The diet of *Pelobates syriacus* BOETTGER, 1889, from the Ghorigol wetland, East Azerbaijan province, Iran
(Anura: Pelobatidae)

Die Nahrung von *Pelobates syriacus* BOETTGER, 1889 im Ghorigol Feuchtgebiet der iranischen Provinz Ost-Aserbaidschan
(Anura: Pelobatidae)

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KURZFASSUNG

Von 138 Exemplaren des Syrischen Schaufelfußes *Pelobates syriacus* BOETTGER, 1889 aus dem Ghorigol Feuchtgebiet in der Nordwestiranischen Provinz Ost-Aserbaidschan wurden die im Frühjahr und Sommer 2016 durch Spülung gewonnen Mageninhalte untersucht. In der Kopf-Rumpf-Länge unterschieden sich die Geschlechter signifikant ($P < 0.05$). Dabei fanden sich Inhalte in 73.19 % der Mägen, 37 waren leer. Die Gesamtzahl festgestellter Mageninhaltsobjekte betrug 480, von denen 392 tierische und 88 keine tierischen Objekte darstellten.

Die Beutetierkategorien, die 16 Ordnungen aus zwei Stämmen (Annelida und Arthropoda) umfaßten, waren ungleichmäßig über die fünf Klassen Arachnida, Chilopoda, Insecta, Malacostraca und Oligochaeta verteilt, wobei Insecta am häufigsten vertreten waren. In allen Mägen lag die relative Häufigkeit der vorkommenden Beutetierkategorien (% FO_i) unter 50 %, ein Hinweis auf eine artlich vielfältige Nahrungszusammensetzung bei *P. syriacus*. Schaufelfüße bevorzugten Coleoptera, Diptera und Hymenoptera als Nahrung. Sowohl das Gesamtvolumen als auch die Stückzahl der Objekte je Magen unterschied sich signifikant zwischen im Frühjahr und im Sommer gefangenen Individuen, nicht aber zwischen Männchen und Weibchen. Mit zunehmender Körpergröße der Schaufelfüße nahmen Stückzahl und Gesamtvolumen der Beuteobjekte in ihren Mägen tendenziell zu.

ABSTRACT

In spring and summer of 2016, a total number of 138 specimens of the Eastern Spadefoot Toad, *Pelobates syriacus* BOETTGER, 1889, were collected in the Ghorigol wetland in the Northwest Iranian Province of East Azerbaijan. Their stomach contents were gained using a stomach flushing technique. Snout-vent length was significantly different between the sexes ($P < 0.05$). The feeding activity index, i.e., the proportion of stomachs containing food items was 73.19 %, 37 stomachs were empty. A total of 480 items were recorded, 88 of which were non-animal objects and 392 animal food items.

Food categories comprised 16 orders belonging to two phyla (Annelida and Arthropoda) and were distributed unevenly within five classes: Arachnida, Chilopoda, Insecta, Malacostraca and Oligochaeta, with Insecta being the most abundant group. In all stomachs, the relative Frequency of Occurrence of a particular category (% FO_i) was less than 50 %, indicating some variability in the diet composition of *P. syriacus*. This species preferred Coleoptera, Diptera and Hymenoptera. Both total volume and number of food items per stomach were significantly different between individuals sampled in spring and summer but not between males and females. As the toads' body size increased, there was a tendency towards consuming larger amounts of food both in volume and number.

KEY WORDS

Amphibia: Anura: Pelobatidae; *Pelobates syriacus*, food ecology, prey taxonomic composition, stomach flushing, Ghorigol Wetland, Province of East Azerbaijan, NW Iran

INTRODUCTION

Amphibians are a major component in food webs due to their role as carnivore predators, herbivores (e. g., tadpoles) and prey, as well as linking terrestrial to aquatic ecosystems for their biphasic life cycles (WILBUR 1997; GIBBONS et al. 2006; REGESTER et al. 2006; WHILES et al. 2006). In general, anuran metamorphs are considered generalist feeders, preying upon a wide range of animal prey when prey availability fluctuates (DUELLMAN & TRUEB 1986).

Dietary composition of many anurans varies between sexes, ages and seasons (e.g., CRNOBRNJA-ISAILOVIĆ et al. 2012; FATHINIA et al. 2016). Biotic and abiotic factors such as seasonal change in food availability, presence or absence of competitors, intrinsic factors (ISACCH & BARG 2002) and sexual size dimorphism may affect the food composition within amphibian species (WELLS 2007).

The Eastern Spadefoot Toad, *Pelobates syriacus* BOETTGER, 1889, is distributed from the southeast Balkans, eastwards to southeastern Transcaucasia and northern Iran, and southwards to the Levant (AGASYAN et al. 2009). In Iran, its range is limited to northern and northwestern regions where it occurs from sea level to 2,000 m a.s.l. (BALOUTCH & KAMI 2007). It shows female-biased sexual size dimorphism (ROT-NIKČEVIĆ 2001). According to the criteria of the IUCN Red List of threatened species, *P. syriacus* has been assessed as Least Concern with decreasing population (AGASYAN et al. 2009). This first published study with the focus on the food ecology of *P. syriacus* aims on providing answers to questions including the effects of age, sex and season on the prey composition.

MATERIALS AND METHODS

Study area and sampling.– Located 45 km south of Tabriz city, the Ghorigol wetland (37°55'01" N, 46°42'10" E) is one of the most important environmental resources of East Azerbaijan province in Iran. In the Ramsar Convention, it has been identified as an international wetland of approximately 200 hectares situated at an altitude of 1,850 m a.s.l. (KHALILIAN et al. 2012; HABIBZADEH et al. 2015). Its water supply includes seasonal precipitation as well as some perpetual subsurface springs (HABIBZADEH et al. 2015). Collecting *Pelobates syriacus* was done around this wetland at night during spring and summer of 2016. Specimens were collected by hand using a flashlight; their stomachs were immediately flushed following SOLÉ at al. (2005). The snout-vent length (SVL) of the specimens was measured to the nearest 0.01 mm using a digital caliper, and gender was identified according to sex-specific external characters such as smaller size, orange spots on flanks and nuptial gland on upper arm in males. Juveniles were distinguished from adults by their small size of 26.30 to 37.9 mm (ROT-NIKČEVIĆ 2001). Based on this data, the authors assigned the specimens to juveniles (SVL < 40 mm) and adults (SVL > 40 mm).

During this survey, 138 specimens (17 juveniles and 121 adults) were stomach-flushed as described in SOLÉ et al. (2005). Of the adult specimens 73 and 48 were male and female, respectively. The number of flushed stomachs in spring and summer was 68 and 70, respectively. The authors used the information provided in GILLOTT (2005) and HICKMAN et al. (2001) to identify the insect prey items of the stomach contents to order rank distinguishing 19 animal food and non-animal categories or components as detailed in the results. Non-animal objects (sand grains and seeds) were considered as swallowed unintentionally and omitted from ecological calculations as were animal objects which had to remain unidentified due to their advanced digesting stage.

Ecological indices.– The feeding activity index was defined as $100 \cdot n/N$ where n, is the number of stomachs containing food and N is the total number of examined stomachs. Sampling adequacy (Q, range 0 – 1) was determined according to Lehner's formula [$Q = 1 - N_{i(1)}/I$; LEHNER 1996], where $N_{i(1)}$ is the number of the food categories represented by one item only in the whole sample, and I is the total number of the food categories. The Berger-Parker Index of Dominance [$d = n_{i\,max}/N$; MAGURRAN 1988] was used to determine the relative abundance of the most abundant category and by this reveal the level of food specialization for each sex. In this formula, $n_{i\,max}$ represents the number of items in the most abundant category, N is the number of items in all categories. The value of the Berger-Parker Index ranges from $1/N$ to 1. Values close to 1 identify highly specialized feeders while those close to $1/N$ highly generalized feeders.

The Shannon-Wiener index [$H' = -\sum(n_i/N)\cdot\log_2\cdot(n_i/N)$] was used to calculate the diversity of the components in the stomachs. The volume of each item was estimated with the formula of prolate ellipsoids [$V = (4/3)\cdot\pi\cdot(1/2L)\cdot(1/2W)^2$]. In this formula, L represents the longest length and W the largest width of the object. Indices of V_i and % V_i were calculated for each category, V_i representing the volume of all items of a given category in all stomachs and % V_i the volume of all items of a given category relative to the volume of all items of all categories, multiplied by 100 [% $V_i = 100\cdot(V_i / \Sigma V_{i...n})$]. Similarly, absolute and relative frequencies of occurrence, FO_i and % FO_i, were calculated for all categories, where FO_i is the number of stomachs containing items of category *i*, and % FO_i is FO_i divided by the number of all stomachs containing ingested items, multiplied by 100. Following OGOANAH & UCHEDIKE (2011), the relative Frequency of Occurrence of a particular category is classified as constant (% $FO_i > 50$), secondary ($25 <$ % $FO_i < 50$) or accidental (% $FO_i < 25$).

The importance of a particular category of stomach content (N_i) relative to the entire range of items was calculated for each category using the index of relative importance (*IRI*) [$IRI_i = (\% N_i + \% V_i) \cdot \% FO_i$; PINKAS et al. (1971)]. The calculation of the trophic niche breadth was done separately for male and female toads, based on the reciprocal value of Simpson's diversity index [$S = 1/\sum P_i^2$] where S is the niche breadth and $P_i = n_i/N$ is the proportion of the number of items of category *i* among the number of all items. To determine dietary niche overlap between sexes (α_{xy}), Schoener's index [$\alpha_{xy} = 1 - 0.5\cdot(\sum | Px_i - Py_i|$; SCHOENER (1970)] was applied, where Px_i and Py_i is the proportion of item *i* among all items found in female and male stomachs, respectively. The output value of Schoener's index ranges from 0 (no dietary overlap) to 1 (complete dietary overlap) (WALLACE & RAMSEY 1983). To determine if any item is preferred over others, the authors used the index *DFP* (Degree of Food Preference) which was developed by BRAGA (1999) who ranked the food categories present in a stomach. If only a single category was present it was assigned the value of 4. In stomachs that contained more than one category, the values 3, 2 and 1 were assigned to the most abundant categories corresponding to their numerical representation in descending order. The index was calculated as $DFP = S_i / N$, where S_i is the sum of values assigned to a particular food category in the stomachs and N the total number of stomachs that contained food. All the ecological indices were calculated using Microsoft Excel 2010.

Statistical analyses.– Both the ingested objects' volume and number as well as SVL of the toads were non-normally distributed, thus requiring nonparametric statistical tests (ZAR 1984). The correlation between the size of the predator and the objects ingested was examined by two simple linear regression analyses: the toads' SVL (i) on the total number of items in the stomach and (ii) on the total volume of the items in the stomach. Empty stomachs were excluded from the analyses, which were performed using the software package IBM SPSS Statistics ver. 16.0.

RESULTS

Animal food items detected comprised the orders Oligochaeta, Acari, Araneae, Blattodea, Chilopoda, Coleoptera, Decapoda, Dermaptera, Diptera, Hemiptera, Hymenoptera, Isopoda, Lepidoptera, Orthoptera, Phthiraptera and Siphonaptera.

Number of prey items.– The rate of feeding activity was 73.19 % in total (37 empty stomachs), 80.88 % in spring (13 empty stomachs), 65.7 % in summer (24 empty stomachs) and 23.53 % for juveniles (13 empty stomachs). This rate was 69.86 % for adult males (20 empty stomachs) and 87.5 % for adult females (6 empty stomachs). Empty stomachs in adults were exclusively recorded in summer.

Mean SVL for *P. syriacus* collected for this survey was 58.66 ± 13.66 mm. Snout-vent length was significantly ($P = 0.001$) longer in females (65.46 ± 14.12 mm) than males (54.19 ± 11.39 mm). The sampling adequacy was 0.79 in total, 0.73 in

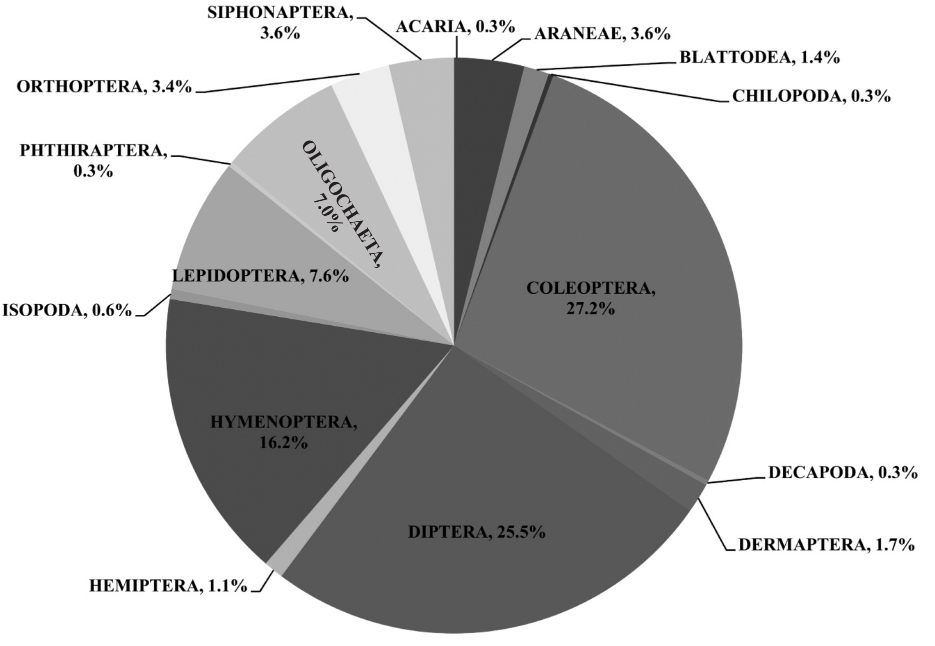

Fig. 1: Diagram showing the numerical proportion of 357 identified organismic items among the prey taxonomic groups flushed from stomachs of 138 NW Iranian *Pelobates syriacus* BOETTGER, 1889.

Abb. 1: Der zahlenmäßige Anteil der 357 bestimmten organismischen Objekte an den Beutetier-Ordnungen in den Mägen von 138 Exemplaren von *Pelobates syriacus* BOETTGER, 1889 aus dem NW-Iran.

females and 0.92 in males, which is sufficient for this study.

A total of 480 food and non-food items were recorded from 138 stomachs, of which 88 were non-food items (85 sand grains, 3 seeds) and 392 were food (35 unidentifiable, 357 identifiable). The number of food items per stomach ranged from 1 to 32 ($\bar{x} = 3.77 \pm 5.06$). Prey categories comprised 16 orders, unevenly distributed within five classes: Arachnida, Chilopoda, Insecta, Malacostraca and Oligochaeta (Table 1), within the phyla Annelida and Arthropoda.

Diet composition.– Only one food item (a crab) was aquatic, the others were terrestrial, representing both adult (N= 342; 96.06 %) and larval (N = 14; 3.94 %) forms. Numerically, insects were the most frequent diet components (314 of 357 = 87.9 %) with Coleoptera (97 of 357 = 27.17 %), Diptera (91 of 357 = 25.49 %) and Hymenoptera (58 of 357 = 16.24 %) as the three most abundant across all food categories (Table 1, Fig. 1). Diet composition changed between the two seasons of spring and summer. Seasonal changes were dramatic in the orders of Hymenoptera and Oligochaeta with increased numbers of Hymenoptera in summer and Oligochaeta in spring (Table 2; Fig. 2). Food specialization based on the Berger-Parker Index was 0.29 for males and 0.38 for females. All food categories turned out to be secondary and accidental because % FO_i was less than 50 % for all food categories. Coleoptera, Diptera and Hymenoptera were regarded as secondary food while the other categories were classified as accidental (Table 1). The degree of food preference (DFP) mirrored the % FO_i results in showing that *P. syriacus* preferred Coleoptera, Diptera and Hymenoptera over other food categories (Table 1).

Table 1: Information on the stomach contents of 138 NW Iranian *Pelobates syriacus* BOETTGER, 1889. N − number of prey items, N % − proportion of N relative to all items, FO − Frequency of Occurrence, % FO − proportion of FO relative to FO of all items, V − sum of volume of prey items in mm³, % V − proportion of V relative to V of all items, DFP − Degree of Food Preference. Data of predominant constituents in bold.

Tab. 1: Die Mageninhalte von 138 nordwestiranischen *Pelobates syriacus* BOETTGER, 1889. N − Anzahl der erbeuteten Objekte, N % − Anteil von N an allen Objekten, FO − Vorkommenshäufigkeit, % FO − Anteil von FO an der Vorkommenshäufigkeit aller Objekte, V − Gesamtvolumen der Beuteobjekte in mm³, % V − Anteil von V am Volumen aller Beuteobjekte, DFP − Ausmaß der Nahrungspräferenz. Zahlenwerte dominierender Bestandteile in Fettschrift.

Food object category Nahrungskategorie	N	% N	FO	% FO	V	% V	DFP
Annelida							
Oligochaeta	**25**	6.38	7	7.07	**6130.64**	**28.18**	0.20
Arthropoda							
Acari	1	0.26	1	1.01	0.21	0.00	0.01
Araneae	13	3.32	11	11.11	427.58	1.97	0.16
Blattodea	5	1.28	4	4.04	55.01	0.25	0.06
Chilopoda	1	0.26	1	1.01	28.09	0.13	0.01
Coleoptera	**97**	**24.74**	**45**	**45.45**	**4869.36**	**22.38**	**1.16**
Decapoda	1	0.26	1	1.01	21.16	0.10	0.01
Dermaptera	6	1.53	4	4.04	737.93	3.39	0.07
Diptera	**91**	**23.21**	**38**	**38.38**	**843.12**	3.88	**0.93**
Hemiptera	4	1.02	3	3.03	155.37	0.71	0.05
Hymenoptera	**58**	**14.80**	**27**	**27.27**	267.16	1.23	**0.66**
Isopoda	2	0.51	2	2.02	100.00	0.46	0.02
Lepidoptera	**27**	6.89	14	14.14	**6665.13**	**30.64**	0.27
Orthoptera	12	3.06	7	8.08	302.57	1.39	0.14
Phthiraptera -Mallophaga	1	0.26	1	1.01	1.37	0.01	0.04
Siphonaptera	13	3.32	8	8.08	235.16	1.08	0.14
Sand grains	85	21.68	30	30.30	330.82	1.52	0.46
Seed	3	0.77	2	2.02	17.58	0.08	0.04
Unidentified organic objects	35	8.93	15	15.15	566.20	2.60	0.34
Total	480	122.48	221	224.22	21754.46	100	4.77

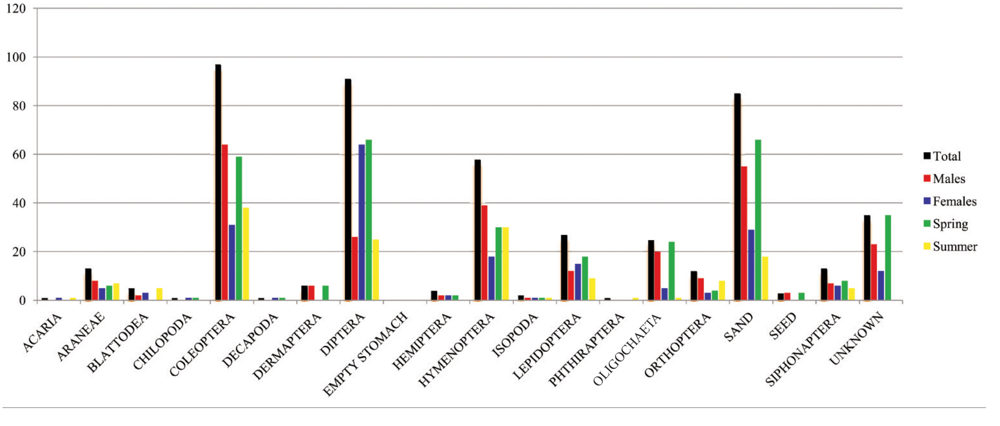

Fig. 2: Diagram comparig the numbers of consumed food components of males, females, in spring and summer with the total number of food components in NW Iranian *Pelobates syriacus* BOETTGER, 1889.

Abb. 2: Vergleich der Stückzahlen aufgenommener Nahrungsobjekte bei Männchen (rot), Weibchen (blau), im Frühjahr (grün) und im Sommer (gelb) mit denen aller Objekte (schwarz) in den Mägen von 138 *Pelobates syriacus* BOETTGER, 1889 aus dem NW-Iran.

Table 2: The Index of Relative Importance (IRI) of the food categories consumed by NW Iranian *Pelobates syriacus* BOETTGER, 1889. The index values are presented separately for males, females, spring, summer and in total (both seasons, both sexes). H − Shannon-Wiener index of diversity. Data of predominant constituents in bold.

Tab. 2: Der Index der relativen Wichtigkeit (IRI) der taxonomischen Beutekategorien, die von nordwestiranischen *Pelobates syriacus* BOETTGER, 1889 gefressen wurden. Die Indexwerte sind getrennt für Männchen, Weibchen, Frühling, Sommer und insgesamt (für beide Jahreszeiten und Geschlechter) angegeben. H − Shannon-Wiener Diversitätsindex. Zahlenwerte dominierender Bestandteile in Fettschrift.

Food object category Nahrungskategorie	Males Männchen	Females Weibchen	Spring Frühling	Summer Sommer	Both seasons and sexes Beide Jahreszeiten und Geschlechter
Annelida					
Oligocheata	**269.69**	**277.77**	**452.48**	22.44	45.14
Arthropoda					
Acari	0.00	2.39	0.00	1.75	0.26
Araneae	79.19	61.64	20.91	**218.22**	58.69
Blattodea	4.57	15.30	0.00	43.36	6.18
Chilopoda	0.00	3.34	0.98	0.00	0.39
Coleoptera	**2511.72**	**2293.81**	**1559.10**	**3367.95**	**2142.19**
Decapoda	0.00	3.10	0.91	0.00	0.36
Dermaptera	60.22	0.00	46.70	0.00	19.89
Diptera	**454.88**	**3415.33**	**1311.51**	**775.81**	**1039.81**
Hemiptera	6.09	6.73	4.76	0.00	7.70
Hymenoptera	**590.31**	**458.17**	**294.92**	**1000.42**	**423.00**
Isopoda	2.09	2.67	0.78	7.11	3.51
Lepidoptera	**621.27**	**542.77**	**581.12**	**298.03**	103.90
Orthoptera	53.10	31.24	7.68	153.38	252.46
Phthiraptera - Mallophaga	0.00	0.00	0.00	1.82	31.21
Siphonaptera	41.03	50.92	34.95	42.96	35.53
Sand grains	1082.36	644.42	981.18	328.38	699.23
Seed	5.72	0.00	4.54	0.00	1.71
Unidentified organic objects	262.00	161.47	482.13	0.00	174.72
Total	6044.24	7971.09	5784.66	6261.62	5045.87
H	3.00	2.84	2.95	2.77	2.95

The Index of Relative Importance (IRI) was indicative of the diverging significance of the food categories ranging from 2,142 for Coleoptera to 0.26 for Acari. Diptera (1,039), Hymenoptera (423) and Orthoptera (252) have also high IRI values (Table 2), however, Coleoptera, Diptera and Hymenoptera form the most abundant food items in both spring and summer as well as among male and female spadefoots. The IRI values of these insect orders vary between sexes and seasons (Table 2).

Based on the Shannon-Wiener index, the diversity of the food components in the stomachs was 2.95 in total, 3.00 for male and 2.84 for female stomach contents. This diversity index was also calculated for the studied seasons, revealing some variation from 2.95 in spring to 2.77 in summer (Table 2). The trophic niche breadth was wider in males (7.96) than females (6.12).

Quantifying dietary niche overlap between males and females, Schoener's index was 0.52 across the entire sampling period and showed a large decrease from spring (0.65) to summer (0.30).

There were significant seasonal differences in the stomach contents regarding total food volume (U = 860, P = 0.008) and number of food items (U = 866, P = 0.008), with higher values in spring than summer. Neither food volume (U = 1585, P = 0.37) nor food number (U = 1563, P = 0.29) were significantly different between males and females. The size of the Eastern Spadefoot Toads was positively correlated with both volume (r^2 = 0.101, P = 0.001) and number (r^2 = 0.047, P = 0.03) of the consumed food items per stomach.

DISCUSSION

The findings of this study reveal that there is a wide variety of food items as well as no constancy in the diet of *P. syriacus*. Hence, this toad can be regarded a generalist feeder preying at least upon animal prey species from 16 different taxa of order rank. Although this study was conducted around a water body (Ghorigol Wetland), almost all of food items (over 99 %) registered for this toad were terrestrial species. These terrestrial food items included mostly adult (96.06 %) and much rarer (3.94 %) larval forms, indicating the importance of motile prey in the diet of this toad. Such a high proportion of adult insects was typical also to other anuran species such as *Pelophylax ridibundus* (PALLAS, 1771) (ÇIÇEK & MERMER 2007; FATHINIA et al. 2016). In *Rana temporaria* (LINNAEUS, 1758), insects were shown to form 74 % regarding the number and 70 % as to the volume of the consumed food, with Diptera dominating among insects (HOUSTON 1973), however, as the second highest contributors following Coleoptera in the present study. The preference towards motile prey objects can be attributed to the feeding behavior of anurans, which is primarily focused on visual detection and subsequent clasping of the prey with the tongue (STEBBINS & COHEN 1995).

Pelobates syriacus showed a decreased rate of feeding activity during summer compared with spring and a lower rate in males than females. Lowered feeding activity in summer has also been shown in other anuran species such as *P. ridibundus* (BOGDAN et al. 2012; FATHINIA et al. 2016). Seasonal changes in the diet were reported also from e.g., *R. temporaria* in England (HOUSTON 1973), numerous anuran species in Peru (TOFT 1980) as well as Central Africa (INGER & MARX 1961). On the other hand, at least three species of *Hyperolius* do not show seasonal changes in diet composition between dry and wet seasons in Nigeria (LUISELLI et al. 2004). Seasonal changes in the feeding activity rate may be the consequence of different factors such as varying energy demands (GRAYSON et al. 2005) and abundance of food resources (DAS 1996; KOVÁCS et al. 2007; LÓPEZ et al. 2009).

There is a positive correlation between predator body size and prey size in many anurans (e.g., HIRAI & MATSUI 2000; MANEYRO et al. 2004; QUIROGA et al. 2009; FATHINIA et al. 2016). This was also shown for *P. syriacus* in this study. Maintaining a positive energy balance is necessary for an animal to grow, reproduce and survive periods of inactivity. To achieve this goal, according to the optimal foraging theory, larger animals must select larger prey than smaller animals (SCHOENER 1979). Moreover, a shift in dietary preferences may occur as the anuran grows older (LIMA & MOREIRA 1993; HIRAI 2002; BLACKBURN & MOREAU 2006). Changes in foraging behavior influence many ontogenetic shifts (SCHOENER & GORMAN 1968). Different reasons have been proposed for shifts in foraging behavior of amphibians. As amphibians grow and attain larger body size they become more effective in their resilience towards water loss, defensive and escaping behavior. These ontogenetic changes allow the larger individuals to increase their foraging amplitude (HODGKISON & HERO 2003).

Non-animal ingested items (including sand grains and plant matter such as seeds) seem to be common in amphibian species such as *P. ridibundus* (FATHINIA et al. 2016), *Rana* spp. (ASZALÓS et al. 2005) and *Ommatotriton ophryticus* (BERTHOLD, 1846) (KUTRUP et al. 2005), whereas only some adult amphibian species were shown to feed on plants (DAS 1996; DA SILVA & DE BRITTO-PEREIRA 2006). In this way the sand particles, whether ingested intentionally or accidentally, may help to mechanically break up the plant materials (EVANS & LAMPO 1996).

The present results revealed the high dietary overlap (52 %) between males and females of the *P. syriacus* population studied. Coexistence of numerous individuals with similar prey spectra (e.g., males and females with high dietary overlap) indicates high prey availability (BRASILEIRO et al. 2010; CALDART et al. 2012).

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