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Seasonal variation and ontogenetic change in the diet of a population of *Bufo gargarizans* from the farmland, Sichuan, China

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Abstract. We examined the diet of *Bufo gargarizans* over its entire activity period (March to early November, 2006) based on the stomach contents of 109 specimens. As the result, it showed consumption of prey types no significant differences in seasonal variation, male and female, ontogenetic change, but there was an evident difference in prey number. The lowest average prey number was observed in July and August suggesting that temperature might be the most important limiting factor for feeding activity. Coleoptera-adults and Myriapoda were the most abundant prey items in the diet of the studied toads. Some of the prey items become abundant in certain parts of the year (e.g. Orthoptera, Lepidoptera larvae, Isopoda). During the period of study an important seasonal change was observed in feeding intensity and in the type of consumed prey. Moreover, the average length of prey items was positively correlated with SVL, proving that toads preferred to choose larger preys as they grew. These results suggest that ontogenetic diet change should be considered when the structure of an anuran community is investigated using food niche analysis. On the other hand, diet composition of the 34 toads in September suggests that prey taxa from the stomach contents are weakly correlated with prey availability. This result indicates that toads might feed opportunistically depending on encounter rates with preys.

Keyword: *Bufo gargarizans*; diet; seasonal variation; ontogenetic change; activity period

Introduction

Dietary information have importance in clarifying anuran life history, population fluctuations, and effects of habitat modification (Smith & Bragg 1949, Toft 1981, Beebee 1996), so understanding feeding relationships in amphibian communities is of fundamental interest for herpetologists and ecologists. Amphibian diet composition is influenced by prey size, mobility, palatability, by prey availability and abundance (eg. Turner 1959, Houston 1973, Yilmaz & Kutrup 2006, Çiçek et al. 2007).

The diet of amphibians has been examined in several studies (e.g. Matsui et al. 2003, Biavati et al. 2004, Aszalós et al. 2005, Covaciu-Marcov et al. 2005, Çiçek & Mermer 2006, Kovács et al. 2007, Mollov et al. 2008, Sas et al. 2007, 2009). However, previous studies have not considered intraspecific diet variation, including ontogenetic diet change. Such results may lead us to partial, and possibly erroneous, conclusions regarding community structure (Christian 1982, Lima & Moreira 1993). Therefore, examination of ontogenetic diet change is necessary before community structure can be analyzed on the basis of food utilization.

In the Chinese mainland, *Bufo gargarizans* is a representative species because of its strong dependence on peanut and vegetable fields during its life history. *B. gargarizans* began to hibernate in mid-November, bred in early- January, then came into dormancy, started to fed in early-March. Little feeding data has been published on this species (e.g. Hu 1979, Hu & Jiang 1983, Gu 1985). For a better understanding of the role of amphibians as predators in ecosystems and anuran community structure, dietary studies through their entire activity season are necessary together with investigating the effects of sex and ontogeny upon feeding.

Materials and methods

We selected a population of *B. gargarizans* comprising about 300 individuals, and inhabiting a large farmland near Xi River (County of Gaoping, China, 30°35′N, 105°45′W, elevation 300 m). The habitat is surrounded by agricultural and hay fields, and uncultivated areas covered by loess. Rich grassy vegetation, shrubs and bushes occur along the Xi River, and there are few other amphibian species present (e.g. *Rana nigromaculata*, *Rana limnochar*).

To detect seasonal diet variations, we collected a total number of 109 individuals of *B. gargarizans*, in March (11), April (14), May (12), June (12), July (10), August (11), September (17), October (13) and November (9) in the area near the slow flowing Xi River. During breeding period the presence or absence of nuptial pads was used to determine toad’s sex.

These faded gradually after breeding still one may distinguish them by scrutiny. Collected toads were divided into five groups based on SVL, (females are larger than males according to growth law of toads). For example: I. - 4.06 ± 0.26, 2-6 cm; II.- 7.03 ± 0.13, ♂6-7.5 cm or ♀6-8.5 cm; III.- 8.57 ± 0.10, ♂7.5-9.0 cm or ♀8.5-10 cm; IV.- 10.06 ± 0.14, ♂9.0-10 cm or ♀10.0-11 cm; V.- 10.95 ± 0.27, ♂10 cm or ♀11 cm- (Zou, 1966; Zhao, 2006). For assessing whether consumed prey items are correlated with prey availability by imbedding plastic cups (vinegar: sugar: absolute alcohol: water = 2:1:1:2) in soil, a second population of *B. gargarizans* was chosen, located at 5 km away from the first. 34 toads from this population were captured.

Because this species is typically nocturnal, we collected them between 06.00 and 08.00 by hand, when they were satiate. Some small toads were found dead by vehicle through activity period.

Stomach contents were collected using the stomach flushing method (about the importance of this method see: Solé et al. 2005) at least three times for each individual to remove all stomach contents and stored in separate airtight test tubes in 4% formaldehyde. Prey items were subsequently identified in the laboratory under binocular anatomical lens.

Food composition was evaluated by percentage number (%N), and frequency of occurrence (%f). In order to determine differences (seasonal, ontogenetic and between sexes 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) and Mann-Whitney U test was used to compare feeding of females and males. Kendall's rank correlation coefficient (τ) was calculated to assess relationship between numerical percentage of preys in the environment and percentage of frequency of the same preys in the stomachs contents, and Pearson's rank correlation coefficient (r) was applied for analyzing relationship between toads' SVL and average length of preys. Statistical analyses were conducted with SPSS 13.0 version.

Results

We extracted and analyzed 1583 prey items representing 40 prey categories from the 109 stomachs in the case of the first population (Table 1), and 566 prey items from the 34 stomachs contents collected from the second population, respectively. Besides plant materials, remains of toad's molted skin were identified, but their proportion was small, so they are not included in the analyses. Tables and figure 2 show only the most important prey items. Coleoptera-adults and Lepidoptera-larvae were few, so we included them into Coleoptera-larvae and Lepidoptera-adult.

The most important prey items for the entire study period were Coleoptera-adults and Myriapoda. Beetles

Table 1. Total number, mean and range of prey items and their frequency of occurrence (%f), separately per month (Number of toads sampled is shown in parentheses).

	Mar. (11)	Apr. (14)	May (12)	Jun (12)	Jul (10)	Aug (11)	Sep (17)	Oct (13)	Nov (9)	χ2 df = 8
Number of prey items	169	274	297	191	56	53	203	196	144	-
Mean	15.36	19.57	24.75	15.92	5.60	4.82	11.94	15.08	16.00	-
Range	2-28	8-57	8-48	3-41	2-13	0-13	0-35	1-29	5-27	-
Coleoptera-adults	90.91	100.00	91.67	91.67	30.00	63.64	76.47	76.92	88.89	27.18**
Lepidoptera-larvae	72.73	64.29	50.00	8.33	-	9.09	17.65	53.85	33.33	30.07***
Heteroptera	54.55	35.71	16.67	16.67	60.00	18.18	17.65	30.77	55.56	13.71 n.s.
Hymenoptera	36.36	64.29	58.33	33.33	40.00	36.36	52.94	46.15	44.44	4.66 n.s.
Dermaptera	27.27	28.57	16.67	-	10.00	18.18	11.76	23.08	33.33	6.78 n.s.
Diptera	9.09	7.14	-	-	10.00	-	-	-	-	0.67 n.s.
Orthoptera	-	21.43	41.67	8.33	10.00	9.09	47.06	69.23	77.78	33.04***
Blattaria	-	-	-	-	10.00	-	5.88	7.69	-	5.723 n.s.
Myriapoda	72.73	28.57	75.00	58.33	80.00	18.18	70.59	30.77	44.44	20.79**
Arachnida	45.45	28.57	25.00	33.33	10.00	27.27	5.88	15.38	77.78	19.91*
Mollusea	36.36	21.43	41.67	16.67	10.00	-	5.88	15.38	-	14.57 n.s.
Amphipoda	-	28.57	25.00	-	10.00	-	-	-	-	20.67**
Isopoda	-	28.57	-	8.33	80.00	9.09	47.06	46.15	44.44	31.99***
Annelida	-	14.29	25.00	16.67	10.00	-	23.53	-	33.33	11.34 n.s.

Legend: (chi²: n.s.-difference not significant, P > 0.05; Significant differences: * -P < 0.05, ** -P < 0.01, *** -P < 0.001).

of 12 families were identified, the most important being Carabidae, Staphylinidae, Elateridae, Curculionidae and Chrysomelidae. Kruskall-Wallis test showed that prey number changed significantly among months (χ^2 = 34.181, *df* = 8, *P* < 0.001, Table 1). For example, we found that fewer prey items were consumed in July and August than in the other months. Moreover, number of empty stomachs was high in August (18.18%). Changes in the consumption of prey types during the study are not significant (Kruskal-Wallis-test χ^2= 4.744, *df* = 8, *P* = 0.785), 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: Lepidoptera-larvae, Orthoptera, Isopoda (these at *P* < 0.001), Coleoptera-adults, Myriapoda, Amphipoda (at *P* < 0.01) and Arachnida (at *P* < 0.05). Differences are not significant (*P* > 0.05) for the other prey categories, for example, Annelida, Mollusca, Heteroptera, Diptera, Dermaptera, Hymenoptera, Blattaria (Table 1). The average length of prey items from the stomach contents was positively correlated with SVL of *B. gargarizans* (*r* = 0.848, *N* = 58, *P* < 0.001, Fig. 1).

The prey types collected in the environment and found in the diet composition were similar but their proportions differed (Fig. 2). The correlation between the prey availability in the environment and the stomach contents of abundant prey taxa (including 11 categories) was significantly positive (τ = 0.661, *P* = 0.006). However, when Heteroptera, Dermaptera, Arachnida, Mollusca and Lepidoptera-larvae were excluded from the analysis because of their small frac-

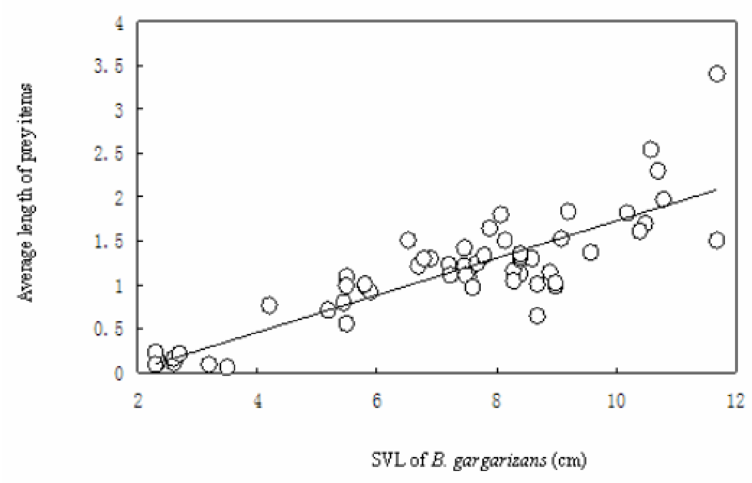

Figure 1. Relationship between SVL and prey size in a natural population of *B. gargarizans*

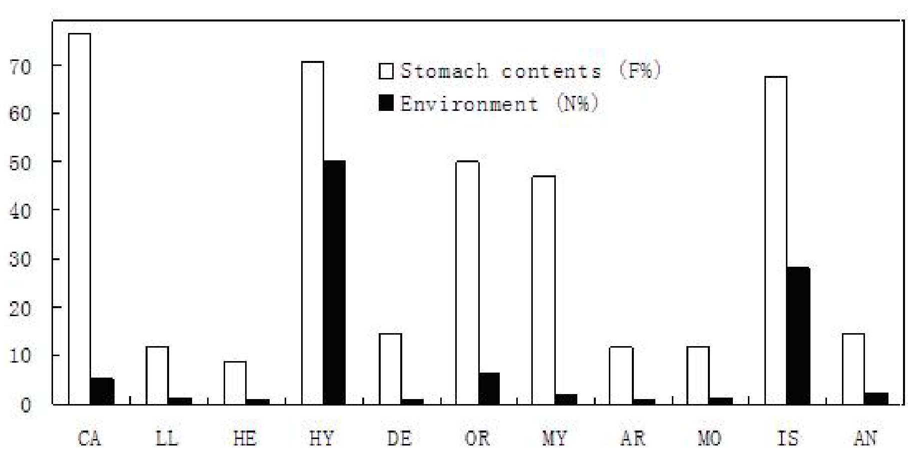

Fig.ure 2. The proportions of some prey taxa in the stomach contents and in the environment (CA, Coleoptera-adults; LL, Lepidoptera-larvae; HE, Heteroptera; HY, Hymenoptera; DE, Dermaptera; OR, Orthoptera; MY, Myriapoda; AR, Arachnida; MO, Mollusca; IS, Isopoda; AN, Annelida) tions in numeric proportions in the environment, the general value of correlation was low and not significant (τ= 0.467, P = 0.188).

There was no significant difference in the consumption of prey types between males and females (Mann-Whitney Test Z = -0.690, P = 0.490), but at Coleoptera-adults and Annelida significant differences regarding intersexual changes were observed (P < 0.05, Table 2).

The most common prey categories were Isopoda, Lepidoptera-larvae, Heteroptera, Hymenoptera, Orthoptera, Myriapoda, Arachnida, Mollusca and Coleoptera-adults in all five SVL groups, but in varying proportions. The significant prey types were Hymenoptera, Isopoda (P < 0.01), Lepidoptera-larvae, Amphipoda and Orthoptera (P < 0.05, Table 2), but the Kruskal-Wallis test suggests that there are no differences among the five SVL groups (χ^2 = 2.363, df = 4, P = 0.669).

Discussion

Our species bred in late December, then, they again came into dormancy, fed diet in early March, so toads' whole feeding period had not breeding behaviour, which differed with other frogs. For example, *Rana nigromaculata* began to breed after hibernation, but these animals' feeding intensity was low in breeding period (Yu unpubl. data). Although animals need large quantities of food after dormancy, available prey were not abundant because of low environment temperature. Thus, the lower rate of feeding activity might also be induced by the scarcity of available food. For example, Annelida and Orthoptera were not found in the stomach contents. From April to June, outside conditions were well (temperature, humidity) and suitable for the activity of animals, so our results showed that feeding intensity was very high, none of the stomachs were empty. We found the important prey categories to be Coleoptera-adults Hymenoptera and Myriapoda. Hu & Jiang (1983) found *Bufo gargarizans* mainly consumed Coleoptera-adults in prey percentage number (98.21%), but others held a small proportion in the diet. However, Lepidoptera-larvae became important prey categories (80.46%), partly because armyworm and cutworm explosively reproduce ever four years (Hu 1979). This result also suggests that toads feed opportunistically depending on encounter rates with prey.

In July and August temperatures were above 40°C during the day and often 30°C at night. So, there were not optimal feeding conditions for the toads' survival. As a result, the number of animals decreased at night and feeding intensity was clearly low. The prey number was the lowest compared to other months and there were found two empty stomachs. This suggests temperature was the most important limiting factor for feeding activity. Isopoda and Myriapoda were the two most important prey categories in July and the frequency of occurrence of all prey categories decreased in August. Hu (1983) found Coleoptera-adults decrease into 39.97%, Lepidoptera-larvae (35.12%) and Diptera (24.91%) were important prey categories. From September to November, climatic conditions assured better circumstances for a higher rate of feeding activity. Moreover, toads must accumulate energy to increase

Table 2. The frequency of occurrence (%f) of prey items in the five SVL groups and intersexual adults.

	I. (30)	II. (25)	III. (31)	IV. (14)	V. (9)	χ^2 df = 4	♂ (37)	♀ (27)	χ^2 df = 1
Coleoptera-adults	93.33	72.00	70.97	85.71	88.89	6.80 n.s.	70.27	92.59	4.81*
Lepidoptera-larvae	16.67	44.00	29.03	57.14	55.56	10.52*	35.14	40.74	0.21 n.s.
Heteroptera	33.33	28.00	22.58	50.00	33.33	3.58 n.s.	32.43	29.63	0.06 n.s.
Hymenoptera	70.00	40.00	29.03	28.57	66.67	14.18**	27.03	44.44	2.10 n.s.
Dermaptera	6.67	20.00	29.03	14.29		7.69 n.s.	29.73	14.81	1.94 n.s.
Diptera	3.33		6.45			2.98 n.s.	5.41		1.51 n.s.
Orthoptera	10.00	32.00	48.39	35.71	44.44	11.21*	40.54	44.44	0.10 n.s.
Blattaria				7.14		6.85 n.s.	5.41	7.41	0.11 n.s.
Myriapoda	43.33	52.00	54.84	71.43	77.78	5.16 n.s.	64.86	55.56	0.57 n.s.
Arachnida	30.00	24.00	6.45	14.29	44.44	8.89 n.s.	18.92	25.93	0.45 n.s.
Mollusea	3.33	20.00	19.35	14.29	44.44	6.30 n.s.	21.62	22.22	0.003 n.s.
Amphipoda		20.00	3.23	7.14		10.89*	10.81	11.11	0.001 n.s.
Isopoda	13.33	16.00	51.61	35.71	33.33	13.61**	29.73	51.85	3.21 n.s.
Annelida		20.00	12.90	28.57	11.11	8.87 n.s.	10.81	33.33	4.89*

survival during the winter hibernation period. Beside these, female toads need to store energy for reproductive costs**.** At the same time, the nuptial pads of male became clear. Coleoptera-adults, Isopoda and Hymenoptera were important categories. In the Guiyang, Gu (1985) found the most heavily exploited prey items were Coleoptera (17.53%), Hymenoptera (15.17%), Dermaptera (14.71%), Orthoptera (12.07%) and Diptera (10.35%).

We found that female SVL was larger than males, but no significant differences regarding the consumed prey items were observed, except for Coleoptera and Annelida. This result was similar with Gu (1985). However, some of the prey items become important in a certain SVL group. Small toads fed only smaller prey, so Formicidae (Hymenoptera) and Coleoptera-larvae became important prey categories (see in: Hirai 2002). The larger ones preferred larger preys, for example, Annelida, Orthoptera and Lepidoptera-larvae, which thus had increased frequency of occurrence. This suggests larger toads may utilize more prey types and large preys for themselves (Gu 1985, Hirai & Matsui 1999, Biavati et al. 2004), but toads with the greatest size had a low number of prey types (Hirai 2002).

B. gargarizans is a species in decline in Asia because of changes in its habitat quality resulting from habitat fragmentation (e.g. spawning sites gradually vanish). In our study area the toads are represented by large local populations, integrated into metapopulations (local populations of up to 200-300 individuals, Yu unpublished data), they have active function for protecting local agriculture. 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 (see in: Kovács et al. 2007).

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