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Feeding Patterns of Fish in Relation to the Trophic Status of Reservoirs: A Case Study of *Rutilus rutilus* (Linnaeus, 1758) in Five Fishing Waters in Serbia

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Abstract: The roach, *Rutilus rutilus* (Linnaeus, 1758), is one of the most common fish species in mesotrophic and eutrophic lakes throughout Europe. In the Serbian reservoirs selected for this study, this species accounts for the majority of juvenile fish biomass. The aim of this study was to investigate the diet composition of juvenile roach to assess their niche based on resource availability in five Serbian reservoirs with different trophic statuses. A modified Costello graph and Kohonen artificial neural network (i.e., a self-organizing map, SOM) were employed to examine the feeding habits of 142 specimens of roach caught in five reservoirs. Our results show that juvenile roach use zooplankton, benthic macroinvertebrates, algae and detritus in their diet. In addition, five neuron clusters (A, B, C, D and E) were isolated in the SOM output network. The SOM identifies specimens that share similar feeding patterns and categorizes them onto the same or adjacent neurons, determined by dominant prey. In terms of the number of specimens, cluster B was the most numerous, and the predominant prey of these specimens were *Daphnia* sp., *Bosmina* sp. and calanoid and cyclopoid copepods. The cluster with the lowest number of specimens is cluster C, and the specimens in it benefited from Chironomidae and Insecta. Due to the different trophic statuses of the reservoirs selected for this study, knowledge of fish feeding habits is essential for the formulation of effective conservation and management strategies for both the species and the reservoirs.

Keywords: roach; diet; eutrophication; self-organizing map; IndVal

Key Contribution: The diet of juvenile roach was examined in five reservoirs in Serbia with varying trophic statuses, aiming for a better understanding of feeding patterns important for the conservation of fish and ecosystems. One approach, critical in light of future global environmental changes, is researching fish feeding as the ultimate link in the food chain of aquatic ecosystems. Roach, particularly in eutrophic lakes, hold significance as they can exploit almost any food source, especially in situations with strong competition. The method of fish feeding analysis presented in this paper is effective and time-saving, offering biologic and ecologic knowledge outcomes.

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1. Introduction

Globally, a variety of stressors induce risks on aquatic habitats [1–5]. Due to these stressors (e.g., eutrophication, habitat fragmentation, climate change, pollution, invasive species, natural resources and services overexploitation, land use change, etc.), fish, which play a main role in the nutrient cycle and energy flow [6–8], are imperiled as a resource and as collateral losses due to the effects of particularly variable human impacts [9–12]. This is one of the main reasons why fish-integrated ecology and biology study outcomes are globally of core concern for scientific and economic purposes.

The richness of the Balkan fauna makes this a special area from this point of view, as the region is one of the topmost biodiversity hotspots on our planet [13,14]. The past two and a half million years' continental and planetary major events played an important role in the appearance and enlargement of such a specific extraordinary biological and ecological variety [15,16].

Water resources play an important role in both the environment and human life [17]. Cultural eutrophication has emerged as the predominant water quality concern for the majority of global aquatic ecosystems [18], altering ecosystems and making these habitats vulnerable as they are exposed to the effects of this factor [19]. Phosphorus is characterized as the key element in controlling eutrophication [20]. The rise in temperature and the constant input of nutrients (phosphorus and nitrogen salts) into aquatic ecosystems can leads to the ageing of ecosystems, i.e., the occurrence of eutrophication. This could alter food webs and affect habitat availability and quality [21].

Fish play a key role in the trophic dynamics of lakes, reservoirs and shallow ecosystems. Because of that knowledge of fish, diet is important to determine the role of fish species in an ecosystem and their function in food webs [22]. In addition, functional diversity measures, such as feeding traits, are more predictive of ecosystem functioning than pure species or taxa diversity measures [23]. Assessing the trophic ecology of fish in natural habitats is essential for understanding biological and ecological requirements and supporting the management and conservation of populations and habitats [24]. Although the diet composition of fish is species-specific, it varies with the availability of food in the environment [25].

The trophic perspective of fishes, their food and their environment is one of the main research approaches to identify fish-integrated biological and ecological aspects [26–30]. Therefore, the aim of this study was to investigate the diet composition of juvenile roach *Rutilus rutilus* (Linnaeus, 1758) in order to assess their niche based on resource availability in five Serbian reservoirs with different trophic statuses. The roach is a fish species that lives in many European lakes in the littoral zone [31], and its occurrence has increased in recent decades [19]. It was selected for this study because it makes up the majority of the biomass of juvenile fish and plays an important role in the food chain as prey for predatory fish [32]. Moreover, the roach is one of the most common fish species in mesotrophic and eutrophic lakes throughout Europe [31]. Another goal was to assess the effectiveness of integrating Kohonen's unsupervised artificial neural network, namely a self-organizing map [33], with the IndVal (Indicator Value) index [34] for analyzing data related to the diet of roach.

2. Materials and Methods

2.1. Study Area and Fish Sampling

The study included five multipurpose reservoirs in Serbia which are used for water supply, hydropower generation, irrigation, recreation and tourism: the Vlasina, Gruža, Gazivode, Šumarice and Vrutci reservoirs (Figure 1).

The morphometric characteristics and trophic statuses of the studied reservoirs are shown in Table 1. Our previous studies showed that the fish community in the researched reservoirs consisted mainly of the following fish species: the Vlasina Reservoir—the Prussian carp *Carassius gibelio* (Bloch, 1782) and the European perch *Perca fluviatilis* (Linnaeus, 1758); the Gruža Reservoir—the Prussian carp, the pikeperch *Sander lucioperca* (Linnaeus, 1758), the roach and the freshwater bream *Abramis brama* (Linnaeus, 1758); the Gazivode Reservoir—freshwater bream, nase *Chondrostoma nasus* (Linnaeus, 1758) and Prussian carp; the Šumarice Reservoir—rudd *Scardinius erythrophthalmus* (Linnaeus, 1758), roach, pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758) and bullhead *Ameiurus* sp; the Vrutci

Reservoir—nase, European perch, freshwater bream and European catfish *Silurus glanis* (Linnaeus, 1758) [35,36].

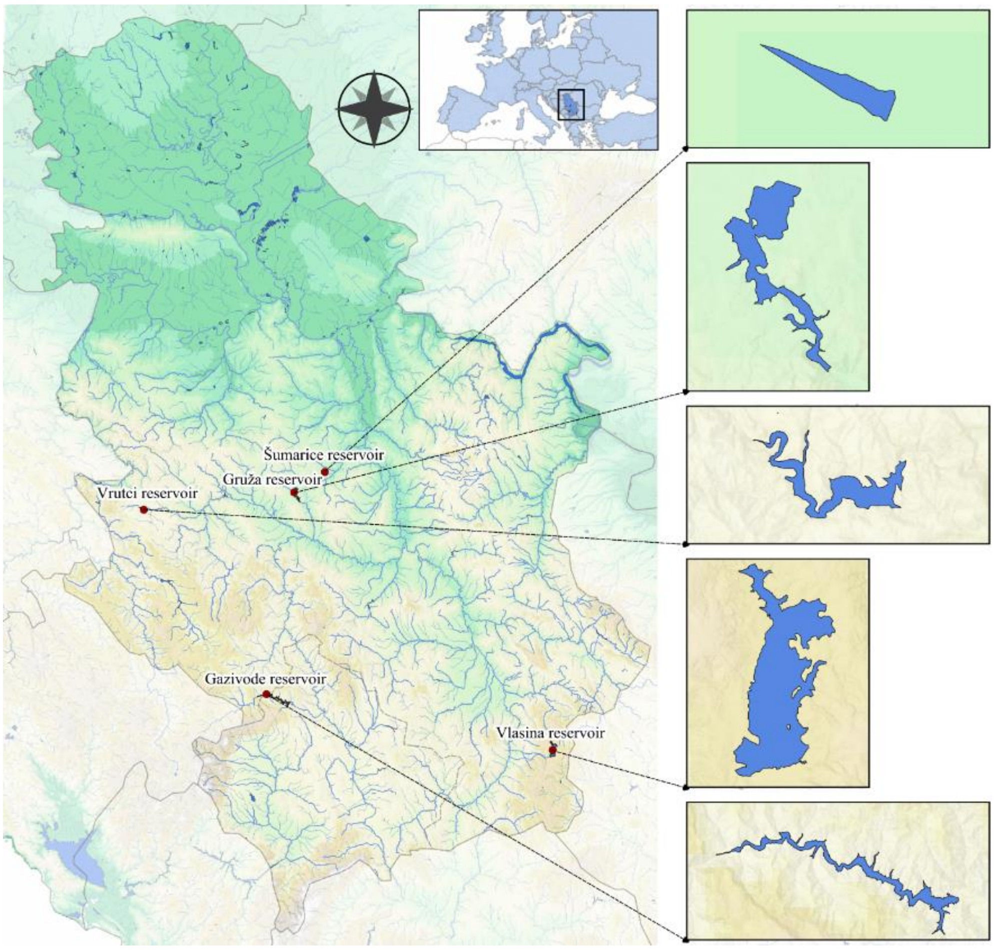

Figure 1. Geographical location of studied reservoirs.

Table 1. Morphometric characteristics and trophic statuses of the studied reservoirs.

	Surface (km^2)	Altitude (m)	Max Depth (m)	Mean Depth (m)	Trophic Status
Vlasina Reservoir	16	1211	35	10.3	oligotrophic [37]
Gruža Reservoir	9.34	269	35	6.5	eutrophic [38]
Gazivode Reservoir	11.9	694	107	36.6	mesotrophic [39]
Šumarice Reservoir	0.22	220	14	6.3	eutrophic [40]; hypereutrophic [41]
Vrutci Reservoir	2.7	700	64	20.9	eutrophic [42]

Fieldwork was conducted from May to September 2017. Roach were sampled with gillnets (with a mesh size of 10 to 120 mm) offshore and with electrofishing using a DC "Aquatech" IG 1300 electrofisher (2.6 kW, 80–470 V) in the littoral zone. Each fish was measured to the nearest mm in total length (TL) and to the nearest g in weight (W). Immediately after capture and measurement, fish selected for analysis, 159 specimens in total, were euthanized with an overdose of 100 mg/L of clove oil for 30 s. Afterward, the fish were dissected and their intestines were removed, preserved in 4% formalin and transported to the laboratory, where the contents of the digestive tract were transferred under a binocular. The prey items were recognized at the most specific level achievable, tallied using binoculars and then stored in 70% ethanol for preservation.

2.2. Content Analysis of the Digestive Tract

To identify the primary prey in the diet, the significance of the dietary components was assessed using the Prominence Value (PV), as computed through the following formulas [43,44]:

$$PV = \%N \times \sqrt{\%FO}$$
$$\%PV = (PV / \sum PV) \times 100.$$

here, %FO represents the frequency of occurrence, indicating the proportion of digestive tracts containing each food item in relation to the total number of digestive tracts with any food. %N stands for relative abundance, reflecting the ratio of the number of specimens for each food item to the total number of specimens [45].

To analyze the feeding strategy of species, we employed a Costello graphical method [46] adapted by Amundsen et al. [47]. This method involved plotting the prey-specific abundance of each food category against the frequency of occurrence (%FO) on a two-dimensional graph. The calculation of prey-specific abundance followed this formula:

$$Pi = 100 \sum Si \times \sum Sti^{-1}.$$

here, Pi represents the prey-specific abundance of prey *i*, Si is the content of prey *i* in the digestive tract (by number) and Sti is the total content of prey in the digestive tract of only those fish that have prey *i* in their digestive tract. In the graphical representation, prey items in the upper part of the graph signify a specialized feeding strategy of the fish, while those in the lower part indicate a generalist feeding strategy. The vacuity index (%VI) was employed to indicate the percentage of empty digestive tracts [45].

2.3. Statistical Data Analysis

An analysis of stomach contents enables one to ascertain the dietary composition of species, providing insights into their feeding habits and trophic roles within the ecosystem [48]. Conversely, data derived from the digestive tract may be prone to noise due to the difficulty in identifying many fragmented or digested elements. To address this issue, we utilized Kohonen's unsupervised artificial neural network, specifically a self-organizing map (SOM) [33], known for its resilience to data noise [49,50]. The SOM technique proves valuable for clustering and visually representing large data sets [51,52]. It can visualize and explore linear and nonlinear relationships in high-dimensional datasets.

In our study, the input data set comprised 142 columns, with each column representing a digestive tract, and 13 rows, where each row represented a prey taxon. Information regarding the relative abundance of prey taxa from the fish digestive tract underwent log transformation (log (x + 1)) and subsequent normalization. The data matrix was successively introduced into the SOM during the learning process. Once the learning process was complete, the data were visualized as a two-dimensional grid of hexagonal neurons. All these neurons constituted the output layer represented by a codebook matrix in which the differences between the neurons, i.e., the models carried by the neurons, increased according to the increase in mutual distance. The clusters of neurons on the trained SOM map were determined using the k-means method [53]. The resolution of the map, denoted by the number of output neurons, serves as a crucial parameter for detecting variations in the data. If the resolution of the network is incorect, such as being either too low or too high, the differences become either too subtle or too exaggerated for a meaningful interpretation [54]. Using the methods proposed by Vesanto et al. [55] and Park et al. [56] and trying to avoid a large number of empty output neurons [51], we determined that a 7 × 8 grid was the most suitable for our study. Using a grey-scale gradient, the SOM Toolbox generated a visualization illustrating the connections between food categories and SOM regions, represented as subclusters of neurons. However, this visualization did not serve the purpose of conducting statistical tests on these associations [57]. The SOM analysis was performed using the algorithm interface of Matlab ver.6.1.0.450 (http://www.cis.hut.fi/projects/somtoolbox, accessed on 27 December 2023).

As SOM primarily serves as a visualization method without statistical capabilities, the indicator value (IndVal) introduced by Dufrêne and Legendre [34] was employed to identify food categories significantly linked with each cluster of SOM output neurons. IndVal for food category *i* within all digestive tracts of a given SOM cluster *j* was computed as the product of *Aij* (relative abundance in %, determined by the mean mass of food category *i* in the digestive tracts of cluster *j* divided by the sum of mean masses of all food categories in all clusters) and *Fij* (relative frequency of occurrence of food category *i* in the digestive tracts of cluster *j*, also expressed in %) as follows:

$$A_{ij} = mean\ mass_{ij}/mean\ mass_i$$

$$F_{ij} = N\ digestive\ tracts_{ij}/N\ digestive\ tracts_j$$

$$IndVal_{ij} = A_{ij} \times F_{ij} \times 100$$

The Monte Carlo significance test, involving 100 permutations, was conducted using the statistical software PC-ORC (ver.6) [58] to detect significant prey taxa. Any indicator species with an IndVal value exceeding 25 was considered indicative of a specific group, provided that the relative abundance and frequency were both at least 50%.

3. Results

For the investigation of diet composition, a total of 142 specimens with TL ranging from 8.5 to 12.1 cm were utilized. The specimens examined per reservoir were as follows: 25 specimens from the Vlasina Reservoir, 47 specimens from the Gruža Reservoir, 30 from the Gazivode Reservoir, 27 from the Šumarice Reservoir and 13 from the Vrutci Reservoir. Fish with empty digestive tracts (17 specimens) were excluded (%VI = 11.97).

The values for relative abundance (%N), frequency of occurrence (%FO) and prominence value (%PV) of each food category in the digestive tracts of the fish studied are shown in Table 2. Prey included 14 different taxa, but not all were represented as prey in every reservoir studied. In addition, detritus was excluded from the calculation, as the remains of animal and plant materials are largely decomposed, so that only their occurrence is available and it was not possible to assign them to any food category. The most diverse diet was found in roach caught in the mesotrophic Gazivode Reservoir, with all 14 prey categories, followed by roach from the hypereutrophic Šumarice Reservoir with 10 and the eutrophic Gruža Reservoir with 8 prey categories, while roach caught in the oligotrophic Vlasina and the eutrophic Vrutci reservoirs had the least diversity (7 prey categories). In all examined reservoirs, roach consumed small crustaceans classified under Calanoida, Cyclopoida and Cladocera, albeit to differing extents. Moreover, roach predominantly consumed cladocerans *Bosmina* sp. and *Daphnia* sp., followed by Insecta and detritus, though the proportion of their diet exhibited variability across different reservoirs. Cladocerans *Daphnia* sp. and *Bosmina* sp. were consistently found in all analyzed digestive tracts of roach from Gruža Reservoir, while algae were present in all examined roach samples from the Šumarice and Gazivode reservoirs. Furthermore, unidentified representatives of the order Cladocera were present in all examined digestive tracts from the Šumarice Reservoir. Only the roach caught in the Gazivode Reservoir fed on organisms classified as Conchostraca and Plecoptera.

The modified Costello graphic predominantly indicated a general feeding strategy in the examined roach, with certain specimens showing specialization in specific prey items (Figure 2). Specifically, the graphical analysis of roach captured in the Vlasina, Gruža and Vrutci reservoirs revealed a generalist feeding strategy, as all prey items were located in the lower part of the graph. The graphical analysis also indicates a generalist feeding strategy of roach caught in the Gazivode and Šumarice reservoirs, as most prey items are located in the lower part of the graph, with the exception of algae in the upper right corner of the graph. Rare preys are also on the menu of roach, located in the lower left corner of the graph.

Table 2. Evaluation of the food composition of roach caught in the studied reservoirs, expressed as relative abundance (%N), frequency of occurrence (%FO) and prominence value (%PV) of food.

	Vlasina			**Gruža**			**Gazivode**			**Šumarice**			**Vrutci**		
	%N	**%FO**	**%PV**	**%N**	**%FO**	**%PV**	**%N**	**%FO**	**%PV**	**%N**	**%FO**	**%PV**	**%N**	**%FO**	**%PV**
Protozoa	-	-	-	2.26	25.53	1.20	2.19	20.00	1.101	3.49	29.62	2.10	-	-	-
Ostracoda	-	-	-	2.42	31.91	1.43	4.49	53.33	3.69	8.31	74.07	7.93	-	-	-
Conchostraca	-	-	-	-	-	-	1.09	10.00	0.38	-	-	-	-	-	-
Cladocera	27.47	48.00	26.95	5.66	63.83	4.75	8.76	83.33	9.001	13.31	100.00	14.77	20.91	76.92	21.23
Daphnia sp.	25.82	56.00	27.36	25.48	100.00	26.78	2.19	16.66	1.005		-		18.49	84.61	19.69
Bosmina sp.	-	-	-	40.53	100.00	42.60	9.96	93.33	10.83	9.98	66.66	9.04	23.59	84.61	25.12
Leptodora kindtii	-	-	-	0.48	4.25	0.10	1.09	26.66	0.63	-	-	-	-	-	-
Calanoida (*Copepoda*)	12.08	40.00	10.82	11.01	91.48	11.06	5.47	56.66	6.63	3.32	29.62	2.04	16.08	61.53	14.61
Cyclopoida (*Copepoda*)	10.98	36.00	9.33	12.13	89.36	12.05	6.90	53.33	5.67	4.99	37.03	3.36	19.30	69.23	18.59
Chironomidae	9.34	40.00	0.08	-	-	-	1.20	13.33	0.49	4.99	74.07	4.76	-	-	-
Plecoptera	-	-	-	-	-	-	0.54	10.00	0.19	-	-	-	-	-	-
Insecta	14.28	72.00	17.16	-	-	-	1.31	23.33	0.71	1.66	11.11	0.61	1.61	15.38	0.73
Algae	-	-	-	-	-	-	54.76	100.00	61.64	49.91	100.00	55.38	-	-	-
Detritus		68.00		-	-	-		33.33			29.63			23.07	

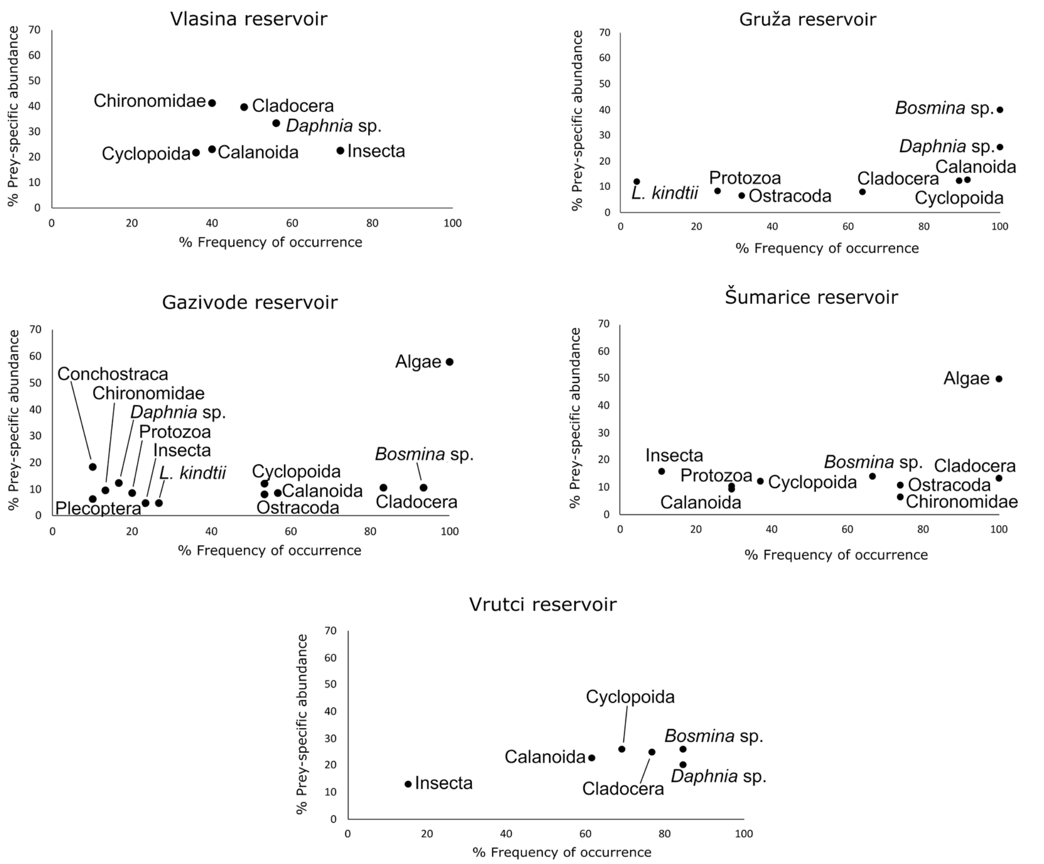

Figure 2. Costello graph. Prey-specific abundance versus frequency of occurrence of the diet of roach collected in five reservoirs in Serbia.

Five neuron clusters (A, B, C, D and E) were isolated in the SOM output network (Figure 3). In cluster A, the digestive tracts of roach sampled in Vlasina Reservoir were the most numerous (12 samples), while the digestive tracts of roach sampled in the Vrutci and Gruža reservoirs had the same number of samples, 3. Cluster B had the largest number of neurons and the largest number of samples. The most numerous in this group were the digestive tracts of roach sampled in the Gruža Reservoir (43 samples). According to the origin of the samples, that is, according to the locality where they were taken and according to the neurons of which they are composed, the most diverse cluster is C. Clusters D and E consist exclusively of samples from the Gazivode and Šumarice reservoirs.

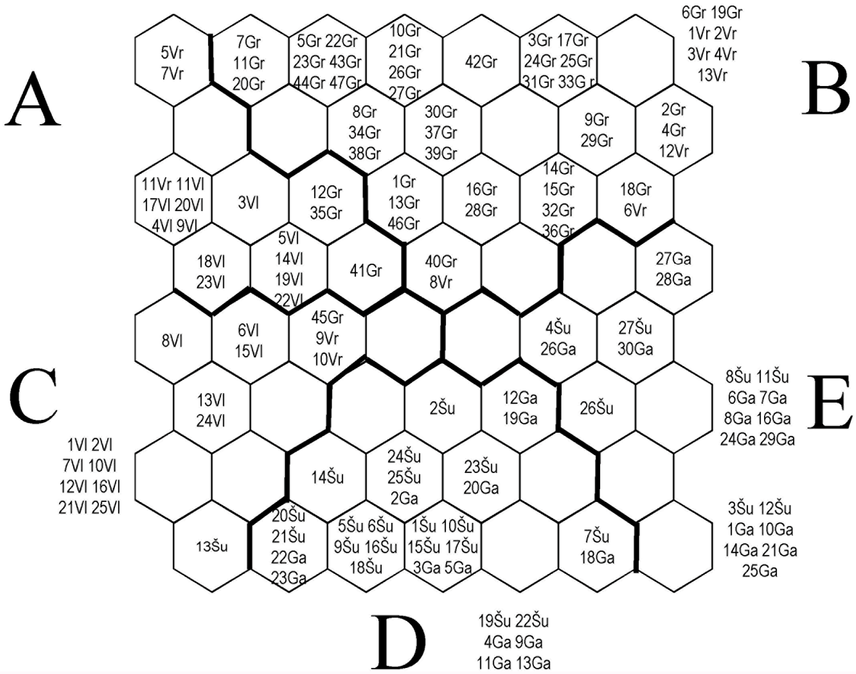

Figure 3. The 142 digestive tracts of roach associated with 56 (7 × 8) SOM output neurons, arranged in five clusters (A, B, C, D and E). The code for each digestive tract consists of the ordinal number of the specimen and two letters for the reservoir studied (Gr—Gruža Reservoir, Šu—Šumarice Reservoir, Ga—Gazivode Reservoir, Vl—Vlasina Reservoir, Vr—Vrutci Reservoir).

Significant IndVal values were found for 8 of 13 food categories, with the exception of detritus (Table 3, Figure 4). This is because detritus contains remains of animal and plant materials that are largely degraded, so it was not possible to assign them to a category. One food category displayed a significant association with the digestive tracts of cluster A, while four food categories were linked to the digestive tracts of clusters B and E. Additionally, two food categories were associated with clusters C and D. Notably, Calanoida and Cyclopoida were identified as significant food categories for specimens whose digestive tracts were classified into clusters B and E, with cladocerans *Daphnia* sp. and *Bosmina* sp. standing out as significant in cluster B, while Ostracoda and algae were significant in cluster *E. Daphnia* sp. were important prey items for specimens from cluster A, Chironomidae and Insecta were important prey items for specimens from cluster C and Chironomidae and algae were important prey items for specimens from cluster D. However, some food categories were important for some groups, e.g., Chironomidae, Insecta and algae were important for some groups, while they were absent from the digestive tracts of specimens from other groups, particularly from cluster B. Ostracoda were absent as prey in specimens from clusters A and C, while they were important prey for other specimens (Table 3).

Table 3. The percentages of relative frequency (%F), relative abundance (%N) and indicator values (IndVal, %I) for different food categories of roach. The highest IndVal values at $p \leq 0.05$ within a specific cluster (A, B, C, D, E) are highlighted in bold, with precise significance levels detailed in Figure 3 (adapted from Dukowska et al. [59,60]).

Food Categories	A			B			C			D			E		
	%F	%N	%I	%F	%N	%I	%F	%N	%I	%F	%N	%I	%F	%N	%I
Protozoa	0	0	0	24	28	7	0	0	0	29	42	12	18	30	5
Ostracoda	0	0	0	29	15	4	0	0	0	59	37	22	73	48	**35**
Conchostraca	0	0	0	0	0	0	0	0	0	6	60	4	5	40	2
Cladocera	78	31	24	67	19	12	29	6	2	97	23	23	82	21	17
Daphnia sp.	94	35	**33**	100	56	**56**	18	1	0	9	1	0	9	6	1
Bosmina sp.	33	7	2	100	61	**61**	6	0	0	76	10	8	91	21	19
Leptodora kindtii	6	30	2	2	5	0	6	16	1	15	24	4	14	25	3
Calanoida (Copepoda)	56	14	8	90	38	**34**	35	14	5	9	1	0	100	33	**33**
Cyclopoida (Copepoda)	50	9	5	90	38	**34**	41	15	6	12	3	0	95	36	**34**
Chironomidae	6	2	0	0	0	0	59	44	**26**	59	48	**28**	14	6	1
Plecoptera	0	0	0	0	0	0	0	0	0	9	100	9	0	0	0
Insecta	39	34	13	0	0	0	82	42	**35**	18	16	3	14	9	1
Algae	0	0	0	0	0	0	6	0	0	100	44	**44**	100	56	**56**

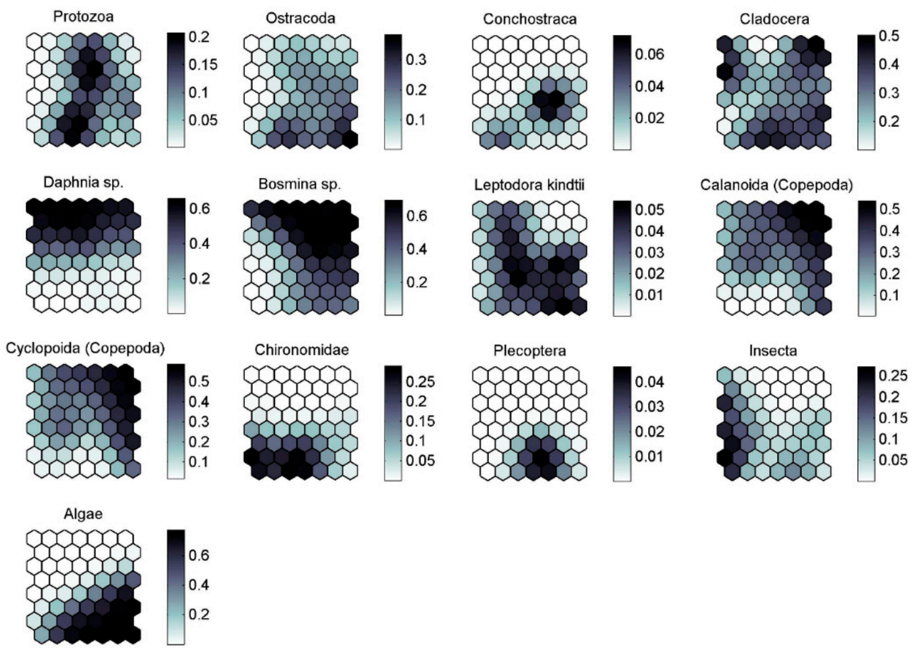

Figure 4. Patterns of distribution for 13 food categories present in the roach diet. The shading is adjusted separately for each food category, with strong correlation to IndVal index values indicated by black shading. A reduction of shading corresponds to a decrease in IndVal index values.

4. Discussion

Dietary analyses have been used for decades in biological and ecological studies on various fish species and in assessing the impact of humans on the aquatic environment [61]. In addition, information from dietary studies, often based on stomach contents, is very useful for a better understanding of trophic pathways, especially when comparing different species or systems [62]. In this study, we analyzed the diet of juvenile roach in five reservoirs with different trophic statuses in Serbia. Roach play an important role, especially in eutrophic lakes, as they are able to exploit almost any type of food source, especially in situations with strong competition [63,64]. Our results indicated that, although the general food categories consumed by roach were similar, roach, which were abundant in all studied reservoirs, especially in the juvenile stage, had their own predominant prey items in different reservoirs.

The roach from the oligotrophic Vlasina Reservoir have the least varied prey, as do the roach from the Vrutci Reservoir, but the roach from the Vlasina Reservoir, unlike the specimens from the other reservoirs, have a fairly similar representation of prey. In the other reservoirs, which are meso- to hypereutrophic, the prey is more diverse, but some of them clearly stand out compared to the others. Cladocera were present in every digestive tract of roach from the Šumarice Reservoir. On the other hand, *Daphnia* sp. and *Bosmina* sp. were present in every digestive tract of roach from the Gruža Reservoir. Although cladocerans were predominant prey, their consumption exceeded that of copepods (Calanoida and Cyclopoida). This observation is supported by Zapletal et al. [65], who noted a lower consumption of copepods by roach, and Kornijów et al. [66], who reported that copepods were absent from the roach diet. The infrequent presence of copepods in the diets of planktivorous fishes like roach is attributed to their ability to evade predators [67,68]. Contrary to our findings, the large cladoceran *Leptodora kindtii* is recognized as a significant component in the roach diet [69,70]. We identified *L. kindtii* in the digestive tracts of roach from two out of the five studied reservoirs (Gruža and Gazivode), with a relatively low frequency of occurrence values. The reason for this could be that it is difficult for visually oriented fish such as roach to catch this species due to its transparency and highly reduced body, which serve as a predator defense strategy [71].

Juvenile roach primarily feed on zooplankton [67,70,72,73]. Our findings support this statement, with the exception of the roach from the oligotrophic Vlasina Reservoir. In the reservoir Vlasina, the highest values for the frequency of occurrence of Insecta and detritus were determined, and a relatively high value for the frequency of occurrence was also found for Chironomidae. In this reservoir, juvenile European perch feed on fish [36], and this could be the reason why the roach retreat to the littoral zone, where they feed on detritus and macroinvertebrates. The protein content of detritus fluctuates, and species must therefore balance their energy requirements by feeding on macroinvertebrates [74]. As per Kornijów et al. [66], only a minority of roach incorporate macroinvertebrates into their diet, despite the substantial biomass of these prey items. However, studies by Bogacka-Kapusta and Kapusta [75] and Adamczuk and Mieczan [76] found that roach in meso-eutrophic reservoirs do include chironomids in their diet. The significance of detritus in the roach diet was emphasized by Zapletal et al. [65] and Kornijów et al. [66]. Detritus, with its higher nutritional value compared to algae, stands out as a crucial food source [77]. Matěna [78,79] suggested that the diet of the roach undergoes changes with the ontogenetic stage, with the proportion of macrophytes and detritus increasing as the fish age. In contrast, Lyagina [80] and Vøllestad [81] proposed that a high detritus content in the roach diet indicates the limited availability of animal prey. According to Brandl [82], roach may consume detritus even before the rise in the abundance of cladocerans.

The Gazivode and Šumarice reservoirs are the only two reservoirs where roach have been identified as algae eaters. At the same time, these are reservoirs in which *Daphnia* sp. are only very rarely (Gazivode Reservoir) or not at all (Šumarice Reservoir) in the diet of roach. On the other hand, the frequency of occurrence of *Bosmina* sp. in the digestive tract of roach is high in both reservoirs. The only one reservoir in which *Bosmina* sp. was not detected in this study in the digestive tract is the Vlasina Reservoir. Although *Bosmina* sp. occurs in the Vlasina Reservoir, its abundance is very low [83]. This speaks in favor of its oligotrophy, as Vodopich and Cowell [84] found that small cladocerans (e.g., *Bosmina* sp.) are abundant under eutrophic conditions, and in our case in the hypereutrophic Šumarice Reservoir.

Since the roach is a successful generalist in European freshwater habitats [85], it is reasonable to assume that the flexible feeding behavior gives it decisive advantages over future-induced changes in the structure of the prey community, as trophic generalists can adapt their diet to the food supply, while trophic specialists are usually dependent on a specific prey [86]. Omnivorous cyprinids such as roach have a more flexible diet and can manage their entire life cycle based on a variety of food resources, including zooplankton, macroinvertebrates and living or dead plant material [87], which was also observed in our study. They show a feeding plasticity by adapting their diet to the prey categories available in certain ecosystems [22]. According to Costello's graph, the algae were of great importance in feeding for the roach from Gazivode and Šumarice reservoirs, as they are closest to the upper right corner of the graph. The rare prey can also be found in the lower left corner [47], in specimens from all reservoirs except the Vlasina Reservoir. The rare prey for the roach from the Gruža Reservoir is *L. kindtii*, for the roach from the Šumarice and Vrutci reservoirs it is Insecta and for the specimens from the Gazivode Reservoir the rare prey includes Plecoptera, Chironomidae and *Daphnia* sp.

Due to the varying levels of digestion, the data pertaining to the contents of the digestive tract may include only general food categories (i.e., higher taxonomic levels) or may be identified to the most specific taxonomic level possible. Opting to standardize the data, whether representing the digestive tract content "coarsely" or in detail, poses the risk of losing information about a substantial portion of the digestive tract content [88,89] and may introduce methodological errors [59]. Consequently, self-organizing maps prove beneficial in the analysis of fish feeding [73], as they adeptly handle nonlinear variables interconnected in complex ways, whether exhibiting normal or skewed distributions [57,59]. Despite their prevalence in biocenology, self-organizing maps and the IndVal index have been utilized sparingly in ecological studies focused on fish feeding [58,59,73]. As shown on the SOM map, roach were divided into five clusters based on the predominant prey in the diet. The specimens in cluster A fed on *Daphnia* sp., which resulted in a significant IndVal. The specimens in cluster B fed most frequently on the cladocerans *Bosmina* sp. and *Daphnia* sp. and copepods Calanoida and Cyclopoida throughout the study, as evidenced by significant IndVal values. All roach from cluster B had *Daphnia* sp. and *Bosmina* sp. in their digestive tracts. It can also be seen that no specimens in cluster B consumed macroinvertebrates. The roach, which were assigned to cluster C, concentrated on Chironomidae and Insecta. Chironomidae and algae played an important role in the diet of roach from cluster D, as indicated by the significant IndVals. The most important prey of the specimens from cluster E were Ostracoda, Calanoida, Cyclopoida and algae. In addition, all specimens from groups D and E had algae in their digestive tracts.

Self-organizing maps are particularly suitable for handling complex and nonlinear ecological data, especially when dealing with large datasets, as in our case [51,90,91]. In comparison to various linear ordination methods, self-organizing maps offer a more comprehensive understanding of community structure in ecological studies [92]. As highlighted by Dukowska et al. [59,60], presenting dietary analysis in this manner enhances the reliability of the obtained data. This is crucial, given that certain food categories were utilized less frequently or were only found in specific specimens. By representing fish diet in this manner, a clearer depiction of trophic relationships within and between species in the studied reservoir is achieved.

Radenković et al. [73] observed that employing self-organizing maps in the analysis of fish feeding yields a more comprehensive understanding of fish feeding habits, offering insight into both similarities and differences among them. This is attributed to the fact that the greater the distance in the network, the more pronounced the distinction in the models assigned to the neurons. Since a neuron can encompass data from multiple samples (i.e., specimens), a high degree of dietary similarity is likely. Ulitimately, the combined use of self-organizing maps and the IndVal index allows for an efficient and time-saving analysis in identifying the contents of the digestive tract. This proves particularly valuable in the case of juveniles, where the process is complex and time-consuming.

Looking at the diet of the roach in the different reservoirs, one gets the impression that the roach in the oligotrophic Vlasina Reservoir feed differently than in other reservoirs whose trophic statuses are less favorable, although the prey diversity was more or less similar. The reason for this could lie in the morphological characteristics of the reservoir because, compared to other reservoirs, it has a larger surface area (several tens of times larger than the Šumarice Reservoir) and lies at the highest altitude. The results of this study showed that the roach from the Vlasina Reservoir consumed *Daphnia* sp. and not *Bosmina* sp. The IndVal also recognized *Daphnia* sp. as significant prey for specimens from cluster A, where half of all samples originated from the Vlasina Reservoir. Indeed, large cladocerans of the genus *Daphnia* are effective phytoplankton filters and play an important role in maintaining water quality by limiting excessive growth of the phytoplankton community [93]. In addition, the IndVal showed that Chironomidae and Insecta were important prey for specimens from cluster C, with most specimens from the Vlasina Reservoir. This is important because roach in this reservoir retreat to the littoral zone and thus, according to Persson et al. [94] and Lammens [95], the population of *Daphnia* sp. can recover, which is important because *Daphnia* sp. play a significant role in maintaining water transparency in numerous reservoirs [96,97].

Intuitively, changes in the diet of fish in the wake of environmental changes can be explained by the fact that new environmental conditions lead to changes in prey communities, which in turn lead to changes in the diet of the fish and to niche variations from bottom to top [86]. Moreover, the number of trophic species, trophic links and the length of the food chain decline with eutrophication [98]. Even though piscivorous fish species were present in all analyzed reservoirs, piscivorous fish species were present only in the Vlasina Reservoir and piscivorous fish species were dominant in abundance compared to the planktivorous and benthivorous fish [36].

5. Conclusions

A better understanding of the problems that occur in aquatic ecosystems is crucial for the conservation of both fish and ecosystems, especially in light of future global environmental changes. It is very important to find the right approach, and research into fish feeding as the last link in the food chain of aquatic ecosystems is one approach. The method of fish feeding analysis presented in this paper is effective and, as already mentioned, time-saving. Due to the different trophic statuses of the reservoirs selected for this study, integrating these results with those already published is essential for formulating effective conservation and management strategies for both the species and the reservoirs.

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