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Diet Features of the Crested Newt *Triturus cristatus* (Laurenti, 1768) at the Eastern Border of Its Range

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Abstract—Dietary spectra of the crested newt (*Triturus cristatus* (Laureti, 1768)) from two habitats at the eastern border of its distribution range have been estimated using a noninvasive study of its stomach contents. The stomachs of *T. cristatus* have been washed out with a syringe with a thin silicone catheter. This method is more accurate than traditional ones, allows extracting large invertebrates, and is less traumatic. After food bolus extraction, the newts have been released back into water bodies. It total, 81 specimens of adult crested newts have been caught: 41 and 40 specimens in the Middle and South Urals, respectively. Differences between the potential and realized dietary spectra of newts in two compared water bodies have been established. For the first time, the nematode *Megalobatrachonema terdentatum* (Linstow, 1890) have been found in the stomach contents of the crested newt.

Keywords: dietary spectrum, oophagia, helminths, noninvasive method, salamanders

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INTRODUCTION

Amphibians, contributing both to aquatic and terrestrial biocenoses and thus linking them, are consumers [1], which make these animals a convenient model for studying biocenotic relationships. The crested newt (*Triturus cristatus* (Laurenti, 1768)), like all salamanders, is a predator throughout its life stages. Consequently, its dietary spectrum does not change dramatically in ontogeny as in anurans [2]. The biocenotic role of *T. cristatus* varies depending on the population number, the structure of a biocenosis, and environmental conditions [3]. In Russia, the studies on the crested newt diet are limited [2, 4–6], while the taxonomic composition of its potential prey is not known at all.

T. cristatus is a protected species included in the Red Data Book of the Sverdlovsk Oblast and Chelyabinsk Oblast (category II) [7, 8]. The studies of its nutritional characteristics and diet patterns are necessary to develop measures for its protection. The crested newt is mosaically distributed and rarely found in the Ural Mountains, which are the eastern boundary of the species range. The features of its diet in this area have not been previously studied. The purpose of our work is to study the specific diet features of adult individuals of *T. cristatus* using a noninvasive research method.

MATERIALS AND METHODS

Habitats

The study material was collected on the territory of the Southern Urals (56°15′2.58″ N, 59°16′36.18″ E) on May 10, 2021 (village Shemakha, northwestern part of Nyazepetrovsky district, Chelyabinsk Oblast) and at the Middle Urals (56°50′26.33″ N, 57°33′13.68″ E) on May 17, 2021 (village Bolshoe Koshaevo, southwestern part of Krasnoufimsky district, Sverdlovsk Oblast). A reservoir in the Southern Urals is of technogenic origin of a slope type; it was located in the forest-steppe zone (southern taiga subzone), 5 m from a country road near a mixed forest, in the floodplain of the Shemakha River. The soil in the reservoir is clayey and peaty. The reservoir is adjacent to low-rise buildings and is often visited by farm animals. Aquatic vegetation is abundant. A reservoir in the Middle Urals is of a karst type, located in the forest-steppe zone (southern taiga subzone), 15 m from the river bed of the Irgina River. The soil is clayey, slightly rocky; aquatic vegetation is scarce.

The water temperature was measured once a day in a daytime with a TP-22 mercury thermometer with a measuring sensitivity of 0.5°C in May before sampling of invertebrates and in August before water sampling. The depth of the reservoir was measured using a DEEPER CHIRP+ sonic echo sounder. Hydrochemical analysis was carried out in the laboratory of engineering and environmental testing of OOO Akva-

Solum. One of the most informative indicators was the COD level (chemical oxygen demand), i.e., the amount of oxygen (or oxidizing agent, equivalent to oxygen, mg/L) necessary for oxidizing organic matter of a sample, at which carbon, sulfur, phosphorus, and other elements (except nitrogen), if they are present in organic matter, are oxidized to CO_2, H_2O, P_2O_5, and SO_3, while nitrogen is converted into ammonium salt. COD was determined by the titrimetric method [9].

Sampling Procedure

In total, 81 specimens of the adult crested newts were studied: 41 specimens from the Middle Urals and 40 from the Southern Urals. Tritons were caught at approximately the same time of day (from 12:00 to 16:00) during the period of maximum activity of amphibians. The stomachs of newts were washed out using a 5-mL syringe filled with water and connected to a silicone catheter (diameter 1.0 mm) [10, 11]. A thin catheter creates a sufficient lumen for the evacuation of the food bolus and stimulates the gag reflex in amphibians. Food particles remaining in the newt's oral cavity after the treatment were removed with entomological tweezers.

The disadvantage of the traditional gastric lavage [2, 12, 13] roots in the procedure of absorption of the food bolus, in which large particles escape the sample. The method we used allows us to extract a larger number of invertebrates, including large food objects (for example, mollusk shells), and thus increases the accuracy of the study, also being less traumatic. After removing the food bolus, the newts were released back into their original habitats.

Sample Analyzing

The food objects of the newts were sampled by a dip net (150 × 126 mm) with a cell size of 0.1 mm. Samples of invertebrates were taken from the coastal, shallow, and deep water parts of the water body in the most frequently visited areas by newts [14]. At each reservoir, the sampling procedure includes ten 1.5-m length sweeps with a dip net.

The extracted stomach content was put in test tubes with 90% ethanol. In order to identify food objects, the samples were examined under a Meiji Techno EMZ-8TR binocular microscope (Japan). Invertebrates and their fragments were separated from other stomach content. Identification of aquatic invertebrates and macroparasites was carried out using standard methods and guide books [15–20].

Infestation with Macroparasites

The infestation of newts with macroparasites was estimated using the following indicators: P, the proportion of infected host individuals in the studied sample, %; A, the average number of parasites of a certain species or group of parasites in all host individuals (specimens per host individual); I, the average number of helminths per one studied host specimen (specimens per host individual) [21].

Data Analysis

The Morisita index [22] was used for estimating the overlapping of potential and realized dietary spectra and for comparison of the diets of newts from different habitats:

$$I\,(\mathrm{m}) = \frac{2\left(\sum P_{ij} \times P_{ik}\right)}{\sum\left(P_{ij}^2 + P_{ik}^2\right)},$$

where P_{ij} is the proportion of the i-th component in the diet of the j-th species; P_{ik} is the proportion of the i-th component in the diet of the k-th species.

The species diversity of the dietary spectra was assessed using the biological diversity indices: Margalef's index, Shannon's diversity index, and the Berger–Parker index [23]. Species diversity indices were calculated for a pooled sample of invertebrates from one water body. Index values and their confidence intervals, except for Margalef's index, were calculated using the Past 4.07d program; the significance of differences was assessed using the permutation test module. This module allows one to calculate a set of diversity indices for two samples and then to compare the differences using random permutations (n = 9999 random two-sample matrices). The bootstrap confidence interval of Margalef's index was calculated in the R software environment, version 4.1.1 (the script is available at: https://github.com/ANSozontov/Berzin_2021). Statistical processing of data on the infestation of newts with macroparasites was carried out using Quantitative Parasitology 3.0 software [24].

RESULTS

In total, 863 invertebrates (including their fragments) from 4 phyla were found in the stomachs of *T. cristatus* from two habitats: Mollusca, Arthropoda, Oligochaeta, and Nematoda. Eleven taxa were common for two habitats (Table 1). Newts' own skin pieces were also found in the stomachs of *T. cristatus*. The occurrence of newts' skin in stomachs was higher in animals from the Middle Urals. Gallert-free eggs of *Rana arvalis* Nilsson, 1842 were found in the stomachs of 40% of the newts caught in the Middle Urals (in 11 females and 5 males). The average number of eggs in the stomach was 17.2 ± 4.4 and 3.2 ± 1.5 in females and males, respectively.

The average number of invertebrates per stomach was 19.61± 0.34 and 1.48 ± 0.16 for the newts from the Southern and Middle Urals, respectively. The stomachs of sexually mature newts from the Middle Urals contained many terrestrial invertebrates; however, 20% of amphibians from this water body lack any

Table 1. Dietary spectrum of *T. cristatus* from the Middle and Southern Urals

Taxon	Southern Urals			Middle Urals		
	proportion of objects in stomachs, %	occurrence in stomachs, %	proportion in the water body, %	proportion of objects in stomachs, %	occurrence in stomachs, %	proportion in the water body, %
Gastropoda Cuvier, 1797	6.0	60.1	16.1	10.1	12.5	60.7
Bivalvia Linnaeus, 1758	0.5	7.3	2.6	–	–	–
Ephemeroptera Hyatt et Arms, 1891	–	–	0.2	–	–	–
Trichoptera Kirby, 1813	0.2	4.9	0.7	3.4	5.0	1.4
Hemiptera Linnaeus, 1758	–	–	0.2	1.7	2.5	5.0
Diptera Linnaeus, 1758						
Chaoboridae Edwards, 1912	–	–	0.2	1.7	2.5	13.9
Culicidae Meigen, 1818	35.4	80.5	6.2	5.1	7.5	4.3
Chironomidae Jacobs, 1900	0.9	7.3	0.4	8.5	7.5	0.7
Stratiomyidae Latreille, 1802	0.5	9.8	0.3	8.5	12.5	0.7
Ceratopogonidae Newman, 1834	0.4	4.9	0.5	1.7	2.5	0.7
Simulidae Newman, 1834	0.1	2.4	–	1.7	2.5	–
Syrphidae Latreille, 1802	0.1	2.4	–	–	–	–
Coleoptera Linnaeus, 1758						
Dytiscidae Latreille, 1802	8.8	68.3	0.7	13.5	3.0	2.9
Curculionidae Latreille, 1802	0.1	2.4	–	–	–	–
Haliplidae Aubé, 1836	–	–	0.2	–	–	–
Gyrinidae Latreille, 1810	–	–	0.1	–	–	–
Elodidae Shuckard, 1840	–	–	0.1	–	–	–
Chrysomelidae Latreille, 1802	–	–	0.2	–	–	–
Noteridae Thomson, 1857			0.1	–	–	2.6
Odonata Fabricius, 1793	0.6	9.8	0.7	–	–	–
Branchiata Lang, 1888 (= Crustacea)						
Cyclopoida Burmeister, 1834	8.6	58.5	18.0	–	–	–
Daphnidae Müller, 1785	4.5	29.3	17.2	3.4	5.0	5.7
Branchinectidae Daday, 1910	17.5	75.6	0.3	–	–	–
Cyclestherida Sars, 1899	2.2	19.5	0.2	–	–	–
Ostracoda Latreille, 1802	11.6	70.7	33.3	–	–	–
Other objects						
Oligochaeta Grube, 1850	0.5	7.3	0.3	37.3	27.5	–
Hirudinea Lamarck, 1818	–	–	0.2	–	–	–
Acari Leach, 1817	0.1	2.4	0.9	–	–	–
Araneae Clerck, 1757	–	–	0.1	–	–	–
Myriapoda Latreille, 1802	–	–	–	3.4	2.5	–
Nematoda Rudolphi, 1808	1.2	14.6	–	–	–	–
Eggs of *R. arvalis*	–	–	–	–	40.0	–
Tadpoles of *R. arvalis*	–	–	–	–	–	1.4
Skin of *T. cristatus*	0.1	2.4		–	22.5	

invertebrates, which was not observed for animals from the Southern Urals. Representatives of 13 taxa were found in the stomach of animals from the Middle Urals, a large proportion of them were representatives of Oligochaeta, Gastropoda, and Diptera (hereinafter in descending order). The dietary spectrum of amphibians was dominated by earthworms; representatives of Diptera from the families Stratiomyidae and Chironomidae were often consumed; representatives of Culicidae were consumed to a lesser extent; representatives of Chaoboridae, Ceratopogonidae, and Simulidae were consumed sporadically.

Representatives of 20 taxa were found in the stomachs of newts from the Southern Urals. The representatives of Crustacea (Branchinectidae, Ostracoda, Cyclopoida), Diptera (Culicidae), Coleoptera (Dytiscidae) predominated in the stomachs. The predominance of representatives of Diptera and Crustacea in the dietary spectrum of amphibians is associated with the abundance of these taxonomic groups in the environment. Frequent detection of representatives of Culicidae in the dietary spectrum is probably due to the high prevalence of these forms in the habitat in the Southern Urals and their large size. According to the frequency of occurrence and the number of individuals in the stomachs of newts, various crustaceans dominate. The order Coleoptera is represented in the dietary spectrum to a lesser extent than Diptera and Crustacea, and mainly by larval forms.

Some taxa of aquatic and terrestrial objects were noted in the diet of amphibians from the Southern Urals, but not in amphibians from the Middle Urals: Odonata, Acari, and Curculionidae (Table 1). Mollusks in the newt stomachs were represented by aquatic forms of the genera *Planorbis*, *Physa*, *Lymnaea*, and *Sphaerium*.

The presence of parasitic nematodes *Megalobatrachonema terdentatum* (Linstow, 1890) (=*Chabaudgolvania terdentatum*) was recorded in adult newts from the Southern Urals. This finding is associated with the presence in the environment of molluscs of the genus *Planorbis*, the intermediate hosts of *M. terdentatum*: $P_{M.\ terdentatum} = 14.6\%$, $A_{M.\ terdentatum} = 0.24$ spec., up to three specimens per newt.

An analysis of dietary spectra overlapping, according to the Morisita index between the studied habitats in terms of the frequency of consumed invertebrates, revealed a low level of similarity in stomach samples (16%) and a slightly higher similarity in the composition of organisms in water samples (37%). A significant level of overlapping of samples from the stomachs with the water body environments in the Southern Urals (48%) is associated with the high frequency of occurrence of invertebrates in the environment, as well as with the degree of their availability. The low overlapping level (26%) of samples from the stomachs with the environment in the Middle Urals can be explained by a significant contribution of terrestrial forms to the diet of the newt.

The indices of species diversity of the dietary spectra were analyzed. The Margalef's and Berger–Parker indices in samples from the stomachs of newts from the Middle Urals turned out to be higher in comparison with those calculated for the newts from the Southern Urals, which is due to the predominance of the moor frog eggs in their diet. The value of the Shannon index in newts from the Southern Urals was significantly higher in comparison with the Middle Urals (Table 2).

The Shannon index, calculated for the samples of invertebrates from the water body of the Southern Urals was significantly higher than that calculated for the water body of the Middle Urals. The value of the Berger–Parker dominance index in the population of newts from the Middle Urals is higher in comparison with the Southern Urals. This difference results from the dominance of one taxon (Gastropoda). No significant differences according to the Shannon index were found between samples from stomachs and water bodies.

DISCUSSION

A comparison of the dietary spectra of the crested newt in the Southern and Middle Urals showed that *T. cristatus* uses different foraging strategies depending on the environmental conditions and the availability of food objects. The number of taxa in the realized and potential spectra, apparently, results from both the phenological features of the studied populations and the physicochemical parameters of both habitats, as well as some food selectivity in conditions of food shortage. The diversity of the potential dietary spectrum of newts depends on a number of features: the specifics of the biotope (abundant aquatic and semiaquatic vegetation and its remains), the level of COD, the location of the water body, and the level of its warming.

Low abundance of prey items leads the crested newt to the more pronounced food selectivity. It is known that newts of the genus *Triturus* (Rafinesque, 1815) quite often eat eggs in spawning areas; this phenomenon was found in *T. cristatus*, *T. dobrogicus* (Kiritzescu, 1903), and *T. carnifex* (Laurenti, 1768) [2, 11]. Salamanders are characterized by interspecies oophagia among females [2, 25]. The adaptive meaning of this phenomenon is the restoration of increased energy consumption for the egg formation and oviposition [2, 26]. The presence of own skin in the stomachs of newts is apparently associated with autocannibalism, which is typical for many salamanders, including *T. cristatus* [2]. Consumption of shed skin is caused by long-term starvation of salamanders, and probably replenishes energy reserves [25, 26].

The deficiency of aquatic food objects leads to an increase of the proportion of large terrestrial food

Table 2. Parameters of water bodies and values of species diversity indices

Index	Southern Urals	Middle Urals	p*
Parameters of water bodies			
Maximum depth, m	1.8	4.0	
Water surface area, m^2	1522	472	
Date**	May 17, 2020/August 7, 2020	May 10, 2020/August 6, 2020	
T, °C	16.9/20.3	18.0/17.0	
pH	7.05/6.99	7.9/7.6	
COD, mg/L	21.4/26.1	5.61/25.2	
Mineralization, mg/L	270.0/960.0	330.0/90.0	
Species diversity indices for the environmental samples			
Margalef's index	4.22 (4.15–4.30***)	2.23 (2.14–2.30)	0.113
Shannon index	1.92 (1.86–1.98)	1.51 (1.29–1.72)	0.004
Berger–Parker index	0.33 (0.31–0.36)	0.61 (0.53–0.69)	0.001
Species diversity indices for the stomach samples			
Margalef's index	2.99 (2.66–3.31)	2.50 (1.62–3.40)	0.428
Shannon index	2.03 (1.96–2.10)	1.05 (0.89–1.21)	0.001
Berger–Parker index	0.35 (0.32–0.39)	0.77 (0.72–0.82)	0.001

*, significance level; **, estimates of hydrochemical and physical indicators obtained on different dates; ***, 95% confidence intervals are given in parentheses.

objects (Oligochaeta) in the dietary spectrum, which, apparently, are more substantial and have a high ratio of protein to their own biomass (59%) [27]. This may also be due to the feeding of amphibians prior to visiting the reservoir, or the accidental capture of some land forms. In the realized dietary spectrum, due to the lack of easily digestible food, the proportion of representatives of aquatic mollusks (Gastropoda) increases. They, apparently, are less digestible, but are more accessible in the environment compared to other taxa. A similar phenomenon was also observed for the common newt in the urbanization gradient of Yekaterinburg [28]. The crested newt does not choose a specific prey; the size of the consumed objects is a significant limitation of its dietary spectrum [29]. However, oophagia may be an exception [2].

The studies in the Middle Urals revealed a proportion of newts that did not feed, while other authors state the opposite [6, 25]. This is probably due to the physicochemical characteristics of the water body. *T. cristatus* consumes organisms that dominate in a particular habitat, as was shown by authors from Italy [29], Great Britain [30], and Mordovia [6]. We have found that the crested newt avoids consumption of its own eggs, preferring the eggs of anurans. It was also noted that females consumed more eggs than males [2, 25, 29, 31]. When food objects are limited, newts moved to oophagy and consumption of their own skin, which is consistent with our data.

The high level of overlapping of the potential dietary spectrum, apparently, results from the similar composition of large taxonomic groups of hydrobionts in water bodies throughout the Ural Mountains. The low level of overlapping of the realized dietary spectrum between the studied water bodies is explained by the high proportion of terrestrial forms in the diet of the newts from the Middle Urals. These differences are due to the physicochemical characteristics of the reservoir (temperature, depth, area, and location). In the Southern Urals, the spawning pond had a shallow depth, was located relatively far from the river (about 300 m), and contained abundant aquatic and semiaquatic vegetation and its remains, as was evidenced by a high level of COD (Table 2). It has been established that in a reservoir with a large area (Southern Urals), the dietary spectrum of newts includes a greater number of aquatic invertebrates, which may be associated with the phenological features of *T. cristatus*; i.e., newts in a reservoir in the Middle Urals begin to feed later. This is also typical for *T. carnifex* [11]. Well-warming (up to 16.9°C and higher) (see Table 2) and the presence of aquatic vegetation create the most optimal conditions for the development of various systematic groups of invertebrates, which is reflected by the indices of their species diversity in the habitat in the Southern Urals.

Favorable temperature conditions, as well as the presence of intermediate hosts of mollusks of the genus *Planorbis* and aquatic forms of Oligochaeta in the water body of the Southern Urals, in comparison with the reservoir of the Middle Urals, create necessary conditions for the development of larvae of the nematode *M. terdentatum*. The third invasive stage of the nematode develops in the aquatic environments at a temperature of about 20°C [32]. *M. terdentatum* is a heteroxenous parasite (needs several intermediate hosts). This nematode can infect sexually mature newts, as evidenced by our data. The low invasion rate of crested newts by the nematode *M. terdentatum* may be associated not only with the peculiarities of the intravital method of extracting the stomach contents, but also with the environmental features of the habitat, as well as with the specific biology of both the parasite and the host [33].

The water body in the Middle Urals is deep and is located close to the river (15 m from the river channel), which does not exclude its replenishment with colder water in spring. This probably results in a later aquatic vegetation development and, accordingly, a lower level of COD in the spring period. So, the dietary spectrum of invertebrates is not rich during this period. It is likely that the highest percentage of empty stomachs is associated with both the greater depth of the reservoir in the Middle Urals and the low content of organic matter in it, as is evidenced by the COD value.

Thus, the dietary spectra of *T. cristatus* in the study areas are represented by invertebrates belonging to four phyla: Mollusca, Arthropoda, Oligochaeta, and Nematoda. The identified interspecific oophagia, the predominance of terrestrial forms in the newt's dietary spectrum, as well as the presence of a certain proportion of empty stomachs result from a shortage of aquatic invertebrates in the environments. The difference between the potential and realized dietary spectra in amphibians in the study areas can be explained by the quantitative and taxonomic richness of *T. cristatus* food objects, which are associated with the specifics of the habitat: good warming of the reservoir, richness of aquatic vegetation, hydrochemical indicators (high level of COD), etc. These features of the habitat probably also explain the findings of macroparasites in the stomach of the crested newt in one habitat.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflicts of interest. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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