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ECOLOGICAL STATUS ASSESSMENT OF LAKE PRESPA BASED ON THE MACROZOOBENTHOS FAUNA

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ABSTRACT

Introduction: *Lake Prespa, a transboundary freshwater ecosystem of high ecological value, is increasingly affected by anthropogenic pressures and fluctuating water levels. This study assesses the ecological status of its littoral zone (North Macedonian side) based on the composition, diversity and distribution of macrozoobenthic communities.*
Methods and results: *Sampling was carried out in autumn 2022 at three sites: Oteshevo, Nakolec and Krani. The research was conducted using standard limnological methods and in accordance with the EU Directive. Samples were collected using a van Ven grab (225 cm^2) at various depths and habitats, using vertical transects extending to the limit of macrophytic vegetation.A total of eleven species from seven systematic groups were registered: Oligochaeta, Hirudinea, Gastropoda, Bivalvia, Amphipoda, Isopoda and Insecta. Nakolec and Krani recorded higher diversity (8 species each), while Oteshevo had only 5. Macrozoobenthos density ranged from 1250 ind/m² (Oteshevo) to 3250 ind/m² (Nakolec), with* Dreissena carinata *peaking at 1875 ind/m² at 2 m depth in Nakolec. A clear decline in biodiversity with depth was observed.*
Discussion: *The results indicate that the invertebrate biodiversity decreases with depth, likely due to increasing sediment uniformity and greater stability of environmental factors with depth. Continued monitoring is recommended for long-term ecological assessment.*

Keywords: macrozoobenthos, biodiversity, ecological status, Lake Prespa, anthropogenic impact.

INTRODUCTION

Benthic macroinvertebrate community structure is most effectively assessed through biodiversity metrics, which encompass both species richness and the relative abundance of taxa within a given assemblage (Rosenberg & Resh 1993; Bonada et al. 2006).

In lake ecosystems, benthic communities are highly responsive to environmental gradients and anthropogenic pressures, with changes typically manifesting in the qualitative and quantitative composition of macrozoobenthos populations (Johnson et al. 2006). This study focused on evaluating structural indices—such as species richness, diversity, and evenness—of benthic fauna across distinct habitats within the littoral zone of Lake Prespa. Sampling was conducted at three littoral localities in the Macedonian part of the lake: Oteshevo, Nakolec, and Krani. These sites were selected based on habitat heterogeneity and exposure to varying degrees of anthropogenic influence.

The research contributes to long-term ecological monitoring and conservation efforts, particularly under conditions of climate variability and nutrient enrichment, which pose increasing threats to Lake Prespa. By analyzing benthic community structure and biodiversity patterns, the study provides critical insights into the ecological integrity of Lake Prespa and supports the development of bioindicator-based management strategies tailored to the lake's unique limnological characteristics.

LITERATURE REVIEW

Earlier studies (2020–2023) showed a strong decline in macrozoobenthos diversity and abundance in Lake Prespa due to water-level fluctuations (Trajanovski et al. 2023). Research from 2023 confirmed this trend across all surveyed localities and again identified *Dreissena carinata* as the dominant species (Trajanovski et al. 2024; Budzakoska-Gjoreska et al. 2024; 2024a). These findings highlight global warming and changes in trophic status as key factors driving species shifts in the lake.

There is limited information on how benthic communities differ across littoral habitats in Lake Prespa, especially among the Macedonian sites Oteshevo, Nakolec and Krani. This study fills that gap by comparing species richness, diversity and evenness across these localities.

METHODS

The field research took place in three localities (Oteshevo, Nakolec and Krani) which, according to previous research, are characterised by varying intensities of anthropogenic pressure and changes in ecological status. The study was designed as a comparative ecological assessment of macrozoobenthic community structures across three distinct littoral habitats in Lake Prespa.The research was conducted using standard limnological methods (Lind 1985; Wetzel 1975; Wetzel & Likens 1979) and in accordance with the EU Directive. Samples were collected using a van Ven grab (225 cm^2) from various depths and habitats with the method of vertical transects extending to the limit of macrophytic vegetation in Lake Prespa. The density values refer to the average density of individuals from two samples per unit surface area (m^2). Macrozobenthos species were determined using keys according to the following authors: Hrabe (1941); Šapkarev (1975); Barnes (1980); Lukin (1976); Lind (1985); Brinkhurst& Jamieson (1978); Hadčišče (1953; 1958 II, 1958 III); Radoman (1983), Kerovec (1986), Kellogg (1994). The determination was done to the level of species. The processed material was preserved in 96% ethyl alcohol, labeled and stored in plastic vials.

To evaluate biodiversity and ecological status, several structural indices were calculated, including species richness, diversity, and evenness.

1) *Index of Species Richness,* according to Margalef (1958), allows the measurement of species richness and the presence of each species in the community, where *n* is the total number of organisms (individuals) of each species, while *N* represents the total number of organisms (individuals) of all macrozoobenthos species in the sample.

$$d = \frac{\sum n(n-1)}{N(N-1)}$$

2) The diversity index (H') according the Shannon-Wiener's formula takes into accountspecies diversityofall species in the community. *S* represents the number of species in the benthic macrozoobenthos communities; *N* is the total number of individuals in the sample, while n_i is the total number of individuals of each macrozoobenthos species.

$$H' = -\sum_{i=1}^{S}\left(\frac{n_i}{N}\right)\ln\left(\frac{n_i}{N}\right)$$ where $N = \Sigma n_i$ or H= - sum (pi·ln(pi)

3) The index of evenness $J_{(e)}$ is calculated according to the formula of Pielou (1966). Using species richness (S) and Shanon-Wiener index (H') we can calculate how similar the density of different types of macrozoobenthos community is. When all registered species have similar densities, the value of the index of evenness equals1, and when species have different densities (some rareand some frequent) the index`s value increases.

$$J_{(e)} = \frac{H'}{\ln S}$$

ASPT (Average Score Per Taxon) represents the average tolerance score of all taxa within the community and is calculated by dividing the BMWP (Table-6 overleaf) by the number of families represented in the sample (Mandaville 2002).

RESULTS

During the field activities at the beginning of December 2022, the qualitative and quantitative composition of the benthic fauna at the Oteshevo locality was assessed. A total of six species belonging to five classes of bottom fauna (Oligochaeta, Hiridinea, Gastropoda, Amphipoda and Insecta) were registered in the samples (Tab.1). The species representing these five classes are indicators of increased water trophic level, indicating the presence of organic pollution in the water. The highest biodiversity, with four species, was recorded at a depth of 2 meters on a muddy bottom with shells and macrophyte vegetation. In terms of density, the highest was found in the population of *Chironomus plumosus* with 375 ind/m^2.

Table 1. Composition and density of bottom fauna in Otesevo (**December** 2022)

Locality Otesevo				
Depth (m)	**Bottom facies**	**Class**	**Species of macrozoobenthos**	**Density (ind/m^2)**
2	Mud with shells and macrophytes (40:50:10%)	Oligochaeta	*Tubifex tubifex*	200
		Gastropoda	*Pyrgohydrobia prespaensis*	25
		Amphipoda	*Gammarus triacanthus prespensis*	175
		Insecta	*Chironomus plumosus*	375
5	Mud withDreissena shells (40:60%)	Oligochaeta	*Tubifex tubifex*	50
		Hirudinea	*Erpobdella octoculata*	25
		Insecta	*Chironomus plumosus*	325
10	Mud with shells (95:5%)	Insecta	*Chironomus plumosus*	75

Table 2. Composition and density of bottom fauna in Nakolec (November 2022)

Locality Nakolec				
Depth (m)	**Bottom facies**	**Class**	**Species of macrozoobenthos**	**Density (ind/m^2)**
2	Mud with shells and macrophyte vegetation	Oligochaeta	*Pothamotrix hammoniensis*	175
			Limnodrilus hoffmeisteri	25
		Bivalvia	*Dreissena carinata*	1875
		Hirudinea	*Erpobdella octoculata*	25
		Amphipoda	*Gammarus triacanthus prespensis*	575
		Insecta	*Chironomus plumosus*	50
5	Mud with shells (1:1)	Oligochaeta	*Limnodrilus hoffmeisteri*	100
			Eiseniella tetraedra	125
		Gastropoda	*Valvata piscinalis*	25
		Bivalvia	*Dreissena carinata*	100
		Amphipoda	*Gammarus triacanthus prespensis*	25
		Insecta	*Chironomus plumosus*	75
10	Mud (90% mud, 10% shells of Dreissena)	Oligochaeta	*Pothamotrix hammoniensis*	50
		Insecta	*Chironomus plumosus*	25

The bottom fauna at the Nakolec locality was represented by eight taxa from six classes of organisms: Oligochaeta, Gastropoda, Bivalvia, Amphipoda, Hirudinea and Insecta. Although the bottom of this profile along the transect showed a fair degree of uniformity (mud and shell facies), the distribution of species was not uniform. Thus, the highest density of organisms was observed at a depth of 2 meters, on a silty facies of crushed shells and snails with vegetation. Six species from five classes were registered on this facies. Quantitatively, the species *Dreissena carinata* was present with the highest density - 1875 ind/m^2.

Table 3. Composition and density of bottom fauna in Krani (November 2022)

Locality Krani				
Depth (m)	**Bottom facies**	**Class**	**Species of macrozoobenthos**	**Density (ind/m^2)**
2	Mud with shells	Oligochaeta	*Pothamotrix hammoniensis*	150
			Limnodrilus hoffmeisteri	25
		Amphipoda	*Gammarus triacanthus prespensis*	25
		Insecta	*Chironomus plumosus*	50
5	Mud with shells (50-50 %)	Oligochaeta	*Limnodrilus hoffmeisteri*	75
		Bivalvia	*Dreissena carinata*	925
		Amphipoda	*Gammarus triacanthusprespensis*	50
		Isopoda	*Asellus aquaticus*	75
		Insecta	*Chironomus plumosus*	50
10.5	Mud with shells (80-20 %)	Oligochaeta	*Pothamotrix hammoniensis*	150
		Gastropoda	*Valvata piscinalis*	25
			Pyrgohydrobia prespensis	25
		Amphipoda	*Gammarus triacanthus prespensis*	25
		Insecta	*Chironomus plumosus*	75

During the autumn campaign, the bottom fauna at the Krani locality was represented by eight taxa from six groups of organisms: Oligochaeta, Gastropoda, Bivalvia, Amphipoda, Isopoda and Insecta. The highest density of organisms was recorded at a depth of 5 meters on a silty facies with shells, where five species were determinate. The most abundant species was *Dreissena carinata*, with a population density of 925 ind/m².

A total of eleven species from seven systematic groups were registered: Oligochaeta, Hirudinea, Gastropoda, Bivalvia, Amphipoda, Isopoda and Insecta (Table 4).

Table 4:**List of macrozoobenthos species composition in Prespa Lake** (**autumn** 2022)

Class	Species
Oligochaeta	*Pothamotrix hammoniensis*
	Limnodrilus hoffmeisteri
	Tubifex tubifex
	Eiseniella tetraedra
Bivalvia	*Dreissena carinata*
Hirudinea	*Erpobdella octoculata*
Amphipoda	*Gammarus triacanthus prespensis*
Insecta	*Chironomus plumosus*
Gastropoda	*Pyrgohydrobia prespaensis*
	Valvata piscinalis
Isopoda	*Asellus aquaticus*

Table 5 presents the ecological status of the sampling sites as assessed by traditional diversity indices. While the Pielou evenness and Simpson diversity indices suggest a wide range of environmental conditions—from very good to poor—their outputs appear inconsistent with other key indicators of ecological degradation.

Table 5. Ecological status of localities according to the indices (autumn 2022)
* Red: very heavily polluted waters; Orange: heavily polluted waters; Yellow: medium polluted waters; Green: slightly polluted waters; Blue: clean unpolluted waters.

Indexes	Otesevo	Nakolec	Krani
N(ind/m^2)	1250	3250	1725
Number of species S	5	8	8
Shannon&Weaner H	2.5	2.2	2.57
Margalef d	2.26	3.7	4.02
Pielou e	0.83	0.58	0.38
Simpson c	0.21	0.37	0.31

The ASPT index served as a sensitive and ecologically meaningful metric for assessing biological integrity, particularly in nutrient-enriched systems. According to its scoring thresholds, none of the surveyed localities met the criteria for good ecological status. Notably, the site at Oteshevo was classified as having a bad ecological status, while the remaining localities were characterized by poor ecological status (Table 6). These findings further reinforce the limitations of traditional diversity indices and underscore the diagnostic value of ASPT in capturing anthropogenic impacts that may be obscured by structural metrics alone.

Table 6. Ecological status of localities according to the ASPT index (autumn 2022)
*Red: bad ecological status; Orange: poor ecological status

Index	Otesevo	Nakolec	Krani
ASPT			

Figure 1. View of an autumn sample from the Nakolec locality at a depth of 5 meters.

DISCUSSION

Macrophytes and macrozoobenthos are important for structure and function of aquatic ecosystems (Varadinova et al. 2023). Aquatic macrophytes increase habitat diversity and provide refuge for invertebrates, thus impacting their species composition and abundance (Pinto et al. 2006). Benthic macroinvertebrates are more sensitive to organic pollution, as they are directly affected by variations in oxygen levels. Anthropogenic stressors, such as altered hydrological regime from hydropower, organic pollution, and nitrogen loads in its inorganic forms, appear to have a considerable effect on river aquatic macrophyte communities (Varadinova et al. 2023).

Macroinvertebrate metrics are useful tools for assessing water quality and the overall health of aquatic ecosystem. Freshwater macroinvertebrate taxa vary in response to organic pollution; thus, their diversity and community composition have been used to make inferences about pollution loads (Tampo et al 2021). Additionally, zoobenthos provide carbon dioxide to promote photosynthesis and improve sediment soil quality, fostering macrophyte growth (Tan et al. 2017).

Table 5 presents the ecological status of the sampling sites as assessed by traditional diversity indices. While the Pielou evenness and Simpson diversity indices suggest a wide range of environmental conditions—from very good to poor—their outputs appear inconsistent with other key indicators of ecological degradation. In contrast, the values of the Shannon–Weaver diversity index (H) and the Margalef richness index (d) provide a more realistic reflection of the actual ecological status across sites. These latter indices better align with the elevated total phosphorus concentrations and the macrophyte-based index (Schneider et al. 2020; Trajanovska et al. 2014,Budzakoska-Gjoreska et al. 2024; Trajanovski et al. 2023), both of which indicate eutrophication and declining ecological integrity in Lake Prespa. This discrepancy underscores the limitations of relying solely on structural evenness metrics in shallow, nutrient-sensitive systems, where compositional and functional indicators offer a more accurate diagnostic of ecological condition.

To address these limitations, the ASPT (Average Score Per Taxon) index has been applied as a more robust and ecologically meaningful metric. Its reliability is supported by previous research on natural lakes in North Macedonia—including Lake Prespa—where

ASPT has consistently demonstrated strong correspondence with nutrient levels, organic pollution, and biological integrity (Schneider et al. 2014, Trajanovska et al. 2019,Budzakoska-Gjoreska et al. 2024a; Trajanovski et al. 2024). As such, ASPT serves as a critical complementary tool for ecological status assessment, particularly in systems where traditional diversity indices may obscure underlying degradation.

CONCLUSION

This study was limited to autumn sampling at three localities, which may not reflect seasonal or spatial variability. Future research should include sampling across different seasons and additional sites to provide a more comprehensive assessment of ecological dynamics and environmental pressures.

Based on the ASPT (Average Score Per Taxon) index, none of the investigated localities in Lake Prespa during the autumn 2022 campaign achieved good ecological status. The site at Oteshevo was classified as having bad ecological status, while all remaining sites were assessed as poor. These results confirm the ASPT index as a sensitive and reliable bioindicator, capable of revealing ecological degradation that may be overlooked by traditional diversity metrics. Its application reinforces the need for functionally grounded indices in assessing nutrient - enriched freshwater systems.

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