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THE FEEDING STUDY OF A *Rana dalmatina* POPULATION FROM CAREI PLAIN

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Abstract. *The study was performed upon a Rana dalmatina population from the skirt of a forested area that is of conservative interest. We compared the food of the population between the adults and juveniles, and between the males and females. The signification test showed that the differences were significant. The most important preys were the caterpillars and spiders for the adults and the cicadas, collembolans and spiders for the juveniles. The adults and juveniles presented the highest differences, due to their different size. This factor restricted the juveniles to smaller-sized preys, such as cicada or collembolans. For these reasons their food was less diverse and the number of prey category was lower. The comparison between the sexes indicated that they used the microhabitats from the level of the forest skirts in a different manner and that the females fed more intensely. They hunted both in the grassy area as well as in the shrub zone. On the other hand, the males were mainly present in the grassy area, having a higher consumption frequency in the case of the caterpillars, and a lower one for the worms and snails that depend on the shadow and humidity given by the bushes. It can be said that the females had a higher ecologic plasticity than the males, connected with a higher mobility and feeding intensity.*

Key words: *food, Rana dalmatina, gender, juveniles, microhabitat use.*

INTRODUCTION

Rana dalmatina is a species of conservative interest that requires a strict protection (2006/105/EC), and is on the Romanian Red List, being considered as a vulnerable species (Iftime 2005). In the present study, we have proposed to analyse the feeding of a *Rana dalmatina* population. It is known that in order to correctly preserve an amphibian species it is necessary to gather data regarding its biology and ecology (Ancona & Capietti,

1995). With regards to understanding an animals' ecology, an important role is played by the study of its feeding (Hodar 1997). Thus, information is obtained regarding the manner in which it uses the feeding resources of the environment (Bellocq et al. 2000).

Only three feeding studies have been performed in Romania upon the agile frog (Aszalos et al. 2005, Kovacs et al. 2010, Hodişan et al. 2010). These compared the feeding of the agile frog with *Rana arvalis* and *Rana temporaria*. One of them also analysed the feeding differences between the age classes (Kovacs et al. 2010). In this study, we have proposed to verify the influence of the sex upon the species' feeding.

MATERIAL AND METHODS

We went to the habitat on the 9th September 2010. The habitat is represented by the skirt of a forested area, being mainly planted with acacia, but in which oaks also survive. A bog is found near the forested area. The area is situated in Carei Plain, near Urziceni de Pădure locality. The animals were directly captured by hand and were immediately analysed after capturing, so as not to digest the consumed food (Caldwell 1996). After having obtained the stomach contents, the frogs were released into the habitat.

We analysed 46 individuals, from which 23 were males, 11 females and 12 juveniles. The drawing of the samples was made using the stomach flushing method (Solé et al. 2005), using a syringe and a flexible perfusion tube. This method is efficient and does not imply killing the animals (Zheng-Jun et al. 2007).

The feeding diversity was calculated using the Shannon-Weaver index (Shannon & Weaver 1949), while the Sørensen index (Sørensen 1948) was applied for its similarity. We established the signification of the food differences between the sexes or age classes using the Mann-Whitney test (Mann & Whitney 1947).

RESULTS

Only one individual did not present stomach contents. This was a male. Thus, the feeding rate is almost maximum in the case of this population (Table 1). The consumption of vegetal parts is also very high (Table 1). All of the females consumed vegetal fragments. From the males and juveniles, only 5 samples did not present vegetal debris in their stomachs. On the other hand, amphibian shed-skin and mineral elements have a much lower frequency (Table 1). The juveniles did not consume them at all. While from the adults, only one individual from each sex consumed shed-skin fragments or minerals. On a whole, it must be underlined that in the case of this population, neither of the samples exclusively consumed vegetal, shed-skin

or mineral debris. Animal preys were permanently identified in the stomach contents together with these elements.

The feeding intensity does not greatly differ from one sex to another, as it differs from an age class to another (Table 1). This fact is reflected in both of the parameters used in this direction. The highest maximum and average number of preys was obtained by the males, while the lowest by the juveniles. The maximum number of preys has double values than the average one.

Table 1. The feeding rate, feeding intensity and frequency of consumption of the vegetal, shed-skin and mineral fragments

	Males	Females	Adults	Juveniles	Total
Stomachs with content (%)	95.65	100	97.05	100	97.50
Stomachs with vegetal parts (%)	82.61	100	88.24	83.33	87.50
Stomachs with shed-skin (%)	4.35	9.09	5.88	-	5
Stomachs with minerals (%)	4.35	9.09	5.88	-	5
Maximum no. of preys / individual	14	12	14	8	14
Average no. of preys / individual	6.47	6.09	6.35	5	6.15

The 281 preys were grouped in 23 taxonomic categories (Table 2). The prey differences between the two sexes are once more lower than between the two age categories. The males registered the highest prey categories, while the juveniles the lowest. The number of the prey category in case of the juveniles is half of the one for the adults.

The caterpillars and the Cicadinea are the most important preys for the studied population (Table 2). The first group has the highest amount and frequency of consumption values in the case of the males and females, while the second one in the case of the juveniles. These two prey types have much higher values than any other prey category. Other important prey categories are the spiders for the males, the worms for the females and the spiders and collembolans for the juveniles (Table 2). It can be once more observed that the differences between the two sexes are lower, in comparison with ones registered by the age classes.

All of the analysed parameters register higher differences between the two age categories than the ones recorded between the sexes The signification test indicates that the food difference is also important in the case of the sexes, not just regarding the age categories (Mann-Whitney U-test, $p<0.05$). The same rules are applied concerning the food diversity (Table 3). Even if the females have a higher food diversity than the males, the differences are much larger between the adults and juveniles. On the other hand, the values that indicate the food similarity are opposite of the ones

that show its diversity. This fact was expectable. The food similarity is higher in the case of the males, respectively in that of the juveniles (Table 3).

Table 2. The amount and frequency of consumption of the preys (A – amount, F – frequency, l - larvae).

	Males		Females		Adults		Juveniles	
	A	F	A	F	A	F	A	F
Oligochaeta Lumbricidae	6.04	21.74	10.45	36.36	7.41	26.47	6.67	33.33
Gastropoda	5.37	26.09	7.46	36.36	6.02	29.41	-	-
Arachnida - Araneae	12.08	52.17	4.48	27.27	9.72	44.12	13.33	33.33
Crustacea - Isopoda	10.74	30.43	-	-	7.41	20.59	3.33	16.67
Diplopoda	2.68	8.70	1.49	9.09	2.31	8.82	-	-
Chilopoda	3.36	21.74	4.48	18.18	3.70	20.59	-	-
Collembola	-	-	1.49	9.09	0.46	2.94	10	50
Blatoidea	1.34	4.35	-	-	0.93	2.94	-	-
Orthoptera	2.68	17.39	4.48	27.27	3.24	20.59	6.67	33.33
Homoptera - Cicadinea	6.71	30.43	4.48	27.27	6.02	29.41	20	83.33
Heteroptera	1.34	8.70	-	-	0.93	5.88	-	-
Lepidoptera (l.)	26.17	73.91	19.40	54.55	24.07	67.65	3.33	16.67
Coleoptera (l.)	0.67	4.35	1.49	9.09	0.93	5.88	-	-
Coleoptera - Carabidae	1.34	8.70	2.99	18.18	1.85	11.76	-	-
Coleoptere - Lampiridae	1.34	4.35	8.96	27.27	3.70	11.76	-	-
Coleoptera - Curculionide	0.67	4.35	1.49	9.09	0.93	5.88	-	-
Coleoptera - Staphylinidae	-	-	4.48	18.18	1.39	5.88	-	-
Coleoptera - Coccinelidae	0.67	4.35	-	-	0.46	2.94	-	-
Coleoptera – others	3.36	17.39	5.97	36.36	4.17	23.53	-	-
Diptera - Brachycera (l.)	9.40	34.78	4.48	27.27	7.87	32.35	13.33	33.33
Diptera - Brachycera	-	-	5.97	27.27	1.85	8.82	6.67	33.33
Hymenoptera - Formicidae	2.68	8.70	-	-	1.85	5.88	10	16.67
Hymenoptera - others	1.34	4.35	5.97	9.09	2.78	5.88	6.67	33.33

DISCUSSIONS

Feeding studies have frequently signalled amphibian populations in which some of the individuals did not feed or exclusively consumed vegetal parts and shed-skin fragments (Sas et al. 2003, Covaciu-Marcov et al. 2004, 2010, Ferenți et al. 2008, Dimancea et al. 2010). The absence of unfed samples indicates the presence of favourable feeding conditions (Sas et al. 2005a, 2009). In our case, the unfed individual represents an exception, which does not characterise the entire population. The presence of animal

preys in the food of all the other individuals indicates optimum conditions for the studied population. The unfavourable climatic conditions from the beginning of spring are usually responsible for the lack of preys from the habitat and from the amphibians' food from our country. This fact has also been recorded in the case of the agile frog population from Livada Plain (Aszalos et al. 2005).

Table 3. The food diversity and similarity

	Males	Females	Adults	Juveniles
Diversity Shannon-Wiener (*H*)	2.24	2.31	2.46	1.91
Similarity Sørrensen (*S*)	0.30	0.22	0.27	0.32

The vegetal matter from the amphibians' food is very rarely considered as having a nutritious value (eg. Núñez et al. 1982, daSilva & deBritto-Pereira 2006, Van-Sluys et al. 2001). There are cases in which the vegetal parts assure an additional water source (eg. Anderson et al. 1999). However, it is generally considered that the consumption of vegetal debris is accidental, these being ingested once with the aimed preys (Lajmanovich 1995, Borczyc 2001, Solé et al. 2009). The accidental consumption can be a good indicator of the feeding intensity, if the frogs hunt their preys from the vegetation of the habitat (Kovács et al. 2007). We consider that the studied population also follows this rule. Almost all of the individuals have vegetal matter in their stomach contents and the habitat has a rich grassy vegetation, which favours its accidental ingestion. Neither of the other agile frog populations studied from our country presented such high values of the vegetal frequency consumption (Aszalos et al. 2005, Kovacs et al. 2010, Hodişan et al. 2010).

The presence of mineral elements in the stomachs of the amphibians is also considered to be accidental (Hodar et al. 1990, Ferenți & Covaciu-Marcov 2009). There is also the possibility that they were consumed in order to digest the exoskeleton of some of the preys (Santana & Juncá 2007). This fact is highly unlikely in our case. From the 19 frogs that consumed coleopterans and gastropods, only 2 had mineral elements in their stomachs. A similar situation is that of the population from Getic Depression (Hodişan et al. 2010), where mineral elements were identified in their stomachs, but with a very low frequency.

The consumption of its own shed-skin is a method of recycling the epidermal proteins (Bustard & Manderson 1965). It is frequently encountered at amphibians and reptiles (Weldon et al. 1993). There have been cases in which amphibians have consumed even the shed-skin of other amphibian species (Cicort-Lucaciu et al. 2004). On many occasions, dermatophagy

within some populations increases, being a solution for the lack of animal preys from the habitat (Covaciu-Marcov et al. 2005, 2010). The analysed population was not found in this situation. On the contrary, the consumption frequency of shed-skin was lower than any of the populations that have been studied in the country. These data confirm that the high frequency of shed-skin indicates unfavourable feeding conditions.

The major difference between the average and maximum number of preys indicates an uneven distribution of preys in the habitat. These results indicate the existence in the area of a high diversity of microhabitats with different trophic resources. This fact has important conservative implications. Thus, the protection of this species must also imply the conservation of the microhabitats in which it feeds, including the grassy areas neighbouring the forest. Some of the frogs will manage to identify areas with many potential preys, while others will not. Usually, the gregarious preys are the ones that mostly favour this possibility. In other cases, like the one of the population from Livada Plain, the difference between these two parameters is too high (Aszalos et al. 2005). Thus, the average number indicates a precarious feeding of the population (average no. = 2.79), while the maximum number indicates an exception of one of the samples from the population (maximum no. = 14). The present study does not present this situation. The average number of preys is much higher, proving that the populations' feeding was carried out in optimum conditions. Moreover, the fact that the maximum number of preys is the same is a coincidence. The feeding greatly differs between the two populations. The most important determining factor is not represented by the habitat, but by the period in which the study was realised. A real influence of the habitat can be analysed in relation with the population from Almăj-Agrij Depression (Kovács et al. 2010). In this case, the feeding intensity was higher than in that of the population studied by us. The food diversity is considered as an indicator of the quality of the environment in which amphibians live (Kovács et al, 2007).

Sex highly influences the feeding of the studied population, according to the Mann-Whitney signification test, but not as much as age class. This fact is suggested by several parameters of the trophic spectrum, such as feeding intensity, food diversity, taxonomic affiliation of the preys or the number of prey categories. One of the most important factors that determined the differences between the age classes is the size of the frogs. A smaller size, such as the juveniles, limits the prey range (Sas et al. 2005b). This is why the most important preys for the juveniles are the small preys. This is also the case of the juveniles from our population. They mostly consumed Cicadinea, collembolans and spiders. In case of the differences between the sexes, the determining factor could be the additional food resource needed

by the females in order to lay their eggs (Biavatti et al. 2004). Gametogenesis is considered responsible for the increase of food intake, oxygen consumption and motor activity in the case of the females (Merkle 1989). The intensification of these physiological activities is connected with the females' ability to store glycogen and lipid in the gonads (Merkle 1989). In case of our population, food mainly differs between the two sexes due to the different method of using the habitat's surface, while secondly due to an increase of the feeding intensity in the case of the females. In our case, the feeding intensification could also be determined by gametogenesis. However, the different usage of the habitat can be harder associated with this process.

There are studies that have shown on the basis of the number and volume of the preys that the sex differences are not significant (eg. Measey 1998, Wheeler et al. 2007). However, analysing the amount of the preys, it has been established, using the signification test, that the differences between the sexes can be important (Measey 1998). In our case, the significant difference based on the prey types is due to the different manner in which the habitat's surface is used. The females used both the shrub and grassy areas from the forest's skirt, increasing thus the feeding diversity. However, the males focused mainly on the grassy area. This difference in using the microhabitats has increased the frequency of the caterpillars in case of the males, which were captured from green surfaces. Moreover, it has led to a decrease of the frequency of the worms and snails, preys that are dependent of the shadow and humidity offered by the shrubs. It is hard to explain why the males have further moved away from the wooden vegetation area, and the females have not. However, considering the importance of the temperature and humidity in the ecology and biology of the amphibians (Wells 2007), it can be assumed that the females have showed a higher need for humidity than males. Thus, considering that females have relayed on the forest's humidity to a much greater extent that the males, we can easily connect the different usage of habitat with the intensification of the other activities from the females.

The vegetal consumption can express in the most accurate manner the intensification of the feeding activity in case of the females. All of the females consumed vegetal matter, while the males did not. Regarding the average and maximum number of preys, which can be used for the evaluation of the feeding activity, we have concluded that the females have close values to the males, even if the latter have higher values. In the case of other amphibian populations, at which it has been observed that the food greatly differs between the sexes, the average number of preys is higher in the case of the females (eg. Brasileiro et al. 2010). Expressing the feeding

activity using the consumed preys or vegetal parts is a relative one. It relays on the correlation between the feeding activity and the ingestion one. In case of the vegetal parts, the fact that the habitat must favour their accidental ingestion must also be taken into consideration. However, there is also a degree of subjectivity in the way in which these parameters are used. A frog can capture two preys at once, especially in the case in which the aimed preys have a gregarious life, which will lead to an overevaluation of the feeding activity. Or a frog can simply miss the aimed prey, activity that will not be quantified in the number of preys. The number of preys does not express those situations in which a frog confuses the preys with plants moved by the air or water currents (Covaciu et al. 2002, 2004). That is why some of these causes that make the evaluation of the feeding activity to become subjective through the method of the number of preys can be eliminated through the vegetal method. This is because the vegetal method is based on counting the frogs and not the consumed elements. Finally, it must be underlined that the highest problem of the vegetal method is its dependence towards the aspect of the habitat. Thus, the higher the vegetal consumption frequency is, the safer the method. In our case the females' percentage of 100% is a real help in this situation. Of course that the amphibian species for which the vegetal parts have a trophic value do not correspond in this context.

In conclusion, it can be said that the studied population had optimum feeding conditions. The fact has conservative implications, in the plain regions the habitats favourable for this species being rare. The proper feeding of this species underlines the value of the natural protected area Carei Plain for the protection of some *Rana dalmatina* populations. The most important preys were the caterpillars and the spiders in case of the adults and the cicadinea, collembolans and spiders in case of the juveniles. The food of the population greatly differs regarding the age class and sex. The highest differences were recorded between the adults and juveniles, due to the smaller size of the latter. This factor limited them to smaller-sized preys, such as cicadinea or collembolans. For these reasons their food was less diverse and their number of prey categories was reduced to a half. The differences between the sexes were determined by the different manner in which the microhabitats were used and by the increase of the feeding activity in case of the females. The females hunted both in the grassy area as well as in the one with shrubs. The males mainly focused on the grassy vegetation area. This is why they had a higher consumption frequency in case of the caterpillars captured from the grassy vegetation and a smaller one in case of the worms and snails, which depend on the shadow and humidity provided by the shrubs. In this manner, the females fed more di-

versely than the males. Therefore, we recorded that the females had a higher ecologic plasticity than the males, connected to a higher mobility and feeding intensity. The most likely cause is the additional food resources required in the egglaying.

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*** Council Directive 2006/105/EC of 20 November 2006 adapting Directives 73/239/EEC, 74/557/EEC and 2002/83/EC in the field of environment, by reason of the accession of Bulgaria and Romania.