On the trophic spectrum of *Pelophylax ridibundus* (Pallas, 1771) (Amphibia: Anura: Ranidae) in western Iran

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Using the stomach flushing technique, a total of 188 specimens of the Marsh Frog, *Pelophylax ridibundus*, were flushed, of which 129 had at least one food item in the stomach. The diet consisted of Annelida, Mollusca, Arthropoda and Chordata with Arthropoda being the most abundant group. Both diet volume and number of food items per stomach were significantly larger in April than in August. The proportion of frequency of occurrence (FOi%) of food categories did not show a constant food item in flushed materials. *Pelophylax ridibundus* prefers Diptera, Coleoptera, Amphipoda and Hymenoptera over other food categories. The Index of Relative Importance (IRI) for food categories differs between sexes and seasons. Differences were found in the food volume and the number of food items between seasons, but not between sexes. Prey volume is positively correlated with frog size.

Keywords: Amphibia; cannibalism; diet; Marsh Frog; Iran

Introduction

The Marsh Frog, *Pelophylax ridibundus*, is widely distributed in Central and Southern Europe and Western Asia (Tok, Atatür & Ayaz, 2000; Çiçek & Mermer, 2006). It is mostly observed in water and at water edges such as the tops of leaves and branches above the water level. Its preferred habitats are densely covered ponds, rivers, lakes and plains (Çiçek & Mermer, 2006). Many studies have been conducted on the diet of *P. ridibundus* in European countries. For example, Popovic, Simic, and Tallósi (1992) compared the feeding spectrum of three species of *Rana* (*R. esculenta*, *R. lessonae* and *R. ridibunda*), showing feeding on different invertebrate groups by these three species, with insects as the predominant group. Ruchin and Ryzhov (2002) documented feeding upon 200 different invertebrate (mainly Arachnida) and vertebrate (fishes, tailless amphibians and mammals) species. Çiçek and Mermer (2006, 2007) demonstrated that *P. ridibundus* feeds mainly upon terrestrial invertebrate groups such as Diptera, Coleoptera and Hymenoptera, while Mollov (2008) showed that *P. ridibundus* is a zoophagous polyphage consuming all mobile objects which it can subdue and swallow. He demonstrated that both sexes have a high trophic niche breadth and show a moderate niche overlap. Cicort-Lucaciuc, Pelle, and Borma (2013) recorded a higher rate of feeding on ants by juvenile in comparison to adult Marsh Frogs.

We assessed the diet of the Marsh Frog in Ilam Province of Iran to determine the trophic spectrum of the species in this part of the distribution area, to find out whether
there are sexual differences in the trophic spectrum, and to find out possible seasonal changes in the diet pattern. We used for our study stomach flushing which is a non-invasive method of dietary analysis (Solé, Beckmann, Pelz, Kwet, & Engels, 2005).

**Material and Methods**

**Study area.** The study was carried out in Ilam Province, western Iran: At Sirvan County (L1: 33°40′N, 46°35′E), which has a Mediterranean climate, and in four counties with a dry and semiarid climate in the southern and western parts of the province: Dareshahr (L2: 33°05′N, 47°20′E), Abdanan (L3: 32°57′N, 47°25′E; and L4: 32°58′N, 47°22′E) and Dehloran (L5: 33°00′N, 46°53′E; L6: 32°57′N, 47°03′E; and L7: 33°03′N, 46°54′E).

**Sampling.** The field studies at L1 were carried out in April 2013 and those of L2-L7 in August 2013. All Marsh Frogs were caught by hand in the daytime, immediately flushed after collecting according to Solé et al. (2005) and measured from snout to vent (SVL) by a digital caliper (0.01 mm accuracy) prior to releasing to the collecting sites. Sex was determined through the presence (in males) or absence (in females) of vocal pouches on both sides of the buccal slit just under tympana (Fathinia et al., 2011). A total of 188 stomachs (133 in summer and 55 in spring) were flushed. Diet components were identified to order ranks according to Gillott (2005).

**Ecological indices.** The rate of feeding activity was defined as $100n/N$ where $n$, is the number of stomachs containing food and $N$, is the total number of examined stomachs. Lehner’s formula (Lehner, 1996) as defined by $Q = 1 – N/I$ was used for calculating sampling adequacy in each sex and in total. $N_i$ is the number of food components recorded just once, and $I$ is the total number of food components. The level of food specialization of each sex was determined using the Berger-Parker Index of Dominance (Magurran, 1988). This index is defined as $d = n_{max}/N$, where $n_{max}$ is the number of the most numerous taxon in the diet, and $N$ is the total number of recorded food items. The Berger-Parker Index ranges from 1/$N$ to 1. The more highly specialised feeder the species and the closer the value to 1, the more general feeder the species.

The diversity of stomach contents was calculated using the Shannon-Wiener index of general diversity as defined by: $H = – \sum (n_i/N) \log_2 (n_i/N)$. The volume of each food item was calculated using the formula of prolate ellipsoid $V = 4/3\pi (1/2L)(1/2W)^2$, where $L$ corresponds to the greatest length and $W$ to the largest width of the prey. The total volume of each prey category ($V_i$) was calculated as the sum of all prey items of the same category. Proportion of $V_i$ relative to the total volume of all calculated food items was also calculated ($V_i% = V_i/\sum V_{i..n}*100$).

Frequency of occurrence (FO) for each food category was estimated as the number of stomachs containing food and $N$, is the total number of examined stomachs. Lehner’s formula (Lehner, 1996) as defined by $Q = 1 – N/I$ was used for calculating sampling adequacy in each sex and in total. $N_i$ is the number of food components recorded just once, and $I$ is the total number of food components. The level of food specialization of each sex was determined using the Berger-Parker Index of Dominance (Magurran, 1988). This index is defined as $d = n_{max}/N$, where $n_{max}$ is the number of the most numerous taxon in the diet, and $N$ is the total number of recorded food items. The Berger-Parker Index ranges from 1/$N$ to 1. The more highly specialised feeder the species and the closer the value to 1, the more general feeder the species.

An index of importance for each prey category related to the entire range of food items was calculated using the index of relative importance (IRI, = (%$N_i$ + %$V_i$) * %FOi) (Pinkas, Oliphant, & Iverson, 1971). The reciprocal value of Simpson’s diversity index ($S = 1/\sum P_i^2$) was used to calculate niche breadth (diversity of the diet) of each sex where $S$, is niche breadth and $P_i$, is proportion of food component $i$. Schoener’s index (1970) ($\alpha = 1 – 0.5(\sum |Pxi – Pyi|)$) was used to determine the dietary niche overlap between males and females, where $\alpha = diet overlap; Pxi = proportion of food item $I$ found in males; $Pyi = proportion of food item $I$ found in females. Schoener’s index ranges from 0 (indicating no diet overlap) to 1 (complete diet overlap) (Wallace & Ramsey, 1983).

Degree of food preference (DFP), developed by Braga (1999), was used to show the degree of preference among numerous food items. Food items were weighted as follow: when only one food group was found in a stomach it was assigned the maximum value of ‘4’, when stomachs contained more than one food type, the values “3”, “2” and “1” were assigned to the most abundant, second most abundant and less abundant groups, respectively. DFP 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 containing food.

Excel 2010 was used to calculate ecological indices.
Table 1. Data of stomach contents of *Pelophylax ridibundus*. N = number of prey items, N% = percentage of N, FO = frequency of occurrence, FO% = percentage of FO, V = sum of volume of prey items in mm³, V% = percentage of V, DFP = degree of food preference.

|                | N   | N%  | FO  | FO%  | V      | V%   | DFP |
|----------------|-----|-----|-----|------|--------|------|-----|
| **Annelida**   |     |     |     |      |        |      |     |
| Lumbriculida   | 5   | 0.89| 2   | 1.55 | 1891.02| 6.27 | 0.04|
| **Mollusca**   |     |     |     |      |        |      |     |
| Gastropoda     | 22  | 3.92| 11  | 8.53 | 735.37 | 2.44 | 0.16|
| **Arthropoda** |     |     |     |      |        |      |     |
| Amphipoda      | 100 | 17.83| 26  | 20.16| 5247.34| 17.40| 0.47|
| Decapoda       | 2   | 0.36| 3   | 2.33 | 306.34 | 1.02 | 0.02|
| Isopoda        | 4   | 0.71| 1   | 0.78 | 44.05  | 0.15 | 0.02|
| Araneae        | 22  | 3.92| 21  | 16.28| 1142.17| 3.79 | 0.24|
| Scorpiones     | 1   | 0.18| 1   | 0.78 | 86.34  | 0.29 | 0.01|
| Opiliones      | 2   | 0.36| 1   | 0.78 | 102.52 | 0.34 | 0.01|
| Chilopoda      | 1   | 0.18| 1   | 0.78 | 94.55  | 0.31 | 0.01|
| Diplopoidea    | 2   | 0.36| 2   | 1.55 | 45.67  | 0.15 | 0.03|
| Blattodea      | 2   | 0.36| 2   | 1.55 | 26.50  | 0.09 | 0.03|
| Coleoptera     | 84  | 14.97| 46  | 35.66| 3265.31| 10.83| 0.64|
| Diptera        | 154 | 27.45| 53  | 41.09| 1327.77| 4.40 | 0.88|
| Hemiptera      | 2   | 0.36| 2   | 1.55 | 44.05  | 0.15 | 0.02|
| Homoptera      | 3   | 0.53| 2   | 1.55 | 3.38   | 0.01 | 0.04|
| Hymenoptera    | 65  | 11.59| 34  | 26.36| 3680.04| 12.20| 0.47|
| Lepidoptera    | 3   | 0.53| 3   | 2.33 | 361.10 | 1.20 | 0.04|
| Mantodea       | 2   | 0.36| 2   | 1.55 | 162.06 | 0.54 | 0.02|
| Megaloptera    | 1   | 0.18| 1   | 0.78 | 11.65  | 0.04 | 0.02|
| Neuroptera     | 1   | 0.18| 1   | 0.78 | 19.82  | 0.07 | 0.01|
| Odonata        | 11  | 1.96| 8   | 6.20 | 661.07 | 2.19 | 0.11|
| Orthoptera     | 14  | 2.50| 10  | 7.75 | 3184.49| 10.56| 0.15|
| Trichoptera    | 1   | 0.18| 1   | 0.78 | 20.08  | 0.07 | 0.01|
| **Chordata**   |     |     |     |      |        |      |     |
| Actinopterygii | 6   | 1.070| 6   | 4.65 | 2580.91| 8.56 | 0.06|
| Anura          | 16  | 2.85| 13  | 10.08| 4805.87| 15.94| 0.13|
| **Unknown**    |     |     |     |      |        |      |     |
| Polystyrene    | 1   | 0.18| 1   | 0.78 | 9.42   | 0.03 | 0.01|
| **Sand**       | 23  | 4.10| 11  | 8.53 | 707.85 | 2.35 | 0.18|
| **Wood**       | 1   | 0.18| 1   | 0.78 | 80.81  | 0.27 | 0.01|
| **Total**      | 561 | 100 | 275 | 213.18| 30745.81| 101.95|     |

**Statistical analyses.** For comparing the volume of the stomach contents and the number of food items, samples were analysed with the Mann-Whitney test (Zar, 1999). Two simple linear regression analyses were performed to reveal if larger frogs consume a greater number and/or greater volume of prey: a) frog’s SVL (snout-vent length) and total number of prey in the stomach and, b) frog’s SVL and total volume of prey in the stomach. The software SPSS 16 was used to perform statistical analyses.
**Results**

**Number of prey items.** 49 of the 55 individuals (89.0%) examined in spring and 80 of the 133 individuals (60.9%) examined in summer contained food items.

The frogs’ mean SVL was 54.3±10.68 mm (range 26.7–86.7 mm). There was no significant difference between males and females (average size of males: 55.7±6.73 mm, n=44 and that of females: 53.6±12.21 mm, n=85) (P = 0.29). The sampling adequacy for males (0.98), females (0.99) and total (0.99) is considered sufficient.

A total of 561 prey items was flushed out from the stomachs of *P. ridibundus* of which 551 items were identified, ranging from 1 to 25 per stomach (average x = 4.35±4.38). Organic materials included 25 different taxa belonging to Annelida, Mollusca, Arthropoda and Chordata. The prey items belonged to nine classes (Oligochaeta, Gastropoda, Malacostraca, Arachnida, Chilopoda, Diplopoda, Insecta, Actinopterygii and Amphibia) and 25 orders (Appendix 1). Indigestible materials included a wood stick, a piece of polystyrene and 24 small pieces of gravel. Ten items were unidentifiable due to decapitation or decaying body parts (Appendix 1).

**Diet composition.** A wide variety of prey items was found in the diet samples. Terrestrial food items were 67.7% and aquatic food items were 32.2% of the total food. Among terrestrial components, insects were numerically the most abundant diet (65%), with Diptera (27.7%) followed by Amphipoda (17.5%), Coleoptera (14.7%) and Hymenoptera (11.4%). All other food categories contributed less than 5% (Table 1, Appendix 2). There were changes in diet composition between spring and summer samples; the most dramatic changes occurred in Amphipoda, Anura, Coleoptera, Diptera, and Actinopterygii (Appendix 1). The Berger-Parker Index, which shows the level of food specialisation, was 0.34 for males and 0.27 for females.

No constant prey item was found among numerous food categories because FOi% of all the categories was less than 50%. Diptera, Coleoptera and Hymenoptera were regarded as secondary food while the other categories were classified as accidental (Table 1). The degree of food preference (DFP) indicates that *P. ridibundus* prefers Diptera followed by Coleoptera, Amphipoda and Hymenoptera over other food categories (Table 1).

In total, the highest and lowest IRI values, among organic items, were 1288.7 for Diptera and 0.17 for Megaloptera. We did not calculate V, V%, and IRI of scorpiones owing to the advanced stage of digestion found in the sole item. Other categories with high IRI values are Coleoptera (905.9), Amphipoda (699.1), and Hymenoptera (617.7) (Table 1). IRI for each food item differs between sexes and seasons (Table 2). Coleoptera, Diptera and Hymenoptera are the most abundant food items during different seasons and between sexes. High IRI values of Amphipoda, Araneae, Orthoptera, Actinopterygii and Anura vary between the sexes and seasons.

The diversity of prey consumed by *P. ridibundus* is 3.3 in total. It varies between the sexes from 3.14 in males to 3.29 in females and between seasons from 2.73 in spring to 3.56 in summer (Table 2). The trophic niche breadth is almost equal for both sexes (5.87 for males and 6.60 for females). Schoener’s Index shows a relatively high value of prey niche overlap (α = 0.75), in both seasons combined, between males and females. Niche overlap shows a decrease from the spring (0.72) towards the summer (0.64).

Both the total food volume (U=1533, P=0.032) and the number of food items (U=328, P=0.0001) per stomach differ between April and August with higher values in April samples. Neither total food volume (U=1779, P=0.654) nor number of food items...
Table 2. A comparison of the Index of Relative Importance (IRI) between the sexes and the time interval for each food item consumed by the Marsh Frog, *Pelophylax ridibundus*. H = Shannon-Wiener index of diversity.

|                | Males  | Females | Spring | Summer | Both seasons |
|----------------|--------|---------|--------|--------|-------------|
| **Annelida**   |        |         |        |        |             |
| Lumbriculida   | 9.21   | 19.56   | 60.07  | 0.00   | 10.93       |
| **Mollusca**   |        |         |        |        |             |
| Gastropoda     | 16.73  | 76.78   | 131.87 | 13.30  | 53.40       |
| **Arthropoda** |        |         |        |        |             |
| Amphipoda      | 297.76 | 841.99  | 3141.77| 0.00   | 699.13      |
| Decapoda       | 0.00   | 4.57    | 0.00   | 7.72   | 3.13        |
| Isopoda        | 0.00   | 1.41    | 2.61   | 0.00   | 0.66        |
| Araneae        | 55.25  | 151.47  | 60.69  | 0.00   | 241.03      |
| Scorpiones     | 0.00   | 0.00    | 0.00   | 0.77   |             |
| Opiliones      | 0.00   | 1.15    | 2.48   | 0.00   | 0.53        |
| Chilopoda      | 0.00   | 0.82    | 1.87   | 0.00   | 0.38        |
| Diplopoda      | 0.00   | 1.67    | 3.30   | 0.00   | 0.77        |
| Blattodea      | 0.00   | 1.46    | 0.00   | 3.47   | 0.78        |
| Coleoptera     | 1101.87| 777.77  | 2089.10| 288.51 | 905.98      |
| Diptera        | 1767.21| 1066.50 | 2575.12| 447.71 | 1288.74     |
| Hemiptera      | 1.97   | 0.38    | 3.25   | 0.00   | 0.77        |
| Homoptera      | 0.00   | 0.00    | 0.50   | 3.08   | 0.83        |
| Hymenoptera    | 758.12 | 504.46  | 408.07 | 840.99 | 617.43      |
| Lepidoptera    | 16.66  | 0.88    | 3.80   | 5.10   | 3.97        |
| Mantodea       | 12.53  | 0.00    | 1.63   | 1.40   | 1.36        |
| Megaloptera    | 1.60   | 0.00    | 0.66   | 0.00   | 0.17        |
| Neuroptera     | 0.00   | 0.40    | 0.00   | 0.92   | 0.19        |
| Odonata        | 12.28  | 32.55   | 120.37 | 0.00   | 25.36       |
| Orthoptera     | 168.62 | 81.95   | 0.00   | 384.34 | 99.66       |
| Trichoptera    | 0.00   | 0.40    | 0.78   | 0.00   | 0.19        |
| **Chordata**   |        |         |        |        |             |
| Actinopterygii | 36.39  | 46.41   | 0.00   | 145.31 | 44.10       |
| Anura          | 238.15 | 215.74  | 0.00   | 634.37 | 186.45      |
| **Unknown**    |        |         |        |        |             |
| Polystyrene    | 6.47   | 25.74   | 0.64   | 74.03  | 16.45       |
| **Sand**       | 2.16   | 0.00    | 0.00   | 1.05   | 0.16        |
| Wood           | 56.41  | 44.57   | 24.56  | 91.33  | 54.14       |
| Total          | 4559.40| 3902.05 | 8634.83| 3184.43| 4139.61     |
| H              | 3.14   | 3.29    | 2.73   | 3.56   | 3.30        |

*(U=1819, P=0.8)* per stomach differ between males and females. Frog size is correlated with prey volume (\(r^2=0.115; P=0.0001\)), but not with the number of prey items (\(r^2=0.001; P=0.728\)).
Discussion

As in previous studies in various parts of the distribution area, our results showed that Pelophylax ridibundus feeds mostly on terrestrial prey. This has also been reported by Ruchin & Ryzhov (2002) from Mordovia, Russia, by Popovic et al. (1992) from Carska bara, Serbia, by Çiçek & Mermer (2006, 2007) from Turkey; by Balint, Citrea, Meteae, Jurj, & Condure (2008) from Romania; by Mollov (2008) from Bulgaria and by Cicort-Lucaciu et al. (2013) from Romania. Pelophylax ridibundus preys more frequently on adult insects (57.2%) than on larvae (3.9%), showing that it primarily forages on active prey. Most anurans detect prey visually, and then try to capture it by using the tongue (Stebbins & Cohen, 1995). The lesser motility of larvae than adult insects is the cause of this difference. The same result has previously been reported by Çiçek & Mermer (2007) from Turkey.

We found a relatively high diversity of food items and no food items were constantly present in the diet. This shows that P. ridibundus is a generalist feeder. The diet is distributed unevenly among 25 different orders belonging to four phyla. Feeding activity is low in summer and fewer food items were obtained. This may be due to the lower energy demands of individual in summer than in spring when individuals start their annual activity and need more energy for reproduction. Bogdan, Covaciu-Marcov, Cupsa, Cicort-Lucaciu, and Sas (2012) noted that the feeding of P. ridibundus is strongly affected by weather conditions (e.g. low temperature and drought) during different seasons of the year.

Vertebrates have also been found in the diet of the Marsh Frog by other authors, for example fish by Angelov and Bacvarov (1972), turtles by Turgay (2001), snakes by Çiçek and Mermer (2007) and small mammals of the genera Apodemus, Sorex and Microtus by Ruchin and Ryzhov (2002). We found fishes and amphibians in the diet only in August.

Food consumption during April is more intense than in August. Numerous factors such as body size or skull shape (Emerson, 1985), energy demand (Grayson et al., 2005) and availability of food resources (Das, 1996; López, Scarabotti, Medrano, & Ghirardi, 2009) affect the diet of anurans. Some studies have shown a positive correlation between predator size and consumed prey size and quantity (Maneyro et al., 2004). Energy demand, which varies between seasons and sexes, can also influence anuran diet (Ryan, 1988).

The presence of 16 post-metamorphic juvenile frogs among the examined food items indicates, once more, that P. ridibundus is a cannibalistic amphibian. Cannibalism was recorded only in August. Cannibalism in P. ridibundus has previously been reported by Çiçek & Mermer (2006, 2007) from Turkey, and by Cicort-Lucaciu et al. (2013) from Romania. Cannibalism occurs in certain conditions: when the number of juveniles in the population increases excessively, ecological conditions in the habitat change, the population outgrows the area it inhabits in time (Stebbins & Cohen, 1995) and food availability in the habitat starts to decrease (Crump, 1992). Whiteman et al. (2003) believe that hunting conspecifics is more beneficial than eating heterospecific prey, because the cost ratio in terms of energy/time is much lower for conspecifics, and Ricklefs and Miller (1999) stated that the low digestibility of the chitinous exoskeleton of heterospecifics further reduces energy gained from this prey type than from conspecifics.

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**Supplementary Material**

The tables are given as a Supplementary Annex, which is available via the “Supplementary” tab on the article’s online page (http://dx.doi.org/10.1080/09397140.2016.1226542).

**Disclosure Statement**

No potential conflict of interest was reported by the authors.

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