

On the diet of recent metamorphs and adults of the Common Toad, *Bufo bufo* (LINNAEUS, 1758) (Anura: Bufonidae)

Zur Nahrung kürzlich verwandelter und adulter Erdkröten, *Bufo bufo* (LINNAEUS, 1758)
(Anura: Bufonidae)

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KURZFASSUNG

Die Autoren untersuchten die taxonomische Zusammensetzung der Beute frisch verwandelter Erdkröten, *Bufo bufo* (LINNAEUS, 1758) in montenegrinischen Populationen des Biogradsees (Biogradsko jezero) und des Schwarzen Sees (Crno jezero) im Vergleich mit jener adulter Exemplare des Biogradsees. Die Jungtiere wurden im Juni und Juli 2012, die adulten im Mai 2004 gefangen. Die Verdauungstrakte der Jungtiere des Schwarzen Sees und der Adulten des Biograd Sees enthielten im Mittel weniger Beutetiere in gleichzeitig artenärmerer Zusammensetzung als jene der Jungtiere des Biogradsees. Vertreter der Klasse der Insekten dominieren in beiden Altersgruppen. Die zahlenmäßig vorherrschende Beute in der Nahrung der Jungtiere waren an beiden Standorten Vertreter der Familien Formicidae, Chironomidae und Byrrhidae (im Biogradsee jeweils 36.36 %, 12.6 % und 11.9 %, im Schwarzen See 41.86 %, 23.26 % beziehungsweise 11.6 %). In der Beute der adulten Männchen überwogen Carabidae (33.34 %) und Formicidae (31 %), während es bei den Weibchen Carabidae (25.5 %) und Curculionidae (21.1 %) waren.

ABSTRACT

The authors analyzed the taxonomic prey composition of Common Toads, *Bufo bufo* (LINNAEUS, 1758), from populations of Lake Biograd (Biogradsko jezero; young and adult specimens) and the Black Lake (Crno jezero; young specimens) in Montenegro, to compare the dietary practices of recent metamorphs and adults. The young were collected in June and July 2012, adults in May 2004. Compared to young metamorphs from the Black Lake and adults from Lake Biograd, the digestive tracts of juveniles from Lake Biograd contained on the average larger numbers of prey items and a more diversified diet in terms of species richness. Insecta constituted the dominant prey in both age categories. In the juveniles of both sites, the numerically dominant prey taxa were Formicidae, Chironomidae and Byrrhidae (Lake Biograd: 36.36 %, 12.6% and 11.9 %, respectively; Black Lake 41.86 %, 23.26 % and 11.6 %), whereas Carabidae (33.34 %) and Formicidae (31 %) prevailed in the diet of adult males, and Carabidae (25.5 %) and Curculionidae (21.1 %) in that of adult females.

KEY WORDS

Amphibia: Anura: Bufonidae: *Bufo bufo*; feeding ecology, adults, young metamorphs, diet, prey, Lake Biograd (Biogradsko jezero), Black Lake (Crno jezero), Montenegro

INTRODUCTION

The Common Toad *Bufo bufo* (LINNAEUS, 1758), is a widespread species inhabiting almost all of Europe, northwesternmost Africa and western Asia, eastwards as far as the region of Irkutsk (BORKIN & VEITH 1997; SINSCH et al. 2009). This ubiquitous, mainly nocturnal amphibian is found in a wide variety of habitats, even fairly dry ones (ARNOLD & OVENDEN 2002; SINSCH et al. 2009). The Common toad is widespread in Montenegro, where it is found from sea level up to 2,000 m a.s.l. (RADOVANOVIĆ 1951).

The diet of the adult *B. bufo* has been the object of numerous studies (WHEATER 1986; GUTOWSKI & KRZYSZTOFIK 1988; VENCES et al. 1998; MOLLOV 2009; MOLLOV & STOJANOVA 2010; CRNOBRNJA-ISAILOVIĆ et al. 2012; many further references in SINSCH et al. 2009: 238), whereas there is lack of information on the diet of recent metamorphs. To cope with this deficit, the authors compared the diet of recently metamorphosed specimens from two locations, and analyzed the dietary dif-

ferences between these and adult individuals. Feeding information is important to understand amphibian populations in terms

of their life histories, fluctuations or the impact of habitat modifications (ANDERSON et al. 1999).

MATERIALS AND METHODS

All collected materials are deposited in the collection of the Natural History Museum of Montenegro (NHMM) in Podgorica. The authors analyzed the prey items in 123 preserved, recently metamorphosed juveniles ($N_{ind} = 85$ individuals from Lake Biograd [Biogradsko jezero] (field numbers N201-N285), and 38 from the Black Lake [Crno jezero] (field numbers N300-N337)) and 31 adult specimens (NHMM 149-179) of *B. bufo* ($N_{ind} = 19$ males and 12 females from Lake Biograd). The adult specimens were collected in May, 2004, and included in a study on morphological differentiation of the Common Toad in the central Balkan Peninsula (ČADENOVIĆ et al. 2013). The juvenile specimens were collected in June and July 2012 for a trophic study (ČADENOVIĆ 2014). Information on the size of the young metamorphs is provided in results. Lake Biograd (1,112 m a.s.l.; 42°52'40"N, 19°36'30"E) lies in an area dominated by beech and beech-fir forests, and the Black Lake (1,422 m a.s.l.; 43°08'30"N, 19°05'35"E) in an area dominated by conifers.

The materials for this study were collected under permits provided by the Environment Protection Agency of Montenegro - EPA, Republic of Montenegro (nos. no. 02 UPI - 1875/5). The juveniles were caught by hand within a belt three meters wide along the shores of the lakes. The collected specimens were sacrificed by quick freezing, preserved and stored in 70 % ethanol and dissected for stomach content analysis only in the present study. The individual stomach contents were examined under a stereomicroscope equipped with an ocular micrometer scale. Prey items were identified to order level (family level, if possible). All of the adult and juvenile specimens studied contained measurable prey items the length (l) and width (w) of which were measured (to the nearest 0.01 mm). Thirty-one categories of prey were identified using published keys (KRUNIĆ 1985; TANASLJEVIĆ & TOŠIĆ 1985; KEROVEC

1986; CHINERY 1973; BELLMANN 1997) and applying current taxonomy (DE JONG et al. 2014).

After taxonomic classification of the prey items, the following parameters were determined: the absolute and relative frequency of a given category among all prey items found (F_{cat} , % F_{cat}) and the absolute and relative frequency of its occurrence given as number and percentage of digestive tracts containing this prey category (F_{dig} , % F_{dig}). For the adult individuals, volumetric percentages (% V) were calculated for each prey category, where the volume of each prey item was estimated using the equation for ellipsoids of revolution ($V = \pi \cdot l \cdot w^2 / 6$). The mean frequency of occurrence

$$MF_{cat} = (\sum F_{dig}) / N_{ind}$$

represents the average number of prey categories per digestive tract. Based on these data an index of importance (POWELL et al. 1990) was computed for each prey taxon

$$I_x = (\% N_{ind} + \% V + \% F_{dig}) / 3$$

as was done before by POLOVIĆ et al. (2013). The taxonomic diversity of the food in adults was calculated using the Shannon-Wiener diversity index (SHANNON 1948),

$$H' = - \sum_{i=1}^S (p_i * \ln p_i)$$

where p_i is the proportion of individuals found in or using resource i ($i = 1, 2, 3, \dots, S$), S = the total number of resource states. The normalized index can range from 0 - 1. In addition, Simpson's index of diversity (D) was calculated according to the formula

$$D = 1 - \sum_{i=1}^S (n_i^2 - n_i) / (N^2 - N)$$

where n_i = the number of individuals of species i in the sample, N = the total number of individuals in the sample and S = the number of species in the sample. Finally,

Simpson's index of Equitability (E_s) was calculated as

$$E_s = 1/(D \cdot S)$$

where D = Simpson's diversity index and S = the number of species in the sample (KREBS 1999). The level of food specialization was determined applying the index of dominance by Berger-Parker (d), calculated using the formula

$$d = n_{\max} / N$$

where n_{\max} is the number of prey items of the most abundant taxon, and N is the total number of prey items in the sample. The index ranges from 0 - 1 (maximum dominance). For calculating the niche breadth (B), the authors used Hurlbert's (1978) standardized niche breadth

$$B_A = (B-1)/(n-1)$$

a modification of Levins' (1968) formula

$$B = 1 / \sum p_i^2$$

where p_i is the proportion of individuals recorded in the resource or using the component i , while n is the number of possible components. Hurlbert's standardized niche breadth varies between 0 (minimum) and 1 (maximum niche breadth).

The software package Statistica 7.0 was employed for statistical data processing, the computer software "Biodiversity Pro" for the calculations of the Berger-Parker index and "EcoSlim 7.0" for the calculation of Hurlbert's standardized niche breadth.

RESULTS

Qualitative and quantitative analyses of digestive tract contents showed that the diet of *B. bufo* is mostly composed of invertebrate species among which adult invertebrates dominated over larval stages. The average snout-vent-length (SVL) of *B. bufo* young metamorphs was 12.08 mm (9.41-14.44 mm) in the Black Lake, corresponding to an estimated age of approximately one month past metamorphosis. The average SVL of the juvenile toadlets at Lake Biograd was 15.44 mm (12.92 - 18.56 mm), corresponding to an estimated age of approximately one and a half months post metamorphosis.

There are several distinctions between the diet of adults and young metamorphs regarding the numbers of prey taxa and consumed prey items as well as food diversity or presence of plant components. The dominant prey of the juveniles in both sites were Formicidae while for adults it were Carabidae, Formicidae and Curculionidae.

Comparison of the components' relative frequencies in the diet of *B. Bufo* revealed that Formicidae, Chironomidae and Byrrhidae were the dominant prey taxa in recent metamorphs (Table 1), whereas Araneae, Carabidae, Curculionidae, Formicidae, Elateridae and Peltidae prevailed in the diet of adult toads (Table 2). Plant components were present in stomachs of adults only, however merely in traces. The adult toads' diet mostly consisted of typical ter-

restrial invertebrates (darkling beetles, ants, isopods, spiders, harvestmen).

According to the average number of prey taxonomic categories per digestive tract (MF_{cat} - mean frequency of occurrence), a diet may be considered rich or poor, relative to the available prey spectrum. In recent metamorphs from Lake Biograd, there were on the average, 1.31 taxonomic prey categories present per digestive tract, whereas it was 0.9 in Lake Crno metamorphs. In adult females MF_{cat} was 4.58, in males 2.89. The more diversified diet of females is also shown by the wider niche breadth ($B_A = 0.36$), compared to males ($B_A = 0.24$) (Table 3).

Coleoptera was one of the most important contents in the diet of adult females ($I_c = 163.04$) and males ($I_c = 80.22$). Following Coleoptera in descending order, the most important groups in the diet of females were Hymenoptera ($I_h = 14.95$), Diplopoda ($I_d = 12.69$) and Araneae ($I_a = 11.49$), in the diet of males Hymenoptera ($I_h = 32.75$), Araneae ($I_a = 12.42$) and Isopoda ($I_i = 7.11$) (Table 4). The level of food specialization was clearly different in males ($d = 0.31$) and females ($d = 0.24$).

The metamorphs of Lake Biograd differed from the metamorphs of the Black Lake and the adults by higher numbers of prey items and taxonomic prey categories per digestive tract and a more diversified diet.

Table 1: Analysis of digestive tract contents of 123 recently metamorphosed *Bufo bufo* (LINNAEUS, 1758). Absolute and relative numbers of prey items by taxon (F_{cat} , % F_{cat}) and frequencies of each taxon's occurrence (number of digestive tracts in which each taxon was found) (F_{dig} , % F_{dig}). MF_{cat} – Mean frequency of occurrence [$(\sum F_{\text{dig}})/N_{\text{ind}}$], larv. – larval.

Tab 1: Analyse der Verdauungstrakt-Inhalte von 123 kürzlich verwandelten *Bufo bufo* (LINNAEUS, 1758). Absolute und relative Anzahl von Beuteobjekten je Taxon (F_{cat} , % F_{cat}) und Vorkommenshäufigkeiten der Taxa (Zahl von Verdauungstrakten, in denen die Taxa jeweils gefunden wurden) (F_{dig} , % F_{dig}). MF_{cat} – Mittlere Häufigkeit des Vorkommens [$(\sum F_{\text{dig}})/N_{\text{ind}}$], larv. – larval, N_{ind} – Anzahl der Jungtiere.

Number of juveniles / Zahl der Jungtiere	Lake Biograd / Biogradsee				Black Lake / Schwarzer See			
	$N_{\text{ind}} = 85$ $MF_{\text{cat}} = 1.31$				$N_{\text{ind}} = 38$ $MF_{\text{cat}} = 0.9$			
31 Prey categories	F_{cat}	% F_{cat}	F_{dig}	% F_{dig}	F_{cat}	% F_{cat}	F_{dig}	% F_{dig}
Arachnida								
Araneae	1	0.7	1	1.18				
Hydrachnidae	2	1.4	1	1.18				
Oribatidae	3	2.1	2	2.35	1	2.32	1	2.63
Gastropoda	3	2.1	3	3.53				
Collembola	7	4.89	4	4.71				
Hirudinea	1	0.7	1	1.18				
Insecta								
Diptera	2	1.4	2	2.35				
Aphididae	6	4.19	3	3.53	2	4.65	2	5.26
Byrrhidae	17	11.89	10	11.76	5	11.63	4	10.53
Chironomidae	18	12.59	15	18.75	10	23.26	8	21.05
Chrysomelidae	1	0.7	1	1.18				
Elmidae	1	0.7	1	1.18				
Ephydriidae	1	0.7	1	1.18	1	2.32	1	2.63
Eurytomidae	4	2.8	3	3.53	3	6.98	3	7.89
Formicidae	52	36.36	40	47.06	18	41.86	13	34.21
Latridiidae	1	0.7	1	1.18				
Mutillidae	1	0.7	1	1.18	1	2.32	1	2.63
Silphidae	1	0.7	1	1.18				
Silvanidae	1	0.7	1	1.18				
Staphylinidae	3	2.1	3	3.53	2	5.26	2	5.26
Tipulidae	1	0.7	1	1.18				
Braconidae	1	0.7	1	1.18				
Trichogrammatidae	1	0.7	1	1.18				
Heteroptera	2	1.4	2	2.35				
larv. Curculionidae	1	0.7	1	1.18				
larv. Dytiscidae	2	1.4	2	2.35				
larv. Homoptera	2	1.4	2	2.35				
larv. Hymenoptera	2	1.4	1	1.18				
larv. Staphylinidae	3	2.1	3	3.53				
larv. Stratyomyidae	1	0.7	1	1.18				
Tardigrada	1	0.7	1	1.18				
Σ	143	~100	111		43	~100	35	

DISCUSSION

No information was available on the prey composition of *B. bufo* toadlets which is why the new alimentary data of recent metamorphs cannot be discussed in view of earlier findings.

The diet of the metamorphs was very similar in both locations examined. The dominant prey items were chiefly small-

sized insects of the families Formicidae, Chironomidae and Byrrhidae. The clear interpopulation differences in the number of prey taxa and average number of components per digestive tract are explained by different sampling periods.

Coleoptera (Carabidae, Curculionidae) were the most abundant prey components in

Table 2: Analysis of digestive tract contents of 31 adult *Bufo bufo* (LINNAEUS, 1758) from Lake Biograd. Absolute and relative numbers of prey items by taxon (F_{cat} , % F_{cat}) and frequencies of each taxon's occurrence (number of digestive tracts in which each taxon was found) (F_{dig} , % F_{dig}). MF_{cat} – Mean frequency of occurrence [$(\sum F_{\text{dig}})/N_{\text{ind}}$].

Tab 2: Analyse der Verdauungstrakt-Inhalte von 31adulten *Bufo bufo* (LINNAEUS, 1758) der Biogradsee-Population. Absolute und relative Anzahl von Beuteobjekten je Taxon (F_{cat} , % F_{cat}) und Vorkommenshäufigkeiten der Taxa (Zahl von Verdauungstrakten, in denen die Taxa jeweils gefunden wurden) (F_{dig} , % F_{dig}). MF_{cat} – Mittlere Häufigkeit des Vorkommens [$(\sum F_{\text{dig}})/N_{\text{ind}}$]. M + W – Männchen + Weibchen.

21 Prey categories	19 Males / Männchen $MF_{\text{cat}} = 2.89$				12 Females / Weibchen $MF_{\text{cat}} = 4.58$				31 Males + Females / M + W $MF_{\text{cat}} = 3.55$			
	F_{cat}	% F_{cat}	F_{dig}	% F_{dig}	F_{cat}	% F_{cat}	F_{dig}	% F_{dig}	F_{cat}	% F_{cat}	F_{dig}	% F_{dig}
Araneae	3	3.45	3	15.80	4	4.44	3	25.00	7	3.95	6	19.35
Diplopoda	1	1.15	1	5.26	3	3.33	3	25.00	4	2.26	4	12.90
Isopoda	2	2.30	2	10.53					2	1.1	2	6.45
Insecta												
Byrrhidae	1	1.15	1	5.26	1	1.11	1	8.33	2	1.1	2	6.45
Carabidae	29	33.34	15	78.95	23	25.55	11	91.67	52	29.38	26	83.85
Chrysomelidae	1	1.15	1	5.26	1	1.11	1	8.33	2	1.1	2	6.45
Curculionidae	8	9.19	5	26.31	19	21.11	10	83.33	27	15.25	15	48.38
Dermaptera					2	2.22	2	16.70	2	1.1	2	6.45
Diptera					1	1.11	1	8.33	1	0.56	1	3.22
Dytiscidae					3	3.33	3	25.00	3	1.69	3	9.67
Elateridae	6	6.90	6	31.58	3	3.33	2	16.70	9	5.08	8	25.80
Formicidae	27	31.00	12	63.16	10	11.11	4	33.33	37	20.9	16	51.60
Heteroptera					3	3.33	2	16.70	3	1.69	2	6.45
Hydrophilidae	1	1.15	1	5.26	1	1.11	1	8.33	2	1.1	2	6.45
Lampyridae	1	1.15	1	5.26					1	0.56	1	3.22
Peltidae					6	6.66	6	50.00	6	3.39	6	19.35
Phalacridae	1	1.15	1	5.26	2	2.22			3	1.69	1	3.22
Scarabeidae	1	1.15	1	5.26	2	2.22			3	1.69	1	3.22
Silphidae					2	2.22	2	16.70	2	1.1	2	6.45
Staphylinidae	4	4.60	4	21.10					4	2.26	4	12.90
Tenebrionidae	1	1.15	1	5.26	4	4.44	3	25.00	5	2.82	4	12.90
Σ	87	100	55		90	100	55		177	100	110	

Table 3: Taxonomic food diversity in adult males and females of *Bufo bufo* (LINNAEUS, 1758) from Lake Biograd (Montenegro), based on Hurlbert's standardized niche breadth (B_A), Shannon-Wiener diversity index (H'), Simpson's equitability index (E_S), Simpson's diversity index (D) and Berger-Parker index of dominance (d).

Table 3: Taxonomische Nahrungsdiversität bei adulten Männchen und Weibchen von *Bufo bufo* (LINNAEUS, 1758) der Biogradsee-Population (Montenegro), dargestellt mittels Hurlberts standardisierter Nischenbreite (B_A), Shannon-Wiener-Diversitätsindex (H'), Simpsons Äquitabilitätsindex (E_H), Simpsons Diversitätsindex (D) und Berger-Parker Dominanzindex (d).

	Males Männchen	Females Weibchen	Males + Females Männchen + Weibchen
B_A (Hurlbert's standardized niche breadth)	0,245337	0,357053	0,248907
H' (Shannon-Wiener diversity index)	2,012721	2,469339	2,3860819
E_S (Simpson's equitability index)	0,725936	0,824286	0,7609907
D (Simpson's Diversity index)	0,809883	0,882178	0,8577768
d (Berger-Parker index of dominance)	0,308511	0,244681	0,2765957

Table 4: Analysis of digestive tract contents of 31 adult *Bufo bufo* (LINNAEUS, 1758) from Lake Biograd. For each main taxonomical prey group the relative numbers of prey items (% F_{cat}), the relative frequencies of its occurrence (proportion of digestive tracts in which each group was found) (% F_{dig}), volumetric percentages (% V) and the index of importance [$I_x = (\% N_{ind} + \% V + \% F_{dig})/3$] are shown.

Tab 4: Analize der Verdauungstrakt-Inhalte von 31 adulten *Bufo bufo* (LINNAEUS, 1758) der Biogradsee-Population. Von jeder taxonomischen Beutetier-Hauptgruppe sind angegeben: die relative Anzahl von Beuteobjekten (F_{cat} , % F_{cat}), ihre relativen Vorkommenshäufigkeiten (Zahl von Verdauungstrakten, in denen die Taxa jeweils gefunden wurden) (F_{dig} , % F_{dig}), ihr prozentualer Volumenanteil (% V) und ein Index zur ihrer Bedeutung im Nahrungsspektrum der Kröte [$I_x = (\% N_{ind} + \% V + \% F_{dig})/3$].

Main prey taxonomical group Beutetier-Hauptgruppen	Males Männchen				Females Weibchen			
	% F_{cat}	% F_{dig}	% V	I_x	% F_{cat}	% F_{dig}	% V	I_x
Coleoptera	194.76	68.20	75.52	80.22	62.06	43.42	333.39	163.04
Araneae	3.45	15.80	18.00	12.42	4.44	25.00	5.03	11.49
Isopoda	2.30	10.53	8.50	7.11	-	-	-	-
Hymenoptera	31.00	63.16	4.10	32.75	11.11	33.33	0.41	14.95
Diplopoda	1.15	5.26	1.20	2.54	3.33	25.00	9.74	12.69
Dermaptera	-	-	-	-	2.22	16.70	1.35	6.76
Heteroptera	-	-	-	-	3.33	16.70	3.24	7.76
Σ	100		100		100		100	

the sample of adult *B. bufo*, followed by Hymenoptera (Formicidae). The findings in Table 2 coincide with the results obtained by e.g., MOLLOV (2009), MOLLOV & STOJANOVA (2010) and CRNOBRNJA-ISAILOVIĆ et al. (2012). No insect larvae were found in the adult *Bufo* prey sample. This is in good accordance with literature data, which report less than 6 % of larvae in the prey of adult *B. bufo* (PELLANTOVA 1978; WHEATER 1986; GITTINS 1987; KUZMIN 1990; MOLLOV 2009; MOLLOV & STOJANOVA 2010; CRNOBRNJA ISAILOVIĆ et al. 2012).

The high percentage of Chironomidae indicates that the recent metamorphs had not yet fully switched to terrestrial lifestyle, whereas the high frequencies of Formicidae and Byrrhidae point to the toadlets' almost accomplished transition to a terrestrial life.

Diversity of prey taxa was clearly more diversified in adult females than males. Similar to WHEATER (1986), GITTINS (1987) and CRNOBRNJA et al. (2012) high diet overlap between males and females was observed in the present study, suggesting intersexual competition for food resources. In the metamorphs however, the percentage of ants (a main prey component) present in the diet was more than twice as high as in adults, which is most likely caused by the toadlets' small size and allows different size

classes to benefit from the exploitation of different resources. The juveniles from the Lake Biograd population had fed on a very diverse spectrum of prey taxa, which is not in line with the results of KOVACS et al. (2010), POLYMERI et al. (2011) and CICORT-LUCACIU et al. (2013), who found that the prey taxonomic diversity of juveniles of other amphibian species is less than that of adults.

Remains of plant material and mud were scarcely present and only in adult animals. Many authors believe that the plant material accidentally enters the digestive tract of metamorphosed anurans during the hunting and swallowing activities (BURESH & TSONKOV 1942; LESCURE 1964; WHEATER 1986; GITTINS 1987; KUZMIN 1999; MOLLOV 2009; MOLLOV & STOJANOVA 2010).

The value of trophic niche breadth in the combined adult sample analyzed ($B_A = 0.25$) was higher than reported in a similar study by CRNOBRNJA-ISAILOVIĆ et al. (2012), when standardized ($B_A = 0.12$).

Although the present results show that *B. bufo* preys on a varied diet, the trophic niche breadth values reveal that the toads of a given population cannot be considered fully polyphagous. Based on the above results, as well as those obtained from the previously mentioned authors, one must conclude that the diet of *B. bufo* varies con-

siderably depending on habitat, season, weather conditions, prey availability as well as the individuals' body size or age. This

rather low alimentary specificity is certainly one of the key traits explaining the wide distribution of the Common Toad.

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