

## Diet and feeding habits of *Triturus cristatus* (Laurenti, 1768) (Amphibia: Salamandridae) in its southernmost locality

Borislav NAUMOV, Emiliya VACHEVA \*,  
and Simeon LUKANOV

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Street, 1113 Sofia, Bulgaria  
\* Corresponding author: E. Vacheva, E-mail: emilia.vacheva@gmail.com

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**Abstract.** Although the Great crested newt (*Triturus cristatus*) is considered a generalist predator, there are few studies on its prey preferences across its wide range. Data on its feeding are virtually lacking for its southernmost populations; this study aims to fill this gap by analyzing the aquatic food spectrum and prey availability from three ponds in Stara Planina Mts., NW Bulgaria. Ponds were simultaneously sampled once per month; consumed prey items were collected using stomach flushing of captured newts, and hydrobiological samples were taken from each pond to establish potential prey diversity. Remains of ingested prey were found in 109 out of the 141 sampled adult newts (71 male and 70 female). Applying the widely used approach of “operational taxonomic units” (OTUs), prey items from stomach flushing were assigned to 27 OTUs (incl. plants and inorganic matter, and for females only - shed skin), while material from the hydrobiological samples – to 28 (20 of them were also found in the stomach content of the newts). The most frequent prey for both sexes were larvae from the family Chironomidae (found in 21% of the studied newts) and the most abundant superorder Cladocera (60% of the identified prey items). Male newts were more likely than females not to have eaten during the summer when the frequency of empty stomachs was higher compared to spring. For both sexes, prey diversity from stomach content was higher in the spring compared to the summer (Simpson’s index values were respectively 0.76 vs. 0.12 for the males and 0.72 vs. 0.54 for the females), and females consumed more diverse prey during the summer, possibly as a result of their breeding behavior.

**Keywords:** Caudata, newt, diet, Balkan Peninsula, Bulgaria.

### Introduction

According to modern taxonomic concepts, the genus *Triturus* Rafinesque, 1815 includes nine species (comprising the crested newts group) distributed in Europe and Western Asia (Frost 2023). The Great crested newt (*Triturus cristatus*) has the largest range, distributed from Scotland, Wales and western France in the west to the Urals in the east (with isolated populations further east of the mountains), and from the middle parts of Norway and Sweden in the north to north-western Bulgaria in the south (Tzankov & Stoyanov 2008, Litvinchuk & Borkin 2009, Wielstra et al. 2014). *Triturus cristatus* reaches about 15–16 cm in total length (in rare cases more), with females slightly larger than males (see Arntzen 2003 and references therein). During the aquatic phase, it inhabits various stagnant water bodies but mostly prefers those of small areas, shallow depths, and without fish (Arntzen 2003, Naumov et al. 2023).

In Europe, *T. cristatus* has a high conservation status (included in Annexes II and IV of Council Directive 92/43/EEC and Appendix II of the Convention on the Conservation of European Wildlife and Natural Habitats, Bern). Detailed knowledge of the species' biology and ecology, including diet, is required for its protection.

The crested newts are more aquatic than most other European newts, and feeding while in the aquatic phase plays an important role in resource acquisition (Jehle et al. 2011). Numerous publications focused on the food spectrum of *T. cristatus* in particular (Avery 1968, Dolmen & Koksvik 1983, Griffiths & Mylotte 1987, Fasola & Canova 1992, Cicort-Lucaciu et al. 2005, Ruchin 2010, Berzin & Burakova 2022), but these studies are mainly from the more northern parts of its range. Most studies on the diet of the species were conducted in Romania: Covaciu-Marcov et al. (2002 a, b), Cicort-Lucaciu et al. (2005, 2007), Bogdan et al. (2013), Roşca et al. (2013),

Sucea et al. (2014). However, the diet and other ecological aspects of populations of *T. cristatus* within the geographical boundaries of the Balkan Peninsula have not yet been studied. Furthermore, data on the seasonal fluctuations in the species' feeding habits and possible differences between the sexes are scarce across its entire range (see Arntzen 2003, Jehle et al. 2011, Fahrbach & Gerlach 2018).

The main goal of the present study was to derive and analyze data on the food spectrum and feeding during the aquatic phase of *T. cristatus* from the population in the southernmost limit of its range (Stara Planina Mts., NW Bulgaria).

### Material and methods

#### Study area

Field studies were conducted in three ponds (Table 1), located close to each other on the ridge of the Vrachanski Balkan Mts., a part of the Stara Planina Mts. in NW Bulgaria. According to Tzankov & Stoyanov (2008), this site represents the southernmost known point in the entire range of *T. cristatus*, and the three ponds were confirmed as separate localities of the species by Naumov et al. (2016, 2023) [as: “Ochindol (3.7 km N)”, “Ochindol (3.8 km N)”, and “Ochindol (3.9 km N)” = “Parshevitsa”; respectively Pond 1, Pond 2, and Pond 3 in this study].

#### Sampling and data collection

The area was visited monthly in April–November 2021, with all three ponds surveyed at the same time during each visit. Newts were caught using funnel traps (see Lukanov et al. 2021 and references therein): 10 in each pond; the average duration of exposure was 16 hours (from late afternoon until late morning or noon the next day). Up to five male and five female adult newts from each pond (or all captured adult newts if the number was less than five per sex) were kept overnight for stomach flushing. These newts were anesthetized by submerging them for 7–8 minutes in a 0.25 g/l water solution of benzocaine (see Cecala et al. 2007 and Naumov & Biserkov 2013).

After the onset of full anesthesia, a single stomach flushing was performed on each individual in the following steps: 1) opening the newt's mouth and inserting a silicone tube (2 mm outer diameter) into the esophagus; 2) connecting the tube to a syringe containing 15 ml of water; 3) injection of the water through 3–4 consecutive presses; 4) collecting the vomited stomach contents in a previously placed petri dish and extracting the remaining components in the oral cavity (if any) using tweezers; 5) placing the extracted material in an Eppendorf

tube with 70% ethanol. After fully recovering normal locomotor activity, the newts were released at the respective capture ponds. This approach (including its variants) is widely used in research focused on amphibian diet and is particularly suitable for studies on species with a high conservation status because it does not sacrifice or harm the animals while providing a sufficiently accurate estimate of the dietary spectrum (Fraser 1976, Griffiths 1986, Wu et al. 2007, Covaciu-Marcov et al. 2010).

Table 1. Characteristics of the studied ponds: Location = geographical coordinates (decimal degrees; datum WGS 1984) and altitude (m); A = water surface area (m<sup>2</sup>); D = maximum depth (m); WCL = water clarity; MC = presence of macrophytes relative to the area of the pond; PF = presence of fish; SV = shore vegetation type; AD = access of domestic animals.

Location	A	D	WCL	MC	PF	SV	AD
Pond 1: N43.1393 E23.4710; 1380	1000	1	to ca. 3 cm	<10%	yes	grasses	yes
Pond 2: N43.1393 E23.4657; 1370	900	1	to the bottom	>80%	no	grasses	no
Pond 3: N43.1395 E23.4626; 1360	1500	1	to ca. 3 cm	<10%	no	grasses	yes

Parallel to the exposure of the traps (on the same dates and at the same locations in the ponds), hydrobiological samples were taken using 10 consecutive two-meter long sweeps with a hydrobiological net (diameter 250 mm; net mesh 0.5 mm) through the bottom and water layer of each pond. All samples were washed and filtered, and the collected material was fixed in 70% ethanol except for caught amphibians and fish (identified, counted, and released at the capture site). This method (or variations thereof) is relatively simple and, although it has some drawbacks (see Discussion), is often used to collect and analyze data on the potential food base of caudate amphibians (Avery 1968, Griffiths 1986, Berzin & Burakova 2022). The taxonomic affiliation of the collected material was determined in laboratory conditions under a stereomicroscope (magnification 10–40×) to the lowest possible rank (usually to order or family, in rare cases to species). In most cases, the ontogenetic stage was also noted. With this in mind, the main grouping was done by referring the collected individuals/remains to the so-called “operational taxonomic units” (OTUs), which is a widely used approach (Sneath & Sokal 1973, Vacheva & Naumov 2020, 2022, Sato et al. 2022). Some authors (e.g., Kuzmin 1992) recommend using the same taxonomic level (usually order) for all material, but in our opinion, the distribution of data to the lowest possible level could provide a more complete understanding of the diet of a species, especially considering the frequent taxonomic changes, incl. at order and even class level. The OTUs of "Insecta (indet.)" and "Amphibia (indet.)" were not included in the food spectrum analysis because as they could not be identified, the individual remains in them may belong to some of the other OTUs; these two OTUs as well as the OTU of "unidentifiable organic remnants" were only used in the analysis of the ratio of full/empty stomachs.

#### Statistical approach

The trophic spectrum (i.e., the qualitative and quantitative composition of the extracted stomach contents) was described and analyzed based on all samples (the combined data from the three ponds). In this way, we could achieve a more complete picture of the species' diet at this location than by analyzing the samples from each pond separately. This approach was also valid for the comparison between the sexes. Possible seasonal variations (including sex-dependent) were analyzed after dividing and referencing the data to the spring (April–June) and summer (July–September) groups, respectively, while the autumn (October–November) samples were excluded due to the small size (only four individuals). The taxonomic diversity of the food was analyzed via Rényi's index family (diversity profiles), which is considered one of the most useful methods for ordering samples according to their diversity (Tóthmérész 1995). The significance of differences in food diversity between the seasons and between the sexes was assessed by a permutation test based on

Simpson's index (D), which gives higher weight to abundant taxa (Krebs 1999).

The feeding intensity was analyzed based on the frequencies of occurrence of full and empty stomachs (we consider this a better indicator than the number of prey items, as it is not affected by prey size). For comparison between sexes and between seasons, the Chi-square test was used, taking into account the fundamental rule that this test is not appropriate when any expected frequency is below 1 or when more than 20% of the table cells have expected cell frequencies less than 5 (Cochran 1954); in cases of  $df = 1$ , the Yates' correction was applied.

The food selection was analyzed based on a comparison of newts' stomach contents with the hydrobiological samples, and this was done separately for each pond since the potential prey items in them are different (see Results). Electivity was described by the index of Vanderploeg & Scavia (1979) ( $E^*$ ), which represents a modification of Ivlev's forage ratio but has a better theoretical justification (Lechowicz 1982). The index ranges from -1 to +1, and it can be interpreted as a measure of the deviation from random feeding ( $E^* = 0$ ) that makes rank order comparison of electivities meaningful. In our case, the index range was divided into three parts, according to which the OTUs were conditionally categorized as preferred ( $E^* > 0.5$ ), neutral ( $0.5 \geq E^* \geq -0.5$ ), and avoided ( $E^* < -0.5$ ). The calculations were made after excluding the OTUs registered only in the stomachs or only in the water samples, as well as those with very low abundance (found in 1 to 3 individuals) because, according to Lechowicz (1982), the index is vulnerable to sampling errors for rare food types.

The statistical procedures were performed using PAST 4.07 (Hammer et al. 2001), except for the electivity index that was calculated in Microsoft Excel (2010) after manual input of the respective formulas.

## Results

### Trophic spectrum

We sampled 141 adult *T. cristatus* (71 males, 70 females) (Appendix 1). The identifiable animal remains in the extracted stomach contents of the studied newts was assigned to 27 OTUs, and the material from the hydrobiological samples was assigned to 28 OTUs (Appendix 2). Twenty OTUs were found both in the hydrobiological samples and newt stomachs, eight OTUs recorded in the hydrobiological samples were not recorded in the newt stomachs [Anisoptera (larva), Araneae, *Carassius gibelio* (ad.), Ephemeroptera (larva), *Galba truncatula* (eggs), Heteroptera (imago),

Hydrachnidia and Ostracoda], and another four OTUs were recorded in the stomachs but not in the hydrobiological samples [*Carassius gibelio* (eggs), Hemiptera (imago), Lumbricidae and Orthoptera (imago)]. The proportion of individual OTUs in newt stomach contents differed in both frequency and abundance, and the latter also applied to individual OTUs in the hydrobiological samples (Table 2, Fig. 1). The most frequently ingested prey were the larvae of species from the family Chironomidae (registered in 21.10% of the stomachs), followed by adult individuals from the

order Diptera (in 15.60% of the stomachs) and individuals in the preimaginal (pupal) stage from the family Chironomidae (in 14.68% of the stomachs). The superorder Cladocera was represented with the greatest abundance (60.34% of the ingested individuals), followed by the family Chironomidae (larva) (6.83%) and the order Diptera (imago) (5.98%). OTUs with the highest abundance in the material from the hydrobiological samples were Chironomidae (larva) (31.35% of all collected individuals), *Galba truncatula* (26.73%), and Naididae (17.33%).

Table 2. Quantitative characterization of recorded OTUs (newt stomachs: all data; hydrobiological samples: excluded OTUs that were not found in stomachs, as well as data from samples collected on days when no newts were caught): number of individual remains in stomachs (N), number of stomachs in which they were found (F) and number of individuals in the hydrobiological samples (H); ad. = adult, e. = egg, i. = imago, l. = larva; p. = pupa, sub. = subadult.

Phylum: Class	OTU	N	F	H
Annelida: Clitellata	Lumbricidae	1	1	-
Annelida: Clitellata	Naididae	47	5	1419
Mollusca: Bivalvia	<i>Pisidium casertanum</i>	10	6	1109
Mollusca: Gastropoda	<i>Galba truncatula</i>	22	10	2189
Arthropoda: Branchiopoda	Cladocera	636	14	15
Arthropoda: Malacostraca	Amphipoda	40	1	3
Arthropoda: Insecta	Aphididae	8	4	21
Arthropoda: Insecta	Chironomidae (l.)	72	23	2567
Arthropoda: Insecta	Chironomidae (p.)	25	16	100
Arthropoda: Insecta	Coleoptera (i.)	7	5	63
Arthropoda: Insecta	Corixidae	18	13	245
Arthropoda: Insecta	Diptera (i.)	63	17	13
Arthropoda: Insecta	Diptera (l.)	5	3	77
Arthropoda: Insecta	Dytiscidae (l.)	13	8	47
Arthropoda: Insecta	Hemiptera (i.)	2	2	-
Arthropoda: Insecta	Hymenoptera (i.)	1	1	6
Arthropoda: Insecta	Insecta (indet.)	10	10	-
Arthropoda: Insecta	Orthoptera (i.)	4	4	-
Arthropoda: Insecta	Trichoptera (l.)	1	1	20
Arthropoda: Insecta	Zygoptera (l.)	7	6	210
Chordata: Actinopterygii	<i>Carassius gibelio</i> (e.)	25	7	-
Chordata: Amphibia	<i>Lissotriton vulgaris</i> (ad.)	1	1	1
Chordata: Amphibia	<i>Triturus cristatus</i> (sub.)	1	1	7
Chordata: Amphibia	<i>Triturus cristatus</i> (l.)	8	7	49
Chordata: Amphibia	Amphibia (indet.)	11	10	-
Chordata: Amphibia	Anura (l.)	36	8	28
Chordata: Amphibia	Ranidae (e.)	1	1	-
	shed skin		3	
	plant remnants		14	
	unidentifiable organic remnants		18	
	inorganic matter		4	

The comparison between the stomach contents of the males and females (Fig. 2) demonstrated that in both sexes, the most frequently ingested prey belonged to the family Chironomidae (larva), followed by the family Corixidae and the order Diptera (imago) in males and by the Chironomidae family (pupa) in females. Cladocera had the highest abundance in both sexes. In males, the number of registered OTUs was 19, two of which were unique [Hymenoptera (imago) and Trichoptera (larva)], and in females, the number

of OTUs was 23, six of which were unique [Amphipoda, Lumbricidae, Diptera (larva), *Lissotriton vulgaris* (ad.), *Triturus cristatus* (sub.), and Ranidae (eggs)]. Remains of ingested own shed skin were recorded only in female individuals. Plant remains, and inorganic particles (small grains of sand, etc.) were found in both sexes with almost equal frequency (plant matter in 13.73% of males and 12.07% of females; inorganic matter in 3.92% of males and 3.45% of females).

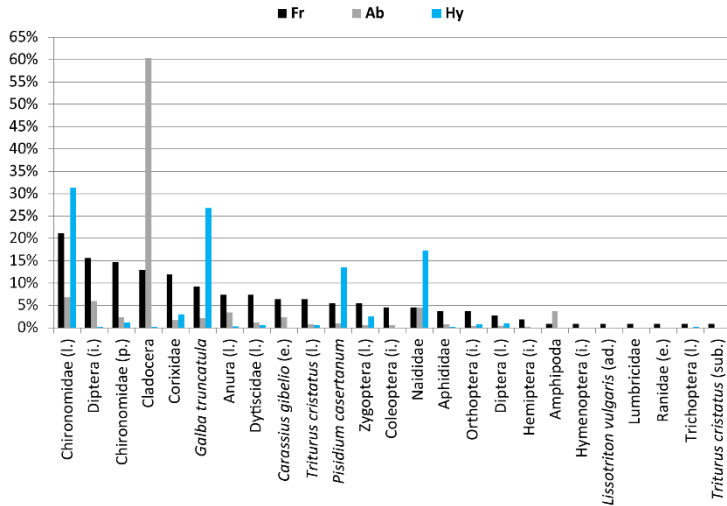


Figure 1. Percentage of OTUs according to their frequency in the stomach contents (Fr), the number of recorded individual remains in the stomachs (Ab), and the number of individuals in the hydrobiological samples (Hy); the order of OTUs is in descending order relative to Fr.

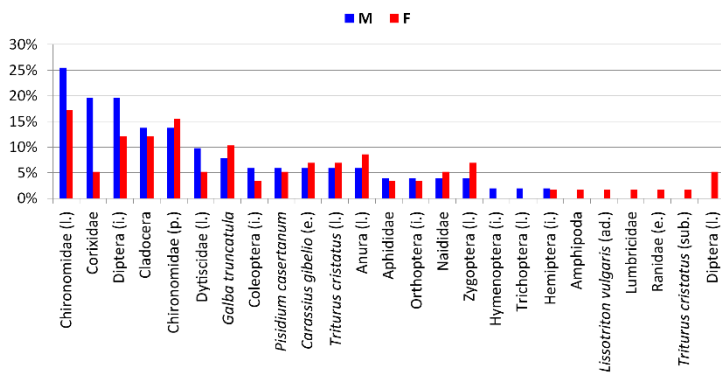


Figure 2. Percentage of OTUs according to their frequency in the stomach contents of male (M) and female (F) newts; ordering of OTUs is in descending order of frequency in males.

Ecological features

There were remains of ingested prey in 109 out of the 141 examined *T. cristatus* individuals (i.e., 77% of all newts had recently consumed prey), but seasonal and sex variations were observed (Fig. 3). In the spring, the frequency of registering empty stomachs was significantly lower than in the summer ( $\chi^2 = 5.478$ ;  $df = 1$ ;  $p = 0.019$ ). When analyzing the full sample (all months in total), the comparison between sexes did not show a significant difference ( $\chi^2 = 1.855$ ;  $df = 1$ ;  $p = 0.173$ ). When dividing the sample by seasons, this remained true for the spring ( $\chi^2 = 0.009$ ;  $df = 1$ ;  $p = 0.924$ ); however, in the summer, the frequency of females with empty stomachs was lower than that of males ( $\chi^2 = 4.792$ ;  $df = 1$ ;  $p = 0.029$ ).

Rényi's profiles demonstrated that the diversity of newt stomach contents was higher in spring than in summer (Fig.

4), both for the entire sample and separately for the two sexes, and the difference between seasons was statistically significant in all cases (Table 3). Comparison between the sexes revealed that the diversity of stomach contents was higher in females for the entire sample (all months in total) and for the summer but not spring (Fig. 4, Table 3).

The analysis of the hydrobiological samples showed that the potential food base of the newts in the three studied ponds differed in terms of the number of recorded OTUs and their abundance (Appendix 2). According to Rényi's profiles (Fig. 5), the diversity of the potential food resource was highest in Pond 2, lower in Pond 3, and lowest in Pond 1 (calculated Simpson's index values were respectively 0.77, 0.66, and 0.36), and differences between ponds were statistically significant (based on Simpson's index in all combinations: Permutation  $p < 0.001$ ).

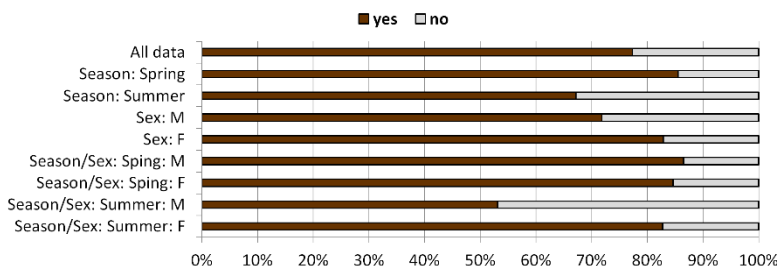


Figure 3. Ratio (%) between newts with full (yes) and empty (no) stomachs in the entire sample (All data) and separately by season, sex (M = males; F = females), and season/sex combination.

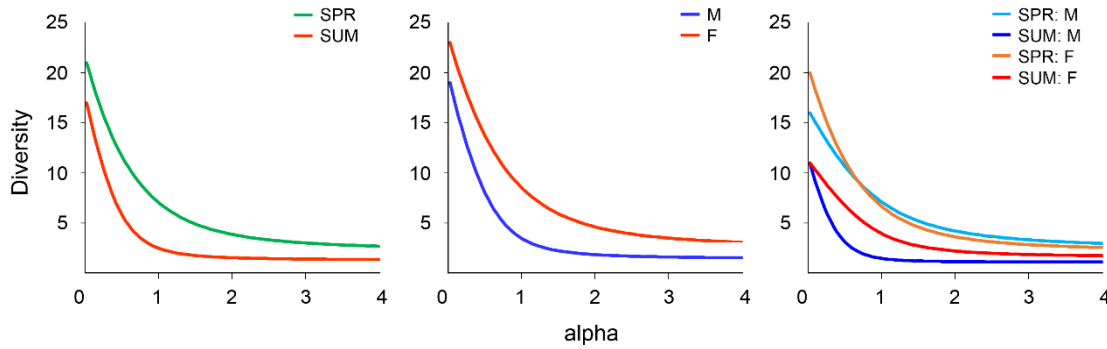


Figure 4. Diversity profiles of *T. cristatus* stomach contents: in spring (SPR) and summer (SUM); in males (M) and females (F) from the entire sample; in spring and summer separately for both sexes.

Table 3. Comparison of stomach contents diversity of *T. cristatus* by season and sex: Simpson's index (D) values and the statistical significance of the differences between them (Permutation p).

		Perm. p
Season	Total: Spring (D = 0.7382) vs. Summer (D = 0.3456)	0.0001
	Males: Spring (D = 0.7590) vs. Summer (D = 0.1159)	0.0001
	Females: Spring (D = 0.7201) vs. Summer (D = 0.5400)	0.0026
Sex	Total: Males (D = 0.4477) vs. Females (D = 0.7797)	0.0001
	Spring: Males (D = 0.7590) vs. Females (D = 0.7201)	0.2473
	Summer: Males (D = 0.1159) vs. Females (D = 0.5400)	0.0001

According to the derived values of the Vanderploeg & Scavia index (based on the aggregated data from the period April-September), the newts' preferred prey in Pond 2 were Dytiscidae (larva) and Diptera (imago), and in Pond 3 - Cladocera (Table 4). When examining the two sexes separately, in Pond 2, Dytiscidae (larva) was the preferred OTU for males, and Diptera (imago) and Anura (larva) - for females, while in Pond 3, it was Cladocera for both sexes (Table 4). The low number of recorded individuals of most OTUs in both stomach and hydrobiological samples from Pond 1 meant that using the index would not be indicative in this case.

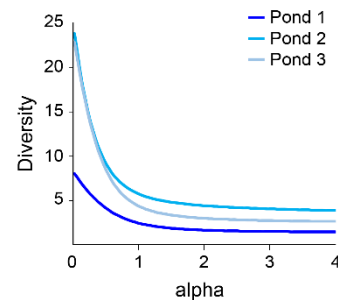


Figure 5. Diversity profiles of the potential food base for *T. cristatus* in the studied ponds.

Table 4. Vanderploeg & Scavia index values for the samples from Pond 2 and Pond 3; symbols >, [=], and < denote preferred, neutral, and avoided prey, respectively (see Material and methods).

OTU	Pond 2			Pond 3		
	Total	Males	Females	Total	Males	Females
Anura (l.)	0.46 [=]	n/a	0.71 [>]	n/a	n/a	n/a
Aphididae	n/a	n/a	n/a	-0.01 [=]	-0.05 [=]	n/a
Chironomidae (l.)	-0.64 [<]	-0.71 [<]	-0.49 [=]	-0.94 [<]	-0.96 [<]	-0.89 [<]
Chironomidae (p.)	0.28 [=]	0.20 [=]	0.43	-0.57 [<]	-0.75 [<]	-0.27 [=]
Cladocera	n/a	n/a	n/a	0.91 [>]	0.91 [>]	0.91 [>]
Coleoptera (i.)	n/a	n/a	n/a	-0.75 [<]	n/a	n/a
Corixidae	n/a	n/a	n/a	-0.89 [<]	-0.88 [<]	-0.91 [<]
Diptera (i.)	0.63 [>]	n/a	0.84 [>]	n/a	n/a	n/a
Dytiscidae (l.)	0.81 [>]	0.89 [>]	n/a	-0.81 [<]	-0.84 [<]	-0.74 [<]
<i>Galba truncatula</i>	-0.76 [<]	-0.83 [<]	-0.64 [<]	n/a	n/a	n/a
Naididae	-0.03 [=]	-0.32 [=]	0.26 [=]	n/a	n/a	n/a
<i>Pisidium casertanum</i>	-0.80 [<]	n/a	-0.66 [<]	n/a	n/a	n/a
<i>Triturus cristatus</i> (l.)	0.43 [=]	0.45 [=]	n/a	n/a	n/a	n/a
Zygoptera (l.)	-0.42 [=]	-0.60 [<]	-0.17 [=]	n/a	n/a	n/a

## Discussion

The diet of *Triturus cristatus* in the studied area consists of representatives of eight classes of four animal phyla (Annelida, Mollusca, Arthropoda, and Chordata), which demonstrates that even at the southernmost limit of its range, this species has a very wide food spectrum, i.e., as Arntzen (2003) has observed, the prey of adult *T. cristatus* in the aquatic phase can be summarized by the term "macrofauna". There are also reports of representatives of three other phyla found in the stomachs of *T. cristatus* from other parts of its range: Nematoda, Nematomorpha, and Platyhelminthes (see Fasola & Canova 1992, Covaciu-Marcov et al. 2002a, Berzin & Burakova 2022), but these are consumed rarely, and in our opinion should not be considered as important components of the food spectrum of the species, at least until more evidence of their presence is accumulated. A comparison at class level with published data on the diet of *T. cristatus* from the areas closest to NW Bulgaria (i.e., Romania, as there are no data from E Serbia), as well as with data from very remote parts of the range (along the northwestern and northeastern borders, respectively more than 2000 km to the NW and about 3000 km to the NE of the Vrachanski Balkan Mts.) it is evident that representatives of six animal classes have been established in each of the compared regions (Table 5). From this, we can suggest that the classes Clitellata, Bivalvia, Gastropoda, Branchiopoda, Insecta, and Amphibia form the main part of the food spectrum of *T. cristatus*. It has to be noted Romania is the only region where all of the thirteen classes are present (Table 5), which is probably related to the fact that the country has the most studies on the feeding of *T. cristatus*, at least to our knowledge.

Table 5. Qualitative composition (presence/absence at class level) of the diet of *T. cristatus* in different parts of its range: TS = this study; RO = combined data from Romania (after Covaciu-Marcov et al. 2002a, b, 2010, Cicort-Lucaciu et al. 2005, 2007, Roşca et al. 2013, Sucea et al. 2014); NW = combined data from several ponds in United Kingdom (after Avery 1968, Griffiths & Mylotte 1987); NE = combined data from some ponds in Chelyabinsk and Sverdlovsk Oblast, Russia (after Berzin & Burakova 2022).

Phylum: Class	TS	RO	NW	NE
Annelida: Clitellata	yes	yes	yes	yes
Nematoda: unknown class	no	yes	no	yes
Mollusca: Bivalvia	yes	yes	yes	yes
Mollusca: Gastropoda	yes	yes	yes	yes
Arthropoda: Branchiopoda	yes	yes	yes	yes
Arthropoda: Copepoda	no	yes	yes	yes
Arthropoda: Malacostraca	yes	yes	yes	no
Arthropoda: Ostracoda	no	yes	yes	yes
Arthropoda: Arachnida	no	yes	no	yes
Arthropoda: Myriapoda s.l.	no	yes	no	yes
Arthropoda: Insecta	yes	yes	yes	yes
Chordata: Actinopterygii	yes	yes	no	no
Chordata: Amphibia	yes	yes	yes	yes

In our studied area, the most frequently ingested prey was the order Diptera (and specifically the larvae of species of the family Chironomidae), while the superorder Cladocera was the most abundant (as number of individuals ingested). The

predominance of mosquitoes (in preimaginal stages) in the diet of *T. cristatus* has been recorded in different parts of the range, e.g., Kühlhorn (1959) noted Chironomidae (larvae and pupae) as the most common prey in Upper Bavaria, Germany, and Covaciu-Marcov et al. (2002b) and Dobre et al. (2007) in Bihor County and "Jiului Gorge" National Park, western Romania, found that mosquito larvae were both the most common prey and the one with the highest number of individuals ingested. In our case, the abundance of ingested Cladocera (greater than that of all other OTUs combined) is likely explained by these animals being much smaller compared to individuals of the other OTUs, and it should also be mentioned that 225 of 636 Cladocera recorded were found in only one stomach (a similarly high abundance of small crustaceans in newt stomachs is relatively common: Dolmen & Koksvik 1983, Ruchin 2010, Sucea et al. 2014). Given this, we believe that the relative frequency of occurrence of individual components in stomachs (considered as OTUs or at the same taxonomic level) is a better indicator for the general characterization of the newt food spectrum than the relative abundance of ingested individuals by OTUs.

The presence of amphibians (eggs, larvae, juveniles, and also adult newts of the smaller species) in the diet of *T. cristatus* has been reported numerous times, and the same applies to the observations of cannibalism (Kuzmin 1999, Arntzen 2003, and references therein). In this regard, our data confirm that amphibians are a common part of the food spectrum of *T. cristatus*. A more peculiar case is the finding of remains of ingested shed skin, as they can hardly be considered a component of the food spectrum. We recorded this phenomenon only in females in August (7.32% of all summer females examined, and more specifically 33.33% of August females examined), which could be explained by some deficiency related to metabolism, e.g., due to pregnancy, but not due to lack of food resources in the ponds. A different temporal trend (decrease in the number of dermatophagy records from the beginning of April to the end of May/beginning of June in both sexes) was recorded for the species in NW Romania, with the authors suggesting that the higher values recorded at the beginning of the aquatic period were probably due to the scarcity of other potential prey (Cicort-Lucaciu et al. 2005, 2007, Covaciu-Marcov et al. 2010). Ingestion of their own shed skin is considered a common phenomenon in caudate amphibians, and although its biological meaning is not fully understood, it is assumed that in this way, the animals acquire some important micronutrients, and it contributes to the body's energy balance during starvation by epidermal proteins recycling (see Kuzmin 1992, Weldon et al. 1993, and references therein).

Of particular interest is the presence of fish eggs (*Carassius gibelio* (Bloch, 1782), family Cyprinidae; the eggs were assigned to this species based on the fact that there are no other fish in the pond) found in the diet of *T. cristatus*, as reports of such cases are very rare. Rosca et al. (2013) mention the presence of the family Cyprinidae in the species' diet in eastern Romania but without mentioning the ontogenetic stage of the ingested fishes. Kinne (2004) mentions *T. cristatus* feeding on juvenile *Leucaspius delineatus* Heckel, 1843 (fam. Cyprinidae) in northern Germany. Iftime & Iftime (2011) reported a case of ingestion of parts of a carcass of *Pseudorasbora parva* (Temminck & Schlegel, 1846) (fam.

Cyprinidae) in Romania, but this is more a manifestation of indiscriminate necrophagy. While predation by fish on newts is a widely known fact, our data and that from Germany and Romania suggest that predation by *T. cristatus* on fish is not uncommon and could be found across its range.

It has been suggested that smell plays an essential role in the feeding behaviour of *T. cristatus* (Cooke & Fulford 1971, Arntzen 2003). This idea is supported by our results, which indicate high frequency of immobile prey in the newt stomachs (the chironomids in pupal stage were the third most frequent prey items, while shells, fish eggs and amphibian eggs can also be considered as immobile prey). In addition, in two of the studied ponds the water was virtually opaque (for the whole duration of the study), limiting the effectiveness of visual cues. The plant remains and inorganic matter (grains of sand) in the newt stomachs (in our case, recorded respectively in 13% and 4% of the examined stomachs) are generally not considered prey, but only artefacts, i.e., accidentally ingested along with the actual prey or as a result of consuming an herbivorous invertebrate whose digestive system contained plant remains (Kuzmin 1992, Cicort-Lucaciu et al. 2005).

According to a number of authors, *T. cristatus* is not selective in regards to its prey. Griffiths & Mylotte (1987) state that the species appears to be a generalist predator with a diet limited by prey size; according to Covaciu-Marcov et al. (2002b), the species selects as a trophic base the most abundant resources in its habitat. Our results show a slightly different trend, as the most abundant OTUs in newt stomachs were not the same as the most abundant in the hydrobiological samples [in Pond 1 the most abundant in the stomachs were Anura (larvae) and in the water samples - Naididae; in Pond 2 - Naididae and *Galba truncatula*, respectively; in Pond 3 - Cladocera and Chironomidae (larvae), respectively]. Furthermore, the electivity index showed that among the analyzed OTUs, some could be defined as neutral (with  $E^*$  values closer to zero than to  $|1|$ ), and others as preferred [ $E^* > 0.5$ ; only three OTUs appeared as such: Dytiscidae (larva) and Diptera (imago) in Pond 2, and Cladocera in Pond 3] or avoided ( $E^* < -0.5$ ). To the best of our knowledge, this type of analysis (via electivity indices) has so far been performed in larvae (Kuzmin 1992), but not in adult *T. cristatus*. It is clear that, as far as selectivity is concerned, the results should be taken with caution due to the limitations of the hydrobiological sampling methodology used: a low probability of capturing very small animals, such as Cladocera, as well as those from the water surface, e.g., accidentally encountered Diptera, Orthoptera, and other potential prey of the newts. However, this also applies to many other similar studies since there is no perfect method by which all potential prey of caudate amphibians can be collected with equal probability in the entire vertical range of the water body (from the bottom substrate to the air above the water surface) regardless of their body size and movement speed (see Kuzmin 1992, Solé & Rödder 2010).

In our case, the difference in the food spectrum between the sexes is mostly expressed in the fact that only females were recorded ingesting frog eggs, adult *Lissotriton vulgaris*, and subadult *Triturus cristatus*. Studies in SW and W Romania by Dobre et al. (2007) and Sucea et al. (2014) found that the prey of female *T. cristatus* was larger and less mobile than that of males. Regarding the consumption of shed skin, Dobre et

al. (2007) recorded such cases only in females (as in our study), while Sucea et al. (2014) found this in both sexes with almost equal frequency. In terms of invertebrates, which are the main part of the food spectrum, our data is largely in accordance with the conclusions of Covaciu-Marcov et al. (2010), who found no difference between males and females.

Relatively little is known about the seasonal nature of feeding in *Triturus cristatus* since, on the one hand, most studies are conducted only during the breeding season, i.e., in the spring, and on the other hand, in a number of publications, the diet is considered without analyzing male and female newts separately. According to our results (based on the ratio of full/empty stomachs), it seems that the adult newts feed more actively in spring than in summer. In their study on 490 individuals from NW Romania for the period April–June, Cicort-Lucaciu et al. (2005) established that the largest proportion of newts with empty stomachs was registered at the beginning of the breeding period and significantly decreased at the end; the authors suggested that this might be due to the change in the abundance of the potential food base in the ponds (from low to high). In monthly observations from March to June (based on 236 individual *T. cristatus*) in SW Romania, Bogdan et al. (2013) found the presence of empty stomachs only in March. Other studies in western Romania in April–May (Cicort-Lucaciu et al. 2007) and April–June (Covaciu-Marcov et al. 2010) did not record even a single individual with an empty stomach, despite their large sample size (208 and 164 individual *T. cristatus*, respectively). Our results indicated that for both sexes, besides the greater quantity of prey in spring, the diet diversity is also higher in spring than in summer. In all probability, this is not due to a seasonal change in the potential food base (since its diversity in the studied ponds was almost the same for spring and summer) but represents a reflection of the feeding behavior of adult newts: more intense and more varied feeding, related to the greater need for energy during the breeding season (i.e., the spring months). Covaciu-Marcov et al. (2010) found higher feeding intensity in female *T. cristatus* in April–June. However, according to our data, no difference between the sexes was observed in the spring, while in the summer, females showed a higher feeding intensity and a more varied diet. It can be assumed that this is also related to reproduction, as females who have become gravid later in the season and have not yet laid eggs (or have only recently laid eggs) might feed more frequently to compensate for the loss of energy spent on egg development.

## Conclusions

Our results confirm that the food spectrum of *Triturus cristatus* is very wide across the species range, both in its main parts and its southernmost distribution limit. During the aquatic phase, adult newts ingest very small and relatively large animals and fast-moving and stationary prey. During the breeding season, both sexes feed more intensively than during the summer months, and the main difference between the sexes is expressed in the higher diet diversity in females and their more intensive feeding in summer. Whether this applies only to the studied population (which, in addition to

being the southernmost, is also at the upper limit of the vertical distribution of the species) or is characteristic of *T. cristatus* in wider geographical limits remains to be clarified by studies in other parts of the range and at lower altitudes. Data on the food spectrum of the species during its terrestrial phase are generally scarce and still lacking in the study area.

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### References

- Arntzen, J. (2003): *Triturus cristatus* Superspecies - Kammolch - Artenkreis (*Triturus cristatus* (Laurenti, 1768) - Nördlicher Kammolch, *Triturus carnifex* (Laurenti, 1768) - Italienischer Kammolch, *Triturus dobrogicus* (Kiritzescu, 1903) - Donau-Kammolch, *Triturus karelinii* (Strauch, 1870) - Südlicher Kammolch). pp. 421–514. In: Grossenbacher, K., Thiesmeier, B. (eds.), Handbuch der Reptilien und Amphibien Europas. Band 4/IIA Schwanzlurche (Urodela) IIA, Salamandridae II: *Triturus* I, Aula-Verlag, Wiesbaden.
- Avery, R.A. (1968): Food and feeding relations of three species of *Triturus* (Amphibia, Urodela) during the aquatic phases. *Oikos* 19: 408–412.
- Berzin, D., Burakova, A. (2022): Diet features of the Crested newt *Triturus cristatus* (Laurenti, 1768) at the eastern border of its range. *Russian Journal of Ecology* 53 (3): 221–227.
- Bogdan, H.V., Covaciu-Marcov, S.D., Gaceu, O., Cicort-Lucaciu, A.S., Ferenc, S., Sas-Kovács, I. (2013): How do we share food? Feeding of four amphibian species from an aquatic habitat in south-western Romania. *Animal Biodiversity and Conservation* 36 (1): 89–99.
- Cecala, K., Price, S., Dorcas, M. (2007): A Comparison of the effectiveness of recommended doses of MS-222 (tricaine methanesulfonate) and orajel® (benzocaine) for amphibian anesthesia. *Herpetological Review* 38 (1): 63–66.
- Cicort-Lucaciu, A.-S., Ardeleanu, A., Cupşa, D., Naghi, N., Dalea, A. (2005): The trophic spectrum of a *Triturus cristatus* (Laurenti 1768) population from Plopiş Mountains area (Bihor County, Romania). *North-Western Journal of Zoology* 1: 31–39.
- Cicort-Lucaciu, A.-S., David, A., Covaci, R., Toader, S., Diaconu, I. (2007): Feeding of some *Triturus cristatus* population in Turţ area (Oas Mountains, Romania). *Herpetologica Romanica* 1: 30–37.
- Cochran, G. (1954): Some methods of strengthening the common chi-square tests. *Biometrics* 10: 417–451.
- Cooke, A., Fulford, W. (1971): Observations on the feeding behaviour of a blind warty newt (*Triturus cristatus*). *British Journal of Herpetology* 4: 216.
- Covaciu-Marcov, S.D., Cupşa, D., Cicort-Lucaciu, A.S., Telcean, I., Sas, I. (2002a): Contribuţii la cunoaşterea spectrului trofic al speciei *Triturus cristatus* (Amphibia, Urodela) din regiunea Marghita şi Munţii Pădurea Craiului (Jud. Bihor, România). *Analele Universităţii din Oradea, Fascicula Biologie* 9: 95–108.
- Covaciu-Marcov, S.D., Cupşa, D., Telcean, I., Cicort-Lucaciu, A.S. (2002b): Spectrul trofic al unei populaţii de *Triturus cristatus* (Amphibia, Urodela) din zona Şerghiş, jud. Bihor, România. *Oltenia - Studii şi Comunicări Ştiinţele Naturii* 18: 188–194.
- Covaciu-Marcov, S.D., Cicort-Lucaciu, A.S., Mitrea, I., Sas, I., Căuş, A.V., Cupşa, D. (2010): Feeding of three syntopic newt species (*Triturus cristatus*, *Mesotriton alpestris* and *Lissotriton vulgare*) from Western Romania. *North-Western Journal of Zoology* 6: 95–108.
- Dobre, F., Bucur, D.-M., Mihut, R., Birceanu, M., Gale, O. (2007): Date asupra compoziţiei hranei a unei populaţii de *Triturus cristatus* (Laur. 1768) de la Parcul Naţional "Defileul Jiului", România. *Bihorean Biologist* 1: 23–28.
- Dolmen, D., Koksvik, J.I. (1983): Food and feeding habits of *Triturus vulgaris* (L.) and *T. cristatus* (Laurenti) (Amphibia) in two bog tarns in central Norway. *Amphibia-Reptilia* 4(1): 17–24.
- Fahrbach, M., Gerlach, U. (2018): Die Gattung *Triturus*. Chimaira, Frankfurt am Main.
- Fasola, M., Canova, L. (1992): Feeding habits of *Triturus vulgaris*, *T. cristatus* and *T. alpestris* (Amphibia, Urodela) in the northern Apennines (Italy). *Bolletino di zoologia* 59: 273–280.
- Fraser, D. (1976): Coexistence of salamanders in the genus *Plethodon*, a variation of the Santa Rosalia theme. *Ecology* 57: 238–251.
- Frost, D. (2023): Amphibian species of the world: an online reference. Version 6.1 (Accessed at 04.11.2022). Electronic Database accessible at <https://amphibiansoftheworld.amnh.org/index.php>. American Museum of Natural History, New York, USA. doi.org/10.5531/db.vz.0001
- Griffiths, R. (1986): Feeding and niche overlap and food selection in smooth and palmate newts, *Triturus vulgaris* and *T. helveticus*, at a pond in mid-Wales. *Journal of Animal Ecology* 55: 201–214.
- Griffiths, R., Mylotte, V. (1987): Microhabitat selection and feeding relations of Smooth and Warty newts, *Triturus vulgaris* and *T. cristatus*, at an Upland Pond in Mid-Wales. *Holarctic Ecology* 10 (1): 1–7.
- Hammer, Ø., Harper, D.A.T., Ryan, P.D. (2001): PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4 (1): 1–9.
- Iftime, A., Iftime, O. (2011): *Triturus cristatus* (Caudata: Salamandridae) feeds upon dead fishes. *Salamandra* 47 (1): 43–44.
- Jehle, R., Thiesmeier, B., Foster, J. (2011): The Crested newt. A dwindling pond-dweller. Laurenti-Verlag, Bielefeld.
- Kinne, O. (2004): Successful re-introduction of the newts *Triturus cristatus* and *T. vulgaris*. *Endangered Species Research* 4: 1–14.
- Krebs, C.J. (1999): Ecological methodology. Second edition. Addison Wesley Longman, Menlo Park, California etc..
- Kühlhorn, F. (1959): Beitrag zur Kenntnis der Ernährungsbiologie unserer heimischen Amphibien. Veröffentlichungen der Zoologischen Staatssammlung München 5: 145–188.
- Kuzmin, S. (1992): Trophology of the tailed amphibians: ecological and evolutionary aspects. Nauka, Moscow. [In Russian].
- Kuzmin, S. (1999): The amphibians of the former Soviet Union. Pensoft, Sofia-Moscow.
- Lechowicz, M. (1982): The sampling characteristics of electivity indices. *Oecologia* 52: 22–30.
- Litvinchuk, S., Borkin, L. (2009): Evolution, systematics and distribution of Crested newts (*Triturus cristatus* complex) in Russia and adjacent countries. Evropeyskiy Dom Press, St. Petersburg. [In Russian, English summary].
- Lukanov, S., Doncheva, T., Kostova, N., Naumov, B. (2021): Effects of selected environmental parameters on the activity and body condition of the Buresch's crested newt (*Triturus ivanbureschi*) with notes on skin secretions. *North-Western Journal of Zoology* 17 (1): 34–38.
- Naumov, B., Biserkov, V. (2013): On the distribution and subspecies affiliation of *Triturus dobrogicus* (Amphibia: Salamandridae) in Bulgaria. *Acta Zoologica Bulgarica* 65 (3): 307–313.
- Naumov, B., Tzankov, N., Donchev, K., Petrov, B., Stojanov, A., Popgeorgiev, G., Mollov, I., Beshkov, V. (2016): The herpetofauna (Amphibia and Reptilia) of Vrachanska Planina mountains - species composition, distribution and conservation. pp. 231–257. In: Bechev, D., Georgiev, D. (eds.), Faunistic diversity of Vrachanski Balkan Nature Park. *ZooNotes, Suppl.* 3.
- Naumov, B., Lukanov, S., Vacheva, E., Slavchev, M. (2023): Distribution and ecology of *Triturus cristatus* (Laurenti, 1768) (Amphibia: Salamandridae) in Bulgaria. *Acta Zoologica Bulgarica* 75 (1): 113–122.
- Roşca, I., Gherghel, I., Strugariu, A., Zamfirescu, Ş.R. (2013): Feeding ecology of two newt species (*Triturus cristatus* and *Lissotriton vulgare*) during the reproduction season. *Knowledge and Management of Aquatic Ecosystems* 408: 05.
- Ruchin, A. (2010): On the distribution and feeding of the Crested Newt, *Triturus cristatus* (Laurenti, 1768), in Mordovia. pp. 166–173. In: Duysebaeva, T. (ed.), Herpetological research in Kazakhstan and neighboring countries, Almaty. [In Russian, English summary].
- Sato, J.J., Ohtsuki, Y., Nishiura, N., Mouri, K. (2022): DNA metabarcoding dietary analyses of the wood mouse *Apodemus speciosus* on Innoshima Island, Japan, and implications for primer choice. *Mammal Research* 67: 109–122.
- Sneath, P., Sokal, R. (1973): Numerical taxonomy. Freeman, San Francisco.
- Solé, M., Rödder, D. (2010): Dietary assessments of adult amphibians. pp. 167–184. In: Dodd Jr., C.K. (ed.), Amphibian ecology and conservation: a handbook of techniques. Oxford University Press, Oxford.
- Sucea, F., Cicort-Lucaciu, A.-F., Covaci, R.F., Dimancea, N. (2014): Note on the diet of two newt species in Jiului Gorge National Park, Romania. *Herpetologica Romanica* 8: 11–27.
- Tóthmérész, B. (1995): Comparison of different methods for diversity ordering. *Journal of Vegetation Science* 6: 283–290.
- Tzankov, N., Stoyanov, A. (2008): *Triturus cristatus* (Laurenti, 1768): a new species for Bulgaria from its southernmost known localities. *Salamandra* 44 (3): 153–162.
- Vacheva, E., Naumov, B. (2020): Diet of the Viviparous lizard *Zootoca vivipara* (Lichtenstein, 1823) (Reptilia: Lacertidae) from its southern range. *North-Western Journal of Zoology* 16 (2): 178–190.
- Vacheva, E., Naumov, B. (2022): A contribution to the knowledge on the diet and food preferences of *Darevskia praticola* (Reptilia: Lacertidae). *Acta Herpetologica* 17 (1): 27–36.

- Vanderploeg, H., Scavia, D. (1979): Two electivity indices for feeding with special reference to zooplankton grazing. *Journal of the Fisheries Research Board of Canada* 36 (4): 362–365.
- Weldon, P., Demeter, B., Rosscoe, R. (1993): A survey of shed skin-eating (dermatophagy) in amphibians and reptiles. *Journal of Herpetology* 27 (2): 219–228.
- Wielstra, B., Sillero, N., Vörös, J., Arntzen, J. (2014): The distribution of the crested and marbled newt species (Amphibia: Salamandridae: *Triturus*) - an addition to the New Atlas of Amphibians and Reptiles of Europe. *Amphibia-Reptilia* 35: 376–381.
- Wu, Z. J., Li, Y. M., Wang, Y. P. (2007): A comparison of stomach flush and stomach dissection in diet analysis of four frog species. *Acta Zoologica Sinica* 53: 364–372.

### + Appendix 1 and Appendix 2

**Appendix 1.** Distribution of the investigated *Triturus cristatus* by the pond, sex (M/F), and date (April–November 2021).

		Spring (n = 76)			Summer (n = 61)			Autumn (n = 4)		Total
		Apr 26	May 19	Jun 14	Jul 13	Aug 11	Sep 15	Oct 14	Nov 11	
Pond 1	M	-	5	5	2	1	-	-	-	13
	F	-	5	5	-	-	-	-	-	10
Pond 2	M	5	5	2	4	4	5	1	1	27
	F	4	5	5	6	5	4	1	1	31
Pond 3	M	5	5	5	5	5	6	-	-	31
	F	5	5	5	5	5	4	-	-	29
Total		19	30	27	22	20	19	2	2	141

**Appendix 2.** Distribution of the prey items from the stomachs of *Triturus cristatus* (by season and sex) and from the hydrobiological samples [OTU = operational taxonomic unit; M = males; F = females; HY = hydrobiological samples; ad. = adult, e. = egg, i. = imago, l. = larva; p. = pupa, sub. = subadult].

#### Pond 1:

Phylum: Class	OTU	Spring			Summer			Autumn		
		M	F	HY	M	F	HY	M	F	HY
Annelida: Clitellata	Lumbricidae	0	1	0	0	n/a	0	n/a	n/a	0
Annelida: Clitellata	Naididae	0	0	43	0	n/a	17	n/a	n/a	63
Arthropoda: Arachnida	Araneae	0	0	0	0	n/a	0	n/a	n/a	1
Arthropoda: Insecta	Aphididae	0	0	6	0	n/a	1	n/a	n/a	0
Arthropoda: Insecta	Chironomidae (l.)	1	0	2	0	n/a	2	n/a	n/a	5
Arthropoda: Insecta	Coleoptera (i.)	0	1	2	0	n/a	0	n/a	n/a	0
Arthropoda: Insecta	Diptera (i.)	9	10	1	0	n/a	0	n/a	n/a	0
Arthropoda: Insecta	Hymenoptera (i.)	0	0	1	0	n/a	1	n/a	n/a	0
Arthropoda: Insecta	Insecta (indet.)	1	0	n/a	0	n/a	n/a	n/a	n/a	n/a
Arthropoda: Insecta	Orthoptera (i.)	0	0	0	1	n/a	0	n/a	n/a	0
Chordata: Actinopterygii	<i>Carassius gibelio</i> (ad.)	0	0	0	0	n/a	10	n/a	n/a	0
Chordata: Actinopterygii	<i>Carassius gibelio</i> (e.)	8	17	0	0	n/a	0	n/a	n/a	0
Chordata: Amphibia	Anura (l.)	3	26	0	0	n/a	0	n/a	n/a	0
Chordata: Amphibia	Ranidae (e.)	0	1	n/a	0	n/a	n/a	n/a	n/a	n/a
n/a	plant remains	yes	yes	n/a	yes	n/a	n/a	n/a	n/a	n/a
n/a	indet. organic remains	yes	yes	n/a	yes	n/a	n/a	n/a	n/a	n/a
n/a	inorganic matter	no	yes	n/a	yes	n/a	n/a	n/a	n/a	n/a

**Pond 2:**

Phylum: Class	OTU	Spring			Summer			Autumn		
		M	F	HY	M	F	HY	M	F	HY
Annelida: Clitellata	Naididae	14	33	78	0	0	533	0	0	317
Arthropoda: Arachnida	Araneae	0	0	1	0	0	1	0	0	0
Arthropoda: Arachnida	Hydrachnidia	0	0	0	0	0	1	0	0	0
Arthropoda: Branchiopoda	Cladocera	8	3	0	0	0	0	0	0	0
Arthropoda: Insecta	Anisoptera (l.)	0	0	0	0	0	7	0	0	0
Arthropoda: Insecta	Aphididae	0	0	9	0	0	0	0	0	2
Arthropoda: Insecta	Chironomidae (l.)	9	16	1142	2	0	334	0	0	382
Arthropoda: Insecta	Chironomidae (p.)	5	5	65	0	1	10	0	0	0
Arthropoda: Insecta	Coleoptera (i.)	0	0	24	1	0	16	0	0	5
Arthropoda: Insecta	Corixidae	0	0	7	2	1	74	0	0	34
Arthropoda: Insecta	Diptera (i.)	0	4	10	0	0	1	1	0	0
Arthropoda: Insecta	Diptera (l.)	0	0	0	0	0	64	0	0	13
Arthropoda: Insecta	Dytiscidae (l.)	4	0	2	0	0	3	0	0	0
Arthropoda: Insecta	Ephemeroptera (l.)	0	0	0	0	0	1	0	0	2
Arthropoda: Insecta	Hemiptera (i.)	0	0	0	0	0	0	0	1	0
Arthropoda: Insecta	Heteroptera (i.)	0	0	0	0	0	1	0	0	0
Arthropoda: Insecta	Hymenoptera (i.)	0	0	3	0	0	0	0	0	0
Arthropoda: Insecta	Insecta (indet.)	2	1	n/a	0	1	n/a	0	0	n/a
Arthropoda: Insecta	Orthoptera (i.)	0	0	0	1	1	0	0	0	0
Arthropoda: Insecta	Trichoptera (l.)	0	0	3	0	0	8	0	0	2
Arthropoda: Insecta	Zygoptera (l.)	2	4	134	0	0	43	0	0	10
Arthropoda: Ostracoda	Ostracoda	0	0	2	0	0	0	0	0	0
Chordata: Amphibia	Amphibia (indet.)	2	4	n/a	1	3	n/a	0	0	n/a
Chordata: Amphibia	Anura (l.)	1	5	7	0	0	20	0	0	0
Chordata: Amphibia	<i>Lissotriton vulgaris</i> (ad.)	0	1	0	0	0	0	0	0	0
Chordata: Amphibia	<i>Triturus cristatus</i> (l.)	2	0	4	2	3	30	0	0	5
Mollusca: Bivalvia	<i>Pisidium casertanum</i>	3	7	807	0	0	273	0	0	23
Mollusca: Gastropoda	<i>Galba truncatula</i>	8	12	812	0	1	1053	0	0	323
Mollusca: Gastropoda	<i>Galba truncatula</i> (e.)	0	0	17	0	0	1	0	0	0
n/a	shed skin	no	no	n/a	no	yes	n/a	no	no	n/a
n/a	plant remains	no	yes	n/a	yes	no	n/a	yes	no	n/a
n/a	indet. organic remains	yes	yes	n/a	yes	yes	n/a	yes	no	n/a

**Pond 3:**

Phylum: Class	OTU	Spring			Summer			Autumn		
		M	F	HY	M	F	HY	M	F	HY
Annelida: Clitellata	Naididae	0	0	224	0	0	230	n/a	n/a	118
Arthropoda: Arachnida	Hydrachnidia	0	0	4	0	0	1	n/a	n/a	0
Arthropoda: Branchiopoda	Cladocera	98	198	15	329	0	0	n/a	n/a	0
Arthropoda: Insecta	Anisoptera (l.)	0	0	3	0	0	2	n/a	n/a	2
Arthropoda: Insecta	Aphididae	4	3	4	1	0	0	n/a	n/a	0
Arthropoda: Insecta	Chironomidae (l.)	17	25	349	2	0	357	n/a	n/a	57
Arthropoda: Insecta	Chironomidae (p.)	3	5	22	2	4	3	n/a	n/a	0
Arthropoda: Insecta	Coleoptera (i.)	2	3	15	0	0	2	n/a	n/a	0
Arthropoda: Insecta	Corixidae	11	4	65	0	0	65	n/a	n/a	0
Arthropoda: Insecta	Diptera (i.)	26	12	1	0	1	0	n/a	n/a	0
Arthropoda: Insecta	Diptera (l.)	0	0	0	0	3	0	n/a	n/a	0
Arthropoda: Insecta	Dytiscidae (l.)	0	3	3	5	1	39	n/a	n/a	1
Arthropoda: Insecta	Hemiptera (i.)	0	0	0	1	0	0	n/a	n/a	0
Arthropoda: Insecta	Heteroptera (i.)	0	0	2	0	0	0	n/a	n/a	0
Arthropoda: Insecta	Hymenoptera (i.)	0	0	1	1	0	0	n/a	n/a	1
Arthropoda: Insecta	Insecta (indet.)	1	2	n/a	0	2	n/a	n/a	n/a	n/a
Arthropoda: Insecta	Orthoptera (i.)	0	0	0	0	1	0	n/a	n/a	0
Arthropoda: Insecta	Trichoptera (l.)	1	0	7	0	0	0	n/a	n/a	0
Arthropoda: Insecta	Zygoptera (l.)	0	0	23	0	1	0	n/a	n/a	1
Arthropoda: Malacostraca	Amphipoda	0	0	3	0	40	0	n/a	n/a	0
Chordata: Amphibia	Amphibia (indet.)	0	1	n/a	0	0	n/a	n/a	n/a	n/a
Chordata: Amphibia	Anura (l.)	0	0	0	0	1	1	n/a	n/a	0
Chordata: Amphibia	<i>Lissotriton vulgaris</i> (ad.)	0	0	1	0	0	0	n/a	n/a	0
Chordata: Amphibia	<i>Triturus cristatus</i> (sub.)	0	1	0	0	0	7	n/a	n/a	0
Chordata: Amphibia	<i>Triturus cristatus</i> (l.)	0	1	0	0	0	10	n/a	n/a	0
Mollusca: Bivalvia	<i>Pisidium casertanum</i>	0	0	1	0	0	5	n/a	n/a	0
Mollusca: Gastropoda	<i>Galba truncatula</i>	0	0	0	0	1	1	n/a	n/a	0
n/a	shed skin	no	no	n/a	no	yes	n/a	n/a	n/a	n/a
n/a	plant remains	yes	no	n/a	yes	yes	n/a	n/a	n/a	n/a
n/a	indet. organic remains	yes	yes	n/a	yes	yes	n/a	n/a	n/a	n/a
n/a	inorganic matter	yes	no	n/a	no	no	n/a	n/a	n/a	n/a