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Food Habit of *Rana* (*Pelophylax*) kl. *esculenta* Females in a New Recorded E-System Population from a Forested Habitat in North-Western Romania

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Abstract: This is the first record of a green frog *Rana* (*Pelophylax*) kl. *esculenta* population from the E-system in the north-western part of Romania. From April to July 2005 we analysed the trophic spectrum of 84 *Rana* kl. *esculenta* females, extracting and identifying a total number of 801 prey items. Beside animal preys, plant materials and remains of shed skin were identified in the stomach contents. We did not find any *Rana* kl. *esculenta* individuals with empty stomachs. The importance of different animal prey categories varied in certain periods of the study. In the stomach contents collected in July we identified 2 adult amphibians. The analysed *Rana* kl. *esculenta* individuals forage mainly on land. The abundance of aquatic preys was higher only at the first sampling event.

Key Words: *Rana* (*Pelophylax*) kl. *esculenta*, feeding, females, E-system, Romania

Introduction

Rana (Pelophylax) kl. *esculenta* is a natural hybrid between *Rana (Pelophylax) lessonae* and *Rana (Pelophylax) ridibunda* species, having a large distribution area in Europe. The 3 green frogs' forms that make up the *Rana (Pelophylax) esculenta* complex are encountered in many system types: L-E, R-E, L-E-R, or E (Tunner and Heppich-Tunner, 1991). Throughout the distribution area of these 3 forms, the L-E and R-E systems are the most common (e.g. Borkin et al., 1986; Tunner and Heppich-Tunner, 1991).

Due to the fact that the taxonomical situation of the green frogs from the *Rana esculenta* complex was clarified only after the 1970s (e.g. Berger, 1973), most of the trophic spectrum studies treat these frogs all together without considering any difference between them (e.g. Kovács and Török, 1995; Lőw and Török, 1998; Cogălniceanu et al., 2000a). Dietary studies discussing separately the feeding of 1 of the 3 forms from their common distribution area were performed for pure populations of *Rana ridibunda* (e.g. Covaciu-Marcov et al., 2000) and for the population of *Rana lessonae* from Reci (inside the Carpathian Mountains), Romania (Sas et al., 2005a).

The aim of the present study was to present an E-system green frog population from the north-western part of Romania and to analyze its feeding.

Materials and Methods

Near the locality of Foieni (47°42'0'' N, 22°23'0'' E, 118 m a.s.l.) in the north-western part of Romania, an E-system *Rana* kl. *esculenta* population was identified. From April to July in 2005 we analysed the feeding of 84 green frogs from this population. The captured frogs were all female; we did not find any male individual. The studied

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individuals were determined after their morphological, chromatic, biometrical, and bioacoustical attributes (in preparation, Sas et al.), using the methods and data indicated in the literature (e.g. Berger, 1973; Wijnands and VanGelder, 1976; Cogălniceanu and Tesio, 1993; Cogălniceanu et al., 2000b).

Stomach contents were collected using the stomach flushing method - without killing the frogs - and stored in separate airtight test tubes in a 4% solution of formaldehyde. Preys were subsequently identified in the laboratory under a binocular microscope. Food composition was evaluated by percentage abundance (%a) and frequency of occurrence (%f). The Kruskal-Wallis test was applied to compare the data sets for all sampling events (Zar, 1999). The Mann-Whitney U-test was used to compare the global month-by-month feeding differences. The Wilcoxon test was used to compare the consumption of aquatic and terrestrial preys. All the analyses were assessed with a 95% confidence interval (Zar, 1999). The changes in prey diversity during the period of our study were estimated using the Shannon-Wiener (1949) diversity index (H).

Results

In April 2005 we recorded a water frog population from the E-system in the north-western part of Romania for the first time. An interesting fact is that during the examination of this population of *Rana* kl. *esculenta*, only female individuals were captured. The recorded habitat is represented by 2 water-collecting channels at the outskirts of an oak forest. These channels are used as breeding sites (observed clutching) by the studied population; the population seems to be a self-maintaining one. After summer (August) the channels dry up rapidly and frogs cannot be seen in the habitat any longer.

In the April-July period of the year 2005, we analysed the trophic spectrum of 84 female frogs from this population. We extracted and determined 801 prey items (Table 1). Beside animal preys, plant materials and remains of molted skin were identified in the stomach contents (Table 2). Regarding the feeding intensity, we did not find any *Rana* kl. *esculenta* individuals with empty stomachs.

Table 1. The number of prey items, the prey percentage (%a) of the most important prey categories, and the prey diversity (Shannon-Wiener H). Number of frogs sampled is shown in parentheses.

	IV. (20)	V. (25)	VI. (19)	VII. (20)
No of preys	356	215	133	97
Mean	17.8	8.6	7.38	4.85
Range	3 - 42	1 - 29	2 - 13	1 - 12
% Aquatic preys	77.81	35.3	6.77	8.25
% Terrestrial preys	22.19	64.7	93.2	91.75
Prey percentage (%a):				
Oligochaeta	-	0.47	3.01	4.12
Gastropoda	1.12	3.26	12.78	14.43
Isopoda	1.12	8.37	6.77	2.06
Araneida	2.81	16.28	28.57	6.19
Diplopoda	0.28	6.51	1.50	11.34
Collembola	5.06	9.30	-	-
Plecoptera [l]	-	20.93	-	-
Plecoptera [a]	-	4.19	0.75	-
Heteroptera	0.56	0.47	3.76	4.12
Coleoptera	3.93	8.37	17.29	30.93
Lepidoptera [l]	-	6.05	15.79	17.53
Nematocera [l]	70.51	2.33	-	-
Brahicera [a]	2.53	2.79	4.51	-
Nematocera [a]	6.46	6.05	2.26	-
Others	5.62	4.65	3.01	7.22
Feeding diversity (H)	1.35	2.65	2.37	2.54

Table 2. The frequency of occurrence (%f) of stomachs with vegetal debris, shad-skin, and animal content. Number of frog sampled is shown in parentheses.

	IV. (20)	V. (25)	VI. (19)	VII. (20)
Occurrence: vegetation	80.00	80.00	84.21	75.00
Occurrence: shed-skin	20.00	8.00	5.26	5.00
Occurrence of animal contents:				
Oligochaeta	-	4.00	15.79	10.00
Gastropoda	15.00	28.00	57.89	60.00
Isopoda	15.00	44.00	31.58	10.00
Araneida	30.00	64.00	73.68	25.00
Diplopoda	5.00	24.00	10.53	40.00
Collembola	5.00	12.00	-	-
Plecoptera [l]	-	48.00	-	-
Plecoptera [a]	-	20.00	5.26	-
Heteroptera	10.00	4.00	26.32	20.00
Coleoptera	55.00	60.00	78.95	95.00
Lepidoptera [l]	-	40.00	57.89	60.00
Nematocera [l]	80.00	4.00	-	-
Brahicera [a]	30.00	20.00	31.58	-
Nematocera [a]	50.00	32.00	15.79	-

Determined prey items were classified into 41 categories. Table 1 and 2 show only the most important ones. Categories with very small proportions, with percentage abundance smaller than 1.00%, were pooled as "others", namely, Hirudinea, Acaria, Chilopoda, Ortoptera, Homoptera, Coleoptera Dytiscidae larvae, Lepidoptera adults, Trichoptera adults, Brachycera larvae, Hymenoptera Formicidae, Panorpata, and Amphibia adults. Beetles of 10 families were identified, and the most important ones are Carabidae, Curculionidae, Elateridae, and Chrysomelidae. The percent abundance of aquatic preys was higher only in the first sampling event (77.81%), and throughout the entire study period we did not record significant differences (Wilcoxon-test Z = 1.09, P > 0.05). Among the aquatic preys we identified Hirudinea, Acaria, Isopoda, Collembola, Plecoptera larvae, Coleoptera Dytiscidae larvae, Nematocera, and Brachycera larvae.

Among the most important prey items consumed by the studied water frogs are Gastropoda, Araneida, Diplopoda, Plecoptera larvae, Coleoptera adults, Lepidoptera larvae, and Nematocera larvae (Tables 1 and 2). These prey categories gain importance in certain periods of the study. However, the global changes in the consumption of prey types during the study are not significant for *Rana* kl. *esculenta* (Kruskal-Wallis-test H = 1.12, P > 0.05). As for the month-by-month differences, we found no significant changes (for all comparison U-test, P > 0.05). The recorded feeding diversity (Shannon-Wiener H) was high (H_{mean} = 2.23, SD = 0.59, SE = 0.29). Only in April, *Rana* kl. *esculenta* exhibited a low dietary diversity (H = 1.73) (Table 1).

Discussion

The E-system population

In Romania, previous to our study, the populations that have been identified were mostly populations of *Rana ridibunda* and *Rana* kl. *esculenta* (R-E system) (Vancea et al., 1989), and the regions were locations where all the 3 forms of the water frog's complex appear (R-E-L system) very rare (Cogălniceanu and Tesio, 1993). In Romania, recently a series of new populations of water frogs from the *Rana esculenta* complex have been identified (Strugariu et al., 2006; Covaciu-Marcov et al., 2007; 2008; Sos et al., 2007). These populations consist exclusively of *Rana ridibunda* or they belong to the R-E or R-E-L systems, no E-system was identified. Generally, towards the southern regions, the R-E systems are present, except an isolated region in the Teuz Valley area and south-western Romania (near the Iron Gate), in which the presence of the species *Rana lessonae* has been recently recorded, so water frogs of this locale form R-E-L systems (Covaciu-Marcov et al., 2007; 2008). In the north region of Romania, all the 3 forms of the water frogs complex (R-E-L system) are present, which makes it the broadest area of the country in which all the 3 forms of the water frogs appear (Covaciu-Marcov et al., 2007).

Although *Rana* kl. *esculenta* is a hybrid, it also frequently appears alone, in the absence of the parental species (e.g. Graf and Polls-Pellaz, 1989; Günther, 1997), which is possible as a consequence of reproduction by hybridogenesis (Uzzell et al., 1980). In Romania, a similar situation, when a hybrid population may persist and perpetuate even in the absence of the parental species, was registered for a hybrid population between *Bombina bombina* and *Bombina variegata*, using the morphological analysis (Sas et al., 2005b).

In 1993, Schmidt worked out a pattern considering that water frogs can reproduce both sexually and asexually. Asexuality characterizes hybridogenesis (Schmidt, 1993). Hotz and collaborators (1992) consider that *Rana* kl. *esculenta* has a reproductive system analogous to the parthenogenetic cycles. It is possible that this population is included in a parthenogenetic cycle, but in order to prove this, beside further field observations, experimental laboratory studies are needed in the future.

The feeding habit

In addition to animal preys, we identified plant materials with high occurrence and shed skins. The absence of empty stomachs throughout the entire studied period shows that there were optimal feeding conditions. The presence of plant material fragments in the stomachs may be considered to be due to accidental intake together with animal preys (see other feeding studies, e.g. Çiçek et al. 2007). The occurrence of shed skins in the stomachs of amphibians has been reported previously (Sas et al., 2005b; Kovács et al., 2007) and might be a habit for epidermal protein recycling (Weldon et al., 1993). Shed skin was found to be a commonly consumed food item in the stomachs of water frogs (*Rana lessonae* – Sas et al., 2005a).

Due to the fact that *Rana* kl. *esculenta* is mostly aquatic, it is expected to forage mainly in aquatic environments. A high consumption of aquatic preys, however, was registered only in the first period of the study (April). For the other months we noticed an increase of the proportion of terrestrial preys in the stomach contents. In the summer period, aquatic preys represented only small proportions of the total number of preys. The fact that the *Rana* kl. *esculenta* hybrid forages mainly on land was reported by other researchers as well (e.g. Lőw et al., 1990; Lőw and Török, 1998). On the other hand, Cogălniceanu et al. (2000b) identified in the stomach contents of *Rana* kl. *esculenta* several aquatic preys. The seasonal differences in the consumption of aquatic and terrestrial preys and the significant differences concerning the consumption of some prey items during the study are results of the seasonal changes in the abundance of the prey type in question. Thus, the consumption in higher amount or the higher occurrence of some food items in certain periods of the study is due to the seasonal variation of the prey item in question (Lőw et al., 1990; Kovács and Török, 1995; Covaciu-Marcov et al., 2000).

In the literature many cases pointed out water frogs consuming vertebrates in addition to invertebrates. Thus, in the stomach contents of the water frogs there were identified items, such as fishes (e.g. *Rana ridibunda* - Covaciu-Marcov et al., 2000), urodelans (e.g. *Rana lessonae* – Sas et al., 2005a), anurans (e.g. *Rana ridibunda* – Çiçek and Mermer, 2006), lizards and even rodents (e.g. *Rana ridibunda* – Sas et al. unpublished data). It should be noted that in the stomach contents collected in July 2 adult amphibians were identified (one specimen of *Rana* kl. *esculenta* and one of *Pelobates fuscus*), as well. The consumption of an individual of *Rana* kl. *esculenta*, beside that of *Pelobates fuscus*, shows the appearance of cannibalism, which is frequently met with the water frogs from the *Rana esculenta* complex.

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