

Trophology of Levant Green Frog, *Pelophylax bedriagae* (Amphibia: Anura: Ranidae) in Choram Township, Iran

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Abstract. A total number of 181 specimens of *Pelophylax bedriagae* were caught in 2017 (summer and autumn) and 2018 (spring) to investigate their stomach contents. Stomach flushing technique was used to obtain stomach contents followed by measuring and recording their dimensions using a digital caliper to the nearest 0.01 mm. Numerous ecological indices were used to compare the stomach contents between male and female individuals as well as across three different seasons. 209 out of 1148 prey items were unidentifiable due to advanced stages of digestion. The identified preys belong to 22 orders of which only two (Crustacea and Gastropoda) were aquatic. Gastropoda, Hymenoptera, Coleoptera, and Diptera were respectively the most frequently eaten prey items by this species in three seasons combined. Some prey items such as Crustacea, Diplopoda, and Isopoda were eaten only by females while Siphonaptera and Opiliones were only consumed by males. Number and volume of food items per stomach ranged from 1 to 178 (7.6 ± 16.8) and 0.08 mm^3 to 5577.2 mm^3 ($206.8 \pm 590 \text{ mm}^3$), respectively. The most abundant food items in both sexes were Hymenoptera, Gastropoda, and Coleoptera, respectively. The volume of consumed food in the three seasons combined was not different between sexes. The number and volume of food items were greater in autumn relative to the other two seasons. Neither food numbers nor food volume per stomach was significantly different between the two sexes in the three seasons combined. The number and volume of food items did not increase as the size of frogs increased. *Pelophylax bedriagae* is a generalist feeder. Hymenoptera and Coleoptera are constant food categories. Approximately 3/4 of food composition is of terrestrial species and 1/4 of aquatic origin. Some ecological factors like habitat consistency and food abundance play an important role in determining the diet of this species.

Key words: Srfaryab, food item, ecology, feeding habit.

Introduction

Levant Green Frog, *Pelophylax bedriagae* (Camerano, 1882) is a widely distributed amphibian species, recorded from Egypt, Greece, Israel, Jordan, Lebanon, Syria, Turkey (AmphibiaWeb 2019), and recently in Iran (Pesarakloo et al. 2017) and Bulgaria (Lukanov et al. 2018). The water frogs in Iran are divided into two different lineages: a clade comprising populations in northwest, west, and southwest belonging to *Pelophylax bedriagae* and northern and northeastern populations comprising *P. bedriagae* as well as *Pelophylax* sp. (Pesarakloo et al. 2017).

Amphibians are mostly opportunistic generalist feeders, but their feeding behavior is affected by extrinsic (food availability) and intrinsic (e.g. body size and shape of the skull) factors (Sugai et al. 2012). Amphibians primarily consume arthropods, snails, annelids and even small vertebrates and differentiate among different types of prey (Freed 1982). Numerous factors such as habitat (Khonyakina 1961, Rodionenko 1972, Makarov & Astradamov 1975, Kuzmin 1985), season (Aleinikova & Utrobina 1951, Markuze 1964, Verzhutzky 1978) and time of day (Rodionenko 1972, Tarkhnishvili & Kuzmin 1987) affect the diet composition of amphibians. Feeding rate of amphibians is highest during larval stages and lowest during metamorphosis period. Reproduction can be regarded as a potential cause for higher feeding activity rate in female compared to male amphibians (Kuzmin 1989). Due to their role in population control of many invertebrates and also as potential prey for many predator organisms, amphibians have an important ecologic position in many aquatic and terrestrial ecosystems (Vitt & Caldwell 2013). Understanding and identifying constant prey items, seasonal differences in diet composition and preference degree over numerous prey items are very im-

portant criteria in recognition of diet composition of amphibians. Such data about diet composition are vital to understanding the longevity of amphibians, population alterations, impacts of habitat changes on amphibians and development of conservation strategies (Anderson 1991).

The current study was performed to determine if: a) diet composition of male and female of this species differed, b) seasons affect diet composition and c) frog size affects volume and number of prey items consumed.

Material and Methods

Study area. This study was conducted in paddy fields of Sarfaryab region ($30^{\circ} 47' 48'' \text{N}$, $50^{\circ} 51' 17'' \text{E}$), Choram township, Kohgiluyeh & Boyer-Ahmad province, southern Iran (in this area, *P. bedriagae* is the only representative of the genus *Pelophylax* according to Pesarakloo et al. 2017). The study area lies in a valley encircled by sparse oak forests of the Zagros Mountains which is characterized by constant water resources originating from permanent springs. In this area rice is cultivated almost every year, lasting from spring to autumn (Fig. 1).

Sampling. All specimens were caught by hand and their sex was determined based on the presence of vocal pouches in males and their absence in females as well as the presence of digital pads in male specimens in the breeding season (Fathinia et al. 2011). Prior to flushing their stomachs, frogs were measured from snout-to-vent using a digital caliper with an accuracy of 0.01 mm. Stomach flushing was performed in the field immediately following the sampling of specimens in summer (28 females, 31 males) and in autumn (45 females, 32 males) 2017 and in spring (27 females, 18 males) 2018. The procedure described by Solé et al. (2005) was followed to obtain stomach contents. The water of the places where the frog lived was used to perform stomach flushing. The specimens were released in their habitat immediately after flushing their stomachs. Stomach contents were identified in Yasouj University zoology lab under an Olympus

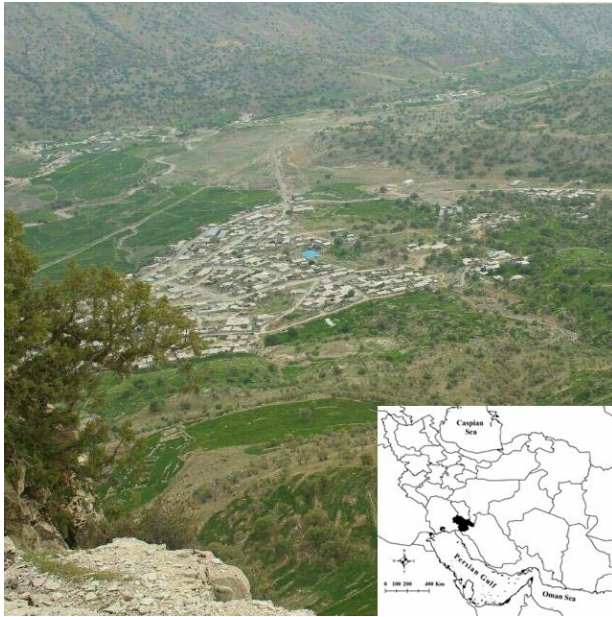


Figure 1. A total view of the study area (Sarfarayab region) within Oak forests of the Zagros Mountains in Choram Township, Kohgiluyeh and Bouyer-Ahmad Province (indicated in black within the Map), Iran.

stereomicroscope to the order rank using the information provided in Gillott (2005) and Hickman et al. (2001).

Ecological indices. We used the formula of $100n/N$, where n is the number of stomachs containing food and N is the total number of examined stomachs, to calculate the rate of feeding activity. We used Lehner's formula (Lehner 1998) $Q = 1 - N_i/I$ to determine sampling adequacy. Level of food specialization for each sex was calculated according to the Berger-Parker Index of Dominance ($d = n_i \max/N_i$; (Magurran 1988)). The value of this index changes from $1/N$ to N . Specialized feeders have values closer to 1 while general feeders have values closer to $1/N$.

The diversity of stomach contents was determined by the Shannon-Wiener index $[H = -\sum(n_i/N) \log_2(n_i/N)]$.

The volume of each stomach component was counted using the formula of prolate ellipsoid $[V = 4/3\pi(1/2L)(1/2W)^2]$ in which L represents the greatest length and W the largest width of the prey. Indices of V_i (total volume of each prey category in all stomachs) and $V_i\%$ ($V_i\% = V_i/\sum V_i \dots n \times 100$) was calculated for each prey category. FOi (frequency of occurrence) calculated as the number of stomachs containing prey category i , and FOi% as a ratio of FOi relative to all stomachs with food item multiplied by 100 (i.e. $FOi\% = FOi/\sum FOi \dots n \times 100$). Following Ogoanah & Uchedike (2011) we categorized the values of FOi% as constant ($FOi\% > 50\%$), secondary ($25\% < FOi\% < 50\%$) and accidental ($FOi\% < 25\%$).

Each of the prey categories was investigated in terms of Index of relative importance (IRI) in relation to the entire range of food items ($IRI_i = (\%N_i + \%V_i) * \%FO_i$) (Pinkas et al. 1971). The reciprocal value of Simpson's diversity index ($S = 1/\sum P_i^2$) was used to calculate niche breadth for each sex, in which S represents niche breadth and P_i is the proportion of food component i . Schoener's index defined as " $\alpha = 1 - 0.5(\sum |P_{xi} - P_{yi}|$ " is an appropriate index which we used to determine dietary niche overlap between males and females. In Schoener's index α = diet overlap; P_{xi} = proportion of food item i found in males; P_{yi} = proportion of food item i found in females. The value of Schoener's index indicates no diet overlap (i.e. $\alpha = 0$) to complete diet overlap (i.e. $\alpha = 1$) (Wallace & Ramsey 1983).

de Souza Braga (1999) developed an index to revealing the degree of food preference (DFP) among numerous prey categories consumed by a given species which was used in this study. A more de-

tailed explanation about these ecological indices is presented in Fathinia et al. (2016). We used Microsoft Excel 2010 to calculate these indices.

Statistical analyses. Our data including food volume, food number and SVL of specimens were analyzed to reveal their normal distribution using Shapiro-Wilk test as a determinant of choosing between parametric and nonparametric tests. Statistic values of this test was significant for SVL ($P = 0.0001$), Food volume ($P = 0.001$) and Food number ($P = 0.001$) revealing non-normal distributions. To reveal the correlation between frogs and prey size two simple linear regression analyses were used: a) frog's SVL (snout-vent length) vs. the total number of preys per stomach and, b) frog's SVL vs. total volume of preys in the stomach. We excluded stomachs with no food items from these statistical analyses. The software SPSS 16 was used to perform statistical analyses.

Results

The rate of feeding activity of *P. bedriagae* in the study area was 87.6% in total (23 empty stomachs), 88.1% in summer (7 empty stomachs), 87% in autumn (10 empty stomachs) and 86.6% in spring (6 empty stomachs). This rate was 93.75% for males (5 empty stomachs) and 82.18% for females (18 empty stomachs).

Mean value of SVL for *P. bedriagae* having at least one item in the stomach was 47.47 ± 14.54 mm (20.07 to 92.15 mm). The Mean values of SVL for male and female specimens did not show significant differences (Mann-Whitney U test; $P = 0.28$). Sampling adequacy was 0.71 in three seasons combined, 0.82 in summer, 0.78 in autumn and 0.75 in spring for both males and females which was deemed sufficient for this study.

The 181 stomachs contained 1125 items, 40 of which were non-food, comprising 9 sand grains, 19 seeds, and 12 small wooden sticks. The remaining 1085 food items comprised of 209 unidentifiable and 876 identifiable items (Fig. 2). The number of food items across all stomachs during the three study seasons ranged from 1 to 178 (mean = 7.6 ± 16.8). The volume of stomach contents ranged from 0.08 mm^3 to 5577.2 mm^3 (mean = $206.8 \pm 590 \text{ mm}^3$). Twenty orders of phylum Arthropoda (classes Arachnida, Chilopoda, Diplopoda, and Insecta) and one order of phylum Mollusca (class Gastropoda) were identified in stomach contents as food categories (Table 1, Fig. 2).

Diet composition. 204 out of 876 identified food items (~23.3%) were from aquatic organisms, comprising crab ($n = 1$) and gastropods ($n = 203$), while the remaining identified items ($n = 672$; ~76.7%) were derived from terrestrial organisms, representing both adult ($n = 642$; ~95.5%) and larval ($n = 30$; ~4.5%) forms. Hymenoptera (~33.7%), Gastropoda (~23.2%) and Coleoptera (~13.6%) were the most frequently consumed diet components while the other 18 food categories ranged from 0.11% to 6.85% (less than 30% combined) (Table 1, Fig. 3). There is a dramatic change in some of the registered food categories between seasons. For instance, Hymenoptera was mostly consumed in summer, Gastropoda in autumn and Diptera and Dermaptera in spring.

Both sexes showed very close values (0.34 and 0.33, respectively) for Berger-Parker index (i.e. Food specialization) in three seasons combined. The highest values for the Ber-

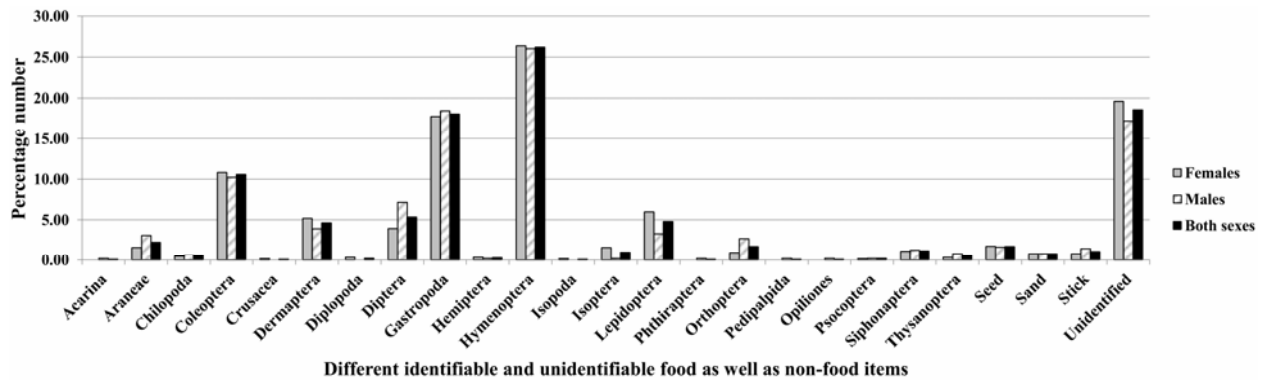


Figure 2. Composition of the diet of male and female *Pelophylax bedriagae*, and male and female combined across all food categories in the three seasons combined.

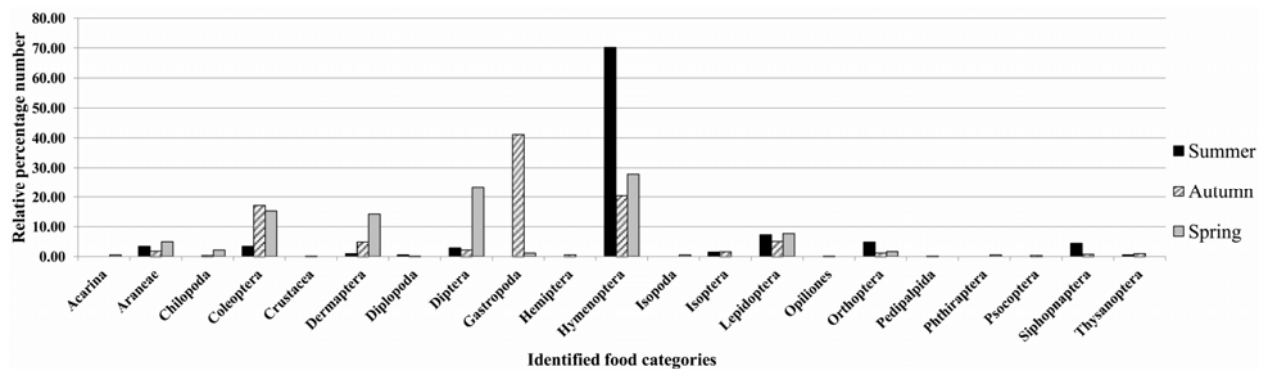


Figure 3. Comparison of dietary identified food items in summer, autumn and spring of *Pelophylax bedriagae*.

ger-Parker index were recorded in summer with 0.76 for females and 0.62 for males, but it was lower than 0.5 for both sexes in autumn and spring. Constant food items were different between male and female frogs in the whole study period. For example order Hymenoptera (FOi% = 57.14) was the constant food item for females and orders Coleoptera (FOi% = 70.42) and Hymenoptera (FOi% = 59.15) were constant for males (Table 1). The degree of food preference (DFP) in three seasons combined, as well as each season, showed that *P. bedriagae* consumes Hymenoptera more than other food categories (Table 1).

Index of relative importance (IRI) for females and males were different in three seasons combined. Gastropoda and Hymenoptera had the highest values for males (IRI = 4376) and females (IRI = 2527), respectively (Table 1). For both sexes, Hymenoptera had the highest IRI value in summer and autumn. Hymenoptera and Coleoptera had the highest values for spring females, while the highest IRI for spring males belonged to Hymenoptera and Diptera.

The diversity of stomach contents was 2.83 (total for both sexes), 2.84 for males and 2.77 for females in three seasons combined. It was 1.76 in summer, 2.5 in autumn and 2.73 in spring for (total for both sexes). Male specimens had greater value ($S = 5.11$) for the trophic niche breadth than females ($S = 4.9$) in three seasons combined. Schoener's index indicating prey niche overlap between males and females in three seasons combined was 0.9. The value of niche overlap was 0.83 for summer, 0.89 for autumn and 0.66 for spring.

There were no significant differences between females and males in the number and volume of consumed food in each of or in three seasons combined (Mann-Whitney U, $P >$

0.05; Table 2). Significant differences among three studied seasons for both total food volume (Chi-Square = 18.22; $P = 0.0001$) and number of food items (Chi-Square = 17.45; $P = 0.0001$) were observed, having higher values (both in number and volume of food items) in autumn and lower values in both volume and number for summer (Table 3). The size of studied frogs did not influence either the number ($r^2 = 0.015$; $P = 0.12$) or volume ($r^2 = 0.001$; $P = 0.66$) of consumed food (Fig. 4).

Discussion

Results of this study show that *Pelophylax bedriagae* consumes numerous food sources, representing 21 different orders with an uneven contribution, which reflects the generalist feeding habit of this frog. Generalist feeding habits have been reported previously for other anuran species in Iran such as *Pelophylax ridibundus* (Fathinia et al. 2016) and *Pelobates syriacus* (Fathinia et al. 2019). Hymenoptera and Coleoptera, amongst other food categories, are constant food items while the others are considered as secondary and accidental. Contrary to this study, no constant food category was identified in *Pelophylax ridibundus* (Fathinia et al. 2016) and *Pelobates syriacus* (Fathinia et al. 2019) in Iran.

A majority of food items consumed by *P. bedriagae* are terrestrial (~76.7%) and the remaining part (~23.3%) are aquatic. Such amphibious feeding habit has reported for other anuran species including *Pelophylax ridibundus* in Russia (Ruchin & Ryzhov 2002), Serbia (Popovic et al. 1992), Turkey (Çiçek & Mermer 2006 & 2007), Romania (Balint et al.

Table 2. Comparison of the number and volume of identified food items of male and female *Pelophylax bedriagae*, in summer, autumn and spring and three seasons combined. Abbreviations: No., number; SD, standard deviation; Min, minimum, Max, maximum, U, Mann-Whitney U test.

Season	Factor	Sex	No.	Mean	SD	Min	Max	U	P value
Summer	Food Number	Male	30.00	3.73	2.84	1.00	11.00	295.00	0.25
		Female	22.00	5.58	15.10	1.00	76.00		
	Food Volume	Male	30.00	224.08	675.59	0.08	3193.33	345.00	0.83
		Female	22.00	41.34	33.19	0.89	131.41		
Autumn	Food Number	Male	29.00	8.87	13.05	2.00	58.00	0.487	0.18
		Female	38.00	11.35	29.15	1.00	178.00		
	Food Volume	Male	29.00	137.97	236.50	5.93	1123.81	0.581	0.82
		Female	38.00	336.86	940.09	8.23	5577.21		
Spring	Food Number	Male	17.00	6.65	4.05	2.00	16.00	0.173	0.70
		Female	22.00	7.27	6.77	1.00	30.00		
	Food Volume	Male	17.00	154.54	217.45	38.68	938.29	0.178	0.80
		Female	22.00	261.47	472.66	14.56	2207.11		
Three seasons combined	Food Number	Male	76.00	6.38	8.76	1.00	58.00	2934.5	0.21
		Female	82.00	8.70	21.67	1.00	178.00		
	Food Volume	Male	76.00	175.18	455.02	0.08	3193.33	3259.00	0.86
		Female	82.00	235.10	690.39	0.89	5577.21		

Table 3. Descriptive statistics and Chi-squared test of number and volume of food items consumed in three seasons of summer, autumn and spring in *Pelophylax bedriagae*. Abbreviations: No., number; SD, standard deviation; Min, minimum, Max, maximum.

	Season	No.	Mean	SD	Min	Max	Chi-Square	P value
Food Number	Summer	52.00	4.56	10.21	1.00	76.00	17.45	0.0001
	Autumn	67.00	10.29	23.52	1.00	178.00		
	Spring	37.00	7.00	5.68	1.00	30.00		
Food Volume	Summer	52.00	142.86	508.54	0.08	3193.33	18.22	0.0001
	Autumn	67.00	251.62	729.97	5.93	5577.21		
	Spring	37.00	214.86	382.44	14.56	2207.11		

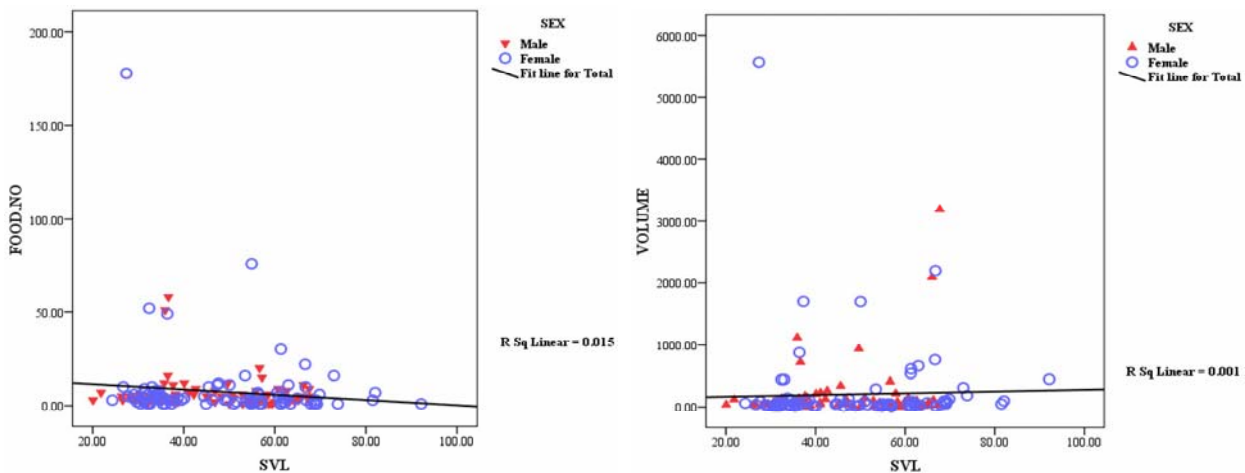


Figure 4. The relationship between the size (SVL) of *Pelophylax bedriagae*, with the number (right) and volume (left) of consumed food items in three seasons combined.

2008) and Iran (Fathinia et al. 2016), *Pelobates syriacus* in northwestern Iran (Fathinia et al. 2019) and *Bufo eichwaldi* in northern Iran (Darvishnia et al. 2018).

More than 72% of all identified food categories in *P. bedriagae* belong to Insecta with the greatest contribution of Hymenoptera amongst other hexapod taxa. Other studies show more or less the same result with a higher proportion of Insecta in comparison to other food items. In *Rana tempo-*

raria, for instance, 74% of food items are insects with Diptera as the highest contributor within Insecta (Houston 1973). In the Syrian spade-foot toad, *Pelobates syriacus*, approximately 88% of food items are insects, with the highest proportion in Diptera (25.5%) (Fathinia et al. 2019). More than 61% of food items of *P. ridibundus* are insects with the highest contribution by Diptera (27%) (Fathinia et al. 2016). Hymenoptera registered as the most frequently used prey in the Asian

Common Toad, *Duttaphrynus melanostictus* (Döring et al. 2017). Diptera composes the highest recorded food item in Common Tree Frog (*Hyla arborea*) with 79.55% and in Common Spadefoot toad (*Pelobates fuscus*) with 69.81%. Hymenoptera (ants) is the most recorded food item in *Bufo viridis* with a frequency of 52.48% (Covaciu-Marcov et al. 2010).

Feeding activity rates are similar in each of the study seasons (88.1% in summer, 87% in autumn, 86.6 in spring). The changes in feeding rate during different seasons might be dependent on numerous factors such as habitat type and abundance of prey. As previously reported for other Iranian anurans, this rate dramatically decreases from spring to summer, i.e. 89% to 60.9% in *P. ridibundus* (Fathinia et al. 2016) and 80.88% to 65.7% in *Pelobates syriacus* (Fathinia et al. 2019). The current study was done in a paddy field, which is retained during spring, summer and autumn, providing good cover across the three seasons and supporting a suitable habitat for the prey of *Pelophylax bedriagae*, which consequently results in a more or less high constant rate of feeding activity across study period, while the study areas for *P. ridibundus* and *P. syriacus* were common habitats that underwent dramatic change during seasons with less cover in summer than spring, leading to less abundant food and finally to a lower rate of feeding activity in summer. On the other hand, frogs in autumn consumed a greater amount and volume of food in comparison to the other two seasons. Such seasonal differences have been reported for other anuran species. In *Pelobates syriacus* (Fathinia et al. 2019) and *Pelophylax ridibundus* (Bogdan et al. 2012, Fathinia et al. 2016) both feeding activity rate and food and volume of consumed food have higher values in spring than in summer. Of numerous factors which may cause seasonal differences in diet composition, we can mention different energy demands (Grayson et al. 2005) and abundance of food resources (Das 1996, Kovács et al. 2007, López et al. 2009). Autumn specimens of Levant Green frogs, despite their smaller size than summer and spring specimens, consume a greater amount of food. This may be due to the fact that greater food consumption leads to larger fat resources and finally to a successful hibernation. The diversity of identified food items in the diet is higher in spring than in summer and autumn. A probable cause in such differences might be the different relative abundance of food items in different seasons.

There is a tendency toward altering main prey taxon among three seasons in this study (i.e. Hymenoptera in summer, Gastropoda in autumn and Diptera in spring). This dietary shift is probably caused by an alteration in prey abundance during different seasons. Such dramatic changes in prey taxa among different seasons have been reported for other anuran species such as *Pelophylax ridibundus* (Fathinia et al. 2016), *Pelobates syriacus* (Fathinia et al. 2019), *Rana hexadactyla* (Das 1996) and *Dendrobates tinctorius* (Born et al. 2010).

Some anuran species have been reported to show cannibalism or feeding on other vertebrates, e.g. *Pelophylax ridibundus* in Iran (Fathinia et al. 2016), Turkey (Çiçek & Mermer 2006 & 2007), and Romania (Cicort-Lucaciu et al. 2013), and *Rana ridibunda* in Mordovia, Russia (Ruchin & Ryzhov 2002). We did not report this behavior for *P. bedriagae* in this study. The lack of conspecific or other small vertebrate prey has also been reported for other anuran spe-

cies such as *Duttaphrynus melanostictus* in Timor Island, Wallacea (Döring et al. 2017). Different causes have been proposed for cannibalism to occur in amphibians. Such conditions like increasing the number of individuals, the change of ecological conditions, outgrowth of the population (Stebbins & Cohen 1995) and decreasing food availability (Crump 1992) are believed to promote cannibalistic behavior. The apparent lack of cannibalism in *P. bedriagae* in this study might be explained by habitat coverage consistency and high levels of food abundance as having been shown in this study by high levels of feeding activity rate.

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