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## FOOD HABITS OF THE ENDEMIC LONG LEGGED WOOD FROG, *RANA PSEUDODALMATINA* (AMPHIBIA, RANIDAE), IN NORTHERN IRAN

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**Food Habits of the Endemic Long Legged Wood Frog, *Rana pseudodalmatina* (Amphibia, Ranidae), in Northern Iran.** Najibzadeh, M., Gharzi, A., Rastegar-Pouyani, N., Rastegar-Pouyani, S., Pesarakloo, A. — Iranian long legged wood frog, *Rana pseudodalmatina* Eiselt & Schmidtler, 1971 is a brown frog species endemic to the Hyrcanian forest. The objective of the present study is to collect detailed information on the feeding habits of 44 specimens of this species (24 ♂, 20 ♀) by analyzing the stomach contents of individuals from 10 populations inhabiting range. The food habit of *R. pseudodalmatina* generally varies by the availability of surrounding prey items, and it is a foraging predator, the food of which consists largely of Coleoptera (mainly Carabidae, Dytiscidae and Haliplidae), Diptera (Muscidae) and Hymenoptera (Formicidae), and no difference was found between females and males in the stomach content.

**Key words:** Iranian long legged wood frog, feeding habits, Coleoptera, Diptera, Hymenoptera.

### Introduction

One of the fundamental interests to herpetologists and ecologists is feeding relationships in amphibian communities (Hirai, Matsui, 2000). Amphibians are among the indispensable elements of the ecosystem as they are a bridge for energy flow between invertebrates and higher trophic levels (Burton, Likens, 1975). Empirical studies suggest that changes in food availability have long-term consequences for various life-history traits due to a reduction in the amount of energy that can be allocated to somatic growth (Yoneda, Wright, 2005; Inat-suchi et al., 2010; Enriquez-Urzelai et al., 2013). There is a relationship between the abundance of prey in the environment and in the diet of anurans (Houston, 1973).

The Western Palearctic brown frogs occur all over Europe and Asia Minor (Gasc et al., 1997; Baran, Atatur, 1998), there are some difficulties in their classification because they include species of similar morphology. Iranian long legged wood frog, *Rana pseudodalmatina* Eiselt & Schmidtler, 1971 is a brown frog species endemic to the Hyrcanian forest. *R. pseudodalmatina* is widely distributed in Golestan, Mazandaran and Gilan provinces, Iran (at the Southern edge of the Caspian Sea) (Veith et al., 2003). This species is threatened by habitat loss arising from urban sprawl along the Caspian coast and foothills, agricultural development (rice cultivation), and logging (Pesarakloo et al., 2009).

There are not studies about food composition of Iranian long legged wood frogs, then, the objective of the present study is to collect detailed information on the feeding habits of this species by analyzing the stomach contents of individuals from ten populations inhabiting range. In this study we present, in a general way, facts on the diet of the Iranian long legged wood frog populations from Iran.

### Material and methods

The study took place between spring 2015–summer 2016, summing up a total number of 44 (24 ♂, 20 ♀) adult *R. pseudodalmatina* collected from 10 stations (fig. 1, table 1). The frogs were captured either by hand, or by using a net. The stomach contents were taken as soon as possible after capturing because the amphibians digest their food very quickly (Caldwell, 1996).

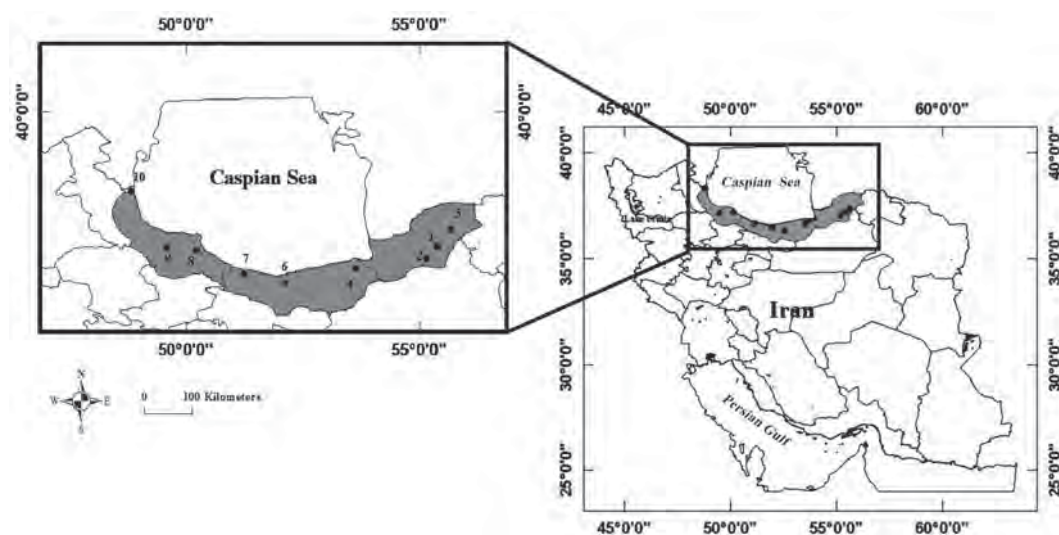


Fig. 1. Map showing the localities of *Rana pseudodalmatina* (Eiselt & Schmidler, 1971) samples from Iran. For identification localities numbers, refer to table 1.

First of all, the stomach contents were collected by using the stomach flushing method (Solé et al., 2005). The stomach contents were preserved in 4 % formalin for later analysis. In addition, the sex of the captured individuals was determined, and snout-urostyle length (SVL, mm), head width length (HWL), femur length (FL), tibia length (TL) were measured, according to Plötner et al., (1994), with a digital calliper. After these procedures, the frogs were subsequently released in the stream.

The preys were determined with a binocular magnifying glass, with the help of specific literature (Oana et al., 2010). Factors such as the general shape and size of the prey, as well as the head, antennae, wings and another pattern's were taken into consideration for identification. As for the distinctive characteristics, the prey items were identified to the lowest possible taxon. After the preys were successfully determined, we calculated more parameters of the feeding, as well as taxonomical appurtenance of the consumed preys, percentage abundance and frequency with which the frogs consume a specific prey taxon.

We also calculated the average number of prey per individual, number of prey items, numeric proportion (percentage proportion from the total number of prey items) and frequency of occurrence (percentage proportion of the frogs that consumed the prey of definite taxon). The results were statistically processed using descriptive statistics and the Mann-Whitney U-test was used to compare the numeric proportion all prey taxa between sexes in order to detect intraspecific differences in the use of food resources, when the data were not normally distributed (Fowler et al., 1998). For the statistical processing of the data we used the software package 'SPSS 16.0'. Vegetal materials, sand and little pebbles were also encountered in the food content. However, these materials were most likely ingested accidentally during foraging and we did not consider them as food (Çiçek, 2011).

## Results

According to morphometric measurements taken from 44 (24 ♂, 20 ♀) adult *R. pseudodalmatina* specimens, the snout-vent length (SVL) of the individuals in the whole population ranged between 34.37–66.15 mm (mean: 49.00). The mouth width (HWL) of the specimens was between 8.34–22.55 mm (mean: 15.87), femur length (FL) of the specimens was between 17.17–37.26 mm (mean: 26.87), and tibia length (TL) of the specimens was between 21.11–39.72 mm (mean: 30.87) in the whole population. We found that, males are slightly bigger than females, (SVL, HWL, FL and TL values), and that statistically significant difference (t.test,  $P < 0.05$ ) was observed between sexes in terms of their sizes.

Overall, the *R. pseudodalmatina* populations in all localities consumed 340 preys belonging to three phyla (Arthropoda, Mollusca and Annelida), five classes (Insecta, Malacostraca, Arachnid, Gastropoda, Oligochaeta) and at least 10 orders (Orthoptera, Diptera, Hemiptera, Hymenoptera, Coleoptera, Lepidoptera, Amphipoda, Araneae, Pulmonata, Megadrilacea (table 1, 2).

**Table 1. Variation in prey taxa in *Rana pseudodalmatina* Eiselt & Schmidtler, 1971 stomach content in all the studied localities**

No	Locality	Phylum	Class	Order	Family
1	Golestan, Minudasht	2	3	7	15
2	Golestan, Azadshahr	1	3	3	6
3	Golestan, Loveh	2	3	3	8
4	Mazanderan, Behshahr	1	3	3	7
5	Mazanderan, Sari	3	3	6	10
6	Mazanderan, Nur	3	3	4	8
7	Mazanderan, Salmanshahr	2	2	3	13
8	Gilan, Langarud	3	5	5	6
9	Gilan, Rasht	2	4	7	12
10	Gilan, Astara	1	3	6	12

**Table 2. Stomach content of both sexes of Iranian long legged wood frog**

Prey taxon	Female			Male			Total		
	n	n, %	f, %	n	n, %	f, %	n	n, %	f, %
Arthropoda	147	90.66	435	166	93.17	408.29	313	93.16	397.15
Insecta	131	80.79	395	149	83.63	362.46	280	84.94	353.97
<b>Orthoptera</b>	8	4.93	25	8	4.49	25	16	4.70	25
Acrididae	8	4.93	25	8	4.49	25	16	4.70	25
<b>Diptera</b>	33	20.37	70	35	19.66	75	68	20	72.72
Muscidae	33	20.37	70	35	19.66	75	68	20	72.72
<b>Hemiptera</b>	6	3.70	20	7	3.93	20.83	13	3.82	20.45
Aphididae	6	3.70	20	7	3.93	20.83	13	3.82	20.45
<b>Hymenoptera</b>	24	14.8	75	27	15.15	70.82	51	16.75	72.21
Formicidae	14	8.64	50	18	10.11	45.83	32	11.17	47.72
Apidae	6	3.70	10	5	2.80	16.66	11	3.23	13.63
Vespidae	4	2.46	15	4	2.24	8.33	8	2.35	11.36
<b>Coleoptera</b>	56	34.53	195	68	38.16	166.65	124	36.42	156.78
Carabidae	14	8.64	50	15	8.42	33.33	29	8.52	40.90
Cicindellidae	7	4.32	20	8	4.49	20.83	15	4.41	20.45
Cucujidae	2	1.23	10	5	2.80	12.5	7	2.05	11.36
Dytiscidae	8	4.93	30	15	8.42	25	23	6.76	27.27
Elateriidae	8	4.93	25	6	3.37	20.83	14	4.11	22.72
Halipilidae	10	6.17	40	11	6.17	37.5	21	6.17	38.63
Hydrophilidae	6	3.70	15	6	3.37	8.33	12	3.52	11.36
Staphylinidae	1	0.61	5	2	1.12	8.33	3	0.88	6.81
<b>Lepidoptera</b>	4	2.46	10	4	2.24	4.16	8	2.35	6.81
Noctuidae	4	2.46	10	4	2.24	4.16	8	2.35	6.81
<b>Malacostraca</b>	9	5.55	25	9	5.05	25	18	4.11	25
<b>Amphipoda</b>	9	5.55	25	9	5.05	25	18	4.11	25
Gammaridae	9	5.55	25	9	5.05	25	18	4.11	25
<b>Arachnida</b>	7	4.32	15	8	4.49	20.83	15	4.41	18.18
Araenae	7	4.32	15	8	4.49	20.83	15	4.41	18.18
<b>Mollusca</b>	5	3.08	20	5	2.80	16.66	10	2.94	18.18
<b>Gastropoda</b>	5	3.08	20	5	2.80	16.66	10	2.94	18.18
Pulmonata	5	3.08	20	5	2.80	16.66	10	2.94	18.18
<b>Annelida</b>	10	6.17	45	7	3.93	25	17	5	34.09
<b>Oligochaeta</b>	10	6.17	45	7	3.93	25	17	5	34.09
<b>Megadrilacea</b>	10	6.17	45	7	3.93	25	17	5	34.09
Lumbricidae	10	6.17	45	7	3.93	25	17	5	34.09

Legend: n — number of prey items; n, % — numeric proportion (percentage proportion from the total number of prey items); f, % — frequency of occurrence (percentage proportion of the frogs that consumed the prey taxon).

Both terrestrial and aquatic prey items were found in the stomach contents of all the specimens. The average number of prey per stomach was 8.94, while the maximum number of prey / individual 35 (male sample, Minudasht locality), whereas the average number of prey per stomach for female and male was 8.52 and 9.36, respectively.

The Insecta containing the highest number of prey groups (number of prey items (n): 280, percentage proportion from the total number of prey items (n, %): 4.94, percentage proportion of the frogs that consumed the prey taxon (f, %): 353.97) (table 2). Six orders were identified within the class Insecta. Among these prey orders the largest groups by frequency of occurrence encountered in the stomach contents were orders Coleoptera (36.422 %), Diptera (20 %) and Hymenoptera (16.75 %), respectively.

Furthermore, the order exhibiting the largest variety among the long legged wood frogs prey groups was the order Coleoptera with eight families. Carabidae (n: 29, n, %: 8.52, f, %: 40.90), Cicindellidae (n: 15, n, %: 4.41, f, %: 20.45), Haliplidae (n: 21, n, %: 6.17, f, %: 38.63), Dytiscidae (n: 23, n, %: 6.76, f, %: 27.27), Hydrophilidae (n: 12, n, %: 3.52, f, %: 11.36), Staphylinidae (n: 3, n, %: 0.88, f, %: 6.81), Cucujidae (n: 7, n, %: 2.05, f, %: 11.36) and Elateridae (n: 14, n, %: 4.11, f, %: 22.72) families belonging to the order Coleoptera. Whereas, the largest family (among all the detected orders) is Muscidae belonging to order Diptera by frequency of occurrence (f, %: 72.72). On the other hand the most important preys for all *R. pseudodalmatina* population were house flies.

Furthermore, table 2 presents the qualitative and quantitative proportion of the trophic spectrum of long legged wood frog. Results of Mann-Whitney U-test show that, the numeric proportion of all prey taxa occurring in the stomachs did not differ significantly between the sexes (U-test,  $Z = 0.69$ ,  $P = 0.48$ ,  $P > 0.05$  for all prey taxa).

In addition to the prey of animal origins, we found vegetal residue, and various inorganic elements including stones.

The majority of the prey taxa identified in the stomach contents registered. Empty stomachs have not been recorded in our survey.

## Discussion

There is no available information concerning food habit of Iranian long legged wood frog in northern Iran. Our study revealed that *Rana pseudodalmatina* feeds largely on various invertebrates and predominantly on the Insecta. The food content consists mainly of Coleoptera (36.42 %), Diptera (20 %) and Hymenoptera (16.75 %). Compared to similar values for prey diversity in other related frogs, Çiçek (2011) have demonstrated that *Rana macrocnemis* Boulenger, 1885 feeds on Coleoptera (62.8 %), Diptera (14.4 %), and Hymenoptera (9.8 %), respectively.

This result also supports the previous studies in other related species, such as *Rana macrocnemis* (Çiçek, 2011), *R. temporaria* (Stoyanova and Mollov, 2008) and *R. dalmatina* (Kovacs et al., 2010). However, Insects also have a significant place in the food of brown and water frogs such as *R. dalmatina* (Aszalos et al., 2005; Kovacs et al., 2010) and *Pelophylax ridibundus* (Mollov, 2008).

Analysis of stomach contents showed that, the average number of prey per stomach was 8.94; whereas the average number of prey per stomach for female and male was 8.52 and 9.36, respectively. However, similar value for Mountain frog (*Rana macrocnemis*) is 8.0 during the breeding period in males and 6.0 in the breeding period in females (Çiçek, 2011).

According to the obtained results, the species feeds on both aquatic and terrestrial prey items. Therefore, this shows that it widely-foraging predator both in water and on land, like many Ranids (e. g. Duellman, Trueb, 1986).

According to Drobenkov et al. (2006) observation flying insects play a noticeable role in the feeding of *R. temporaria*. In the present study, the largest family (among all the detected prey orders) is Muscidae belonging to order Diptera by frequency of occurrence

(f, %: 72.72). In other hand, the most important preys for all *R. pseudodalmatina* population were house flies.

Besides Arthropods, two other phylum were recovered from the stomachs — Annelida (n: 17, n, %: 5, f, %: 34.09) and Mollusca (n: 10, n, %: 2.94, f, %: 18.18). Annelida and Mollusca recovered from the stomach contents showed that Iranian long legged wood frogs did not limit their diet to the Phylum Arthropoda and that they could easily consume different prey groups too.

Results of Mann-Whitney U-test show absence of differences between females and males in the stomach content. This is probably associated that females and males use the same area for foraging. The overlapping of the food composition indicates that it does not vary by sex and that individuals use the same habitat in order to forage (e. g. Hirai and Matsui, 2000). Other researches in other species also revealed no difference between females and males with respect to feeding (Measey, 1998; Mollov, 2008; Hirai, Matsui, 2000; Parker, Goldstein, 2004).

## Conclusion

The food habit of *R. pseudodalmatina* generally varies by the availability of surrounding prey items (table 1, 2), and it is a foraging predator, the food of which consists largely of Coleoptera (mainly Carabidae, Dytiscidae and Haliplidae), Diptera (Muscidae) and Hymenoptera (Formicidae), and no difference was found between females and males in the stomach content.

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