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Seasonal variation in the diet of a population of *Hyla arborea* from Romania

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Abstract. We examined the diet of *Hyla arborea* over its entire activity period (March to late September, 2004), and analysed a total of 585 adult samples. From the stomach contents we identified plant remains, shed-skin fragments, and animals. We identified a total of 2976 prey items, almost all of which originated from the terrestrial environment. Adult araneans and coleopterans were the most abundant prey items in the diet of the studied tree frogs. Some of the prey items become abundant in certain parts of the year (e.g. Homoptera, Lepidoptera larvae, Trichoptera). The dietary diversity index is high and exhibits seasonal changes. During the period of study an important seasonal change was observed in feeding intensity and in the type of consumed prey. Our results show that *Hyla arborea* has a broad dietary diversity which was expected as a consequence of exploiting the habitat both vertically and horizontally, possibly allowing access to a broader spectrum of prey.

Introduction

Understanding the position of amphibians in the trophic network is extremely important because the composition of their diet may be an indicator of the quality of their habitat (Gunzburger, 1999). Moreover, amphibians are important components of ecosystems, in part because they assure direct energy flow from invertebrates to higher trophic levels (Burton and Likens, 1975), and because they can be important consumers in both aquatic and terrestrial habitats (Whiles et al., 2006).

The diet of amphibians has been examined in several studies (e.g. Larsen, 1992; Denoël and Joly, 2001; Denoël and Andreone, 2003; Cicek and Mermer, 2006; for studies carried out in Romania see: Cogălniceanu, Palmer and Ciubuc, 2000; Aszalós et al., 2005; Cicort-Lucaciu et al., 2005; Sas et al., 2005 and the references cited therein). In a recent review, Whiles et al. (2006) emphasized the importance of quantitative information relating to the ecological role of amphibians in ecosystems. Lack of such information makes our understanding of the consequences of amphibian declines for ecosystem function more difficult.

Some feeding data has been published on the species of the genus *Hyla* (e.g. Nishikawa, 1932; Kilby, 1945; Johnson and Bury, 1965; Oplinger, 1967; Freed, 1982; Hirai and Matsui, 2000), including *Hyla arborea* (Juszczyk, 1974; Bannikov et al., 1985; Clausnitzer, 1986; Kovács and Török, 1997a; Cogălniceanu, Palmer and Ciubuc, 2000). All of these studies, however, were based upon a small sample of tree frogs, and addressed feeding only in the breeding season. An important component of the year round life cycle of European anurans is spent in terrestrial habitats, where these animals feed, grow, migrate and disperse. Having both aquatic and terrestrial life stages, pond-breeding amphibians may have important impacts on the food webs of the different ecosystems they occupy. For a better understanding of the role of amphibians as predators in ecosystems, study of their entire activity season throughout a year, including both aquatic and terrestrial life stages, is necessary.

This study documents the diet of the European Tree Frog, *Hyla arborea*, in a swampland

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throughout an entire one year activity period, including both the breeding and postbreeding seasons.

Materials and methods

The samples were taken near Resighea (County of Satu-Mare, Romania, 47°36′N/22°19′E, 113 m a.s.l.), in the hydrographic basin of the Ier Valley, in which an area of 1.2 km^2 was selected for this study. The studied habitat consists of a network of wet areas represented by swamps in open, sunny areas, situated at the edge of a locust tree plantation and some ditches that drain waters in the agricultural fields. The level of water is highest during spring, exceeding 0.5 m, but dries up during the summer. Usually, after autumn rains, the level of water rises again, but 2004 was a dry autumn. Rich grassy vegetation, shrubs and bushes surround the swamp. The habitat is bordered by agricultural and hay fields, and uncultivated areas covered by loess.

The breeding period of the tree frog in this area lasts from the end of March until the end of May, and recruits appear only in the end of June (E.H. Kovács and I. Sas, personal observations). Samples were taken monthly, from March to September in 2004 (except for July, when we did not manage to catch any adult frogs). 385 adult tree frogs were collected from the swamp's water and surroundings during their breeding period (March-May 1) and 200 adult tree frogs were collected after that (May 2-September) from shrubs and trees situated in the damper zones of the habitat.

Stomach contents were collected using the stomach flushing method (e.g. Opatrnỳ, 1980; Griffiths, 1986) and stored in separate airtight test tubes in 4% formaldehyde. Prey items were subsequently identified in the laboratory under a binocular microscope.

Food composition was evaluated by percentage abundance (%A), and frequency of occurrence (%f). In order to determine differences and seasonal variations in the frequency of occurrence of prey consumed, the Chi-square test was used. The Kruskal-Wallis test was applied to compare the data sets from all sampling events (Zar, 1999). The Wilcoxon test was used to compare the consumption of aquatic and terrestrial prey. All the analyses were assessed with a 95% confidence interval (Zar, 1999). The temporally dependent dietary diversity was estimated with the Shannon-Wiener (1949) diversity index (H). Niche overlap was estimated using Pianka's index (Pianka, 1973), using EcoSim 7.0 (Gotelli and Enstminger, 2001). Rarefaction analysis was done for dietary diversity to evaluate whether the sample size of each event was sufficient for the study (the minimum sample size varied between 17 and 21).

Results

We extracted and analyzed 2976 prey items from 585 stomachs (table 1). Beside invertebrate prey, plant materials, remains of molted skin and amphibian eggs (other than those of *Hyla*) were identified. Feeding intensity (occurrence of empty stomachs) showed major changes during the study period ($\chi^2_6 = 0.8$, $P < 0.001$). The occurrence of empty stomachs was higher at the beginning of the breeding period (March: %f = 14.63) compared to the end of it (end of May: %f = 2.29) or the post-reproductive period (e.g. June: %f = 3.22) (table 2). Remains of molted skin were identified only in the samples originating from the aquatic habitat ($\chi^2_6 = 89.41$, $P > 0.05$) (table 2). Amphibian eggs (*Rana arvalis*) were found only at the beginning of the breeding period (%f = 4.87). The variation in the frequency of stomachs containing plant material is connected with the changes in feeding intensity (table 2).

Table 1. Seasonal changes in the number of prey items and in dietary diversity (Shannon-Wiener H). Number of frogs sampled is shown in parentheses. (III – March 28; IV – April 17; V1 – May 6; V2 – May 29; VI – June 14; VIII – August 16; IX – September 22.)

	III (41)	IV (178)	V1 (166)	V2 (131)	VI (31)	VIII (18)	IX (20)
Number of prey items	173	982	976	561	145	44	95
Mean	4.94	5.88	5.94	4.38	4.83	2.62	4.75
Range	1-16	1-21	1-18	1-16	1-10	1-4	1-10
Shannon-Wiener H	2.21	2.3	2.31	2.15	2.23	1.45	2.23

Prey items were determined to belong to 53 categories. Table 2 and figure 1 show only the most important ones. Categories with very small proportions were pooled as "Others" (these are: Gastropoda, Pseudoscorpiones, Acarea, Myriapoda, Colembolla, Odonata, Orthoptera, Dermaptera, Raphidioptera, Neuroptera, Mecoptera, Diptera-larvae). Overall the prey is represented almost exclusively by terrestrial invertebrates, the number of aquatic organisms (Gastropoda, Isopoda, Nematocera larvae) being found only in the stomach of frogs captured in March (5.2%). Thus, the difference of aquatic prey in comparison to terrestrial prey is significant (Wilcoxon-test $Z = 2.36$, $P < 0.05$). The most important prey items for the entire

Table 2. The frequency of occurrence (%f) of empty stomachs, of stomachs with plant debris, shed-skin and animal content (chi^2: n.s.-difference not significant, $P > 0.05$; Significant differences: * – $P < 0.05$, ** – $P < 0.01$, *** $P < 0.001$). Number of frogs sampled is shown in parentheses. (III – March 28; IV – April 17; V1 – May 6; V2 – May 29; VI – June 14; VIII – August 16; IX – September 22.)

	III (41)	IV (178)	V1 (166)	V2 (131)	VI (31)	VIII (18)	IX (20)	x^2 (d.f. = 6)
Occurrence: empty stomachs	14.63	6.17	4.54	2.29	3.22	0	0	0.8^{***}
Occurrence: vegetation	31.7	27.52	14.2	13.74	9.67	12.5	35	17.64^{*}
Occurrence: shed-skin	24.39	1.12	0.56	0.76	0	0	0	$89.41^{n.s.}$
Occurrence of animal contents:								
Araneida	68.29	47.75	75.56	64.12	51.61	62.5	20	17.85^{**}
Isopoda	29.26	2.24	3.97			12.5	25	$77.01^{n.s.}$
Heteroptera	4.87	11.79	20.45	12.21				17.76^{*}
Homoptera	2.43	1.68	1.13	16.03	51.61	50	50	$135.14^{n.s.}$
Coleoptera-larvae	21.95	11.23	24.43	13.74	12.9		5	14.41^{**}
Coleoptera-imago	53.65	60.11	47.15	41.98	70.96	12.5	40	10.78^{**}
Trichoptera-imago			15.34	35.11	0		65	$109.53^{n.s.}$
Lepidoptera-larvae	17.07	45.5	43.18	22.9	6.45		10	32.66^{*}
Brachycera-imago	2.43	22.47	10.79	11.45	16.12	12.5	30	17.31^{*}
Nematocera-imago		21.34	26.13	29.77	25.8	25	15	13.52^{**}
Hymenoptera	7.31	19.66	12.5	9.92	35.48		25	18.1^{*}
Others	14.63	10.67	5.68	0.76	6.45		10	15.73^{*}

study period are adults of Araneae and Coleoptera. Beetles of 11 families were identified, the most important being Carabidae, Staphylinidae, Elateridae and Chrysomelidae. Changes in the consumption of prey types during the study are not significant (Kruskal-Wallis-test $H = 5.1$, $P > 0.05$), but it is evident that some of the prey items become important in certain periods of the year. Significant differences regarding seasonal changes were observed at the following prey: Araneida, Coleoptera-larvae, Coleoptera-imago, Nematocera-imago (these at $P < 0.01$) and Heteroptera, Lepidoptera-larvae, Brahicera-imago, Hymenoptera (at $P < 0.05$) (table 2). Differences are not significant ($P > 0.05$) for the other prey categories; these gain importance only in some periods of the study: Isopoda, Homoptera, Trichoptera-imago (table 2).

Dietary diversity (Shannon-Wiener $H_{\text{mean}} = 2.12$, SD $= 0.3$, SE $= 0.11$) exhibits high values for every period of the study, except August (table 1). Seasonal changes (monthly differences) analysed using Pianka`s index are presented in figure 2. It can be observed that significant differences appear in the diet of the tree frogs between seasons (but not significant within a season) (e.g. March-September $Q = 0.22$, May-September $Q = 0.25$).

Discussion

The results of this study indicate that there are seasonal feeding changes of the *Hyla arborea* population we studied. In the stomach contents we identified plant materials (with a relative high occurrence) and shed skins in addition to invertebrate prey. The low number of empty stomachs throughout the entire study shows that there were optimal feeding conditions for the frogs' survival, although there is a certain level of human influence because the habitat is surrounded by agricultural fields.

During the reproductive period (March-end of May) the number of empty stomachs was higher than later in the season. This may be the result of energetic constraints associated with the reproductive behaviour of the frogs. However, *Hyla arborea* is an anuran with a prolonged breeding season (Grafe and Meuche, 2005), so it forages even in its breeding period, a fact previously confirmed for males (Tester, 1990). Thus, the lower rate of feeding activity

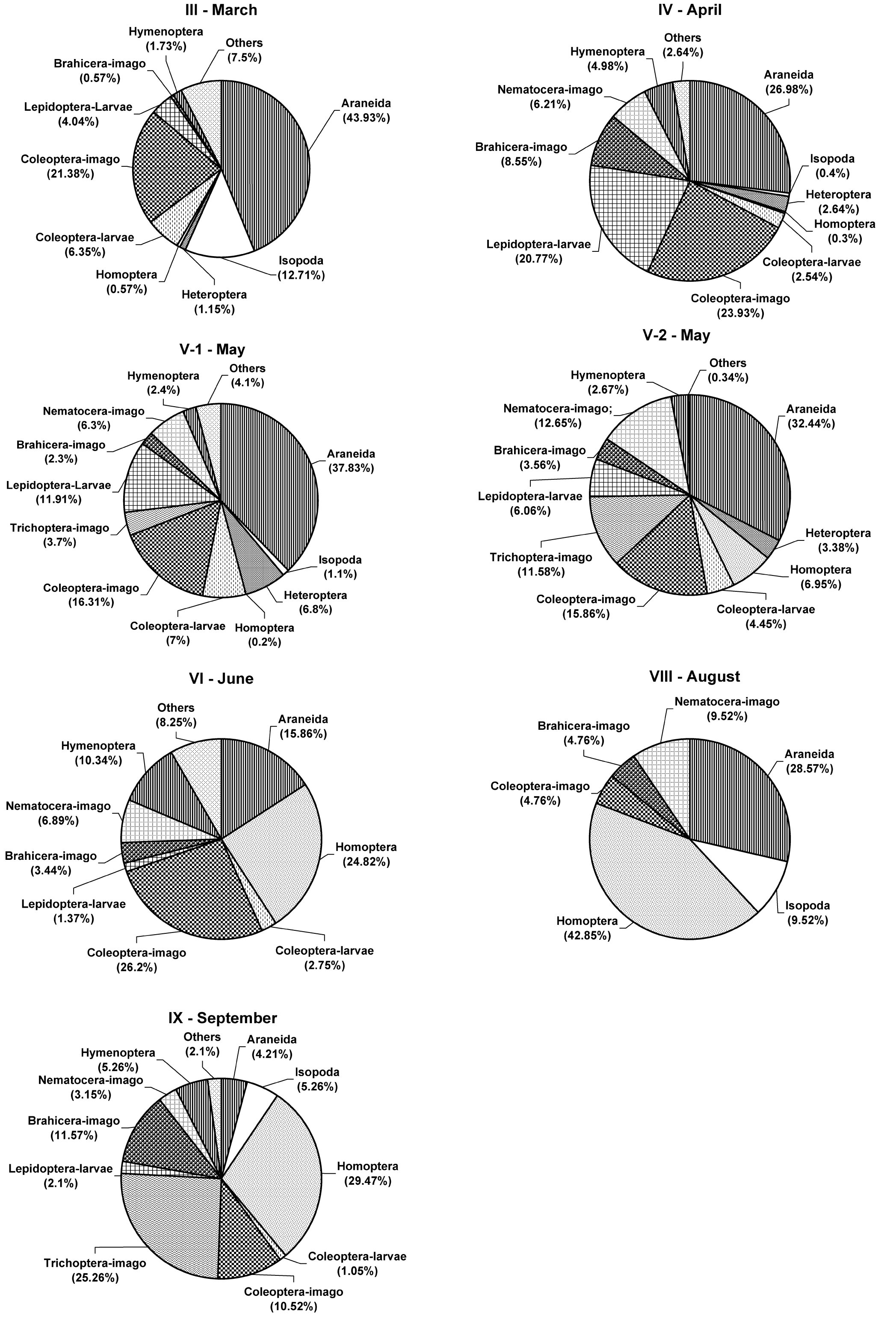

Figure 1. The prey percentage (%A) of the most important prey categories (III – March 28; IV – April 17; V1 – May 6; V2 – May 29; VI – June 14; VIII – August 16; IX – September 22).

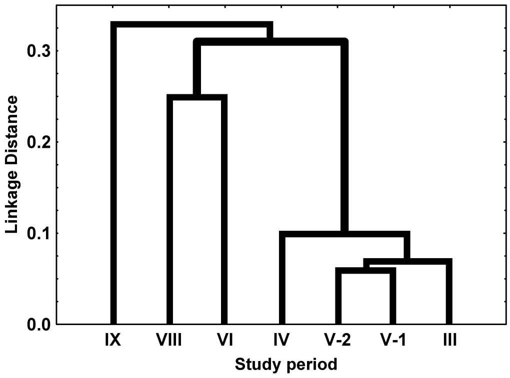

Figure 2. Tree diagram for food niche similarity (Pianka's *Q*) between months (Single linkage, 1 – Pearson r) (III – March 28; IV – April 17; V1 – May 6; V2 – May 29; VI – June 14; VIII – August 16; IX – September 22).

might also be induced by the scarcity of available food. In the period before hibernation (August, September) our results show that feeding intensity is very high; none of the stomachs analysed were empty. Frogs must accumulate energy to increase survival during the winter hibernation period (e.g. see Holenweg and Reyer, 2000). Beside this, frogs need to store energy for "reproductive costs": egg mass production and deposition (females) (e.g. see Loumbourdis and Kyriakopoulousklavounou, 1996), and the very costly activity of calling and mating following the hibernation period (males) (e.g. see Smith, 2003).

The percentage of different prey items exhibits certain seasonal variations. In the breeding period we found the important prey categories to be Araneida, Coleoptera-imago, Lepidoptera-larvae and Isopoda (fig. 1). Kovács and Török (1997b), in western Hungary, found that in the breeding period *Hyla arborea* mainly consumes Diptera (48.3%), but Araneida (11.7%) and Coleoptera (17.26%) were also important. For the summer period we found Homoptera to be an important prey category, along with Araneida and Coleoptera-imago (fig. 1). Previously only Cogălniceanu and collaborators (2000) have found Homoptera to represent greatest prey percentage (45.9%) in the diet of *Hyla arborea*. In western Hungary, Kovács and Török (1997b) found Diptera (58%) to exhibit the greatest percentage, Homoptera was second in prey percentage abundance (15%) for the summer. In autumn (September) we found Homoptera, Coleoptera-imago, Trichoptera-imago and Brachycera-imago (fig. 1) to be important categories. In western Hungary the most heavily exploited prey items were Lepidoptera-larvae (13.3%), Diptera (12.6%) and Araneida (16.1%) (Kovács and Török, 1997b). It is important to note that, given the results of Kovács and Török (1997b), we found Lepidoptera-larvae in high proportion only in spring (April, 20.77%) whereas in autumn they were consumed in lower numbers (2.1%). The food niche similarity (after Pianka's *Q*) is presented in figure 2, showing the feeding differences between spring (breeding period) and summer, autumn (post-breeding period).

The seasonal changes in prey percentage may have resulted from the use of different habitat types (aquatic and terrestrial) and habitat parts (in the case of terrestrial habitats) in different periods of the year. On the other hand, seasonal changes of diet composition may be induced by climatic conditions, such as humidity (e.g. the appearance of Isopoda in March and September), or by seasonal variation of the prey as reflected in their life cycles (Török and Csörgő, 1992) (in our study, Homoptera, Lepidoptera larvae, Trichoptera, – fig. 1, table 2). A good example of this is that other feeding studies conducted on *Hyla arborea* found that homopterans begin to be an important prey category beginning in the summer period (Cogălniceanu, Palmer and Ciubuc, 2000; Kovács and Török, 1997b). The presence or absence of certain prey types in the stomach contents might also be influenced by the fact that *Hyla arborea* uses various microhabitats, distributed vertically ("top-canopy direction"), during different periods of the year (Kovács and Török, 1997a).

Prey of aquatic provenance was identified only for the samples taken in March, at the beginning of the breeding period. That was also the only time we found eggs of *Rana arvalis* in the stomach contents. The fact that prey originating from the aquatic environment was identified only in March, although frogs do not leave the pond used for reproduction until the end of May, reveals that those prey were consumed accidentally.

The presence of fragments of vegetation in the stomachs may be considered to be due to accidental intake, being consumed together with animal prey (mobile prey). This statement is supported by the fact that once the feeding intensity and the rate of feeding activity of the frogs increased, the occurrence of stomachs with plant materials became higher (table 2). The occurrence of shed skins in the stomachs of frogs has been reported previously (Guidali et al., 1999; Hirai and Matsui, 2000; Sas et al., 2003a) and might be reflective of epidermal protein recycling (Weldon et al., 1993). Shed skin was found to be the most commonly consumed food item in the stomachs of *Hyla* from March to May (Oplinger, 1967).

Hyla arborea seems to be a species with low dietary diversity (Chiminello and Generani, 1992; Kovács and Török, 1997a) when compared to other amphibians (Kovács and Török, 1997b; Cogălniceanu, Palmer and Ciubuc, 2000). We obtained a higher diet diversity than those reported by other authors (table 1). Dietary diversity is closely connected with the quality of the habitat in which amphibians live. For the area reported on in this study there are reports relating to the diet of other species of anurans (e.g. *Bombina bombina* – Sas et al., 2003b, *Rana arvalis* – Sas et al., 2003a). Comparing our results with those indicates that *Hyla arborea* has higher dietary diversity, although they share the same habitat. This was expected, considering that tree frogs use not only horizontal spatial niches but also vertical ones, thus encountering a wide variety of prey taxa.

Hyla arborea is a species in decline in different parts of Europe because of changes in its habitat quality resulting from fish introductions (Brönmark and Edenhamn, 1994) and habitat fragmentation (Andersen, Fog and Damgaard, 2004). In our study area the tree frog is represented by large local populations, integrated into metapopulations (local populations of up to 500 individuals, Kovács and Sas, unpublished data), and is an important component of the aquatic and terrestrial habitats in both biomass and as an energetic link between aquatic and terrestrial habitats (Whiles et al., 2006). Further studies regarding the diet of amphibians at the community level, in parallel with studies of spatial and temporal dynamics of populations and habitat use, will help us to better understand the role of amphibian communities in these human-modified ecosystems.

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