

## Article

# Zooplankton as Indicator of Ecological Status in the Streževo Reservoir (North Macedonia)

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**Abstract:** This study examined the ecological status of the Streževo Reservoir in North Macedonia, focusing on zooplankton as an indicator of water quality. Built in 1982, the Streževo Reservoir serves several purposes, including irrigation, water supply, and hydropower generation. The research project investigated the seasonal and vertical variation in zooplankton abundance and biomass as well as the influence of environmental factors. Sampling was conducted seasonally (spring, summer, and autumn) in 2010 and 2011 across the longitudinal profile (epilimnion, metalimnion, and hypolimnion) of the reservoir at three sampling stations: the inflow of the Šemnica River, a central station in open water, and a site near the dam. The Streževo Reservoir is characterized by significantly pronounced seasonal and vertical temperature stratifications. The species diversity of the zooplankton was low, with only 21 taxa identified. Seasonal oscillations in abundance were statistically significant, with maximum values in the summer period and minimum values in spring. The Shannon diversity index displayed the lowest diversity values in the autumn, in the hypolimnion, and the highest values in the summer, in the metalimnion. The RDA analysis showed that temperature was the most important predictor of zooplankton abundance distribution, followed by Chl *a* concentration and TN. According to the Zooplankton Index of Quality Assessment (Zoo-IQ), during the investigated period the reservoir had good water quality in all three studied seasons, as well as through the whole profile. Overall, the study highlights the importance of zooplankton as an indicator of water quality and provides valuable insights into the ecological status of the Streževo Reservoir. The novelty of this study lies in its comprehensive examination of the interconnected dynamics affecting reservoir ecology, particularly as the present study is the first to perform such an analysis for the Streževo Reservoir. It highlights the impacts of thermal stratification on biochemical processes, the seasonal variations in dissolved oxygen and phosphorus levels due to phytoplankton activity, and the influences of temperature on zooplankton diversity and abundance. Furthermore, it introduces the Zoo-IQ index as an innovative tool for assessing water quality through zooplankton analysis, emphasizing its relevance as an early indicator of ecological changes in freshwater systems. Moreover, this multi-faceted approach underscores the complexity of reservoir ecosystems and the importance of proactive management strategies to the mitigation of water quality fluctuations. This study underlines the need for continuous monitoring and proactive management strategies to address the aging of reservoirs.



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## 1. Introduction

Reservoirs are complex ecosystems that provide a transitional environment between lakes and rivers and are geologically much younger than natural lakes [1]. In response to the growing human need for water resource management, reservoirs with multifunctional purposes—such as flood control, hydropower generation, water supply, irrigation, technological water supply for large industrial facilities, tourism, and sports—have been created by damming water in the valleys of larger rivers [2]. However, reservoirs are primarily built to harness hydropower, which utilizes renewable energy sources to generate electricity, a desirable and important resource for all of humanity, as this method of electricity production is known to have a much smaller impact on climate change. Therefore, damming is one of the most effective methods of regulating the water balance, with the aim of redistributing the water in the catchment area over time. Reservoirs are used when users need to be supplied with the required quantities of water with the appropriate water quality. The reservoirs neutralize sudden torrents and help to reduce soil erosion [3]. In addition, reservoirs are used to supply water to the population and to industry, which improves the standard of living of the local population and the growth of agriculture and accordingly reduces migration from the countryside to the city. Reservoirs are also important for fishing and contribute to the development of tourism, sports, and recreation [4].

Despite their many benefits, reservoirs also bring numerous disadvantages, particularly those related to their relatively short lifespans, which range from 30 to 200 years [5]. Damming river courses modifies ecosystem functioning and community structures [6] through artificial changes in hydrological conditions [7], habitat modification [8], and altered limnological characteristics [9–11]. Simoes et al. [12] showed that damming negatively affected the alpha diversity and species composition of zooplankton communities in 30 reservoirs and 29 natural lakes located in the Neotropics. A reservoir's aging process begins very soon after construction and depends on the water residence time, which is usually shorter than those found in lakes [13]. Juracek, during his research on the aging of America's reservoirs, noticed that short- and medium-term (<50 years) environmental consequences of reservoir construction and operation include an altered flow regime, lost connectivity (longitudinal and floodplain), an altered sediment regime, substrate compositional change, and downstream channel degradation [14].

Each reservoir exhibits a unique biology that develops through a complex and gradual process. Over time, the nutrient load from the basin and its effects on accumulation-based processes and pelagic components become more pronounced and accelerate. Straškrábová et al. [15] studied changes in pelagic food webs and their microbial components in the Rimov reservoir in the Czech Republic, and they noticed that seasonal changes of the higher trophic level—zooplankton—determined the changes in the seasonal variation of the microbial loop—both protozoans and bacteria. So, after zooplankton development, the bacteria and phytoplankton are controlled. They concluded that changes observed along the longitudinal profile of the transition zone are similar to the time-succession of pelagic biota observed during the initial period of reservoir ageing. At the same time, the anthropogenic impact is particularly pronounced, as reflected in the more frequent and extreme variations in environmental conditions, including seasonal changes in meteorological (temperature and precipitation) and hydrological (discharge, inflows, and outflows) factors, which also affect the structuring and functioning of biocoenoses in reservoirs [15–17]. For instance,

changes in phytoplankton assemblages are driven mainly by hydrochemical and hydrodynamic variables in freshwater reservoirs [18].

As well, human-induced negative impacts on lentic waters strongly affect algal biomass and significantly reduce the abundance, density, and species richness of invertebrates. This was confirmed by a meta-analysis conducted by Sabater et al. [19] which examined the potential effects of stressors on nutrients, microcontaminants, biological communities, and ecosystem functions, including organic matter breakdown, gross primary production, and respiration.

The use of bioindicators, such as macroinvertebrates and phytoplankton, in the evaluation of water quality and ecosystem functioning is crucial for national policies and water management, particularly under the Water Framework Directive (WFD) [20]. Many studies [21–23] have shown that zooplankton, although omitted from the WFD, is suitable for long-term monitoring of environmental stressors in surface waters. Zooplankton are an essential component of aquatic food webs and serve as key indicators of ecosystem health. Changes in these populations can reflect broader environmental shifts and water quality issues. Zooplankton, with its main groups, Rotifera, Cladocera, and Copepoda, is known as a very sensitive indicator of environmental alterations [24–27]. The characterization of the degree of pollution of water bodies by the determination of the sizes of zooplankton communities has been successfully used since the beginning of the 20th century [28]; also, several bioindication methods have been developed [29–31].

Despite their importance in the trophic web, zooplankton were not included as one of the Biological Quality Elements (BQE) used to determine water quality. In the research of Munoz-Colmenares et al. [32], zooplankton species were studied that could be considered to be indicators of trophic status and ecological potential for more than 60 water reservoirs associated with the Ebro River in Spain. According to physicochemical and biological elements, the trophic status and ecological potential of these reservoirs were established. The results from this research and similar work indicate that specific zooplankton species are related to water quality and suggest that zooplankton can be used as a valuable tool to determine water-quality status. And it is also well known that zooplankton species contribute significantly to freshwater ecosystem diversity and play a pivotal role between primary producers and higher-level consumers [33–35].

Many indices based on zooplankton traits, such as diversity; abundance; biomass; body size; and functional, ecological, and feeding guilds, are commonly used in ecological status assessments [36–40]. Zooplankton was studied in Lake Ohrid, Northern Macedonia, near the site of the present study, to determine the seasonal and spatial patterns of the zooplankton functional feeding guilds in relation to the environmental conditions; the metalimnion of the lake was determined to be the most productive environment, one where the biomass of the phytoplankton and abundance of the zooplankton reached their maxima [41]. Another important index based on zooplankton was proposed by [42], and named the Zooplankton Index of Quality Assessment (Zoo-IQ). This is a multi-metric index that combines four metrics in a complementary way: total zooplankton abundance ( $\text{ind L}^{-1}$ ), total zooplankton biomass ( $\mu\text{g L}^{-1}$ ), the ratio of zooplankton biomass to abundance, and the ratio of large-sized cladocerans ( $>500 \mu\text{m}$ ) to total cladoceran abundance. Multi-metric indices reflect the complex dimensions of biological systems and, therefore, are significantly more useful and provide a more realistic representation of the ecosystem's state, compared to simpler indices [43]. All of these studies emphasize the importance of analyzing the functional characteristics of biocoenoses, and assessing the influences of nutrient loading and food resources on these characteristics. This approach is essential for determining the ecological status and potential of aquatic ecosystems. Changes in zooplankton in water reservoirs are important due to its ability to sensitively respond to changes

in water conditions, such as nutrient levels, temperature, and pollution. Monitoring its abundance and diversity can provide valuable insights into water quality and potential problems like eutrophication [44]. Zooplankton diversity is crucial for maintaining ecological balance. Changes in these communities can indicate shifts in biodiversity that may affect other organisms in the ecosystem [45]. Changes in zooplankton populations can provide indicators of how climate change is impacting aquatic ecosystems, including shifts in species distributions and alterations in seasonal cycles [46]. Studying zooplankton dynamics assists in developing effective management strategies for water reservoirs, helping to maintain biodiversity, and ensuring the sustainability of aquatic resources. Shen et al. [47] observe that monitoring zooplankton in water reservoirs is also crucial for understanding ecosystem dynamics, assessing environmental changes, and informing management practices.

In the European Union, according to the Water Framework Directive [20], reservoirs are classified as heavily modified water bodies [1] and are strongly affected by anthropogenic drivers. Since the functional characteristics of zooplankton serve as indicators of the ecological status and functioning of aquatic ecosystems [36–40], the primary aim of this study was to utilize zooplankton traits, such as diversity, abundance, and biomass, as indicators of water quality and environmental conditions in the reservoir. Additionally, we used the zooplankton multi-metric index Zoo-IQ [42] in order to test the application and usefulness of this index for monitoring the ecological status of human-made reservoirs.

## 2. Materials and Methods

The Streževo Reservoir (Republic of North Macedonia, Europe, Figure 1) was built in 1982 by damming the Šemnica River; the reservoir has an earth–rock dam 76 m high, a storage capacity of 120 million cubic meters, and a surface area of 4 km<sup>2</sup> (Table 1). The reservoir is 5 km long, with a maximum width of 750 m. Due to its geometric features and the winds from Mount Baba, it stratifies once a year, in summer. The reservoir serves multiple purposes: irrigating agricultural fields in the Pelagonia Plain, supplying water to the city of Bitola and the adjacent large thermal power plant, treating waste, generating small-scale hydropower, controlling floods, and supporting local fishing. Additionally, it contributes to the development of tourism, sports, and recreation.

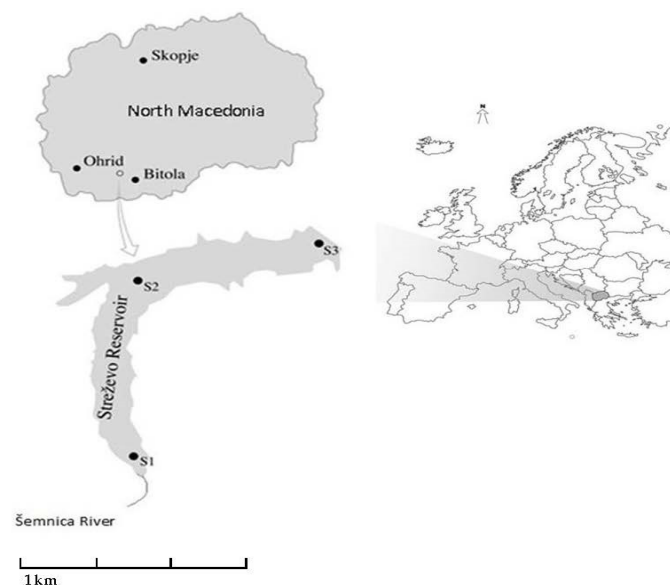


**Figure 1.** Map of Streževo Reservoir (the investigated reservoir) and three sampling stations (S1, S2, and S3).

**Table 1.** Hydromorphometrical features of the Streževo Reservoir.

| Parameter                                      | Value                |
|------------------------------------------------|----------------------|
| Coordinates:                                   |                      |
| S1                                             | 41°07'30"; 21°09'53" |
| S2                                             | 41°08'15"; 21°10'07" |
| S3                                             | 41°08'35"; 21°11'47" |
| Total length (m)                               | 5200                 |
| Width <sub>max</sub> (m)                       | 750                  |
| Depth <sub>max</sub> (m)                       | 40                   |
| Total volume (10 <sup>6</sup> m <sup>3</sup> ) | 120                  |
| Main inflow                                    | Šemnica River        |

Sampling was conducted seasonally in 2010 and 2011 across the longitudinal profile of the reservoir at three sampling stations: S1—the inflow of the Šemnica River; S2—the central station, in open water (pelagial); and S3—the site near the dam (Figure 1). Samples from S2 and S3 were collected at each station along the vertical profile at depths of 1 m (epilimnion, E), 8 m (metalimnion, M), and 20 m (hypolimnion, H). At S1, only surface samples, from a depth of 1 m, were taken. The three seasons considered in the temporal analyses were spring (Sp), summer (Su), and autumn (Au). There were no statistically significant differences between the sampled years in environmental parameters or in zooplankton abundance, so the data from 2010 and 2011 were combined. Water temperature and dissolved oxygen were measured in the field using a Hach HQ30d (Hach, Company, Loveland, CO, USA), while other parameters were analyzed in the laboratory. Total nitrogen (TN) and total phosphorus (TP) were analyzed according to the procedures described in [48]. The concentration of chlorophyll *a* (Chl *a*), as an indicator of phytoplankton biomass, was measured by spectrophotometry, following its extraction in 90% ethanol [49]. For organic matter (OM), chemical oxygen demand was measured by the oxidation of dissolved organic matter, using KMnO<sub>4</sub> (results expressed as mg O<sub>2(Mn)</sub> L<sup>−1</sup>).

Zooplankton was collected by a 5 L Ruttner sampler, and 30 L of water was filtered in situ through a net (45 µm mesh-size) and preserved in 4% formalin. For quantitative analysis, samples were concentrated by centrifugation (2000 rpm for 5 min) to a volume of 15–20 mL, mixed thoroughly, and then 3 × 1 mL aliquots were counted in their entirety using a Sedgewick–Rafter cell under a Leica DM IRB microscope (100× to 600× magnification). Specimens were identified to the lowest possible taxonomic level, based on [50–52].

The obtained data were logarithmically transformed [ $\log(x + 1)$ ], checked by the Shapiro–Wilk test, and if they did not follow a normal distribution, they were analyzed by nonparametric tests using Statistica 13.0 software [53]. The Kruskal–Wallis H test was conducted for the analysis of spatial and seasonal differences in the environmental parameters and zooplankton assemblage. The Shannon diversity index was calculated in the analytical package PRIMER v6 [54]. The influences of the environmental variables on the zooplankton were analyzed with CANOCO 5 software v6 [55]. A detrended correspondence analysis showed that the data could be regarded as linear (maximum length of gradient < 1); we used the most suitable redundancy analysis (RDA). In the RDA, the ordination axes were constrained to be linear combinations of the environmental factors provided and were used to investigate the relationships between these factors and the distribution of zooplankton. The statistical significance of environmental variables was tested with the Monte Carlo (MC) permutation test (499 permutations). Additionally, we used nonmetric multidimensional scaling (nMDS) analysis in order to enable visualization of differences between sites and seasons, based on zooplankton abundance. We used the Bray–Curtis distance for computing zooplankton similarities or differences. This index

considers both the number of shared species and their abundances in order to calculate the percentages of similarity between depth samples and seasons. Before calculating similarities, we  $\log(x)$ -transformed data, because the presence of highly abundant species could mask the influences of low-abundance species.

The collected zooplankton data related to the abundance and biomass of the Rotifera, Cladocera, and Copepoda were used to calculate the Zoo-IQ index [42]. This multi-metric index is calculated using the following formula:

$$\text{Zoo-IQ} = A_{\text{Zoo}} + B_{\text{Zoo}} + \text{MW}_{\text{Zoo}} + R_{\text{Clad}}$$

where  $A_{\text{Zoo}}$  is the total abundance (ind  $\text{L}^{-1}$ ) of zooplankton;  $B_{\text{Zoo}}$  is the total biomass ( $\mu\text{g L}^{-1}$ ) of zooplankton;  $\text{MW}_{\text{Zoo}}$  is the ratio of zooplankton biomass to abundance; and  $R_{\text{Clad}}$  is the abundance ratio of large-sized cladocerans ( $>500 \mu\text{m}$ ) to total cladocerans. The range of the index is from 4 to 20. This range was divided into five classes indicating the water quality according to the following scale: bad  $\leq 6$ , 6 < poor  $\leq 10$ , 10 < moderate  $\leq 14$ , 14 < good  $\leq 18$ , 18 < high.

### 3. Results

#### 3.1. Spatio-Seasonal Patterns of Environmental Parameters

The Strežovo Reservoir is characterized by significantly pronounced seasonal and vertical temperature stratifications in summer (Table 2). As expected, the highest water temperatures, of 24 °C, were measured during the summer in the epilimnion, and the minimum of 6 °C was in spring, in the hypolimnion. (Kruskal–Wallis test,  $p < 0.05$ ; Table 2). The concentrations of dissolved oxygen and total phosphorus were significantly higher in the spring than in summer (Table 2); to the contrary, there were no significant differences in their vertical distributions (Table 3). Chlorophyll *a* showed strong spatial (i.e., vertical) differences, with its significantly lower concentrations in the hypolimnion than in the epilimnion and metalimnion (Table 2).

**Table 2.** Seasonal and vertical values (mean  $\pm$  SD) of measured abiotic parameters in the Strežovo. Abbreviations: Chl *a*—Chlorophyll *a* ( $\mu\text{g L}^{-1}$ ); OM—Dissolved organic matter ( $\text{mg O}_{2(\text{Mn})} \text{L}^{-1}$ );  $\text{O}_2$ —Dissolved oxygen ( $\text{mg L}^{-1}$ ); T—Temperature (°C); TP—Total phosphorus ( $\mu\text{g L}^{-1}$ ); TN—Total nitrogen ( $\mu\text{g L}^{-1}$ ); SD—Standard deviation.

| Season | Depth | T (°C)           | $\text{O}_2$ ( $\text{mg L}^{-1}$ ) | TP ( $\mu\text{g L}^{-1}$ ) | TN ( $\mu\text{g L}^{-1}$ ) | OM ( $\text{mg O}_{2(\text{Mn})} \text{L}^{-1}$ ) | Chl <i>a</i> ( $\mu\text{g L}^{-1}$ ) |
|--------|-------|------------------|-------------------------------------|-----------------------------|-----------------------------|---------------------------------------------------|---------------------------------------|
| Sp     | 1     | 10.06 $\pm$ 6.96 | 10.51 $\pm$ 1.55                    | 17.34 $\pm$ 4.46            | 490.01 $\pm$ 293.84         | 8.43 $\pm$ 1.24                                   | 6.16 $\pm$ 2.83                       |
|        | 8     | 7.08 $\pm$ 3.33  | 10.84 $\pm$ 0.86                    | 16.74 $\pm$ 2.30            | 406.58 $\pm$ 184.30         | 9.38 $\pm$ 1.30                                   | 8.83 $\pm$ 5.85                       |
|        | 20    | 6.05 $\pm$ 1.10  | 10.86 $\pm$ 0.76                    | 17.33 $\pm$ 5.26            | 367.55 $\pm$ 97.40          | 9.26 $\pm$ 1.45                                   | 5.41 $\pm$ 3.70                       |
| Su     | 1     | 23.84 $\pm$ 0.32 | 9.07 $\pm$ 0.39                     | 15.97 $\pm$ 4.64            | 327.14 $\pm$ 153.57         | 11.31 $\pm$ 2.19                                  | 6.48 $\pm$ 3.68                       |
|        | 8     | 13.13 $\pm$ 1.65 | 8.84 $\pm$ 3.51                     | 13.81 $\pm$ 3.83            | 276.31 $\pm$ 49.81          | 10.19 $\pm$ 1.25                                  | 13.14 $\pm$ 11.49                     |
|        | 20    | 7.90 $\pm$ 1.04  | 8.56 $\pm$ 0.57                     | 11.48 $\pm$ 1.32            | 367.41 $\pm$ 176.01         | 8.59 $\pm$ 0.80                                   | 1.15 $\pm$ 0.28                       |
| Au     | 1     | 11.07 $\pm$ 0.12 | 10.03 $\pm$ 0.06                    | 15.93 $\pm$ 1.75            | 469.10 $\pm$ 136.28         | 10.87 $\pm$ 1.83                                  | 11.44 $\pm$ 0.58                      |
|        | 8     | 10.75 $\pm$ 0.21 | 9.67 $\pm$ 0.11                     | 13.16 $\pm$ 1.21            | 304.34 $\pm$ 7.04           | 11.14 $\pm$ 2.02                                  | 7.22 $\pm$ 3.83                       |
|        | 20    | 8.45 $\pm$ 0.07  | 8.58 $\pm$ 0.08                     | 9.78 $\pm$ 0.76             | 579.51 $\pm$ 68.96          | 8.28 $\pm$ 0.01                                   | 1.21 $\pm$ 0.15                       |

**Table 3.** Seasonal and vertical differences in environmental parameters, zooplankton abundances (ind L<sup>−1</sup>), and biomass (μg L<sup>−1</sup>). Kruskal–Wallis test, H (2, n = 34,  $p < 0.05$ ), with accompanying results of the post hoc multiple comparison test. Abbreviation: n.s. = non-significant.

|                                                    | Seasonal Differences |        |               | Vertical Differences |        |               |
|----------------------------------------------------|----------------------|--------|---------------|----------------------|--------|---------------|
|                                                    | H                    | $p$    | Post-Hoc Test | H                    | $p$    | Post-Hoc Test |
| <b>Environmental variables</b>                     |                      |        |               |                      |        |               |
| Temperature (°C)                                   | 12.25                | 0.002  | Su>Sp         | 5.83                 | 0.05   | H < E         |
| Dissolved oxygen (mg L <sup>−1</sup> )             | 9.29                 | 0.01   | Sp>Su         | n.s                  |        |               |
| Total Phosphorus (μg L <sup>−1</sup> )             | 7.07                 |        | Sp>Su         | n.s                  |        |               |
| Chlorophyll <i>a</i> (μg L <sup>−1</sup> )         |                      |        |               | 12.57                | 0.002  | H < E, M      |
| <b>Zooplankton</b>                                 |                      |        |               |                      |        |               |
| Total zooplankton abundance (Ind L <sup>−1</sup> ) | 11.2                 | 0.0037 | Su>Sp         | n.s                  |        |               |
| Rotifera abundance (Ind L <sup>−1</sup> )          | 18.09                | 0.0001 | Au>Sp         | n.s                  |        |               |
| Cladocera abundance (Ind L <sup>−1</sup> )         |                      | n.s.   |               | n.s                  |        |               |
| Copepoda abundance (Ind L <sup>−1</sup> )          |                      | n.s.   |               | 9.85                 | 0.0073 | H < E, M      |
| Total zooplankton biomass (μg L <sup>−1</sup> )    | 10.63                | 0.0049 | Su>Sp; Au>Sp  | n.s                  |        |               |
| Rotifera biomass (μg L <sup>−1</sup> )             | 7.1                  | 0.0288 | Su>Sp; Au>Sp  | n.s                  |        |               |
| Cladocera biomass (μg L <sup>−1</sup> )            | 9.44                 | 0.0089 | Su>Sp         | n.s                  |        |               |
| Copepoda biomass (μg L <sup>−1</sup> )             |                      | n.s.   |               | 6.71                 | 0.035  | H < E, M      |
| Shannon diversity index (H')                       | 5.91                 | 0.0522 | Su>Au         | n.s                  |        |               |
| Number of taxa (S)                                 | 7.23                 | 0.027  | Su>Au         | n.s                  |        |               |

### 3.2. Seasonal and Vertical Oscillations in Zooplankton

The species diversity of the zooplankton was low, with only 21 taxa identified. Rotifers were the most species-rich group, comprising 10 different taxa, followed by Copepods with 6 taxa, and Cladocera with 5 taxa. Of all recorded taxa, four dominated in abundance (*Daphnia cuculata*, *Eudiaptomus gracilis*, *Asplancha* sp., and *Keratella quadrata*), constituting 96% of the total abundance, while the remaining zooplankton taxa each accounted for less than 1% of the total abundance. No invasive zooplankton species were found during this investigation.

Seasonal oscillations in abundance were statistically significant (Table 2), with maximum values found in the summer period ( $148 \pm 77$  ind L<sup>−1</sup>) and minimum in spring ( $10 \pm 2$  ind L<sup>−1</sup>), while vertical variations in zooplankton abundance were not statistically significant (Table 2). Copepoda dominated in abundance during spring and autumn, whereas Rotifera outnumbered them in the summer period (Figure 2a). The significant increase in abundance during the summer compared to spring and autumn is primarily attributed to the species *K. quadrata*. The dominance of this species of Rotifera in the autumn period was replaced by the increase of the predatory species of the genus *Asplancha*. The dominant species of Copepoda, *E. gracilis*, reached its highest abundance during the summer period in the metalimnion. Also, in the same period in the metalimnion, the highest abundance of Cladocera was recorded, primarily the species *D. cuculata*.

Zooplankton biomass exhibited similar dynamics, with statistically significant seasonal differences, and the highest biomass being recorded during the summer period (Table 2; Figure 2b). In the vertical distribution, only Copepoda showed statistically significant differences in abundance and biomass, with lower values observed in the deeper parts of the reservoir (Table 2). The Shannon diversity index displayed both seasonal and vertical differences. The lowest diversity values were recorded in the autumn period, and these findings were statistically significant (Table 2; Figure 3). Vertically, the highest values were observed in the metalimnion, while the lowest were in the hypolimnion of the Streževu Reservoir.

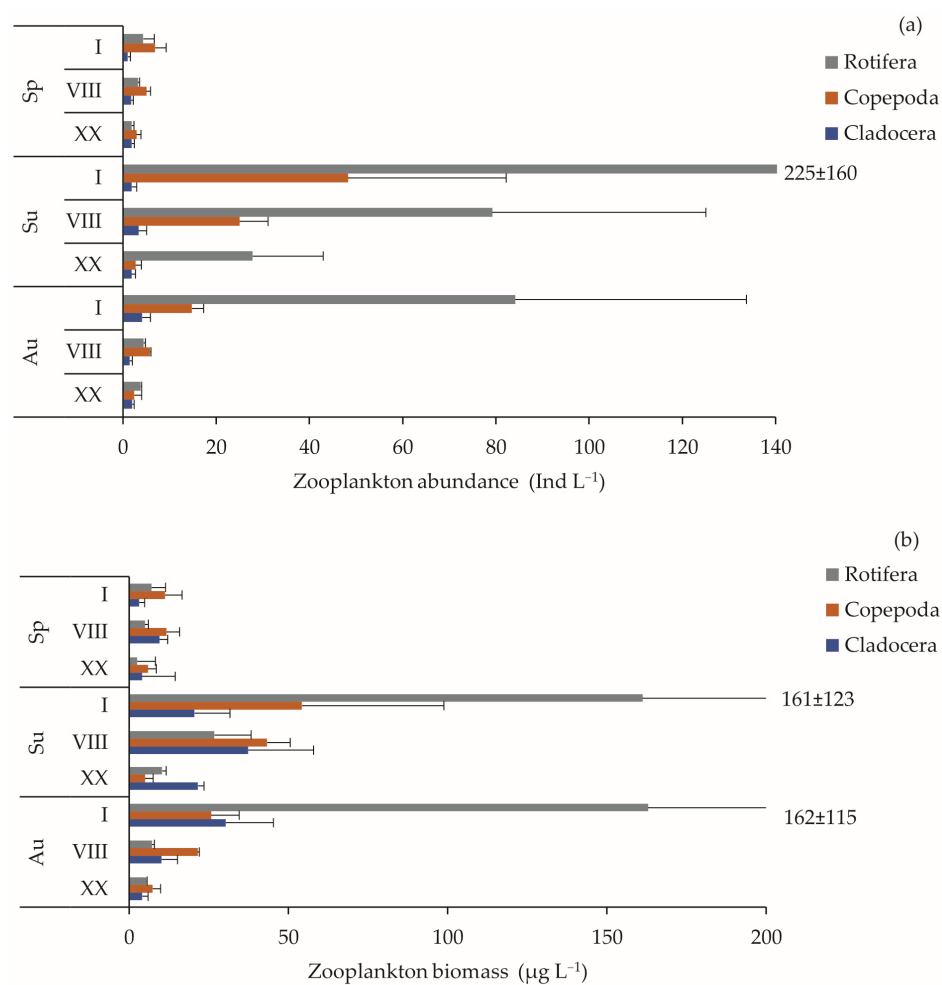


**Figure 2.** Spatial and temporal values ( $\pm$ SE) for a zooplankton: (a) abundance and (b) biomass in the Streževo Reservoir. Abbreviations: Sp—spring; Su—summer; Au—autumn; I—epilimnion; VIII—metalimnion; XX—hypolimnion.

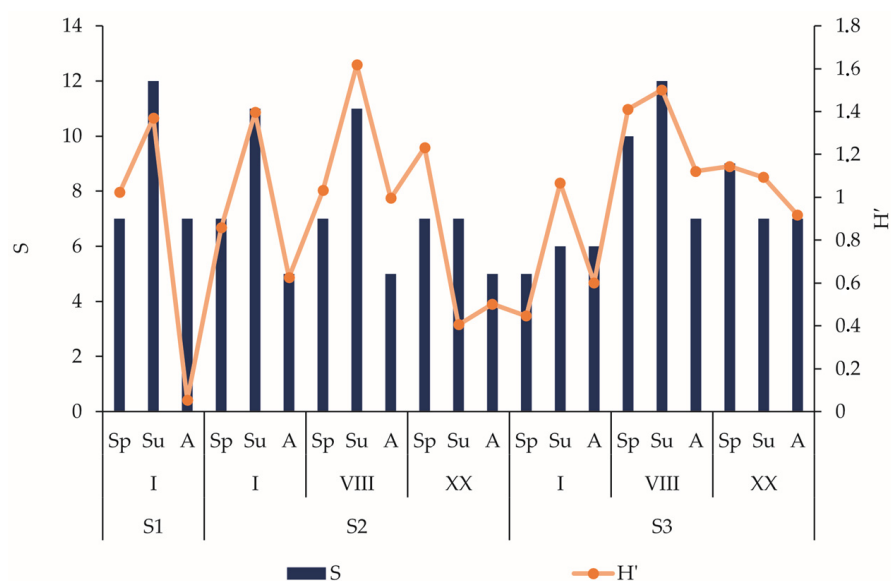


**Figure 3.** Shannon index of diversity  $H'$ , and taxa number ( $S$ ) of zooplankton in the Streževo Reservoir. Abbreviations: Sp—spring; Su—summer; A—autumn; I—epilimnion; VIII—metalimnion; XX—hypolimnion.



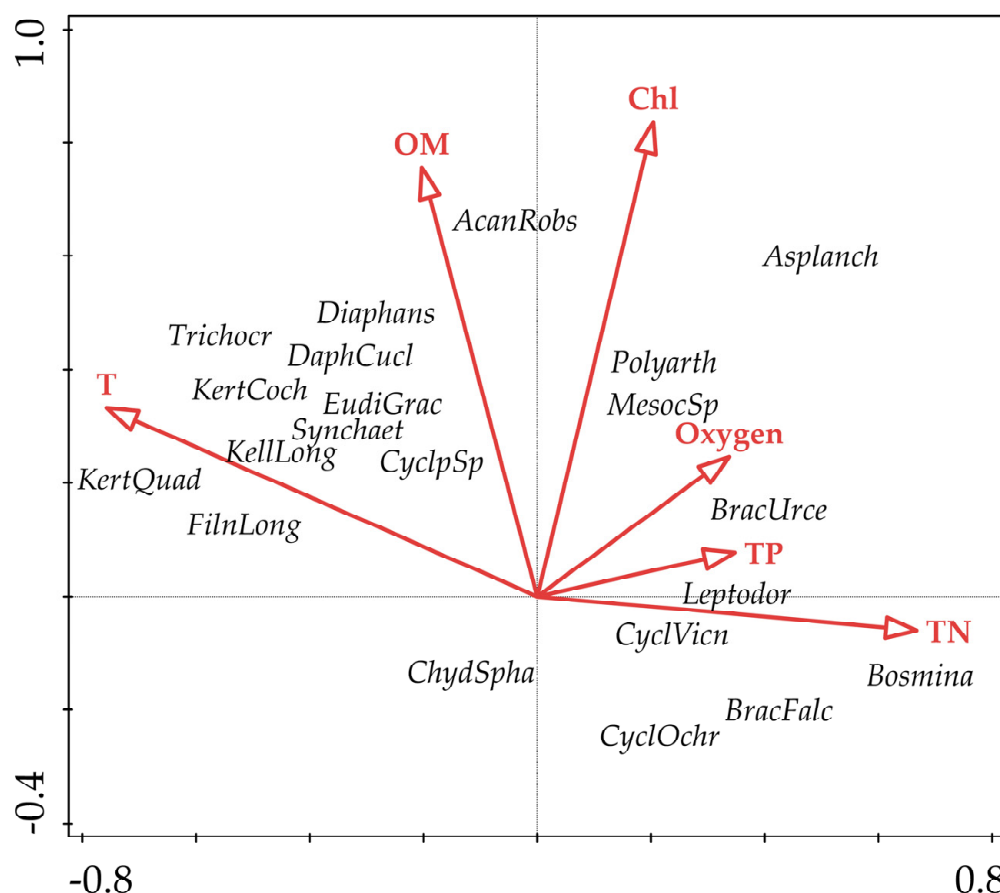


**Figure 5.** Redundancy analysis (RDA) showing the zooplankton abundance and the influence of environmental factors. RDA1 = 22%; RDA2 = 17%. Abbreviations for zooplankton: AcanRob—*Acanthocyclops robustus* (G. O. Sars, 1863); Asplanch—*Asplanchna* sp.; Bosmina—*Bosmina* sp.; BracFalc—*Brachionus falcatus* Zacharias, 1898; BracUrce—*Brachionus urceolaris* Müller, 1773; ChydSpha—*Chydorus sphaericus* (O. F. Müller, 1776); CyclOchr—*Cyclops ochridanus* Kiefer, 1932; CyclVicn—*Cyclops vicinus* Uljanin, 1875; CylpSp—*Cyclops* sp.; DaphCucl—*Daphnia cuculata* Sars, 1862; Diaphans—*Diaphanosoma* sp.; EudiGrac—*Eudiaptomus gracilis* (Sars, 1863); FinLong—*Filinia longiseta* (Ehrenberg, 1834); KellLong—*Kellicottia longispina* (Kellicott, 1879); KertCoch—*Keratella cochlearis* (Gosse, 1851); KertQuad—*Keratella quadrata* (Müller, 1786); Leptodor—*Leptodora kindtii* (Focke, 1844); Polyarth—*Polyarthra* sp.; MesoSp—*Mesocyclops* sp.; Trichocr—*Trichocerca* sp. Abbreviations for environmental factors: Chl—Chlorophyll *a* ( $\mu\text{g L}^{-1}$ ); OM—Dissolved organic matter ( $\text{mg O}_{2(\text{Mn})} \text{L}^{-1}$ ); Oxygen—Dissolved oxygen ( $\text{mg L}^{-1}$ ); T—Temperature ( $^{\circ}\text{C}$ ); TP—Total phosphorus ( $\mu\text{g L}^{-1}$ ); TN—Total nitrogen ( $\mu\text{g L}^{-1}$ ).

### 3.4. Zoo-IQ Index and Water Quality Assessment

The results obtained using the Zoo-IQ index are shown in Figure 6. The index values are generally high (14 and above), regardless of the sampling depth, indicating good reservoir conditions throughout the entire vertical water column. Additionally, seasonal changes in the abundance and biomass of zooplankton did not cause significant fluctuations in the Zoo-IQ index. During the investigated period, the reservoir had good water quality during all three studied seasons, with maximum values being recorded in the autumn period.

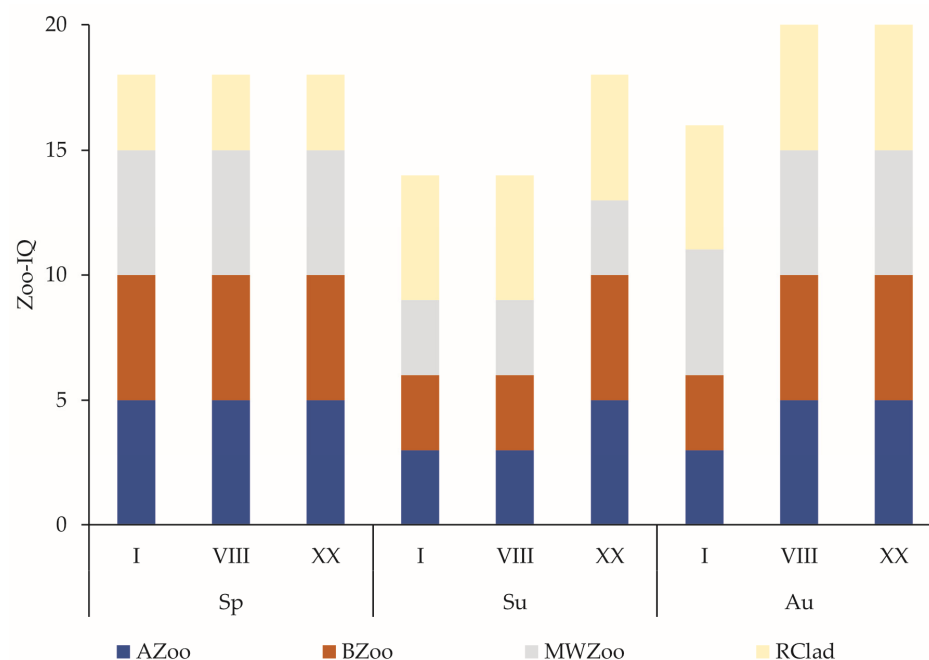


**Figure 6.** Zoo-IQ index values in the Streževo Reservoir. Abbreviations: Sp—spring; Su—summer; Au—autumn; I—epilimnion; VIII—metalimnion; XX—hypolimnion. For  $A_{Zoo}$ ,  $B_{Zoo}$ ,  $MW_{Zoo}$ , and  $R_{Clad}$  and the range for water quality, please see the detailed description in Materials and Methods.

## 4. Discussion

### 4.1. Challenges of Aging Reservoirs and Water Quality Fluctuations

We are entering an era in which we will be confronted with more extreme consequences of aging reservoirs. These challenges can be addressed on a case-by-case basis with reactive measures, or they can be tackled with a more consistent, proactive mindset. The water quality of reservoirs fluctuates throughout the annual hydrological cycle and depends on factors such as rainfall, soil erosion in the protected zone, sediments, the number of human settlements, and the level of industrial development in the catchment area [52].

### 4.2. Thermal Stratification and Its Impact on the Reservoir

Thermal stratification occurs in lakes and reservoirs because water at different temperatures has different densities. Solar radiation is the largest heat source, with most of this heat being absorbed directly by the water [56,57]. The amount of heat absorbed strongly depends on the geographical location of the water body and factors such as air temperature, water depth, water inflow, wind strength, etc. The Streževo Reservoir is a monomictic reservoir in which thermal stratification is maintained from early spring to late fall. The Streževo Reservoir exhibits significant seasonal and vertical temperature stratification, with the highest water temperatures (24 °C) being observed during summer and the lowest (5 °C) during spring, as expected. The mean temperature of the water column in the epilimnion reached its maximum value in the summer. A decrease in temperature led to a decrease in the rate of biochemical processes, while an increase in temperature increased the rate and decreased the solubility of gasses, which became clear when analyzing the oxygen levels in the water [58].

### 4.3. Dissolved Oxygen and Phosphorus Dynamics

In the Streževo, higher oxygen concentrations in spring were likely the result of the accumulation of photosynthesizing phytoplankton on the surface, but also due to the lower water temperature, which allows for greater solubility of atmospheric oxygen [58,59]. Simi-

lar observations were reported for Lake Pamvotis by [60]. The concentrations of dissolved oxygen and total phosphorus in the Streževo Reservoir were significantly higher in the spring than in summer. This indicates the influx of biogenic phosphorus and other pollutants from the catchment area, which is a source of diffuse pollution. Point sources of pollution located upstream and transboundary transport from these sources could also have had a negative effect. However, there were no significant differences in their vertical distribution, a finding which can be attributed to the initial boost in phytoplankton growth, nutrient release from sediments, and changes in temperature and biological activity that occur throughout the seasons [61].

#### 4.4. Chlorophyll *a* as an Indicator of Ecological Health

Chlorophyll *a* concentrations showed strong vertical fluctuations and were lower in the hypolimnion than in the epilimnion and metalimnion, which can be explained by the phytoplankton's requirements for light. The epilimnion receives more sunlight, which can lead to higher concentrations of phytoplankton and, consequently, also of chlorophyll *a*. In the hypolimnion, on the other hand, the light decreases, which leads to lower photosynthetic activity and lower chlorophyll *a* level. Monitoring chlorophyll *a* helps inform water management practices, enabling the implementation of measures to control nutrient loading, maintain water quality, and protect aquatic ecosystems. So, chlorophyll *a* is a crucial metric for assessing the ecological health, productivity, and water quality of reservoirs, guiding effective management and conservation efforts.

#### 4.5. Zooplankton Dynamics and Environmental Influences

Temperature was the factor that had the greatest influence on zooplankton dynamics (both abundance and biomass), a finding which is also characteristic of other lakes [62]. Latitude affects a number of climatic variables over time, significantly influencing the initial conditions and structure of the biocenosis [63]. Zooplankton diversity was low, with 21 taxa identified. A similar low species diversity was also recorded in Lake Ohrid, Northern Macedonia, where 16 species of zooplankton were observed [64]. Furthermore, in three investigated reservoirs (also in Northern Macedonia), a relatively small number of plankton species was recorded [26], which is consistent with our results. A low number of zooplankton species in lakes can be caused by a combination of natural factors (e.g., trophic state, habitat availability, and temperature) and anthropogenic influences (e.g., pollution, invasive species, and habitat destruction). The interactions between these factors often create conditions where only a limited number of species are able to survive and thrive, leading to low biodiversity in the lake ecosystem. For example, in nutrient-rich eutrophic lakes, the high levels of nitrogen and phosphorus promote the overgrowth of algae, leading to algal blooms. These blooms can reduce water clarity, deplete oxygen levels (hypoxia), and create an unfavorable environment for many species, especially those that require clear, oxygen-rich waters [65]. Oligotrophic lakes also often support fewer species due to limited food availability for primary producers (phytoplankton) and primary consumers (zooplankton) [66]. The depth of the lake can also influence species diversity. Shallow lakes tend to have higher species diversity because they support more varied habitats and allow for the mixing of nutrients, while deeper lakes may have less diversity due to thermal stratification [66]. Furthermore, lakes with homogeneous habitats (e.g., uniform depth, bottom substrate, or lack of vegetative cover) typically support fewer species because there are fewer ecological niches available for different organisms. The number of species often increases when there is a variety of habitats, such as littoral zones, pelagic zones, and benthic zones. Complex habitats, such as submerged plants, rocks, or woody debris, provide more microhabitats and resources for various species [67]. In lakes, the presence of

predators (such as fish) can significantly reduce zooplankton diversity and abundance, especially for Cladocera [68]. The novelty here lies in the integrative analysis of the factors that influence zooplankton dynamics and biodiversity in freshwater ecosystems. Multiple interactions between natural and anthropogenic factors, such as trophic states and pollution, contribute to the observed low diversity and provide a comprehensive understanding of ecosystem health. This holistic perspective on the ecological dynamics at play improves the understanding of freshwater ecosystems and provides insights that can inform conservation and management strategies to preserve biodiversity.

The Shannon diversity index revealed both seasonal and vertical differences, with the lowest diversity found in autumn and the highest in the metalimnion. Autumn typically represents a transitional period in aquatic ecosystems. As temperatures begin to drop and the amount of sunlight decreases, conditions in the lake may become less favorable for some species, especially plankton and algae, which are sensitive to temperature and light. During spring and summer, conditions may be more favorable for a wider range of species due to warmer temperatures, longer daylight hours, and higher levels of primary production. This could result in higher diversity, as species have access to abundant food and suitable conditions [66]. The metalimnion is capable of demonstrating a unique set of environmental conditions, such as higher oxygen levels compared to the hypolimnion, and more stable temperatures compared to the epilimnion. Thus, metalimnion can provide a balanced environment that supports a high diversity of species because it can offer a range of niches that cater to different organisms. Species that prefer cooler temperatures or more oxygenated waters may thrive in this layer, leading to greater diversity [66]. Considering the metalimnion to be a unique habitat with a pronounced oxygen content and temperature stability opens up a new perspective on how structured water layers can promote greater biodiversity. This aspect emphasizes the importance of specific ecological niches for the survival of zooplankton, especially those adapted to cooler, oxygen-rich conditions. Zooplankton abundance in the Streževu reaches its peak during the summer, driven by warmer temperatures and an increased availability of food sources like phytoplankton and organic detritus. This trend, with the highest zooplankton levels being recorded in summer, while the lowest occur in spring, has been previously observed in other reservoirs and lakes [69,70]. The novelty here lies in the detailed study of seasonal and vertical variations in zooplankton diversity in relation to environmental conditions in the Streževu Reservoir. By using the Shannon diversity index, a quantitative measure of biodiversity is provided that highlights the influence of seasonal changes, with a notable contrast between fall and summer. This insight into the impacts of transitional seasons on species dynamics emphasizes the sensitivity of aquatic organisms to temperature and light fluctuations and highlights the complexity of ecological interactions.

Copepoda dominated in spring and autumn, while Rotifera prevailed in summer. This can be explained by several ecological factors, including food availability, temperature, and reproductive cycles. Copepods are often more abundant in the cooler spring and autumn months because they tend to thrive in these temperatures. Copepods have slower reproductive rates in warmer waters, so the cooler temperatures of spring and autumn may provide more favorable conditions for their growth and reproduction [66,68]. Rotifers tend to dominate during the warmer summer months because they reproduce more quickly in warmer waters. The increased temperatures in summer allow for faster metabolic rates, which leads to rapid population growth. Additionally, rotifers are generally better adapted to handle the stress of the warm, nutrient-rich environments which are typical in summer [71]. The low abundance and diversity of Cladocera in the Streževu indicate a high predation pressure. Namely, cladocerans, being slow swimmers, were the first selected by fish predators, and thus cladocerans populations declined first in habitats

with high fish predation [72]. Zooplankton biomass followed similar seasonal and vertical trends as abundance, and similar significant differences were observed.

The increase in zooplankton abundance typically lags behind the increase in phytoplankton, caused by the rise in phytoplankton biomass due to increased food availability [73]. Despite the fact that the rotifer fauna is the most diverse component of the zooplankton in this reservoir, the abundance of pelagic rotifer species was relatively low. According to field investigations and laboratory studies conducted by many authors, rotifer distribution in lakes is related to both abiotic factors, such as temperature [74] and oxygen content [75], as well as biotic factors, such as food availability [76] and predation [73].

#### *4.6. Zoo-IQ Index: A Tool for Assessing Water Quality*

The Zoo-IQ index, which we tested based on the collected data, showed good water column quality in the Streževno reservoir. Thus, the Zoo-IQ is very useful biological index that use zooplankton to reflect ecological changes in lentic aquatic environments. Its basis in the analysis of the zooplankton community enables a differentiated understanding of aquatic ecosystems, as zooplankton is an essential component of the food web and is highly responsive to environmental changes. By assessing the zooplankton (Rotifera, Cladocera, and Copepoda) abundance, biomass, and size categories of Cladocera, the Zoo-IQ gives a snapshot of the water quality, since zooplankton communities are sensitive to changes in their environment, particularly factors such as nutrient pollution, oxygen levels, and temperature [42]. In addition, long-term application of the Zoo-IQ index can help establish baseline conditions, track seasonal and annual changes, and implement adaptive management strategies for the reservoir. This biological index not only improves our understanding of ecological dynamics, but also helps to make informed decisions for conservation and restoration actions, ensuring the sustainability of freshwater resources in the face of changing environmental conditions.

## **5. Conclusions**

Policymakers and researchers are facing the challenge of developing balanced approaches to ensure sound exit strategies, given the complex nature of reservoirs. In particular, long-term monitoring of reservoir conditions would provide valuable insights to assist in future policy development and lay the foundations for proactive reservoir management. The zooplankton community in the Streževno Reservoir is intricately tied to the trophic level of the system (which influences food resources), the availability of food (especially phytoplankton), and the dynamic biotic interactions (such as predation and competition) that shape the assemblages across different seasons and depth zones. It is of great importance to maintain the good condition of the Streževno Reservoir and mitigate the processes of eutrophication. In this regard, it is essential to undertake measures to eliminate the causes leading to eutrophication, such as restricting nutrient influx at the reservoir's entrance. Each reservoir has unique ecological characteristics and responds to specific needs of the local communities, so there is no one-size-fits-all solution, nor should there be. Developing a strategy to address the aging of reservoirs is one way to extend the lifespans of these extremely important and sensitive water bodies. The novelty of this study lies in the detailed assessment of seasonal and vertical dynamics of temperature, dissolved oxygen, phosphorus, chlorophyll *a*, and zooplankton communities, which were systematically analyzed for the first time in the Streževno Reservoir. The study presents significant quantitative results, such as the marked differences in temperature and dissolved oxygen levels between seasons; the low species diversity of zooplankton, with emphasis on the dominance and abundance of certain taxa; and the seasonal variation in zooplankton biomass and community composition. The application of advanced statistical analyses, including NMDS and RDA, highlights

the influences of environmental factors on zooplankton distribution. In addition, the consistent assessment of water quality through the Zoo-IQ index provides a novel, integrated metric that indicates good reservoir conditions across seasons, emphasizing the importance of zooplankton as an ecological indicator for freshwater management.

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