




Ecological and morphological traits of the invasive pumpkinseed (*Lepomis gibbosus*) in Serbian lowland reservoirs

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Abstract: This study investigates the ecological and morphological traits of invasive pumpkinseed (*Lepomis gibbosus*) in five lowland reservoirs in Serbia. A total of 434 specimens were collected in October 2020 and analyzed for population structure and growth parameters, body shape, condition, and diet. The population consisted of seven age classes, with three distinct age structures identified across the analyzed reservoirs. The highest growth increment occurred during the first year of life. Compared to other European populations, this cohort exhibited the slowest overall growth, characterized by the lowest k values. However, the specimens maintained a robust body condition ($K > 1$) and displayed positive allometric growth ($b > 3$). The autumn diet included 14 prey categories, with Chironomidae larvae being the most prominent. Mean dietary similarity between reservoirs was 75.7%, indicating significant dietary niche overlap. Niche breadth analysis showed greater dietary specialization in Topola and Kudreč 2, and lower specialization in Jatagan, where prey diversity was highest. These findings offer valuable insights into the adaptive potential and ecological impact of this invasive species and can support future management and monitoring strategies.

Keywords: age structure; growth; condition; body shape; diet

INTRODUCTION

Pumpkinseed, *Lepomis gibbosus* (Linnaeus, 1758), a species native to North America, was introduced to Europe in the 1880s as a pond and aquarium fish [1]. The species is a flexible generalist with high phenotypic plasticity, which enables it to alter its overall life-history traits [2], with populations shifting from an opportunistic to an equilibrium strategy during the course of invasion [3]. It inhabits almost every type of freshwater ecosystem (large rivers, lakes, ponds, canals, backwaters) [4] but is considerably less frequent in riverine environments [2]. This benthopelagic species can live up to eight years and typically begins spawning between one and three years of age [4]. It is considered an opportunistic feeder [2] with a diet that includes a broad

spectrum of prey, such as zooplankton [5], arthropods [6], insects [6], mollusks [5], and plant material [6]. In Europe, the negative effects of this species have been documented on macroinvertebrate fauna [7], native ichthyofauna [8], and native batrachofauna [9].

In Serbia, pumpkinseed is listed among 23 non-native fish species [10], with the first recorded specimen captured in the Tisza River during the 1930s [11]. Since then, it has spread across more than 50% of the territory of the Republic of Serbia [11]. It is regarded as an invasive species with negative impacts on native ecosystems [12], and its FISK (Fish Invasiveness Screening Kit) score categorizes it as a moderately high-risk species [13].

The main objectives of this research were to: (i) determine the population structure and growth parameters of the pumpkinseed, identifying potential differences between reservoir populations; (ii) analyze individual body shape across different reservoirs; (iii) assess fish condition through established body indices; and (iv) characterize the pumpkinseed diet and compare dietary composition between reservoirs.

MATERIALS AND METHODS

Ethics statement

The Ethics Committee for the Protection of the Welfare of Experimental Animals at the Institute for Biological Research “Siniša Stanković” – National Institute of the Republic of Serbia confirms (No. 01-1954) that ethics committee approval is not mandatory for fishing for scientific research purposes (Official Gazette of the RS, No. 41/2009, paragraph 9, article 2).

Sampling locations

Fish sampling was carried out from 2-13 October 2020, at five small eutrophic reservoirs in central Serbia (Supplementary Fig. S1). All sampled reservoirs are artificial and have similar physicochemical parameters (Supplementary Table S1). The reservoir areas range from 1.2 ha (Topola) to 18 ha (Markovačko Lake), and all are used for recreational fishing. Detailed information on the sampled reservoirs and their ichthyofauna is provided in Supplementary Table S2.

Fish sampling and sample preparation

Fish sampling was conducted using the electrofishing device Villager VGI2400 (230 V, 8.7 A, 2.0 kW). At each reservoir, the electrofishing method was performed from a boat for 30 min along coastal vegetation, covering a 100-m transect. The electrofishing device was operated using the same output settings at all locations. A total of 434 pumpkinseed was collected: 88 from Vlaški Do, 39 from Jatagan, 79 from Topola, 88 from Markovačko Lake, and 140 from Kudreč 2. Prior to dissection, fish were overdosed with 2-phenoxyethanol, and euthanasia was performed in accordance with ethical norms. For each individual, total length (TL, cm) and body weight (BW, g) were measured. Sex was

determined by macroscopic examination of the gonads; however, as sampling was conducted in the post-spawn period, only 143 individuals (32.9% of the total sample) had determinable sex, so further indices and statistical analyses were excluded. After dissection, the weight of fish without intestines (BW', g) was measured, and intestines were placed in 70% ethanol for subsequent diet analysis. For age analysis, scales were taken from between the lateral line and the dorsal fin. Readable scales (not damaged or regenerated) were cleaned, mounted on microscope slides, and photographed using a stereo microscope (BRESSER Advance ICD). Scales were read by three independent readers and re-read by the same readers after several months. In cases of differing interpretations, age was determined by the most experienced reader. Age was determined by counting scale annuli. Subsequent measurements of the total scale radius and the radii to each annulus were performed using ImageJ analysis software.

Growth parameters and length-weight relationship

Length back-calculation was performed using the Monastyrsky equation. The von Bertalanffy growth function (VBGF) was determined according to Beverton and Holt, with parameters estimated using the Walford finite difference plot. Growth curves were generated with the Growth II (Simply Growth) program (©PISCES Conservation Ltd, 1995-2025), which estimates parameters by non-linear regression using the Levenberg-Marquardt method. To test overall growth performance and address the correlation between growth parameters k and L_{∞} , the phi-prime index (φ') was calculated. The total length-body weight relationship was calculated, and the curve was generated with the Simply Growth program. The Supplementary Material contains all equations and references used for the growth calculations. Length back-calculation, von Bertalanffy growth parameters, and total length-body weight relationship were calculated for both the total pumpkinseed sample and samples from each analyzed reservoir.

Geometric morphometry

The external body shapes of 419 pumpkinseeds were quantified using geometric morphometrics. Digital images of the left side, fins extended, of each fish were taken with a ruler for calibrating against a known size

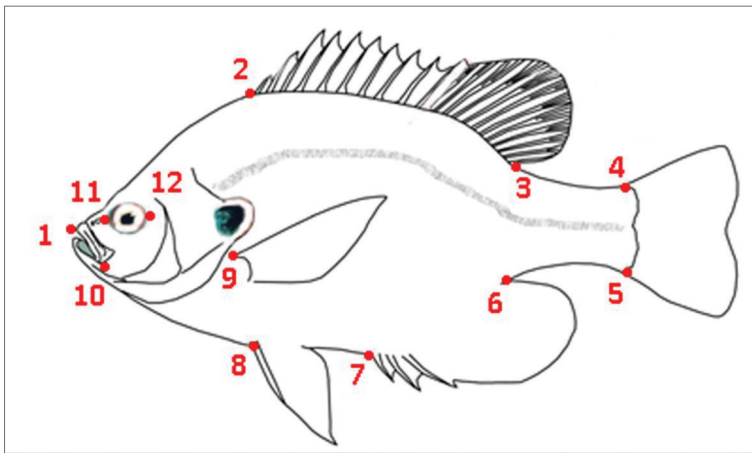


Fig. 1. Locations of 12 two-dimensional (2D) landmarks for geometric morphometric analysis of pumpkinseeds. 1 – tip of snout; 2 – anterior attachment of dorsal fin; 3 – termination of dorsal fin attachment; 4 – upper insertion of caudal fin; 5 – lower insertion of caudal fin; 6 – posterior terminal attachment of anal fin; 7 – anterior attachment of anal fin; 8 – ventral insertion of pelvic fin; 9 – dorsal insertion of pectoral fin; 10 – termination of upper jaw; 11 – anterior edge of orbit; 12 – posterior edge of orbit

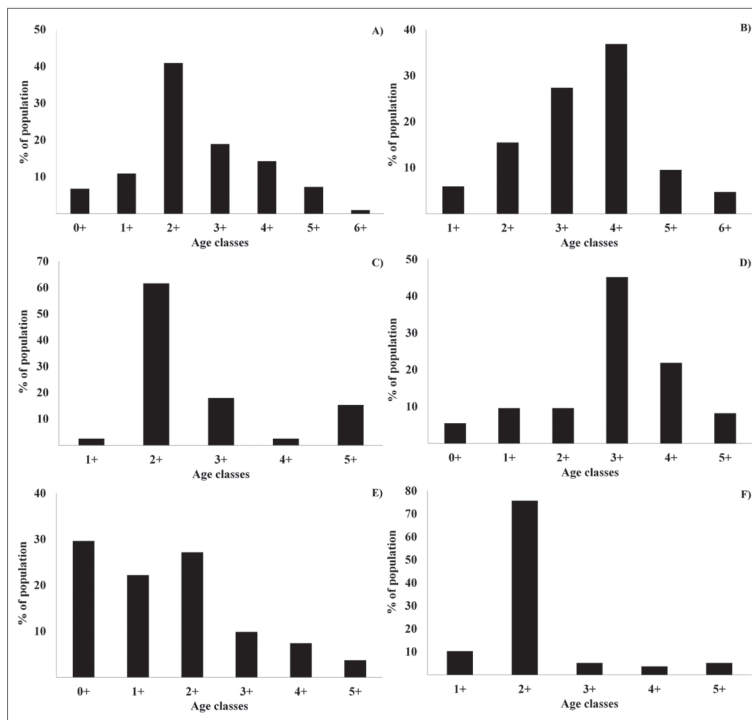


Fig. 2. Age classes of analyzed pumpkinseeds; A – total sample, B – Vlaški Do, C – Jatagan, D – Topola, E – Markovačko Lake, F – Kudreč 2

standard. The tpsDig2 program was used to digitize 12 homologous landmarks (Fig. 1). For shape analyses, generalized Procrustes analysis (GPA) was applied, which preserves all information about shape variability

among the individuals, removing only information unrelated to shape (orientation, position, and scale).

Body indices

Fulton's condition factor (K) was calculated using the equation presented in the Supplementary Material.

Diet analysis

Intestinal contents were examined under a binocular microscope (Zeiss Stemi 508, Germany), and prey remains were identified to the lowest possible taxon using identification keys [14-18]. The vacuity index (VI) was calculated according to Berg. Frequency of occurrence (F) was determined using the equation of Ahlbeck Bergendahl et al. Numerical abundance (N) was calculated as a percentage using the equation of Hyslop. The relative importance of each prey item was estimated as the prominence value. Trophic niche breadth was assessed using Shannon's and Levin's indices. For Levin's index, the standardised value was also calculated. Pielou's evenness was calculated using Pielou's equation by Pielou. Diet similarities between fish from different locations were analyzed using Renkonen's index. The Supplementary Material contains all equations and references used for the calculation of diet indices.

Statistical analysis

Data normality was tested using the Shapiro-Wilk test. When data did not follow a normal distribution, the Kruskal-Wallis H test was applied, followed by Mann-Whitney U post hoc tests. When data were normally distributed, independent samples t-tests or one-way ANOVA with Tukey post hoc tests were used. To determine the maximum variation in body shape among age classes, a principal component analysis (PCA) was performed. To evaluate

the relative variation in body shape among individuals, a Procrustes ANOVA grouped by age was applied. Data were analysed using MorphoJ software (version 1.08) [19]. Centroid size (CS), defined as the square root of the sum of squared distances between the center of the landmark configuration and each landmark, was used to analyze differences in fish size. Statistical analyses were performed using Microsoft Excel, IBM SPSS 22 Statistics, and Statistica software (ver. 8.0).

RESULTS

Population and growth parameters

The mean TL of the analyzed pumpkinseeds was 7.0 ± 1.9 cm, and the mean BW was 6.2 ± 5.9 g. Pumpkinseed individuals with the highest mean TL were from Vlaški Do and Topola. Fish from other sites formed distinct groups, with the lowest mean TL observed in fish from Markovačko Lake. The highest mean BW was recorded for fish sampled at Vlaški Do. Pumpkinseeds caught at Jatagan and Topola had similar BW, as did those from Markovačko Lake and Kudreč 2. Fish from the latter two reservoirs had the lowest values of mean BW values (Supplementary Table S3).

The pumpkinseed population across the sampled reservoirs comprised seven age classes (0+ to 6+), with the highest abundance observed in the 2+ age class (Fig. 2A). In each reservoir, local populations were classified into five or six age classes. The most abundant age classes were 4+ at Vlaški Do (Fig. 2B), 2+ at Jatagan (Fig. 2C), 3+ at Topola (Fig. 2D), and 2+ at Kudreč 2 (Fig. 2F). The population at Markovačko Lake was characterized by a similar percentage of age classes 0+-2+ (Fig. 2E), without a dominant class, unlike the other reservoirs.

The highest relative growth increment was recorded during the first year of life across all reservoirs (Supplementary Table S4A-F). The von Bertalanffy growth curve is presented in Supplementary Fig. S2, and the equation parameters, along with the estimated phi-prime growth performance index (ϕ'), are presented in Supplementary Table S5. Although growth parameters were similar across the sampled reservoirs, individuals from Markovačko Lake exhibited the lowest growth rate and the highest asymptotic length, as well

as the highest phi-prime growth performance index. The exponent (b) of the length-weight relationship exceeded 3.0, indicating positive allometric growth (Supplementary Fig. S3), which was consistent across all reservoir populations (Supplementary Table S5). As back-calculated lengths, von Bertalanffy growth parameters, and length-weight parameters were similar across reservoirs, the following discussion focuses on values obtained for the total sample.

Geometric morphometry

Procrustes ANOVA revealed significant differences in both centroid size ($F=45.82$, $P<0.0001$) and shape ($F=18.51$, $P<0.0001$) among individuals from different reservoirs. Significant differences in centroid size ($F=298.72$, $P<0.0001$) and shape ($F=13.52$, $P<0.0001$) were also detected among individuals of different age classes. CVA slightly but significantly separated specimens from different reservoirs. The first two canonical axes (CV1 and CV2) accounted for 78% of the observed variation (55.6% and 22.8%, respectively), while the third axis accounted for a further 14.7% (Fig. 3).

Body indices

Fulton's condition factor (K) exceeded 1 in fish from all reservoirs; however, pumpkinseeds from Vlaški Do had a significantly higher K value than those from Jatagan, Topola, and Kudreč 2 (Supplementary Table S6).

Diet analysis

The vacuity index for the entire sample was 26.1%. The highest vacuity index was observed in fish sampled from Jatagan (41.0%), and the lowest in fish from Vlaški Do (18.2%). Fourteen prey types were identified in the diet of pumpkinseeds, four of which (Chironomidae larvae, Trichoptera, Ephemeroptera, and Odonata) were present across all sampling locations. Fish from Topola had the greatest dietary diversity (14 prey types), while those from Markovačko Lake had the lowest (7 prey types). Chironomidae larvae were the most dominant prey type in the pumpkinseed diet, followed by Trichoptera (Table 1, Supplementary Table S7). Few significant differences in diet composition were detected among locations, with no discernible pattern (Supplementary Tables S8-19).

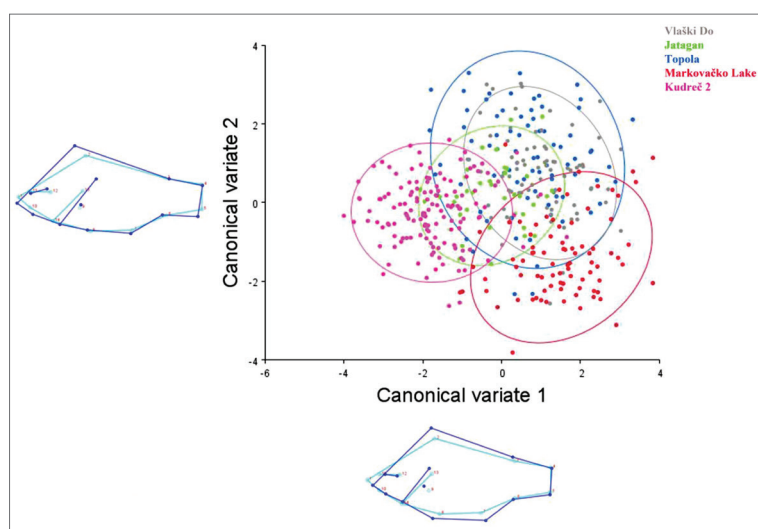


Fig. 3. Scatterplot of canonical variate analysis, with morphometric overlap in pumpkinseed body shape along the first two canonical variates (CV1 and CV2)

Table 1. Prey in pumpkinseed diet expressed as frequency of occurrence (%F), numerical abundance (%N), prominence value (PV), and percentage prominence value (%PV)

Prey type	%F	%N	PV	%PV
Chironomidae larvae	68.4	54.8	453.7	67.51
Chironomidae pupae	2.2	1.1	1.6	0.24
Ceratopogonidae	0.3	0.2	0.1	0.02
Diptera	0.9	0.2	0.2	0.03
Trichoptera	36.9	27.1	164.4	24.46
Ephemeroptera	16.9	9.4	38.5	5.72
Odonata	4.1	1.3	2.5	0.38
Heteroptera	0.6	0.2	0.2	0.03
Coleoptera	0.9	0.2	0.2	0.03
Gammaridae	2.8	0.9	1.6	0.24
Unidentified Mollusca	6.9	2.2	5.8	0.86
Bivalvia	1.3	0.7	0.8	0.12
Gastropoda	1.9	0.7	1.0	0.14
Terrestrial insects	2.8	0.9	1.5	0.22

Based on Renkonen's index, mean dietary similarity among pumpkinseeds from different reservoirs was 75.7%, ranging from 60.6% (Vlaški Do and Topola) to 86.9% (Topola and Kudreč 2). In terms of standardized niche breadth, similar values were observed for pumpkinseeds from Vlaški Do, Jatagan, and Markovačko Lake. Regarding prey diversity in the diet, fish from Vlaški Do and Jatagan had a similar evenness index (Supplementary Table S20).

DISCUSSION

High abundances of non-native species may pose an ecological problem, as they can act as a filter excluding native species, especially in reservoirs, where non-native species tend to show greater habitat preference [20] and competitive aggression for food and territory [21]. Artificially created waterbodies can support abundant pumpkinseed populations [7], which may have adverse effects on local native fauna. Copp et al. [8] reported that the presence of pumpkinseed can reduce the dietary niche breadth and trophic position of native fish species, as well as negatively affect their somatic growth rates. Almeida et al. [21] reported that the feeding habits of pumpkinseed, together with competition for habitat and spawning sites, could have a negative impact on populations of native fish species. Pumpkinseed has been shown to drastically decrease macroinvertebrate abundance by up to 83% [7]. Exclusion of native species could potentially include some amphibian species, such as *Hyla arborea* (European tree frog) and the Pelophylax (water frog) complex [9].

Douligeri et al. [22] reported that pumpkinseed populations in Europe comprise seven age classes, consistent with the findings observed in Serbian reservoirs. Pumpkinseed is a species whose population growth rates are most sensitive to changes in juvenile survival [23]. In the present study, three types of age structure were identified. In Vlaški Do and Topola, the population structure was skewed towards older individuals (age 3+ and above). High densities of older fish and good representation of all age classes suggest high survival rates and a limited influence of 0+ abundance on the fecundity of older individuals [24]. In Markovačko Lake, the population structure was skewed toward younger individuals (ages 2+ and below). A high proportion of the population belonging to 0+ and 1+ age groups could be related to a higher natural mortality rate and food resources [25]. In Jatagan and Kudreč 2, there is a

dominant age class (individuals of age 2+ in both cases). While in Jatagan, this could be the result of a smaller sample size compared to other analyzed reservoirs, in Kudreč 2, the predominant abundance of one age class could indicate a cycling population. This occurs when the abundance of the 0+ age class influences the fecundity of the next generation [24], and has been documented in percid species (e.g., European perch, *Perca fluviatilis*) in reservoirs of Central and Eastern Europe [26].

Pumpkinseeds from Serbian reservoirs were smaller in size in their first year of life compared to those in other European studies [1,27,28,29]. The first year of life appears to be critical, as subsequent annual growth increments were similar to or greater than those reported in most European studies [1,27,28], with the exception of Cucherousset et al. [29]. Due to these smaller first-year sizes, back-calculated lengths in subsequent years were generally below European means [1,27,28,29]. Variation in growth rates among non-native European pumpkinseed populations, particularly during juvenile life stages, may reflect differences in environmental conditions [2], while smaller body size could result from strong intraspecific competition for food and reduced predation pressure [1]. Uzunova et al. [30] observed that the presence of predators significantly influences the size (mean length) of pumpkinseed. In our study, predatory species were present in all analyzed reservoirs, but there was no discernible pattern between the abundance of predators and mean pumpkinseed length. Fish from Markovačko Lake had the lowest mean length in an assemblage with the lowest predator abundance. Pumpkinseed from Topola and Kudreč 2 showed a significant difference in mean length but were part of assemblages with similar abundances of predatory fish. It should be noted that predatory fish abundance was also assessed by electrofishing, which may underestimate their true numbers. The absence of consistent patterns may reflect differences in environmental and anthropogenic pressures across regions, which in turn influence interactions between native and non-native fish species [31]. In its native range, a higher abundance of pumpkinseed in the ecosystem can lead to smaller body size in the population due to lower prey availability [32]. Results from the Serbian reservoirs do not support this observation. Populations with the largest mean body size were from Vlaški Do and Topola, which had different pumpkinseed abundances.

Pumpkinseed populations examined in several European studies [1,22,25,27,28,33] are characterized by higher k and correspondingly lower L_{∞} values than populations from Serbian reservoirs, while ϕ' values were either similar to [1,22] or higher [25,33] than European findings. Pumpkinseed populations in Serbia are consistent with the findings of Copp et al. [34], who reported that slower-growing populations with small body size and reduced fecundity undergo an ontogenetic shift from a specialist (dispersive) phenotype to a generalist (maintenance) phenotype. Among populations from different reservoirs, pumpkinseeds from Markovačko Lake exhibited the slowest growth, which may be partly attributable to the high abundance of the youngest age classes in that population. Uzunova et al. [28] stated that a high abundance of younger cohorts leads to increased intraspecific competition, resulting in slower growth. Different sample sizes of the populations, however, do not appear to influence life-history traits [35]. Villeneuve et al. [27] reported that lower summer temperatures and resource limitations are the main drivers of slow growth and small adult body size. As sampling was conducted in autumn, further studies are needed to evaluate the effects of seasonal and environmental factors on pumpkinseed ecological traits. Additionally, results from Serbian reservoirs should be interpreted with caution, as L_{∞} values exceed 20% of the maximum mean back-calculated total length [1], which may render these estimates unreliable for comparative purposes. Furthermore, Copp et al. [1] proposed that size at age is a better parameter than von Bertalanffy growth parameters when comparing different populations. Most European studies [22,25,27,28,29] have reported values of b exceeding 3, indicating positive allometric growth and potentially favourable habitat conditions, as Treer et al. [36] suggested that the length-weight relationship may reflect the environmental conditions of the inhabited waterbody. All pumpkinseed populations in the present study likewise exhibited positive allometric growth.

Sex differences were not further analyzed in this research, as only one-third of the sample had discernible gonads. This could affect the results obtained. While there appears to be no difference in mortality rates between sexes [37], some authors [38] have found seasonal differences in somatic parameters (TL, BW, and K) between females and males. Furthermore, there

seems to exist an among-individual variability in growth and sexual maturation in males [39]. These small, “cuckolder” males allocate energy to gonad growth at the expense of somatic growth [39]. The onset and duration of the spawning period in Serbian reservoirs remain unknown, which may also influence growth, as [40] demonstrated that asynchronous maturation and spawning can result in variation in individual life histories. These gaps highlight the need for in-depth somatic analysis of both sexes and detailed characterisation of the spawning season.

Significant differences in both centroid size and body shape among reservoirs and age classes were identified by Procrustes ANOVA. Although the CVA shows statistically significant shape divergence, it is relatively modest due to substantial overlapping among reservoir groups. Similar patterns have been reported in lake- and stream-dwelling pumpkinseed populations in the Iberian Peninsula [41], which exhibited significant morphological differences but limited correspondence between morphometric traits and habitat-specific functional expectations. Additionally, our ANOVA results are consistent with findings from other Balkan reservoir populations of pumpkinseed [42], which reported considerable morphometric differences among sites, although not all populations were distinguishable, and differences were restricted to a subset of traits. Ontogenetic effects were particularly pronounced in the present study, with centroid size explaining a larger proportion of variation than reservoir, indicating that growth-related allometry is the dominant driver of morphological variation. This is consistent with patterns reported in introduced European pumpkinseed populations, where life-history traits and size-structured morphology differ among populations [29], as well as with ontogenetic shape differences between juvenile and adult pumpkinseed observed in both native and non-native ranges [43]. Pumpkinseed populations from the analyzed reservoirs exhibited detectable differences in body depth and head morphology, reflecting the species' phenotypic plasticity in response to environmental factors such as water temperature, prey availability, and habitat complexity [2,43]. However, given the overlap between groups, our results should be considered alongside those of [44], who reported no significant variation in body depth. In the present study, all individuals were sampled from the littoral zone, suggesting that

habitat microvariability is unlikely to fully explain the observed shape differences. Additionally, previous research has emphasized that statistical significance in morphological variation does not necessarily imply functional or ecological significance [45]. Potential differences in diet composition among reservoirs may partly contribute to the observed morphological variation. However, despite some variation in prey diversity, diets were broadly similar across reservoirs, consistent with the overlap in body shape. While dietary differences may influence subtle morphological variation, the ecological significance of reservoir-related shape differences remains modest, in line with previous studies on pumpkinseed morphology [41,43,44].

Condition indices, together with information on other life-history traits, can enhance understanding of species population ecology [46]. Fulton's condition factor can be used to assess fish energy reserves and health [47]. Several studies have reported overall good body condition in pumpkinseeds [3,22,25,27,35], also observed in populations from the analyzed Serbian reservoirs. Mozsár et al. [47] reported a positive relationship between fish body condition and total length. In the present research, the highest mean K value was indeed observed in the pumpkinseed population with the highest mean length (Vlaški Do). However, the population from Topola, which did not differ significantly in mean length from the Vlaški Do population, had a significantly lower condition factor. Klaar et al. [48] and Konečná et al. [35] suggested that pumpkinseed populations with higher body condition tend to occur at lower population densities with reduced intraspecific food competition. This could explain the significantly higher condition of fish from Vlaški Do compared to those from Jatagan, Topola, and Kudreč, as the abundance of pumpkinseeds in Vlaški Do is much lower than in the aforementioned reservoirs. Almeida et al. [49] observed that higher food availability could lead to differences in body condition. Although no clear pattern emerged in the statistical differences among dietary prey items, the significantly higher condition factor of pumpkinseeds from Vlaški Do compared to those from Jatagan, Markovačko Lake, and Kudreč 2 may reflect the greater prominence of Trichoptera and Ephemeroptera in their diet. These insects are considered high quality in fish diet due to their content of EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid), and n-6/n-3 polyunsaturated fatty acids [50].

The vacuity index is considered an inverse measure of feeding intensity [51]. This suggests that the pumpkinseed population from Vlaški Do has the highest feeding intensity, which could contribute to the higher growth coefficient k observed in this population. In all the reservoirs analyzed, the main prey items were larvae of non-biting midges (Chironomidae), caddisflies (Trichoptera), and mayflies (Ephemeroptera). It should be noted that these findings represent a seasonal (autumn) diet, which could change substantially at other times of the year. The prominence of Chironomidae larvae in the pumpkinseed diet may reflect several factors. They may represent the most readily available prey in the habitat, as van Kleef et al. [7] reported that pumpkinseeds feed on the most frequent and abundant prey, and Tomeček et al. [2] similarly noted that pumpkinseed diet is strongly correlated with macroinvertebrate abundance. Another reason could be the presence of European perch in all analyzed reservoirs. Fobert et al. [52] observed diet separation between pumpkinseed and European perch as a means to avoid competition, with pumpkinseed showing increased reliance on Chironomidae under such conditions. Additionally, even moderate abundances of cyprinid species appear to influence pumpkinseed diet composition [53]. Gastropods featured little in the autumn diet of pumpkinseed. Wolfram-Wais et al. [54] suggested that low representation of this prey type may reflect either the small body size of pumpkinseeds (limiting their ability to exploit this resource) or limited prey availability.

Renkonen's index is considered one of the most reliable measures of dietary similarity, as it is unaffected by sample size or size displacements [55]. In the analyzed reservoirs, even the lowest observed similarity in the autumn diet (60.6% between populations from Vlaški Do and Topola) is above the threshold of 60%, which is considered a biologically significant dietary niche overlap [56].

Species with standardised niche breadth closer to zero have a more specialized diet [57]. Based on the autumn niche breadth, the least specialized pumpkinseed populations were those from Vlaški Do, Jatagan, and Markovačko Lake, while the most specialized were from Topola and Kudreč 2. The prominence of chironomid larvae appears to be a key factor, as their PV% exceeded 70% in both Topola and Kudreč 2.

Populations from Vlaški Do and Jatagan exhibited the greatest dietary diversity based on the evenness index, while Kudreč 2 showed the lowest. Almeida et al. [49] found that Diptera larvae (including Chironomidae) were the most abundant prey in the pumpkinseed diet when electivity for this prey type was highest.

CONCLUSIONS

This study presents the first comprehensive analysis of pumpkinseed populations in Serbian reservoirs and one of the most detailed investigations in the Balkans. The pumpkinseed populations exhibited slow growth, positive allometric patterns, and good overall condition. Age structure varied among reservoirs, with three distinct patterns apparent, potentially reflecting differences in population dynamics. Morphometric analyses revealed significant shape variation across sites. The autumn diet consisted primarily of aquatic invertebrates, especially Chironomidae larvae, with high similarity and biologically relevant overlap among populations. Niche breadth varied among reservoirs, with signs of dietary specialization in certain populations. These findings highlight the adaptive flexibility of pumpkinseed in novel environments and emphasize its potential to affect local ecosystems at multiple trophic levels. As one of the most widely distributed invasive fish species in Europe, understanding its ecological traits is essential for predicting its spread and developing effective monitoring and management strategies under changing environmental conditions.

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Vesna Đikanović: Methodology, Writing – review and editing;
Katarina Jovičić: Methodology, Writing – review and editing;
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SUPPLEMENTARY MATERIAL

Information on sampled reservoirs

Vlaški Do (44°28'17" N, E, 20°56'21" E, 117 m a.s.l.) was created by damming the streams Trskar and Bobovik. The length of the reservoir is about 1.200 m and the width is 120 m, with a total area of about 14.5 ha. It is primarily a reservoir for irrigation, whose water level regime primarily depends on local precipitation.

Jatagan (44°36'11" N, 21°03'32" E, 69 m a.s.l.), with length of 400 m, width of 300 m, and a total area of about 10.5 ha, is one of 18 lakes that were created as a result of the exploitation of gravel on the coast of Velika Morava. Unlike Vlaški Do, Markovačko Lake, Topola, and Kudreč 2, which were created by damming smaller streams and primarily represent reservoirs for irrigation, whose water level regime primarily depends on local precipitation, the Jatagan reservoir are primarily filled with underground water, so their water level regime depends on the water level of Velika Morava River. The water in this reservoir is of high quality, because it is actually filtered through the soil before it appears in the pit itself. In the process of formation, they are overgrown with sedge on the coast (Phragmites reeds

and Typha rushes) and various floating and submerged aquatic vegetation (usually species from the genera Potamogeton, Ceratophyllum, Myriophyllum).

Topola (44°16'09" N, 20°41'30" E, 195 m a.s.l.) was created by damming the Rij stream. The length of the reservoir is 250 m and the width is 50 m, with a total area of about 1.2 ha. It is primarily a reservoir for recreation, whose water level regime primarily depends on local precipitation.

Markovačko Lake (44°23'20" N, 20°39'05" E, 146 m a.s.l.) is 1.000 m long, 180 m wide, 12 m deep, and covers an area of 18 ha. It was formed at 1969 by damming the Košarna River and serves for irrigation of the nearby agricultural combine and apple orchard. Its water level regime also depends on local precipitation.

Kudreč 2 (44°23'13" N, 20°59'01" E, 140 m a.s.l.) has length of 450 m, width of 120 m, and a surface area of 5.5 ha. This reservoir serves for irrigation, whose water level regime primarily depends on local precipitation.

Supplementary Table S1. Water temperature, pH, oxygen concentration, oxygen saturation, and conductivity measured at five reservoirs

Reservoir	Temperature (°C)	pH	O ₂ concentration (mg/L)	O ₂ saturation (%)	Conductivity (µS/cm)
Vlaški Do	22.5	8.3	12.5	99	470
Jatagan	19.8	8.0	13.9	115	397
Topola	21.1	8.5	12.8	104	445
Markovačko Lake	22.0	8.4	12.9	112	449
Kudreč 2	20.7	8.2	13.4	109	411

Supplementary Table S2. Fish assemblages obtained through electrofishing at five reservoirs, with abundances (A, %) and mass fraction (M, %)

Species / Reservoir	Vlaški Do		Jatagan		Topola		Markovačko Lake		Kudreč 2	
	A	M	A	M	A	M	A	M	A	M
Native										
Wels catfish, <i>Silurus glanis</i>							0.7	9.0	1.5	18.5
Northern pike, <i>Esox lucius</i>	0.9	3.1	2.1	23.5						
Common carp, <i>Cyprinus carpio</i>	0.6	17.0			1.2	7.5	3.8	9.0	0.5	13.3
Tench, <i>Tinca tinca</i>			2.1	1.1						
Roach, <i>Rutilus rutilus</i>	8.6	13.9	4.2	6.6	9.9	5.7	39.2	18.8	9.1	1.2
Rudd, <i>Scardinius erythrophthalmus</i>	8.3	8.9	4.2	7.0	0.6	0.1	0.3	0.5	6.6	1.5
Bleak, <i>Alburnus alburnus</i>					11.0	1.7	1.0	0.3		
Perch, <i>Perca fluviatilis</i>	4.7	1.5	12.6	24.6	2.9	5.3	1.7	2.4	2.5	0.4
Pikeperch, <i>Sander lucioperca</i>					4.1	51.1	1.0	22.6	3.0	50.8
Non-native										
Prussian carp, <i>Carassius gibelio</i>	10.9	31.7			4.7	15.2	7.2	26.8	5.1	25.1
Topmouth gudgeon, <i>Pseudorasbora parva</i>	9.4	1.0					10.0	3.0		
Black bullhead, <i>Ameiurus melas</i>	30.7	18.5	32.6	18.9	19.8	8.2	3.1	5.2	2.5	0.8
Monkey goby, <i>Neogobius fluviatilis</i>			1.1	0.3			2.4	0.4		
Pumpkinseed, <i>Lepomis gibbosus</i>	26.0	4.3	41.1	18.0	45.9	5.1	30.2	11.1	70.7	6.8

Equations

Length back-calculation [1]:

$$L_n = ((S_n/S)^b) \times TL$$

L_n is the total length at age n , S_n is scale radius at age n , S is the scale radius at time of catch, and b is the slope from the body length-scale radius regression.

Von Bertalanffy growth equation [2]:

$$L_t = L_\infty \times [1 - e^{(-k \times (t - t_0))}]$$

L_t is expected length at time t , L_∞ is the asymptotic length, k is the growth coefficient, and t_0 is the theoretical age at which L_t would be zero.

Phi-prime index [3]:

$$\phi' = \log_{10} k + 2 \times \log_{10} L_\infty$$

Total length-body weight relationship:

$$BW = a \times TL^b$$

a is the intercept and b is the slope of the length-weight regression.

Fulton's condition factor [4]:

$$K = BW' \times TL^{-3} \times 100$$

Vacuity index [5]:

$$\%VI = \frac{N_{ei}}{N_t} \times 100$$

N_{ei} is number of fish with empty intestines and N_t is the total number of analyzed fish.

Frequency of occurrence [6]:

$$\%F = \frac{N_{ix}}{N_{it}} \times 100$$

N_{ix} is number of intestines with prey item x and N_{it} is total number of intestines with any prey item.

Percentage numerical abundance [7]:

$$\%N = \frac{N_{px}}{N_{pt}} \times 100$$

N_{px} is total number of individuals of prey item x and N_{pt} is total number of individuals of all prey items.

Prominence value [8]:

$$PV = N \times \sqrt{F}$$

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

Shannon's index [9]:

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

$$p_i = \frac{N_{px}}{N_{pt}}$$

Levin's index [9]:

$$B = \frac{1}{\sum p_i^2}$$

$$p_i = \frac{N_{px}}{N_{pt}}$$

Standardised Levin's index [10]:

$$B_s = \frac{B - 1}{n - 1}$$

n is the number of possible resource states.

Pielou's evenness [11]:

$$E = \frac{H}{H_{max}}$$

H_{max} represents maximum value of Shannon's index, calculated as [9]:

$$H_{max} = \ln S_t$$

where S_t is total number of prey categories.

Renkonen's index [12]:

$$P = \% \sum \text{minimum}(P_{1i}P_{2i})$$

P_{1i} is the percentage of species in sample 1 and P_{2i} is the percentage of species in sample 2.

Supplementary References

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Supplementary Table S3. Total length (TL) and body weight (BW) of pumpkinseeds in the total sample and by reservoir. Values are presented as mean \pm SD

Reservoir	TL (cm)	BW (g)
Vlaški Do	8.4 \pm 1.5 ^a	9.5 \pm 4.9 ^a
Jatagan	7.7 \pm 1.6 ^b	7.3 \pm 6.6 ^b
Topola	7.8 \pm 2.0 ^a	8.0 \pm 6.0 ^b
Markovačko Lake	6.0 \pm 2.1 ^d	5.0 \pm 7.1 ^c
Kudreč 2	6.1 \pm 1.1 ^c	3.7 \pm 3.4 ^c

Significant differences in pumpkinseed total sample between reservoirs are marked by symbols **a, b, c, d**

Supplementary Table S4. Back-calculated length-at-age of pumpkinseeds from different localities

Supplementary Table S4A. Back-calculated length-at-age of pumpkinseeds (total sample).

Total sample	0+	1+	2+	3+	4+	5+	6+
0+	41.6 \pm 4.5						
1+	35.4 \pm 9.5	52.6 \pm 4.4					
2+	26.9 \pm 7.4	52.3 \pm 8.5	62.2 \pm 7.5				
3+	27.6 \pm 9.7	53.1 \pm 9.7	70.9 \pm 8.4	79.1 \pm 8.4			
4+	30.5 \pm 11.8	52.6 \pm 9.4	70.3 \pm 7.3	84.6 \pm 6.3	91.7 \pm 6.6		
5+	25.4 \pm 8.8	48.1 \pm 9.2	67.1 \pm 8.4	84.9 \pm 10.0	98.4 \pm 9.9	106.7 \pm 10.0	
6+	24.8 \pm 10.4	49.4 \pm 8.4	66.1 \pm 8.5	80.5 \pm 4.8	93.8 \pm 6.5	103.2 \pm 4.6	110.0 \pm 4.7
average total	30.3 \pm 6.1	51.3 \pm 2.1	67.3 \pm 3.5	82.3 \pm 2.9	94.6 \pm 3.4	104.9 \pm 2.5	110.00
yearly mm		21.0	16.0	15.0	12.4	10.3	5.1
yearly %		69.3	31.1	22.2	15.0	10.9	4.8

Supplementary Table S4B. Back-calculated length-at-age of pumpkinseeds (Vlaški Do)

Vlaški Do	0+	1+	2+	3+	4+	5+	6+
1+	41.5 \pm 5.5	54.0 \pm 1.2					
2+	35.0 \pm 5.7	61.1 \pm 9.6	71.1 \pm 8.1				
3+	35.1 \pm 7.4	57.9 \pm 7.9	73.0 \pm 7.4	81.0 \pm 7.0			
4+	37.7 \pm 9.6	57.3 \pm 6.9	73.0 \pm 5.6	85.5 \pm 5.3	91.9 \pm 6.0		
5+	27.1 \pm 12.3	50.3 \pm 8.3	65.3 \pm 6.7	82.0 \pm 6.6	93.5 \pm 4.4	100.8 \pm 5.5	
6+	24.2 \pm 10.4	48.7 \pm 8.5	65.5 \pm 8.6	80.1 \pm 4.8	93.5 \pm 6.5	103.1 \pm 4.6	110.0 \pm 4.7
average total MON	33.4 \pm 6.5	54.9 \pm 4.8	69.6 \pm 3.9	82.2 \pm 2.4	92.9 \pm 0.9	101.9 \pm 1.6	110.0
yearly mm		21.5	14.7	12.6	10.8	9.0	8.1
yearly %		64.2	26.7	18.1	13.1	9.6	7.9

Supplementary Table S4C. Back-calculated length-at-age of pumpkinseeds (Jatagan)

Jatagan	0+	1+	2+	3+	4+	5+
1+	54.1	65.0				
2+	36.8 \pm 4.2	63.8 \pm 4.5	70.3 \pm 4.4			
3+	33.2 \pm 5.9	50.8 \pm 7.9	63.9 \pm 5.5	69.1 \pm 4.4		
4+	23.3	42.4	62.9	77.8	82.0	
5+	34.6 \pm 6.8	53.7 \pm 8.6	73.9 \pm 8.3	91.5 \pm 8.0	105.7 \pm 7.0	111.3 \pm 7.7
average total MON	36.4 \pm 11.2	55.1 \pm 9.4	67.8 \pm 5.3	79.5 \pm 11.3	93.9 \pm 16.8	111.3
yearly mm		18.7	12.6	11.7	14.4	17.5
yearly %		51.5	22.9	17.3	18.1	18.6

Supplementary Table S4D. Back-calculated length-at-age of pumpkinseeds (Topola)

Topola	0+	1+	2+	3+	4+	5+
0+	46.3 ± 2.6					
1+	36.5 ± 8.0	51.9 ± 5.1				
2+	30.5 ± 6.3	59.3 ± 11.3	66.9 ± 12.6			
3+	24.7 ± 9.4	54.9 ± 10.4	73.9 ± 8.5	81.6 ± 8.0		
4+	24.2 ± 7.8	48.2 ± 7.4	67.8 ± 5.5	84.9 ± 6.0	92.0 ± 6.2	
5+	25.7 ± 4.1	49.2 ± 8.3	71.4 ± 8.3	89.5 ± 9.8	104.8 ± 8.5	112.3 ± 8.2
average total MON	31.3 ± 8.7	52.7 ± 4.5	70.0 ± 3.3	85.4 ± 4.0	98.4 ± 9.1	112.3
yearly mm		21.4	17.3	15.4	13.0	13.9
yearly %		68.2	32.8	22.0	15.3	14.2

Supplementary Table S4E. Back-calculated length-at-age of pumpkinseeds (Markovačko Lake)

Markovačko Lake	0+	1+	2+	3+	4+	5+
0+	40.8 ± 4.2					
1+	38.7 ± 6.6	52.9 ± 4.4				
2+	28.4 ± 6.6	54.2 ± 7.1	64.8 ± 6.7			
3+	23.1 ± 6.0	46.5 ± 8.9	69.4 ± 8.6	80.9 ± 7.1		
4+	24.4 ± 6.9	55.5 ± 9.1	74.8 ± 7.6	86.8 ± 6.3	98.2 ± 5.0	
5+	22.52 ± 6.0	60.6 ± 2.6	75.5 ± 3.7	97.3 ± 11.2	109.1 ± 8.5	121.0 ± 6.2
average total MON	29.66 ± 8.1	53.9 ± 5.1	71.1 ± 5.0	88.3 ± 8.3	103.6 ± 7.7	121.0
yearly mm		24.3	17.2	17.2	15.3	17.4
yearly %		81.8	31.9	24.2	17.4	16.7

Supplementary Table S4F. Back-calculated length-at-age of pumpkinseeds (Kudreč 2)

Kudreč 2	0+	1+	2+	3+	4+	5+
1+	26.3 ± 9.9	51.1 ± 3.4				
2+	21.4 ± 5.8	46.7 ± 4.8	58.3 ± 4.2			
3+	27.4 ± 2.3	47.6 ± 5.8	62.0 ± 4.0	69.1 ± 2.9		
4+	18.8 ± 7.6	41.3 ± 7.1	61.4 ± 10.2	77.3 ± 10.0	84.4 ± 6.1	
5+	18.5 ± 6.9	35.9 ± 4.2	57.1 ± 6.7	74.0 ± 6.2	87.6 ± 7.1	98.4 ± 6.6
average total MON	22.5 ± 4.1	44.5 ± 6.0	59.7 ± 2.4	73.5 ± 4.1	86.0 ± 2.3	98.4
yearly mm		22.1	15.2	13.8	12.5	12.4
yearly %		98.2	34.1	23.1	17.0	14.5

Supplementary Table S5. Equation parameters of von Bertalanffy growth curves, and estimated phi-prime growth performance index of pumpkinseeds from five reservoirs

	Total length – body weight relationship	k	L _∞ (mm)	t ₀	ϕ'
Total sample	$BW = 0.009 \times TL^{3.23}$	0.07	252.04	- 1.05	3.67
Vlaški Do	$BW = 0.008 \times TL^{3.28}$	0.14	161.50	- 1.02	3.56
Jatagan	$BW = 0.006 \times TL^{3.36}$	0.08	236.68	- 1.33	3.65
Topola	$BW = 0.008 \times TL^{3.25}$	0.09	228.36	- 1.18	3.65
Markovačko Lake	$BW = 0.007 \times TL^{3.40}$	0.05	336.24	- 1.25	3.78
Kudreč 2	$BW = 0.008 \times TL^{3.27}$	0.10	193.94	- 0.76	3.56

Supplementary Table S6. Fulton's condition factor (K) of pumpkinseeds (total sample, age classes) from five reservoirs. Values are presented as mean ± SD

	Total sample	Vlaški Do	Jatagan	Topola	Markovačko Lake	Kudreč 2
K	1.30 ± 0.2	1.34 ± 0.2 A	1.25 ± 0.2 B	1.27 ± 0.2 B	1.31 ± 0.3 AB	1.28 ± 0.1 B

Significant differences in pumpkinseeds total sample between reservoirs are marked by symbols **A**, **B**

Tables S8-S19. Differences in pumpkinseed diet**Supplementary Table S8A.** Differences in Chironomidae larvae proportion in pumpkinseed diet from 5 reservoirs

Kruskal-Wallis H	12.357
df	4
P	0.015

Supplementary Table S8B. Differences in Chironomidae larvae proportion in pumpkinseed diet (pairwise comparisons)

		Vlaški Do	Jatagan	Topola	Markovačko Lake
Jatagan	Mann-Whitney U Z P	725.500 -1.525 0.127			
Topola	Mann-Whitney U Z P	1886.500 -1.632 0.103	509.500 -2.643 0.008		
Markovačko Lake	Mann-Whitney U Z P	1937.500 -0.217 0.828	537.000 -1.651 0.099	1467.000 -1.374 0.169	
Kudreč 2	Mann-Whitney U Z P	3239.500 -2.017 0.044	874.500 -2.925 0.003	3299.500 -0.169 0.866	2512.000 -1.711 0.087

Supplementary Table S10B. Differences in Trichoptera proportion in pumpkinseed diet (pairwise comparisons)

		Vlaški Do	Jatagan	Topola	Markovačko Lake
Jatagan	Mann-Whitney U Z P	774.000 -1.334 0.182			
Topola	Mann-Whitney U Z P	1960.000 -1.588 0.112	761.500 -0.187 0.851		
Markovačko Lake	Mann-Whitney U Z P	1428.500 -2.976 0.003	391.000 -3.358 0.001	1002.000 -4.391 0.000	
Kudreč 2	Mann-Whitney U Z P	3405.000 -1.616 0.106	984.500 -2.410 0.016	2507.000 -3.210 0.001	2501.500 -1.773 0.076

Supplementary Table S11A. Differences in Ephemeroptera proportion in pumpkinseed diet from 5 reservoirs.

Kruskal-Wallis H	24.272
df	4
P	0.000

Supplementary Table S11B. Differences in Ephemeroptera proportion in pumpkinseed diet (pairwise comparisons)

		Vlaški Do	Jatagan	Topola	Markovačko Lake
Jatagan	Mann-Whitney U	859.500			
	Z	-0.424			
	P	0.672			
Topola	Mann-Whitney U	1764.000	621.500		
	Z	-3.076	-2.402		
	P	0.002	0.016		
Markovačko Lake	Mann-Whitney U	1614.000	567.500	1657.000	
	Z	-2.529	-1.940	-0.518	
	P	0.011	0.052	0.605	
Kudreč 2	Mann-Whitney U	3037.500	1059.000	3296.000	2840.000
	Z	-4.001	-3.147	-0.379	-0.971
	P	0.000	0.002	0.705	0.331

Supplementary Table S12. Differences in Odonata proportion in pumpkinseed diet from 5 reservoirs

Kruskal-Wallis H	8.012
df	4
P	0.091

Supplementary Table S13. Differences in Odonata proportion in pumpkinseed diet between Jatagan and Topola reservoirs

Mann-Whitney U	756.000
Z	-0.686
P	0.492

Supplementary Table S14. Differences in Coleoptera proportion in pumpkinseed diet from 3 reservoirs

Kruskal-Wallis H	1.263
df	2
P	0.532

Supplementary Table S15. Differences in Gammaridae proportion in pumpkinseed diet from 3 reservoirs

Kruskal-Wallis H	0.577
df	2
P	0.749

Supplementary Table S16. Differences in unidentified Mollusca proportion in pumpkinseed diet from 4 reservoirs

Kruskal-Wallis H	2.921
df	3
P	0.404

Supplementary Table S17. Differences in Bivalvia proportion in pumpkinseed diet between Topola and Markovačko Lake reservoirs

Mann-Whitney U	1654.000
Z	-0.885
P	0.376

Supplementary Table S18. Differences in Gastropoda proportion in pumpkinseed diet from 4 reservoirs

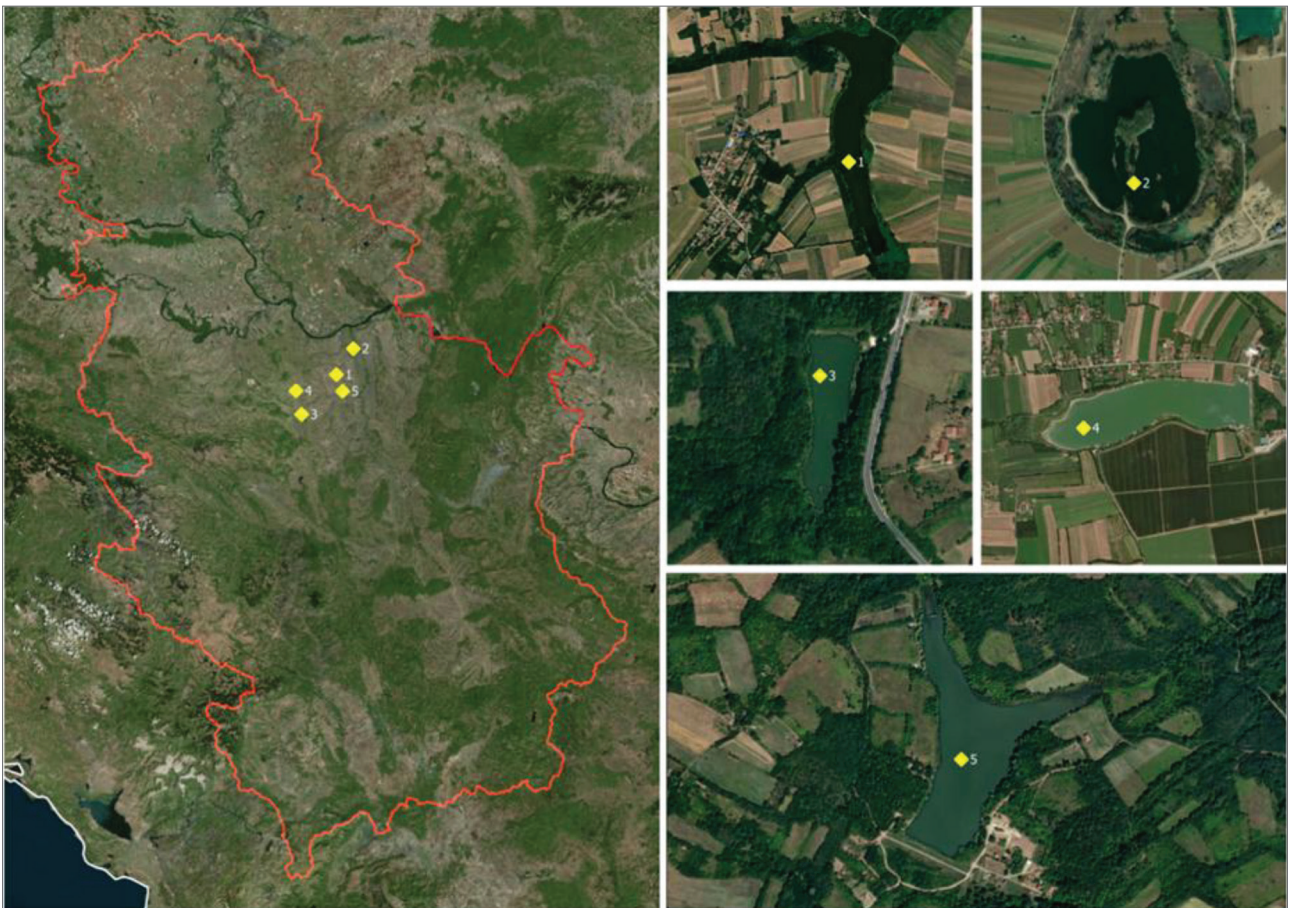
Kruskal-Wallis H	2.921
df	3
P	0.404

Supplementary Table S19. Differences in the proportion of terrestrial insects in pumpkinseed diet from 3 reservoirs.

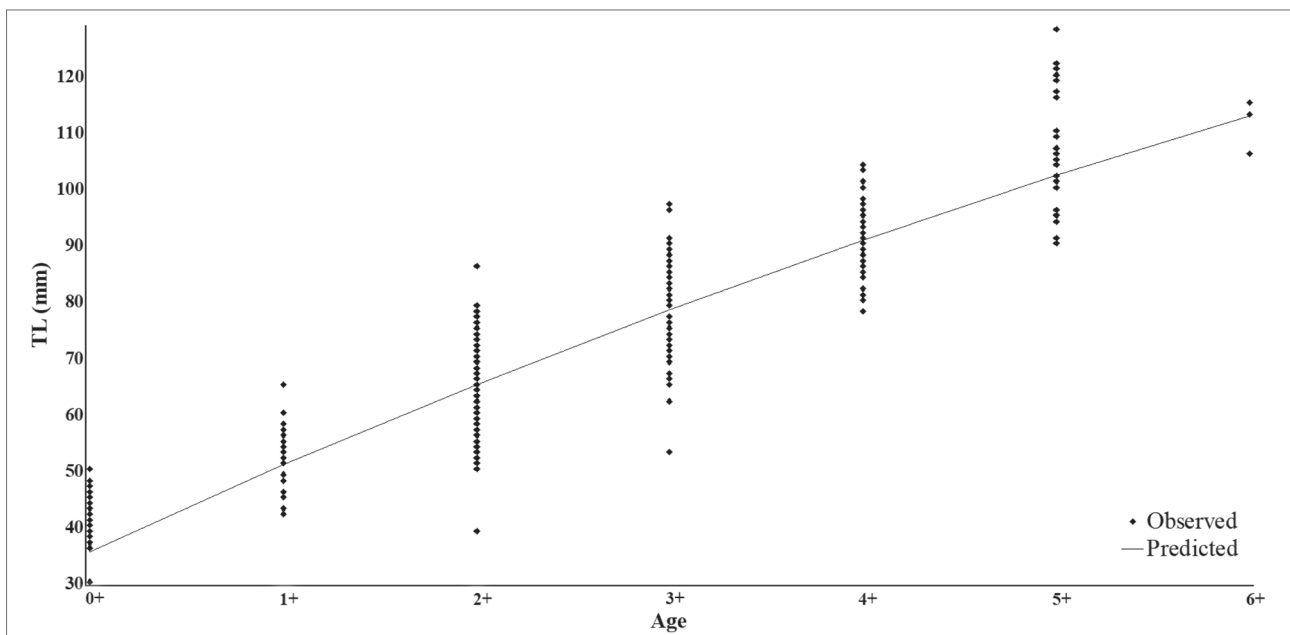
Kruskal-Wallis H	2.875
df	2
P	0.238

Supplementary Table S20. Values of Levin's index (B), standardised value Levin's index (Bs), Shannon's index (H), and equitability index (E) of prey items in pumpkinseed diet from five reservoirs

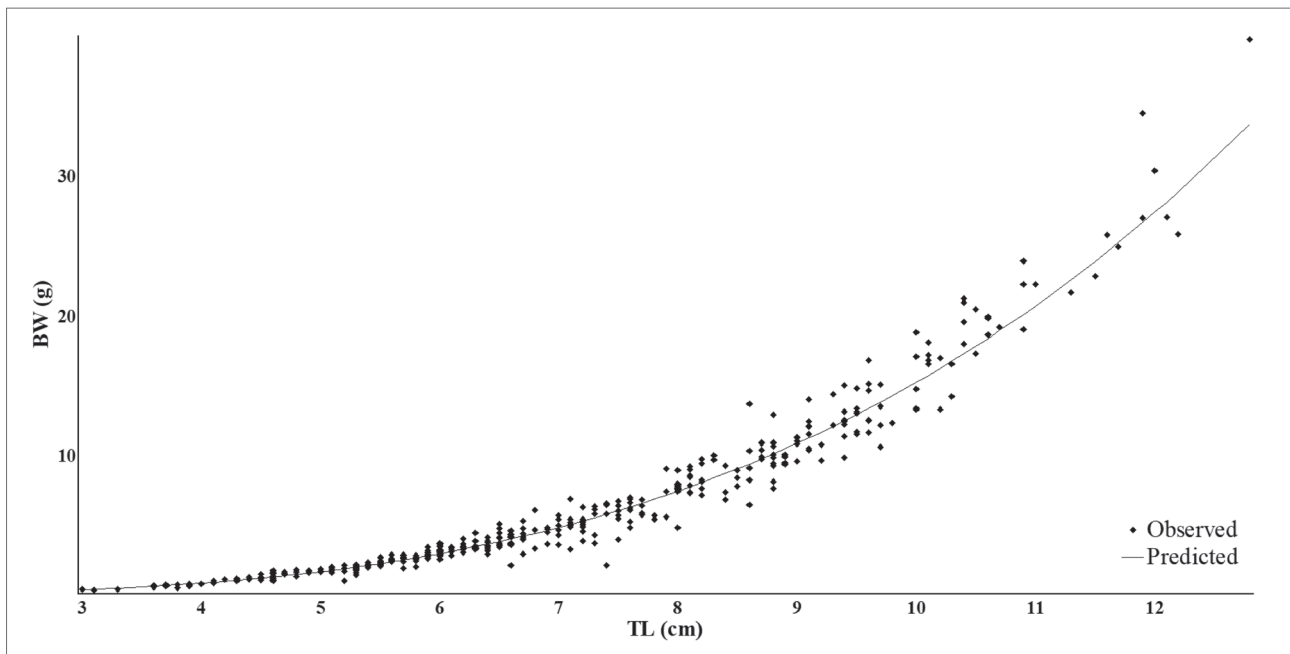
	B	Bs	H	E
Vlaški Do	3.29	0.29	1.34	0.64
Jatagan	3.19	0.27	1.51	0.69
Topola	2.39	0.11	1.45	0.55
Markovačko Lake	2.50	0.25	1.11	0.57
Kudreč 2	1.89	0.13	0.87	0.42
Total sample	2.61	0.12	1.31	0.49



Supplementary Fig. S1. Sampled reservoirs. 1 – Vlaški Do; 2 – Jatagan; 3 – Topola; 4 – Markovačko Lake; 5 – Kudreč



Supplementary Fig. S2. Von Bertalanffy growth curve of pumpkinseeds



Supplementary Fig. S3. Length-weight relationship curve of pumpkinseeds