

## Seasonal feeding habits and ontogenetic diet shift of black bullhead (*Ameiurus melas*) in Lake Sava (Serbia)

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**Received:** September 9, 2021; **Revised:** October 8, 2021; **Accepted:** October 11, 2021; **Published online:** November 3, 2021

**Abstract:** The black bullhead is an invasive species that forms dense populations and has a negative impact on the native ichthyofauna. Recent studies indicate that it is one of the most dominant invasive fish species in Serbian waters. Feeding habits based on stomach contents of individuals caught in Lake Sava were analyzed as a first step towards evaluating its possible negative impact on the native fish fauna of the lake. The following indices for diet analysis were applied: vacuity index, frequency of occurrence, numerical abundance and prominence value. Trophic niche breadth and seasonal trophic diversity were calculated using the Shannon's index. The diet spectrum comprised 16 different prey categories from five groups: Mollusca, Crustacea, Insecta, Teleostei and plants. Fish were the main prey in all seasons, followed by aquatic invertebrates. Plant material and terrestrial insects were used as food in relatively small quantities. Predation on fish eggs was also detected. Our research confirmed that this species is an opportunistic generalist. An ontogenetic diet shift was also detected. The lowest value of trophic diversity was found in age 1+ individuals ( $H=1.39$ ), while the highest was found in age 3+ individuals ( $H=2.00$ ). The widest niche breadth was recorded in spring 2011 ( $H=2.12$ ).

**Keywords:** non-native fish; opportunistic generalist; trophic niche breadth; diet analysis

### INTRODUCTION

According to the International Union for Conservation of Nature (IUCN) [1], the impacts of non-indigenous invasive species are immense, insidious and usually irreversible, and can be compared to the loss and degradation of habitats on the global scale. The negative effects of introductions of non-native species reflect on the environment [2-4], diversity of native species, economic resources and human health [5,6]. On the global scale, freshwater ecosystems are inhabited by many non-indigenous fishes [7], which causes behavioral shifts in native species and completely rearranges food webs, leading to the potential extinction of entire faunas [8].

The black bullhead, *Ameiurus melas* (Rafinesque, 1820), which is native to North America, is one of the most abundant and successful non-native fish species in European freshwater ecosystems [9-11]. Possible

reasons for the success of black bullhead populations are its potential to survive in the sediment during short periods of drought, tolerance to pollution and low oxygen levels, its high flexibility in life history [12], the high degree of parental care and ability to erect dorsal and pectoral spines as a defense against predators [13]. Black bullhead is an opportunistic and nocturnal feeder [9]. Black bullhead occurrence can have a negative impact on native fish populations [14]. It poses a major problem for fishery management throughout Europe, which has led to many attempts by fishery managers to implement selective fishing of this species [10,15,16]. The first introduction of this species in Europe occurred in 1871 for the purpose of aquaculture [17]. The black bullhead is currently considered invasive in most European countries, where this species has established self-sustaining populations [18]. As regards the Balkan Peninsula and neighboring countries, the species was introduced as early as

1905, and this first introduction occurred in Croatia [19]. It was also recorded in Bosnia and Herzegovina [20], Hungary [21], Romania [22], and Slovenia [23]. In Bosnia and Herzegovina [20], Croatia [24,25] and Romania [22], it is considered an invasive species. The first record of the black bullhead in Serbian waters was in 2005 [26], and the species is now abundant in Serbian waters [27].

To date, there are still no published data on the feeding habits of the black bullhead from the Balkan Peninsula. The aim of the research was to evaluate the threat of the exotic black bullhead by direct predation on and competition with the native fish fauna, and to test for seasonal and ontogenetic dietary differences. The research was carried out in Lake Sava, which is one of the waterbodies with an established population of the black bullhead.

## MATERIALS AND METHODS

### Study area

With a capacity of  $4 \times 10^6 \text{ m}^3$ , Lake Sava is a small reservoir (86 ha). It was formed in 1967 as a water management, sport and recreational facility by damming the right-hand branch of the Sava River near the Ada Ciganlija river island. It is positioned at an altitude of 72 m, with an average depth of 4.5 m; for the most part, it is about 200 m wide, and its length is 4.2 km [28]. It is a mesotrophic to eutrophic marsh ecosystem in the process of ecological succession on the site of a former riverine ecosystem [29]. The lake has the status of a special fishing waterbody ("catch-and-release fishing"). It is located in the urban area of Belgrade (N –  $44^\circ 47' 17.1''$ , E –  $20^\circ 24' 49.3''$ ).

Among the 20 fish species currently present in the lake, based on the total biomass the most significant are the Gibel carp *Carassius gibelio* ( $115.61 \text{ kg km}^{-1}$ ), European catfish *Silurus glanis* ( $80.83 \text{ kg km}^{-1}$ ), European perch *Perca fluviatilis* ( $60.33 \text{ kg km}^{-1}$ ), common carp *Cyprinus carpio* ( $35.6 \text{ kg km}^{-1}$ ), common bream *Abramis brama* ( $26.7 \text{ kg km}^{-1}$ ), northern pike *Esox lucius* ( $21.52 \text{ kg km}^{-1}$ ), common rudd *Scardinius erythrophthalmus* ( $22.25 \text{ kg km}^{-1}$ ), and pikeperch *Sander lucioperca* ( $10.5 \text{ kg km}^{-1}$ ). The total biomass of the black bullhead population in the reservoir is  $15.51 \text{ kg km}^{-1}$  and its annual production is  $21.56 \text{ kg km}^{-1}$  [30].

### Sampling procedure

Field research was approved by the Ministry of Environment, Mining and Spatial Planning, which issued the appropriate fishing permit for scientific and research purposes. According to the Law on Protection and Sustainable Use of Fish Resources [31], unlimited fishing of all non-native species is allowed. Black bullhead samples were collected monthly from April 2011 to December 2012, using double fyke nets (8-mm mesh size). Nets were positioned in three rows, with five nets placed in each row at 3 m, 10 m, 18 m, 25 m and 35 m distance from the shore, and at depths of 1.5 m, 4 m, 5.5 m, 7.5 m and 8 m, respectively. The distance between the rows was 15 m. Nets were set for 24 h and checked daily for three days in a row. The nets were lifted, emptied and put back into the water every day at the same time. Individuals of other fish species caught during the research were returned unharmed to the lake and only their numbers were recorded. For species identification, we used the relevant keys [26,32]. Monthly results were combined into seasons as follows: April and May 2011 were merged into spring 2011, June, July and August 2011 into summer 2011, September, October and November 2011 into autumn 2011, June and August 2012 into summer 2012 and December 2012 represented winter 2012.

Sampled black bullheads were anaesthetized by administering clove oil in the water until they were determined to be unconscious (i.e., by the loss of reflexes) [33]. Total length (TL) and mass (W) were measured to the nearest 0.5 cm and 1 g. To determine the age of individuals, two methods were combined: length-frequency analysis [34,35] and inspection of otoliths. Sagittal otoliths were removed from the vestibular apparatus of 50 individuals and the age was assessed from growth rings using the method described by [36].

### Diet analysis

The stomach contents were analyzed under a dissecting microscope or macroscopically where possible. Prey categories were identified to the lowest possible taxonomic level and the number of consumed prey items was noted. In this analysis, we used the following indices [37]: frequency of occurrence (F), calculated as the number of stomachs containing a prey item

**Table 1.** Black bullhead diet in Lake Sava expressed as percentage frequency of occurrence (%F), numerical abundance (%Cn), and percentage prominence value (PV%) of the main prey types.

	Spring 2011 (n = 22)				Summer 2011 (n = 16)				Autumn 2011 (n = 18)				Summer 2012 (n = 5)				Autumn 2012 (n = 25)				Winter 2012 (n = 3)							
	F	Cn	PV	PV%	F	Cn	PV	PV%	F	Cn	PV	PV%	F	Cn	PV	PV%	F	Cn	PV	PV%	F	Cn	PV	PV%				
<b>Gastropoda</b>																												
<i>Esperiana daudebartii</i>	9.5	12.5	38.6	11.3	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
<i>Physella acuta</i>	4.8	4.2	9.1	2.7	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Undet. snails	14.3	12.5	47.2	13.8	7.1	5.6	14.9	3.4	/	/	/	/	40.0	20.0	126.5	21.2	/	/	/	/	/	/	/	/	/	/	/	/
<b>Malacostraca</b>																												
<i>Astacus astacus</i>	/	/	/	/	/	/	/	/	8.3	7.1	20.6	4.2	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
<b>Insecta</b>																												
Hemiptera	/	/	/	/	7.1	5.6	14.9	3.4	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Coleoptera - other	/	/	/	/	7.1	5.6	14.9	3.4	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Coleoptera - Staphylinidae	/	/	/	/	7.1	11.1	29.7	6.8	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Coleoptera - Coccinellidae	/	/	/	/	/	/	/	/	8.3	7.1	20.6	4.2	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Undet. insects	4.8	4.2	9.1	2.7	14.3	11.1	42.0	9.7	8.3	7.1	20.6	4.2	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
<b>Teleostei</b>																												
<b>Percidae</b>																												
<i>Perca fluviatilis</i>	14.3	20.8	78.7	23.0	21.4	16.7	77.2	17.8	41.7	35.7	230.5	46.7	40.0	30.0	189.7	31.9	50.0	50.0	353.6	50.0	/	/	/	/	/	/	/	/
<b>Centrarchidae</b>																												
<i>Lepomis gibbosus</i>	9.5	8.3	25.7	7.5	35.7	33.3	199.2	45.8	16.7	14.3	58.3	11.8	40.0	30.0	189.7	31.9	/	/	/	/	66.7	81.8	668.0	81.8	/	/	/	/
<b>Gobiidae</b>																												
<i>Neogobius fluviatilis</i>	/	/	/	/	/	/	/	/	25.0	28.6	142.9	28.9	20.0	10.0	44.7	7.5	/	/	/	/	66.7	18.2	148.5	18.2	/	/	/	/
<i>Babka gymnotrachelus</i>	9.5	8.3	25.7	7.5	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Undet. fish	9.5	8.3	25.7	7.5	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Fish eggs	4.8	4.2	9.1	2.7	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
<b>Plant residues</b>	19.0	16.7	72.7	21.3	14.3	11.1	42.0	9.7	8.3	7.1	20.6	4.2	/	/	/	/	50.0	50.0	353.6	50.0	/	/	/	/	/	/	/	/

divided by the total number of non-empty stomachs  $\times 100$ ; numerical abundance ( $C_n$ ) calculated as the number of individuals of a particular prey item in all stomachs divided by the total number of individuals of all prey categories in all stomachs  $\times 100$ , and the seasonal number of prey categories. Considering most of the prey was partially digested, its mass could not be accurately measured.

The most important prey categories were determined using the prominence value (PV) [38,39]:

$$PV = C_n \sqrt{F} \quad (1)$$

Percentage prominence value is expressed as:

$$PV\% = (PV / \sum PV) \times 100 \quad (2)$$

The average number of prey items per individual fish was calculated as the sum of individuals in all prey categories divided by the number of stomachs.

The trophic niche breadth was calculated according to Shannon's diversity index [40]:

$$H = -\sum_{i=1}^n (p_i)(\ln p_i) \quad (3)$$

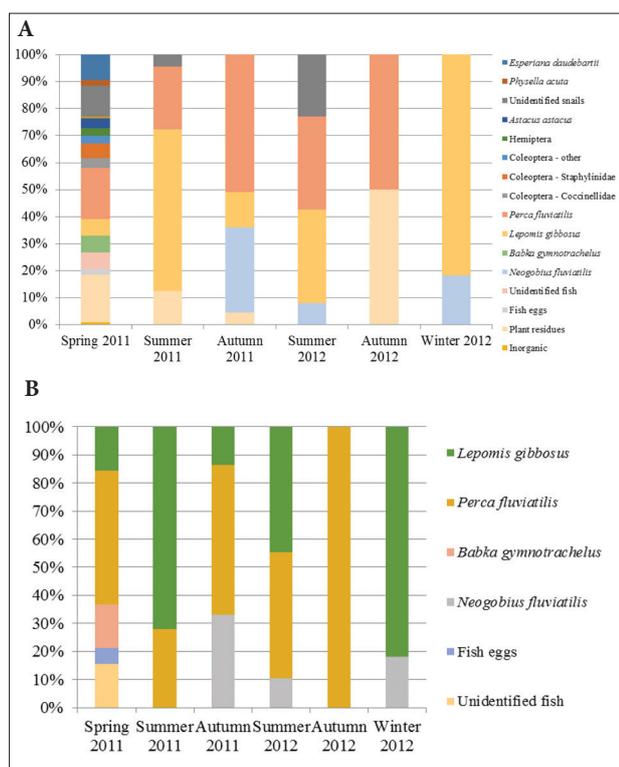
where  $p_i$  is the proportion of a particular prey category to the total number of prey categories. Trophic

diversity  $H$  is calculated using the PV value. The index is calculated for each month separately.

## RESULTS

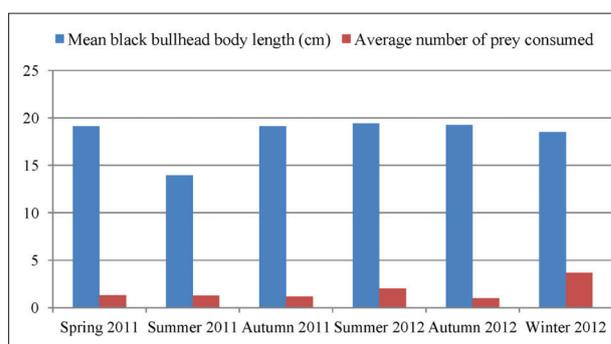
The stomach contents of 89 individuals were analyzed. The diet was comprised of 16 different prey types categorized into five basic groups: Mollusca, Crustacea, Insecta, Teleostei and plants (Table 1). Seasonal variation of the black bullhead diet spectrum was observed. The highest prey diversity was recorded during spring of 2011, followed by summer of 2011. Fish were the most common prey during all seasons (Fig. 1A). The term "inorganic" refers to stones and sand, with a wad of chewing gum found in one case. As regards the consumed fish species, *Lepomis gibbosus* was the most important prey item during the summers of 2011 and 2012 and winter of 2012, and *Perca fluviatilis* during spring and autumn of 2011 and summer and autumn of 2012 (Fig. 1B).

The mean body length of analyzed black bullhead individuals was similar during all seasons and ranged between 17.5 cm and 19.2 cm TL, with a predominance of individuals aged 2+ and 3+, except in the summer of 2011 when it was 14.1 cm TL, with a predominance of individuals aged 1+ (Table 2).

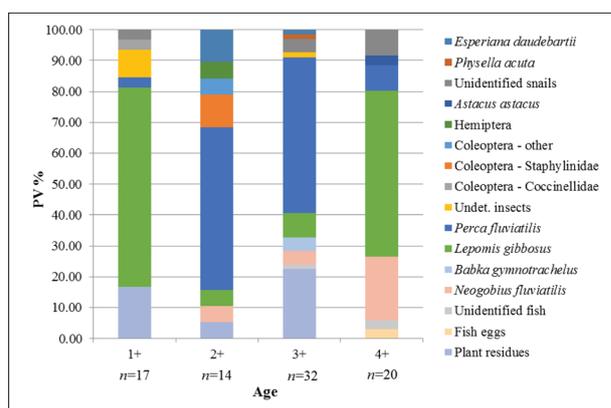


**Fig 1.** Seasonal variation of diet expressed as PV% for all prey types (A) and fish prey species (B).

The average number of prey items per stomach was also similar between seasons and ranged from 1 to 2; the only difference was recorded for the winter of 2012 when the average number of prey items was 3.7 (Fig. 2). Individuals aged 1+ fed mainly on invertebrates and plant material, but also on fish ( $H=1.39$ ). Individuals aged 2+ fed primarily on fish, but also on invertebrates and plant residues ( $H=1.87$ ). The most diverse diet had individuals aged 3+ ( $H=2.00$ ) that also fed on fish as well as on insects and plant material. Individuals aged 4+ mostly fed on fish and a very few invertebrates, while plant material was not detected ( $H=1.59$ ).



**Fig 2.** Seasonal predator mean length and average number of prey items.



**Fig 3.** Prey spectrum of different age classes presented as PV% for each prey category.

The chi-square test showed that there is a significant difference between the age classes as regards the cumulative PV% of consumed *P. fluviatilis* and *L. gibbosus* ( $\chi^2=160.34$ ,  $P<0.00001$ ) (Fig. 3). *P. fluviatilis* was the most dominant prey item for age classes 2+ (PV%=52.63) and 3+ (PV%=49.71), while *L. gibbosus* was the most dominant prey item for age classes 1+ (PV%=64.61) and 4+ (PV%=53.6).

**Table 2.** Number of individuals ( $n$ ), total body length (TL, cm), body weight (W, g) and age of sampled black bullhead in Lake Sava.

Sampling period		n	TL (cm)		W (g)		AGE	
			min-max	mean $\pm$ SD	min-max	mean $\pm$ SD	min-max	mode
2011	Spring	22	13.5 - 23.0	19.2 $\pm$ 2.1	33 - 184	112 $\pm$ 39	1+ - 4+	3+
	Summer	16	11.0 - 19.5	14.1 $\pm$ 2.4	16 - 120	44 $\pm$ 26	1+ - 3+	1+
	Autumn	18	10.0 - 25.0	17.8 $\pm$ 3.5	50 - 238	93 $\pm$ 50	1+ - 4+	2+
2012	Spring	5	15.5 - 21.5	19.0 $\pm$ 2.4	57 - 135	102 $\pm$ 37	2+ - 4+	3+
	Summer	25	15.0 - 22.0	18.7 $\pm$ 1.9	47 - 146	93 $\pm$ 31	2+ - 4+	3+
	Autumn	3	13.0 - 21.5	17.5 $\pm$ 4.3	31 - 131	80 $\pm$ 50	1+ - 4+	2+

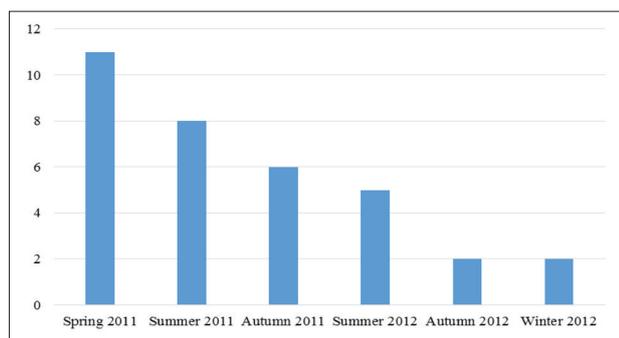


Fig 4. Seasonal number of prey categories.

The trophic niche breadth, as a measure of dietary diversity, was highest during the spring of 2011 ( $H=2.12\pm 0.09$ ). Slightly lower values were recorded during the summer of 2011 and the downward trend continued in autumn and winter 2011. During the summer of 2012, the trophic niche width was higher than during the rest of 2012, but lower than in the summer of 2011. The lowest value was recorded in the winter of 2012 ( $H=0.47\pm 0.1$ ). The seasonal number of prey categories was highest during the spring of 2011, followed by the summer of 2011. The lowest values were recorded during autumn and winter of 2012 (Fig. 4).

## DISCUSSION

There is some disagreement regarding the most appropriate sampling method for fish dietary analyses. A study on the Iberian Peninsula [41] showed that individuals of black bullhead captured by gillnets had empty stomachs, while those that were captured by electrofishing had prey in their stomachs, which suggests that this method does not cause regurgitation. Also, there are several factors that can affect the quantity and type of food found in fish stomachs, such as the diel cycle, seasonal and interannual changes in feeding ecology and prey availability, territorial behavior and different digestion rates [42]. The black bullheads in Lake Sava were collected with double fyke nets. It cannot be said with certainty how long the individuals were trapped in the nets because the nets were underwater for 24 h. It is possible that in this way some trapped fish had enough time to digest the whole prey.

Residues of plant material, terrestrial and aquatic invertebrates, as well as fish, both native and exotic,

were identified in black bullhead stomachs in several Iberian basins [41]. In Lake Sava, fish were the main prey in all seasons, as opposed to black bullhead on the Iberian Peninsula where aquatic macroinvertebrates (mainly Chironomidae) dominated its diet in all size-classes. The choice of fish prey is logical because of its higher energy content [43]. On the Iberian Peninsula there was no positive relationship between black bullhead size and fish prey size, probably indicating piscivory on dead or dying vulnerable fishes, as well as predation on smaller-sized active fishes. In Lake Sava, the second most frequent prey were aquatic invertebrates, which is in accordance with the results from the Iberian Peninsula [41], England [45] and Vojvodina [44] for both lentic and lotic habitats. Black bullhead individuals used plant material in relatively small quantities both in Lake Sava and the Iberian Peninsula, most likely because of its low-calorie value and poor absorption rate [46]. In other ictalurids, consumption of a larger amount of plant material occurs only when there is no other food available [47]. The black bullhead in Vojvodina also consumed plant residues and algae throughout the year, probably accidentally while consuming epiphytic fauna [44]. Nevertheless, in England, it largely consumed plant material, with a frequency of occurrence of 98% [45]. In some stomachs we also found residues of sand and stones, probably accidentally ingested during the search for prey in the benthic zone.

Studies of the only black bullhead wild population in the UK [45] as well as of populations from several Iberian basins [41] showed that chironomid larvae were the main prey item. Chironomid larvae were also a very important diet component of black bullhead individuals in Vojvodina, especially during May and June [44]. However, chironomid larvae are poorly represented in the macroinvertebrate fauna of Lake Sava where Oligochaeta predominate [48]. Very few terrestrial insects were found in black bullhead stomachs in Lake Sava, unlike in individuals from the Iberian Peninsula [41], probably due to the poorly developed coastal vegetation and the presence of sport and recreational facilities along the coast. The low consumption of terrestrial prey is likely the result of poorly developed riparian vegetation [41].

The predominance of fish in black bullhead diet in Lake Sava in all seasons suggests that the black bullhead

can negatively affect native fish fauna either through direct predation or competition for food. Considering the voraciousness and aggressive behavior of the black bullhead, it is a potential competitor for native fish species that feed on a similar prey spectrum [41]. There are several ways in which black bullhead could displace native fish to suboptimal food resources, by preying on the same species it reduces the amount of available prey [49], it can increase water turbidity [50] and impede the visual efficiency of other fish [51,52]. Considering its high local abundance, the behavior of black bullhead can affect both feeding patterns of predatory species and the anti-predator behavior of native prey [49]. The black bullhead in Lake Sava fed on both native (European perch) and non-native species (pumpkinseed, gobies). Pumpkinseed were the most important category of prey during the sampling period, along with European perch. Also, the results from the spring of 2011 indicate that black bullhead fed on fish eggs as well. Rapid digestion is the probable reason why there were not more fish eggs present in the sampled black bullhead stomachs. Predation on fish eggs is often underestimated because of the abovementioned reason, yet this occurrence is highly significant because it has strong effects even at low predation rates [53]. On the Iberian Peninsula, however, only 10% of black bullhead individuals fed on fish, revealed by the very low abundance of this prey item (<2%) in black bullhead stomachs [41]. In the Ečka fishpond in Vojvodina, the fish appear as prey throughout the year, more in colder and less in warmer months [44]. Black bullhead individuals in England also fed on fish with a frequency of occurrence of 30% [45]. The diet analysis of a related exotic species, the channel catfish *Ictalurus punctatus* in the River Arno (central Italy) showed that detritus, algae and phytoplankton were predominant in immature individuals [54]. On the other hand, in larger fish ( $\geq 30$  cm TL), two invasive exotic species, the topmouth gudgeon *Pseudorasbora parva* and the red swamp crayfish *Procambarus clarkii*, were predominant prey items [54].

An ontogenetic diet shift is a common phenomenon that occurs in many freshwater fish [53,55,56], including the black bullhead, with juveniles feeding on insect larvae, leeches and crustaceans, and adults on various bivalves, snails, chironomid larvae, plant material and fish [16,41]. Changes occur in terms of food composition, as well as in the size of prey [9]. These ontogenetic diet differences could also be the result of

an ontogenetic shift in habitat use since smaller fish are more pelagic and adults are more benthic [10]. The research in Lake Sava also showed that black bullhead changes its diet with age. Smaller and younger individuals feed on small and soft-bodied prey as well as on plant residues. As they grow, morphological changes allow them to capture and consume larger prey, such as fish and larger crustaceans, while smaller invertebrates and plant residue become rare. The results on the Iberian Peninsula have also shown that variation in diet occurs during the ontogenetic development of black bullheads [41]. As already mentioned, chironomid larvae were the dominant prey regardless of age classes and sites, however, an ontogenetic shift was present as regards other (secondary) prey items, such as microcrustaceans after which consumption decreased as the fish grew larger and were replaced by larger prey, such as mayflies, caddisflies, oligochaetes, terrestrial prey and fishes, depending on the sampling location. Results from Vojvodina also confirmed that black bullhead diet changes with the age of individuals. Juveniles mostly fed on bottom fauna, primarily chironomids, followed by cladocerans, copepods, ostracods, plants and insects, while adults fed mostly on fish [44]. An ontogenetic shift was also detected in a related ictalurid species, the channel catfish in the River Arno, where diet composition significantly varied among size classes [54].

Trophic diversity also varies in relation to age and length of the individuals, with the lowest diversity detected in the diet of the largest black bullheads and the highest in medium-size individuals [41]. The research in Lake Sava confirms this conclusion since 3+ individuals had the most diverse diet. A possible explanation for this phenomenon lies in the fact that as fish grow larger, they capture and ingest a wider range of prey items and therefore trophic diversity can increase. However, beyond a specific size, individuals either cannot effectively capture smaller prey, or such prey is no longer energy-efficient [41]. This hypothesis should be taken with caution because during the research in Lake Sava we did not catch enough large individuals to fully confirm it. Considering that in 2012 the black bullhead population in Lake Sava was in the recovery phase after a mass mortality event [57], this could be the reason for the smaller niche width in 2012. In that period, especially during the summer, juveniles dominated, having a less diverse diet spectrum.

After more than 40 years of this species' presence in Serbia, the only previous study on black bullhead feeding habits in this area was conducted in Vojvodina in the Danube River, the Danube-Tisza-Danube canal and in the Ečka fishpond [44]. The present work is the first study of its type in a lake ecosystem in Serbia. The results of diet analysis of the black bullhead individuals from Lake Sava indicate that this species is a generalist and opportunist.

**Funding:** This study was supported by the Ministry of Education, Science, and Technological Development of the Republic of Serbia, Project No. TR37009 and Project No. OI173045.

**Acknowledgements:** The authors express their gratitude to the public company "Ada Ciganlija" for providing logistic support during the field work.

**Author contributions:** Milica Jaćimović performed the field work, performed the statistical and lab analysis, interpreted the data, analyzed the results and prepared the manuscript and illustrations; Jasmina Krpo-Ćetković was responsible for the study design, conducted the statistical analysis, interpreted the data, analyzed the results and prepared the manuscript; Stefan Skorić performed the field work and lab analysis and prepared the manuscript and illustrations; Marija Smederevac-Lalić performed the lab analysis and prepared the manuscript and illustrations; Aleksandar Hegediš performed the lab analysis and prepared the manuscript.

**Conflict of interest disclosure:** All authors confirm no actual or potential conflicts of interest, including any financial, personal or other relationships with individuals or organizations.

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