3	2.3	0	0	0	0	0	0	1	2.27	5	0.94	2	2.70
Diplura larvae	1	0.1	1	0.8	1	0.87	1	9.09	1	0.32	0	0	0	0	0	0
Thysanura	2	0.21	2	1.5	0	0	0	0	1	0.32	1	2.27	1	0.19	1	1.35
Odonata	1	0.1	1	0.8	0	0	0	0	1	0.32	1	2.27	0	0	0	0
Ephemeroptera	9	0.94	1	0.8	0	0	0	0	9	2.90	1	2.27	0	0	0	0
Plecoptera	4	0.41	4	3.1	0	0	0	0	1	0.32	1	2.27	3	0.56	3	4.05
Plecoptera larvae	6	0.62	4	2.3	0	0	0	0	2	0.64	2	4.54	4	0.75	2	2.70
Orthoptera	28	2.92	21	16.2	0	0	0	0	5	1.61	5	11.36	23	4.31	16	21.62
Orthoptera larvae	1	0.1	1	0.8	0	0	0	0	1	0.32	1	2.27	0	0	0	0
Dictyoptera	16	1.66	5	3.8	2	1.74	1	9.09	2	0.64	2	5.54	12	2.25	2	2.70
Dictyoptera larvae	1	0.1	1	0.8	0	0	0	0	1	0.32	1	2.27	0	0	0	0
Dermaptera	4	0.41	4	3.1	0	0	0	0	1	0.32	1	2.27	3	0.56	3	4.05
Dermaptera larvae	1	0.1	1	0.8	0	0	0	0	1	0.32	1	2.27	0	0	0	0
Phasmida	7	0.73	1	0.8	0	0	0	0	0	0	0	0	7	1.31	1	1.35
Embioptera	1	0.1	1	0.8	0	0	0	0	0	0	0	0	1	0.19	1	1.35
Thysanoptera	4	0.41	4	3.1	2	1.74	2	18.18	0	0	0	0	2	0.37	2	2.70
Homoptera	28	2.92	25	19.2	1	0.87	1	9.09	12	3.87	12	27.27	15	2.81	12	16.22
Homoptera larvae	3	0.31	3	2.3	0	0	0	0	2	0.64	2	4.54	1	0.19	1	1.35
Heteroptera	45	4.69	29	22.3	2	1.74	2	18.18	20	6.45	12	27.27	23	4.31	15	20.27
Heteroptera larvae	1	0.1	1	0.8	0	0	0	0	1	0.32	1	2.27	0	0	0	0
Diptera	245	25.52	87	66.9	18	15.65	7	63.64	97	31.29	33	75	129	24.16	47	63.51
Diptera larvae	19	1.98	14	10.8	1	0.87	1	9.09	5	1.61	4	9.09	13	2.43	9	12.16
Trichoptera larvae	1	0.1	1	0.8	0	0	0	0	0	0	0	0	1	0.19	1	1.35
Lepidoptera	9	0.94	7	5.4	0	0	0	0	5	1.61	4	9.09	4	0.75	3	4.05
Lepidoptera larvae	1	0.1	1	0.8	0	0	0	0	0	0	0	0	1	0.19	1	1.35
Coleoptera	149	15.52	65	50	6	5.22	3	27.27	50	16.13	23	52.27	93	17.42	39	52.70
Coleoptera larvae	29	3.02	11	8.5	6	5.22	3	27.27	2	0.64	2	4.54	21	3.93	6	8.11
Hymenoptera	155	16.14	55	42.3	68	59.13	8	72.73	27	8.71	17	38.64	60	11.24	30	40.54
Formicidae	74	7.71	42	32.3	2	1.74	2	18.18	30	9.68	15	34.09	42	7.86	25	33.78
Undet. Arthropoda	5	0.52	5	3.8	0	0	0	0	1	0.32	1	2.27	4	0.75	4	5.40
Undet. Larvae	18	1.87	14	10.8	2	1.74	2	18.18	7	2.26	6	13.64	9	1.68	6	8.11
Birds	1	0.1	1	0.8	0	0	0	0	0	0	0	0	1	0.19	1	1.35
Total	960	130			115	11			310	44			534	74		

± 0.24, n = 268 for adult males, and mean = 6.08 ± 0.29 mm, n = 427 for adult females).

For the discriminant analysis, the correlations between the variables and the two discriminant axes are provided in Table 3. The discriminant function is able to correctly classify the 64.5% of individuals as adult males, adult females or juveniles according to their diet, so the goodness of fit is acceptable. Differences in the diet of

Table 2. Simpson's diversity values of the diet of males, females and juveniles of *P. saharicus* and p-values from pairwise comparisons of Hill's numbers (see more details in the text)

	Adult males	Adult females	Juveniles
diversity values	$0.8521 \pm 1.80 \times 10^{-4}$	$0.8818 \pm 5.51 \times 10^{-5}$	$0.6273 \pm 2.19 \times 10^{-3}$
Hill's numbers	males-females	females-juveniles	juveniles-males
q = 0	0.8398	0.9134	0.6960
q = 1	0.7928	0.7014	0.4258
q = 2	0.8300	0.7280	0.4742

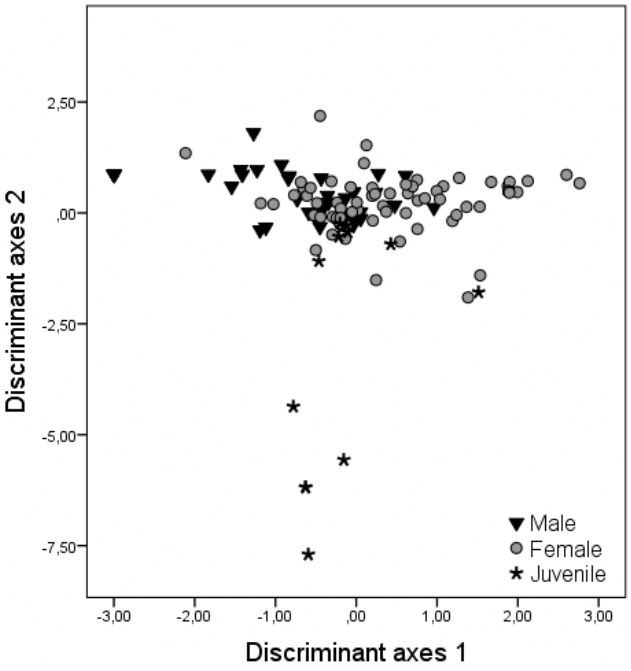

Fig. 1. Values of each dimension selected in the discriminant function analysis of the diet of *Pelophylax saharicus* are plotted for each studied frog. Individuals are marked regarding age and sex in order to visualize the age and sex differences in the trophic ecology of the Sahara frog.

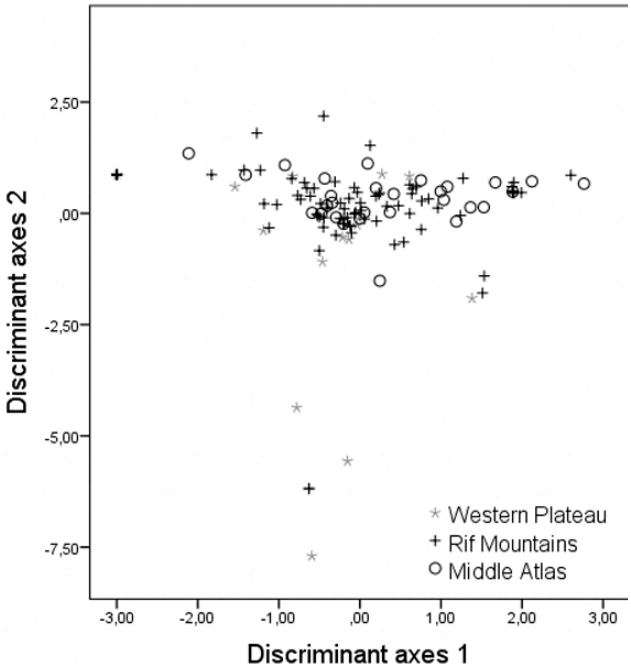

Fig. 2. Values of each dimension selected in the discriminant function analysis of the diet of *Pelophylax saharicus* are plotted for each studied frog. Individuals are marked regarding the area of study: the Western Plateau, the Rif Mountains, and the Middle Atlas.

males, females and juveniles are plotted in Figure 1. On one hand, the discriminant axes 1 somewhat divides diet of males (negative values) from diet of females (positive values), and it is mainly positively correlated with the presence of Formicidae and Coleoptera, and negatively correlated with the presence of Hymenoptera, larvae of Diplura, Acarina and Tysanoptera (Fig. 1, Table 3). On the other hand, the discriminant axes 2 divides the diet of juveniles (negative values) from the diet of adults (positive values), and it is mainly positively correlated with the presence of Orthoptera, Gastropoda, larvae of Isopoda, Ostracoda and larvae of Coleoptera, among others, and mainly negatively correlated with the presence of Araneae, Ephemenoptera, larvae of Orthoptera larvae, Diplopoda, larvae of Dermaptera, or larvae of Dictyoptera, among others (Fig. 1, Table 3). Regarding the area of study, we did not detect with the discriminant analysis any clear pattern in the composition of the diet (Fig. 2).

The diet of juvenile individuals is clearly different, being less diverse than the diet of adults (Table 2). Young frogs use to hunt smaller prey than adults, mainly small Hymenoptera. Hirai and Matsui (1999) found a significant correlation between SVL and prey size of *Pelophylax nigromaculatus*, as we observed in *P. saharicus*, suggesting that individuals of green frogs tend to eat larger prey as they grow.

Table 3. Pooled values of within-groups correlations between the discriminating variables (the prey items) and the standardized canonical discriminant functions (the two discriminant axes). Discriminating variables are ordered by absolute size of correlation within the discriminant axes 1.

Group	Discriminant axes 1	Discriminant axes 2
Hymenoptera	-0.481*	-0.019
Diplura larvae	-0.302*	-0.043
Acarina	-0.302*	-0.043
Thysanoptera	-0.281*	0.087
Formicidae	0.119*	-0.059
Coleoptera	0.088*	0.052
Plecoptera larvae	0.050*	0.030
Araneae	0.144	-0.319*
Orthoptera	0.109	0.280*
Gastropoda	0.049	0.211*
Ephemenoptera	0.041	-0.200*
Orthoptera larvae	0.041	-0.200*
Diplopoda	0.041	-0.200*
Dermaptera larvae[a]	0.041	-0.200*
Dictyoptera larvae[a]	0.041	-0.200*
Crustacea	0.041	-0.200*
Odonata	0.041	-0.200*
Heteroptera larvae	0.041	-0.200*
Isopoda larvae	0.061	0.188*
Ostracoda	0.032	0.174*
Homoptera larvae	0.061	-0.159*
Lepidoptera	0.084	-0.140*
Coleoptera larvae	-0.084	0.140*
Diptera	0.049	-0.139*
Undet. Arthropoda	0.060	0.137*
Lepidoptera larvae	0.023	0.125*
Embioptera	0.023	0.125*
Trichoptera larvae	0.023	0.125*
Phasmida	0.023	0.125*
Bird	0.023	0.125*
Diptera larvae	0.076	-0.119*
Diplura larvae	0.040	0.110*
Chilopoda	0.037	0.101*
Homoptera	0.093	-0.095*
Dictyoptera	-0.020	0.090*
Plecoptera	0.055	0.089*
Dermaptera	0.055	0.089*
Undet. Larvae	-0.018	-0.069*
Thysanura	0.045	-0.052*

* Largest absolute correlation between each variable and any discriminant function

[a] This variable not used in the analysis.

Pelophylax saharicus has a similar feeding ecology composition that its sister taxon, *P. perezi,* from the Iberian Peninsula (Lizana et al., 1989), and other species of the genus, as *P. ridibundus* (Çiçek et al., 2006; Mollov et al., 2010). Diptera predominates as the main prey item of adult individuals of both species, followed in abundance by Coleoptera prey (aquatic species mostly) and Hymenoptera, often Formicidae. The diet of *P. saharicus* in Morocco has some differences with the diet of other species of *Pelophylax*, as *P. kurtmuelleri* in Greece, which actively selects arachnids over other types of prey (Plitsi et al., in press). Nonetheless, we lack data about availability of prey in the habitat of Sahara frogs, which limits our results. Thus, our results about the differences in the diet of sexes and ages should be taken with caution, since it is possible that the electability of each type of prey would be similar to their availability in the environment. Therefore, future research in the diet of *P. saharicus* frogs, including the availability of prey in their habitats and seasonal comparisons would be useful to get deeper knowledge about the ecology of the species.

Furthermore, the diet of *P. kurtmuelleri* frogs is highly influenced by their habitat (Plitsi et al., in press), and the diets of *P. ridibundus* and *P. esculenta* are also influenced by seasonality (Sas et al., 2009; Mollov et al., 2010) and weather conditions (Bogdan et al., 2012). Thus, we cannot exclude that *P. saharicus* could also employ a variable foraging strategy.

Lizana et al. (1989) observed that the females of the Iberian green frog ate significantly larger prey than adult males, as we observed in *P. saharicus*. The ingestion of larger prey by females and their more diverse diet could be the reason of the slightly bigger parasite load of this sex. In this sense, and working with the same frogs, Navarro and Lluch (2006) found that females showed more diverse helminth infracommunities, even if differences with males were not statistically significant. The inclusion of a large amount of flying prey in the diet reinforces the hypothesis that *P. saharicus* is a sit-and-wait forager. Gastropoda were only important in the Rif sample, with a similar proportion as the reported for the Tunisian studied population (Hassine and Nouira, 2009). In *P. ridibunda* of Turkey no differences of diet regarding sex were found (Çiçek et al., 2006).

The consumption of Formicidae is not higher in *P. saharicus* than in *P. perezi* of the Iberian Peninsula, and it is consistent with the diet of the Tunisian population (Hassine and Nouira, 2009). The consumption of ants and other prey groups could be due to a foraging behaviour near the water or at more terrestrial habitats. In fact, many rivers and natural ponds of Morocco scarcely have riverside edges, forcing the individuals to stand close to water shore. Sas et al. (2009) found that *P. esculenta* of Romania changes the proportion of aquatic and terrestrial preys along the year activity period, which, although unknown yet, would be also possible for *P. saharicus* of Morocco.

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