

Diet Analysis of the Amur Sleeper (*Perccottus glenii*) from the Danube River Drainage Channel (Serbia)

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Abstract

The stomach content of 277 Amur sleeper individuals has been analyzed to present its feeding habits. Fish were sampled using electrofishing from July to November 2017 in the Danube River drainage channel near Veliko Gradište (Serbia). Fish age was estimated by otoliths examination. Ingested prey organisms were identified to the lowest reliable taxonomic level. The biotic indices: vacuity index (VI), frequency of occurrence (F), abundance (C_n), index of importance (PV), Shannon's diversity index (H'), and equitability index (E_h) were calculated. A total of 18 prey categories and 1144 individual prey were identified in the fish diet, dominantly aquatic macroinvertebrates. There were no significant difference in diet composition between sampling months as well as 0⁺, 1⁺, and 2⁺ age groups (both 3⁺ and 4⁺ had one individual). Only eight individuals were found with empty intestines, thus VI was low (2.9). In average, each fish had 4.1 prey items in their intestines. For whole sample, H' was 1.84 and E_h was 0.64. Trichoptera, Ephemeroptera, and Gastropoda were the most frequent, dominant, and abundant. The greatest diversity of prey items was recorded for fish sampled in October as well as for 2⁺ individuals, and the lowest for fish from August and 0⁺ individuals.

Introduction

Invasion of alien fish species may affect the structure and functioning of native communities and ecosystems, throw their predation and competition with native species (Zaret & Paine, 1973; Lodge, 1993; Khan & Panikkar, 2009). One of the possible ways to assess the impact of invasive species is analysis of their dietary characteristics using numerous methods (Hynes 1950; Hyslop 1980; Pierce & Boyle 1991). The main used methods are based on numbers, biomasses or volumes, and frequency of occurrence of prey (Hynes, 1950; Pinkas *et al.*, 1971; Cortés, 1997). The Amur (Chinese) sleeper, *Perccottus glenii* Dybowski, 1877 (Odontobutidae), known also as rotan, is one of the most widespread invasive fish in Eurasia (Reshetnikov, 2010). It is a medium-sized fish (total length up to 27 cm)

native to the Far East region of Eurasia in Russia, north-eastern China and northern North Korea. The Amur sleeper has been recently recorded in the Serbian part of the Danube and Tisza Rivers in the regions having strong commercial fishery activities (Gergely & Tucakov, 2004; Šipoš *et al.*, 2004; Simonović *et al.*, 2006; Hegediš *et al.*, 2007; Lenhardt *et al.*, 2010; Skorić *et al.*, 2017).

It is a limnophilic species, inhabiting freshwater channels, gravel pits, flood plains, oxbow lakes and fish ponds. It prefers stagnant waters with dense aquatic vegetation and muddy substrate (Kottelat & Freyhoff, 2007) and tolerates low water oxygen levels and habitat degradation (Reshetnikov, 2004; Nastase *et al.*, 2019). It shows highly flexible feeding strategy using locally available food resources. Also, the Amur sleeper is able to avoid predators by inhabiting waters unsuitable for most other freshwater fishes (Kati *et al.*, 2015). All listed

biological characteristics, together with prolonged reproductive period, are characteristic for highly invasive species (Puesink, 2005). Analysing morphometric characteristics of the Amur sleeper, Nikolić *et al.* (2021) indicated that older individuals had longer anterior parts of the body and more robust jaws and heads compared to younger individuals, which could be a strategy for avoiding intraspecific competition for food. In this kind of lentic locality, in the drainage channel near Danube River (Serbia), covered by riparian vegetation, samples of this invasive fish have been collected and analyzed. In investigated channel, existing fish community consisted of common species like roach (*Rutilus rutilus*), tench (*Tinca tinca*), northern pike (*Esox lucius*), pumpkinseed (*Lepomis gibbosus*), common rudd (*Scardinius erythrophthalmus*), weatherfish (*Misgurnus fossilis*), and European bitterling (*Rhodeus amarus*).

The Amur sleeper is predator which diet consists of a wide range of prey. It uses different food resources ranging from zooplankton (Cladocera, Copepoda) consumed by juveniles, through aquatic macroinvertebrates (larvae of Insecta, Mollusca, Crustacea and Oligochaeta) to vertebrates (larvae and eggs of amphibians and fish) (Sinelnikov, 1976; Manteifel & Reshetnikov, 2002; Reshetnikov, 2003, 2008; Koščo *et al.*, 2008; Grabowska *et al.*, 2009).

The objective of this study was to investigate the feeding ecology of the Amur sleeper from invaded channel in Serbia and to determine whether food composition vary depending on sampling period and age of fish.

Materials & Methods

Fish sampling and stomach content analysis

The fish samples were collected during July, August, October and November 2017, from the drainage channel near Veliko Gradište (N 44° 44.322'; E 21° 29.240'; 69 m altitude), the Danube River (Figure 1). The total of 277 fish individuals were collected with electrofishing device Elemax SHX 2000 (Sawafuji, 220V, 8.5A). Total length (TL, in cm) and total weight (W, in g) of each individual were measured. The age of the analyzed fish was estimated by otoliths examination. From each individual both otoliths were extracted, cleaned, dried and put in paper envelopes. Each otolith was ground with sandpaper in order to facilitate age reading. Three independent researches used a binocular microscope "Zeiss Stemi 508" for reading the anuli. After measuring, fish were dissected with a plastic laboratory set. The eviscerated guts were then preserved in 70% ethanol for further identification of dietary items. The fish intestines were examined under a binocular microscope "Zeiss Stemi 508" and appropriate identification keys (Müller-Liebenan, 1969; Belfiore, 1983; Malicky, 1983; Pflieger, 1990; Fres 1997; Hynes, 1997; Nilsson, 1997a,b) were used for the identification of intestinal contents to the lowest taxon possible. Quantification of stomach contents was based on the number of all identifiable prey items. Nevertheless, in some samples, intestine content analysis was difficult, for the reason that prey have been masticated or digested.

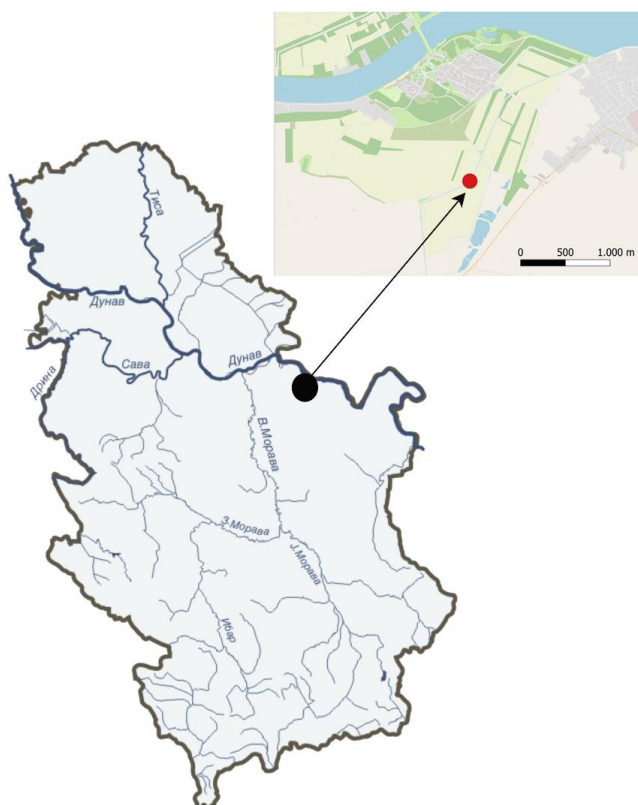


Figure 1. Study area was located in eastern Serbia, near the city Veliko Gradište (geographical coordinates: N 44° 44.322'; E 21° 29.240')

Biotic Indices

The following indices were used for the data analyses of stomach content of the Amur sleeper:

Vacuity index (VI):

$$VI(\%) = \frac{N_{es}}{N_t} \times 100 \quad (1)$$

where, N_{es} is number of fish with empty stomach and N_t is total number of fish (Berg, 1949).

Frequency of occurrence (F):

$$F(\%) = \frac{N_{iy}}{N_i} \times 100 \quad (2)$$

where N_{iy} is number of fish intestines with a certain prey category and N_i refers to the total number of full intestines (Hyslop, 1980; Ahlbeck *et al.*, 2012).

Percentage share (C_n):

$$C_n = \frac{N_{prey\ category\ i}}{N_{prey\ categories}} \times 100 \quad (3)$$

where $N_{prey\ category\ i}$ is calculated as the total number of individuals in all stomachs of a specific prey item (i) and $N_{prey\ categories}$ is the total number of individuals in all stomachs of all prey categories (Hyslop, 1980).

Index of importance or prominence value (PV), the most important prey categories are determined as (Hickley *et al.*, 1994; Lorenzoni *et al.*, 2002):

$$PV = C_n \times \sqrt{F} \quad (4)$$

$$PV(\%) = \frac{PV}{\sum PV} \times 100$$

Shannon's diversity index (H') was used to calculate the trophic niche breadth:

$$H' = - \sum_{i=1}^{S_t} p_i \ln p_i \quad (5)$$

$$p_i = \frac{n_i}{N}$$

$$H'_{max} = \ln S_t$$

where p_i proportion of the i -th prey category in the sample, n_i - number of individuals of the i -th prey category, N total number of prey items, and S_t total number of prey categories (Krebs, 2001).

Equitability index (E_h):

$$E_h = \frac{H'}{H'_{max}} \quad (6)$$

It shows the uniformity of the number of items of each prey category; refers to how close in numbers each item in an intestinal content is, to know the abundance of prey item relative to other items identified in fish intestinal content. It is ranges from 0 (only one prey item) and 1 (more than one prey item with the same abundance in fish intestine) (Washington, 1984).

Statistical Analyses

In order to test the normality of the data distribution we used Shapiro-Wilk's test. Since data sets lacked normality of distribution, comparisons of dietary characteristics (diet composition and prey abundance) between sampling months/age groups were performed using the non-parametric Kruskal-Wallis H test, followed by the Mann-Whitney U test. We excluded age group 3⁺ and 4⁺ from the analysis, because both groups were presented by only one individual. Significance level (α) for all conducted tests was at 5%. Statistica 7.0 Software (StatSoft, Inc., Tulsa, OK, USA) was used to perform all statistical analyses.

Result & Discussions

A total of 277 individuals of the Amur sleeper have been collected and examined – 52 in July, 75 in August, 94 in October, and 56 in November. Age of investigated fish varied between 0⁺ and 4⁺ (Table 1). The most dominant were 0⁺ and 1⁺ age groups, while 3⁺ and 4⁺ age groups were both presented by only one individual. In average, older individuals were longer and heavier compared to the youngest ones (Table 1). List of all identified prey items in fish intestinal content is presented in Supplementary Material.

Table 1. Descriptive statistics of defined age groups. Values are presented as mean ± standard deviation, range from min to max values are given for total length (TL, cm) and body weight (W, g), while N refers to number of individuals.

N	Age group	July		August		October		November	
		TL	W	TL	W	TL	W	TL	W
135	0 ⁺	2.86±0.34	2.31±0.25	3.74±0.53	3.05±0.48	4.24±0.57	0.69±0.21	4.57±0.60	1.13±0.41
	range	2.30-3.32	0.19-0.39	2.58-4.77	0.20-1.10	3.35-5.52	0.36-1.21	2.85-5.47	0.29-2.10
102	1 ⁺	7.67±0.59	6.30±0.55	6.81±2.33	5.70±2.00	7.85±1.09	5.75±2.08	6.91±1.29	4.32±2.67
	range	4.97-7.38	3.10-8.97	7.34-10.44	4.20-13.10	4.55-10.05	0.84-10.75	5.03-9.66	1.50-11.26
38	2 ⁺	9.37±0.84	7.76±0.67	10.77±0.91	9.11±0.98	9.70±1.11	11.61±5.96	11.21±1.52	19.64±8.72
	range	6.92-8.53	6.12-12.59	9.86-11.67	11.6-19.2	8.09-12.16	6.40-33.31	8.83-12.94	7.80-31.40
1	3 ⁺							14.07	40.00
1	4 ⁺	17.06	70.19						

Understanding of diet habits and feeding preference is important in ecological study. There are some studies on food composition of the Amur sleeper (Szito & Harka, 2000; Koščo & Manko, 2003), but they describe the diet of only a few particular samples at a time. The detailed studies on feeding ecology of Amur sleeper populations are available for its native range (Kirpichnikov, 1945; Nikolskii, 1956; Sinelnikov, 1976) as well as for some invaded habitats (Szito & Harka 2000; Reshetnikov 2001, 2003, 2008; Bogutskaya & Naseka 2002; Orlova *et al.*, 2006; Koščo *et al.*, 2008; Grabowska *et al.*, 2009; Kati *et al.*, 2015). The mentioned studies on its non-native range revealed that the Amur sleeper is a predator with a wide-ranging diet habits consisting of crustaceans (Cladocera, Copepoda, Malacostraca), larvae of insects (Ephemeroptera, Odonata, Hemiptera, Diptera, Trichoptera, Coleoptera), mollusks, fish, and even larvae of amphibians (frogs and newts) that are rarely consumed by other fish.

The wide diet spectrum of the Amur sleeper indicates that it is a non-selective, opportunistic predator and that several taxonomic groups of native aquatic fauna as aquatic macroinvertebrates as well as fish and amphibian may be affected by its presence (Grabowska *et al.* 2009). The presence of this fish in small waterbodies leads to a great decrease of aquatic macroinvertebrates diversity (Reshetnikov, 2008). A total of 1144 individual prey items belonging to 18 prey categories were identified in the fish diet (Table 2). Only eight individuals (6 of 0⁺ and 2 of 1⁺) were found with empty intestines. Therefore, the value of vacuity index was low (VI=2.9). In average, each fish had 4.1 prey items in their intestines. A detailed overview of identified prey items is given in Supplementary Material.

The diet of Amur sleeper included a wide variety of hydrobionts of all trophic levels. They were represented mostly by aquatic macroinvertebrates (insects, mollusks, gammarids, annelids), followed by small fish

and terrestrial insects. Analyzing the distribution of prey items per fish age class and sampling month, the results are as follows: 434, 493, 179, 18, and 6 for 0⁺, 1⁺, 2⁺, 3⁺, and 4⁺, respectively, as well as 301, 230, 362, and 238 for July, August, October, and November, respectively. However, there were no significant difference in diet composition between sampling months as well as between 0⁺, 1⁺, and 2⁺ age groups. This was also noted by Koščo *et al.* (2008) who found out size-dependent food composition. Studies on the feeding habits of the Amur sleeper from drainage channels in East Slovakia investigated that food composition of the Amur sleeper was highly size-dependent. The diet composition of this species from lotic and lentic ecosystems of Central Europe was also mainly regulated by fish body size that had stronger effect than the habitat and the season (Kati *et al.*, 2015). Spanovskaya *et al.* (1964) stated that the Amur sleeper fed more on benthos individuals compared to fishes, rather choosing the less mobile prey which prefers the bottom or vegetation.

It is found that the Amur sleeper could serve as a vector for the introduction of various parasites (more than a 100 species) (Nastase *et al.*, 2019). In the intestinal content of fish, we found the representatives of two helminth groups – Nematoda (two individuals) and Cestoda (one individual). Intestinal food items identified as aquatic macroinvertebrates are recognize as an intermediate hosts for recorded helminths. In the detailed data on parasites of the amur sleeper published by Sokolov *et al.* (2014), for Serbia has been determined *Eustrongylides sp.* (Nematoda) (Nikolić *et al.*, 2007).

Selected biotic indices indicated which prey category has the largest share in diet of the Amur sleeper. Insect component represented by Trichoptera and Ephemeroptera, followed by Plecoptera and Odonata as well as mollusk component represented by gastropods were the most frequent, dominant, and abundant food items in the intestines of the Amur

Table 2. Frequency of occurrence (F), numerical abundance (C_n), and index of importance (PV and PV%) of identified intestinal prey items of the Amur sleeper.

Prey item/Indices	Frequency of occurrence	Numerical abundance	Index of importance	
	F	C _n	PV	%PV
Ephemeroptera	39.78	24.04	151.61	27.87
Trichoptera	61.34	22.38	175.26	32.21
Odonata	23.79	8.04	39.23	7.21
Hemiptera	3.35	0.87	1.60	0.29
Heteroptera	0.74	0.17	0.15	0.03
Plecoptera	21.19	10.23	47.08	8.65
Diptera	0.37	0.09	0.05	0.01
Chironomidae	10.78	6.21	20.38	3.75
Coleoptera	0.37	0.09	0.05	0.01
Unidentified.insect	2.60	0.79	1.27	0.23
Mollusca (digested)	2.60	0.79	1.27	0.23
Gastropoda	50.93	14.25	101.68	18.69
Bivalvia	0.37	0.09	0.05	0.01
Gammaridae	3.35	1.49	2.72	0.50
Myriapoda	0.37	0.09	0.05	0.01
Oligochaeta	1.49	0.35	0.43	0.08
Hirudinea	2.60	0.70	1.13	0.21
Juvenile Percottus sp.	0.37	0.09	0.05	0.01

sleeper (Table 2). The domination of insect larvae and mollusks in the rotan diet content was confirmed also in the study of Kati *et al.* (2015). For whole sample, Shannon's diversity index (H') was 1.84 showing that food resource is quite large, and equitability index (E_h) for fish intestinal content was 0.64.

The greatest diversity of prey taxa ($n=14$) was found in individuals caught in October, and the lowest ($n=9$) was recorded in individuals caught in August (Table 3; Figure 2). Prey items belonging to Ephemeroptera, Trichoptera, Odonata, Gastropoda, Hemiptera, and Plecoptera were recorded in each of the sampling months. Trichoptera and Gastropoda dominated in diet of the Amur sleeper caught in August, while Ephemeroptera dominated in November.

The greatest number of prey taxa ($n=15$) was found in 1^+ individuals, and the lowest ($n=12$) was recorded in 2^+ individuals (Table 4). On contrary, the greatest H' (1.95) and E_h (0.67) was recorded for 2^+ individuals

(Figure 3). Prey items belonging to Trichoptera, Gastropoda, Ephemeroptera, Odonata, Plecoptera, Chironomidae, Gammaridae, Hemiptera, Mollusca, and Oligochaeta were recorded in the intestines of all age groups. Trichoptera, Gastropoda, and Ephemeroptera dominated in diet of all age. Nonetheless, in intestine of 1^+ fish specimen one small Amur sleeper has been identified, which is an indication of cannibalism.

Generally, the results presented in this paper are in accordance with the results of previous work on feeding habits of the Amur sleeper. The large number of food categories found in intestines of the Amur sleeper confirms previous findings that this fish species is a non-selective predator with a broad diet spectrum, covering several trophic levels of the aquatic food chain. However, Trichoptera, Ephemeroptera, and Gastropoda dominated in its diet regarding the sampling month and/or age of fish. This could be a result of their great abundance in local macroinvertebrate community,

Table 3. Values of applied biotic indices on the fish intestinal content presented by sampling months.

Prey item/Indices	Frequency of occurrence				Numerical abundance				Index of importance							
	F				C _n				PV				%PV			
	July	Aug.	Oct.	Nov.	July	Aug.	Oct.	Nov.	July	Aug.	Oct.	Nov.	July	Aug.	Oct.	Nov.
Ephemeroptera	57.69	9.72	36.67	67.27	37.21	3.04	15.19	42.44	282.62	9.49	92.00	348.07	41.62	1.16	15.83	55.88
Trichoptera	48.08	98.61	54.44	36.36	15.28	36.52	22.10	19.33	105.96	362.67	163.06	116.55	15.61	44.35	28.06	18.71
Odonata	38.46	11.11	31.11	14.55	9.97	3.91	12.43	3.36	61.81	13.04	69.34	12.82	9.10	1.59	11.93	2.06
Hemiptera	9.62	2.78	1.11	1.82	1.66	0.87	0.55	0.42	5.15	1.45	0.58	0.57	0.76	0.18	0.10	0.09
Heteroptera	3.85				0.66				1.30				0.19			
Plecoptera	32.69	19.44	27.78	1.82	13.62	6.96	14.92	2.52	77.88	30.68	78.62	3.40	11.47	3.75	13.53	0.55
Diptera	1.92				0.33				0.46				0.07			
Chironomidae		1.39	20.00	18.18		0.43	10.22	13.87		0.51	45.71	59.12		0.06	7.87	9.49
Coleoptera			1.11				0.28				0.29			0.05		
undeterm.insect	5.77			7.27	1.00			2.52	2.39			6.80	0.35			1.09
Mollusca (digested)		6.94	2.22			3.04	0.55			8.02	0.82			0.98	0.14	
Gastropoda	50.00	76.39	43.33	30.91	19.93	44.78	17.68	12.18	140.95	391.40	116.38	67.74	20.76	47.86	20.03	10.88
Bivalvia			1.11				0.28				0.29			0.05		
Gammaridae			7.78	3.64			4.14	0.84			11.56	1.60		1.99	0.26	
Myriapoda			1.11				0.28				0.29			0.05		
Oligochaeta			3.33	1.82			0.83	0.42			1.51	0.57		0.26	0.09	
Hirudinea	1.92	1.39	1.11	7.27	0.33	0.43	0.28	2.10	0.46	0.51	0.29	5.67	0.07	0.06	0.05	0.91
Juvenile Percottus sp.			1.11				0.28				0.29			0.05		

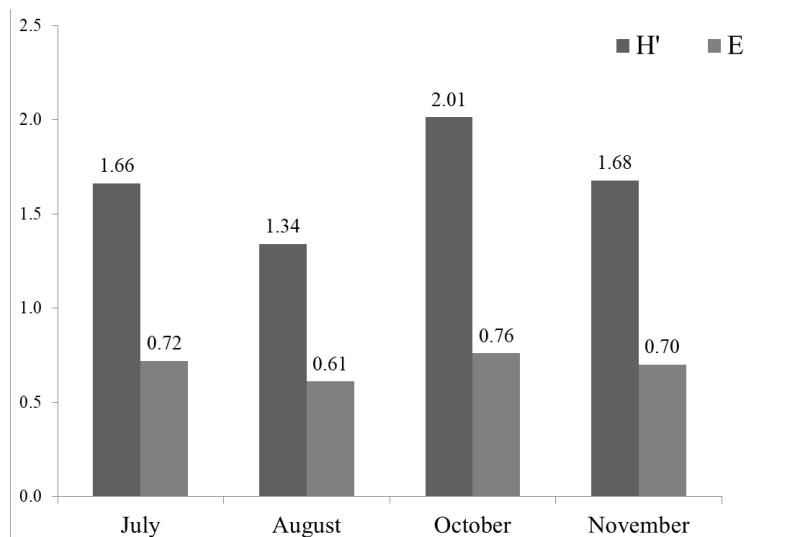


Figure 2. Values of Shannon's diversity index (H') and equitability index (E_h) presented by sampling months

Table 4. Values of applied biotic indices on the fish intestinal content presented by age groups.

Prey item/Indices	Frequency of occurrence			Numerical abundance			Index of importance					
	F			C _n			PV			PV%		
	0 ⁺	1 ⁺	2 ⁺	0 ⁺	1 ⁺	2 ⁺	0 ⁺	1 ⁺	2 ⁺	0 ⁺	1 ⁺	2 ⁺
Ephemeroptera	36.30	49.02	39.47	19.91	30.97	17.88	119.94	216.85	112.31	19.01	35.41	18.02
Trichoptera	67.41	41.18	57.89	27.78	16.80	29.61	228.07	107.82	225.28	36.14	17.61	36.14
Odonata	8.15	33.33	47.37	2.55	12.15	11.17	7.27	70.12	76.90	1.15	11.45	12.34
Hemiptera	0.74	4.90	7.89	0.23	1.21	1.68	0.20	2.69	4.71	0.03	0.44	0.76
Heteroptera		0.98			0.20			0.20			0.03	
Plecoptera	29.63	12.74	7.89	16.90	6.68	4.47	91.98	23.84	12.55	14.58	3.89	2.01
Diptera		0.98			0.20			0.20			0.03	
Chironomidae	5.93	15.69	7.89	4.86	6.88	2.79	11.84	27.26	7.85	1.88	4.45	1.26
Coleoptera			2.63			0.56			0.91			0.15
udeterm.insect	2.97	2.94		1.39	0.61		2.39	1.04		0.38	0.17	
Mollusca (digested)	0.74	3.92	5.26	0.23	0.81	2.23	0.20	1.60	5.13	0.03	0.26	0.82
Gastropoda	45.18	52.94	52.63	25.00	21.66	19.55	168.04	157.60	141.85	26.63	25.73	22.76
Bivalvia	0.74			0.23			0.20			0.03		
Gammaridae	1.48	0.98	15.79	0.46	0.20	7.82	0.56	0.20	31.08	0.09	0.03	4.99
Myriapoda	0.74			0.23			0.20			0.03		
Oligochaeta	0.74	1.96	2.63	0.23	0.40	0.56	0.20	0.57	0.91	0.03	0.09	0.15
Hirudinea		4.90	5.26		1.01	1.68		2.24	3.84		0.37	0.62
Juvenile Perccottus sp.		0.98			0.20			0.20			0.03	

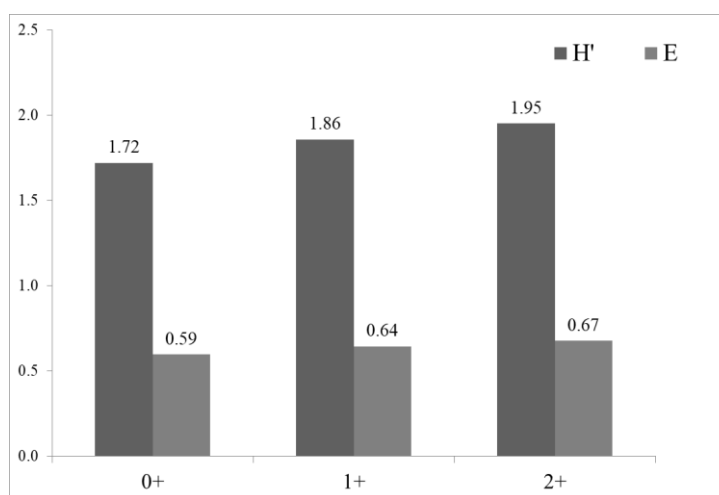


Figure 3. Values of Shannon's diversity index (H') and equitability index (E_n) presented by age groups

indicating that diet characteristics of the Amur sleeper is influenced by the availability of certain prey.

These findings could be the base for further investigation in order to determine the role of the invasive fish species in the food web of shallow waters, considering the fact that specimens are taking diverse food categories, including plankton, as well as benthic and terrestrial species.

Ethical Statement

Not applicable

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Author Contribution

VDj: conceptualization, analysis of fish diet, writing original draft; SS: fish sampling, visualization; BM: conceptualization, supervision; DN: fish sampling, laboratory analyses, statistical analyses, writing original draft.

Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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