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# CHANGES OF PLANKTON COMPOSITION IN WINTER CONDITIONS IN AN URBAN LAKE

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***Abstract***

*The winter season, characterized by unfavorable conditions for aquatic biota, determined by low temperatures, instigates a series of ecological shifts that intricately shape the phytoplankton and zooplankton communities' structure and function. Our study in Văcărești Lake focuses on the effect of the winter season on plankton communities, highlighting structural and functional changes in these communities. During the winter, environmental parameters significantly changed indicating organic matter decomposition and eutrophic conditions. The winter conditions functioned as ecological stressors on plankton communities. Phytoplankton responded by changing composition, with green algae and diatoms becoming prominent as cyanobacteria declined. Zooplankton, especially Rotifera and Cladocera, showed increased diversity and abundance, while Ciliata and Copepoda decreased. The RDA analysis highlighted phytoplankton's responsiveness to TDS, conductivity, turbidity, pH, and zooplankton's correlation with oxygen and temperature. The Diversity t-test indicated significant changes in both phytoplankton and zooplankton communities in terms of diversity. Zooplankton diversity was higher than phytoplankton, with moderate species composition changes, reflecting cold-tolerant species development and the ecosystem's resilience and adaptability to winter conditions.*

***Key words***: *abundance, cold-season changes, phytoplankton, zooplankton structure, Văcărești Natural Park.*

## INTRODUCTION

Winter poses numerous challenges to the biotic components of aquatic ecosystems, particularly the so structurally diverse communities of the zooplankton. The cold season starting with November heralds a multitude of physicochemical transformations determined by temperature and light that shape the dynamics of these vital processes (Molles, 2016). Water temperature undergoes a significant drop, significantly impacting the metabolic activities of plankton. In tandem with temperature, light emerges as another critical factor, its diminishing intensity intricately linked to the seasonal changes in aquatic ecosystems. The reduced light availability during winter precipitates a decline in phytoplankton production, the primary food source for many zooplankton species. The interaction between temperature and light dynamics makes the biotic response of phytoplankton and zooplankton to winter conditions crucial. This scarcity of food resources can influence the composition and abundance of zooplankton communities, as some species may exhibit adaptations to low food availability. In contrast, others may struggle to find sufficient food. Furthermore, changes in winter conditions in temperate zones have significantly impacted the ice and snow regime. During winter, chlorophyll-a content can reach an average of only 43% of its summer level (Negrete-Garcia et al., 2024). Grazing by zooplankton is responsible for phytoplankton losses and changes in community composition. Due to ongoing climate change, warmer winters may also influence the development of overwintering phytoplankton (Hrycik & Stockwell, 2021). Fott et al., 2020 consider that light regime and water temperature significantly positively influence chlorophyll-a concentrations in the cold season. Instead, zooplankton tend to slow down, influencing their growth rates, feeding behaviors, and overall life cycles. Within the diverse structure of zooplankton, many species show pronounced sensitivity to temperature fluctuations, prompting the evolution of adaptive strategies for survival during winter. These strategies manifest through various forms of resistance, allowing certain zooplankton species to endure harsh conditions. According to Litchman et al., 2013, zooplankton exhibits a high diversity of traits and ecological strategies that impact other trophic levels and the cycles of matter and energy.

Understanding these seasonal shifts provides invaluable insights into the adaptive mechanisms, resilience, and ecological aspects of phytoplankton and zooplankton communities, underscoring the significance of comprehending their responses to the challenges posed by the cold season (Wollschläger et al., 2021; Bramm et al., 2009; Davis & Baird, 2022).

In the seasonal dynamics, the winter period is not only an inactive period of rest but also defines the plankton dynamics of the following year. In temperate eutrophic lakes, there are usually two or more peaks of zooplankton activity during the warm season, typically following the spring and summer/autumn phytoplankton blooms.

Warming climate conditions have led to biodiversity loss, the proliferation of opportunistic and resistant species, and the emergence of invasive species, negatively impacting ecosystems. Ecosystems in temperate zones are particularly affected due to complex seasonal regulation processes and a diminished capacity for resilience (Lenard et al., 2019). However, mild winter temperatures and nutrient availability influence phytoplankton development, demonstrating their temperature sensitivity, which can increase productivity. In urban areas, lakes are exposed to heightened nutrient loading and temperatures, leading to severe phytoplankton blooms. Understanding the role of urbanization and its interaction with climate change in producing these blooms is crucial to preventing negative effects on urban water sources (Li et al., 2023). Consequently, zooplankton peak numbers may vary seasonally and annually, reflecting fluctuations in environmental conditions and food web interactions (Sutton et al., 2021; Jensen, 2019). Our study aimed to identify changes in the structural composition of phyto- and zooplankton, highlighting key species such as *Moina* spp. and *Daphnia* spp., and their potential cascading effects on the overall aquatic food webs. It is known that the winter period directly influences the structure and diversity at the plankton level. Due to the low temperatures, the phytoplankton undergoes significant changes in species composition and density. The changes at this level together with other environmental factors, define the traits of the zooplankton consumers. Our study hypothesizes that winter is a period of decline in plankton communities, characterized by a poor representation of species, even in urban areas, where temperatures are slightly higher as a result of heat islands. We assume that in November, when the cold season starts, we will encounter a higher diversity, with species that have accumulated during the year, while in February both the density and richness of species are much more reduced. This was a pilot study testing the structural changes of the phyto- and zooplankton evaluated at the end of the growing season, late autumn, followed by an evaluation during the cold period of winter and the beginning of the next growing season.

In this aim, we proposed the following (1) to identify the changes in the water physicochemical parameters throughout the cold period, (2) to describe the structure and diversity of phytoplankton and zooplankton communities, (3) to establish the relationships between phyto- and zooplankton and environmental variables and (4) to identify which factors defined the structure of these communities.

## MATERIALS AND METHODS

### *Sample Collection and Laboratory Analyses*

The Văcărești Natural Park has its origins in an unfinished hydrological project, initially conceived as a component of the development of the Dâmbovița River. Before 1989, Ceaușescu planned to turn the area into a recreational place with a lake and sports facilities. The project failed, due to the risk of flooding and the lake basin remained unused thus the area became known as "Văcărești Wetlands". Over the previous 20 years, the abandoned area has been transformed into a unique lacustrine ecosystem that appeared in the former Văcărești Pit. The area has become a habitat for various species of birds, reptiles, and mammals. The place attracted the attention of NGOs and the Ministry of the Environment so, in 2016, "Văcărești Wetlands " was declared a Natural Park. The Văcărești Natural Park became Romania’s first urban natural park and the only protected area in Bucharest. This initiative has been supported and recognized by international environmental organizations, considering the project as an outstanding example of cohabitation between urban and wild nature (Atanasiu et al., 2017; Manea et al., 2013). Văcărești Natural Park (Figure 1) has an area of approximately 200 hectares and is located at an altitude of 60-80 meters (Merciu et al., 2017).

Figure 1. The Văcărești Park map with the sampling points (Google Earth app, 2022)

The park is fed primarily by springs from the Dâmbovița River, which support its aquatic ecosystem. This includes lakes, marshes, extensive reed beds, and areas with rich vegetation such as meadows, willows, and various aquatic plants (Danta, 1993).

Bucharest's climate is characterized by hot summers, cold winters, moderate precipitation, and occasional cold snaps. Văcărești experiences typical urban climate effects, including the urban heat island phenomenon. Under these conditions, the wetlands play a crucial role in moderating temperatures, maintaining local humidity, and creating a more favorable microclimate, despite variability caused by urbanization and seasonal changes. These meteorological factors can influence species distribution within the wetland and affect water quality (Legutko-Kobus et al., 2023).

The park is surrounded by residential areas, commercial zones, and busy roads. However, due to the remnants of old communist-era constructions, the wetland remains relatively isolated. Despite its proximity to urban development, Văcărești has become a wildlife sanctuary, supporting over 100 bird species as well as various mammals and reptiles (Manea et al., 2016).

The area is exposed to urban runoff, including contaminants from roads and waste from surrounding areas, which poses a challenge to the park's water quality, noise, and light pollution.

The sampling took place in November 2021 and February 2022 in six stations to cover a representative proportion of the lake in the evaluation of zooplankton communities (Table 1).

Table 1. The GPS coordinates of the sampling points

| Sampling points | Latitude | Longitude |
|---|---|---|
| S1 | 44°24'7.82"N | 26° 8'18.26"E |
| S2 | 44°24'4.19"N | 26° 8'20.58"E |
| S3 | 44°24'6.48"N | 26° 8'13.76"E |
| S4 | 44°24'9.99"N | 26° 8'13.09"E |
| S5 | 44°24'8.38"N | 26° 8'11.19"E |
| S6 | 4°24'6.58"N | 26° 8'9.71"E |

### *Environmental data*

The measurements of the water physicochemical factors were done using a Hanna Instruments HI 9829 multiparameter. The device is equipped with sensors for temperature - °C, pH, ORP (Oxidation-reduction potential) - mV, % DO (Dissolved Oxygen Saturation); DO (Dissolved Oxygen) - mg/L; Conductivity - µS/cm; TDS (Total Dissolved Solids) - mg/L, Salinity, and turbidity - NTU. In addition, the Secchi disc was used for Depth and Transparency (m) assessment.

### *Phytoplankton sampling*

The phytoplankton sampling was done on the water column using a Schindler-Patalas device (4 L) and mixed into a 10 L bucket. In 500 mL plastic bottles samples were fixed with formaldehyde (4%) and stored for a period of sedimentation. The taxonomical identification was done using a Zeiss inverted microscope for *Cyanobacteria* (Komárek & Anagnostidis, 1998; Komárek & Anagnostidis, 2005*),* *Chlorophyceae* (Ettl & Gärtner, 1983; Ettl & Gärtner, 1988), *Bacillariophyceae* (Krammer & Lange-Bertalot, 1986; Krammer & Lange-Bertalot, 1988; Krammer & Lange-Bertalot, 1991a; Krammer & Lange-Bertalot, 1991b) *Dinophyceae* (Huber-Pestalozzi, 1950), and *Xanthophyceae* (Gottfried, 1976) with an additional check on https://www.algaebase.org 2019). Cell densities (cells/L) were determined at the species or genus level using the Utermöhl method (Edler & Elbrächter, 2010). Three aliquots were analyzed microscopically for each sample.

### *Zooplankton sampling*

The samples were taken on a water column, using a Patalas - Schindler plankton trap (4 L) device, and filtered through a standard plankton net (mesh size 50 µm Ø). The filtered samples were collected in 10 ml bottles, preserved in 4% formalin, kept for 2 weeks for sedimentation, and later filtered for the second time to reduce the excess water. Using a Kolkwitz counting chamber, three aliquots (1 mL) from the final sample were analyzed using a Zeiss inverted microscope for taxonomic determination and counting of individuals for abundance as individuals per liter (ind. $L^{-1}$) estimation according to the Edmonson & Winberg (1971) method. The taxonomic determinations were made using the following taxonomic keys *Ciliata* (Foissner et al., 1991; Foissner et al., 1992; Foissner et al. 1994; Foissner et al., 1995), *Testate Amoeba* or *Testacea* (Bartoš, 1954; Grospietsch, 1972), *Rotifera* (Rudescu, 1960), *Cladocera* (Negrea, 1983). *Copepoda* were identified as the main groups. The juvenile stages were counted together and the adult individuals were classified into *Cyclopida* g. sp.; *Harpacticoida* g. sp. and *Calanoida* g. sp. For both, phytoplankton and zooplankton in the microscopic analyses, three sample aliquots were performed.

### *Statistical analysis*

***Descriptive statistics*** were used to analyze and summarize the data. For this purpose, Minimum (Min), Maximum (Max) Mean, and Standard deviation (n-1) were selected.

The ***IndVal*** measures the strength of the association of a species with period environment conditions, based on abundance and frequency depending on the site and period.

The results include: ***IndVal %*** - is the percentage representation of the IndVal, revealing the relative importance of the species in the ecosystem context. The mathematical formula:

$$IndVal\ \% = \frac{\text{INDVALij}}{\sum \text{INDVAL}} \text{ x } 100$$

IndVal is calculated from the following formula:

$$INDVAL_{ij} = 100A_{ij}B_{ij}$$

The indicator value of species *i* in group *j*, values range from 0 to 100 (percentage). ***p -*** indicates the statistical significance of the association. A value under $p < 0.05$ suggests a strong significant association, while a higher value indicates no significance. The p-value (statistical significance) of the indicator values is assessed through 9999 permutations of sites across groups.

***Specificity (A) -*** refers to how exclusively a species is found in a particular habitat. The mathematical formula:

$$A_{ij} = N_{ij}/N$$

where:

- Nij - is the mean of individuals of species *i* across sites in group *j*;
- Ni is the sum of the mean numbers of individuals of species *i* over all groups.

A value of 1.00 indicates that the species is only found in that habitat, while lower values indicate broader habitat use.

***Fidelity (B)*** represents the reliability of the species as an indicator of the habitat.

The mathematical formula:

$$B_{ij} = Nsites_{ij}/Nsites_j$$

where:

- $Nsites_{ij}$ - is the number of sites in group j where species *i* is present;
- $Nsites_j$ is the total number of sites in group j. A value of 1.00 indicates the species is consistently found in that habitat when it is present.

The analysis was performed using Past 4.13 software (Hammer & Harper, 2024).

***Diversity indices***, such as Species richness (SR), Shannon index (H), Evenness (E), and Simpson index (D) are commonly applied measures for biodiversity and are crucial to quantifying the variety and distribution of species within a community. ***Species richness*** refers to the total number of different taxa (species).

***Simpson's Dominance Index*** quantifies how dominant certain species are within a community. A higher value indicates lower diversity and one or a few species dominate the population.

$$\text{D} = \sum_i \left(\frac{ni}{n}\right)^2$$

where *ni* is the number of individuals of taxon *i*. The index ranges from 0 (infinite diversity) to 1 (no diversity).

The ***Shannon Index (H)*** measures the uncertainty in predicting the species identity of a randomly chosen individual from a community. It takes into account both the number of species and their relative abundances:

$$H = -\sum_i \frac{ni}{n} \ln \frac{ni}{n}$$

where:

- ni is the number of individuals of taxon I;
- n is the sum of all ni.

***Buzas and Gibson's Evenness Index (E)*** is a measure of species' evenness derived from the Shannon diversity index. It quantifies how evenly individuals are distributed among the species in a community, providing insights into biodiversity beyond mere species richness.

$$\mathrm{E} = \frac{e^H}{S}$$

where:

- e - is Euler's number, approximately equal to 2.71;
- H - is the Shannon index;
- S is the total number of taxa.

The evenness index ranges from 0 to 1, where 0 indicates that all individuals belong to one species (complete dominance) and 1 indicates that all species have equal abundance (maximum evenness).

The ***Diversity t-test (Hutcheson's t-test)*** is based on an adapted variant of the classic t-test, allowing for the comparison of Shannon and Simpson indices values between two samples. This test provides an assessment of the statistical significance of observed differences. The formula used in Hutcheson's t-test is similar to that of the standard t-test but includes adjustments for the variance of the Shannon and Simpson indices (Hammer & Harper, 2006).

***Redundancy Analysis (RDA)*** is a multivariate statistical technique used to evaluate the relationships between plankton groups and physicochemical parameters. Before analysis, data were log-transformed to meet the assumptions of the method. The graphical output of RDA includes quantitative environmental variables represented by lines (vectors) and plankton group variables represented by squares. The first two axes, F1 and F2, explain the data's total variation, providing a robust representation of the explanatory variables. The environmental predictors, shown by red arrows, indicate the magnitude and direction of the parameters. The positioning of the squares (representing plankton groups) and the environmental predictors along the different axes illustrate the strength and direction of the relationships between the variables. This visual representation enables the identification of key environmental factors influencing the distribution and composition of plankton communities. The length and orientation of the arrows in the plot signify the magnitude and direction of the environmental gradients, aiding in interpreting complex ecological interactions (Legendre & Legendre, 2012). XLSTAT pro was used for statistical analysis (XLSTAT pro. 2013; PAST software (Hammer et al., 2001).

## RESULTS AND DISCUSSIONS

### *Abiotic parameters*

Environmental parameters showed increasing trends from November to February, except for temperature, oxygen saturation, and ORP. During this period, depth ranged from 1 to 2.35 meters, transparency from 1 to 2 meters, and temperature from 6 to 10.44°C. A redox potential (ORP) variation was also observed, indicating unfavorable ecological conditions. In November, the ORP was positive but low (29.2 mV - 54.3 mV), reflecting organic matter decomposition processes, eutrophic conditions, and high oxygen consumption. By February, the decrease in oxygen content was associated with negative ORP values (Table 2), suggesting ongoing decomposition processes through reducing mechanisms. These conditions can negatively affect both biota and natural nutrient cycling processes.

Table 2. Descriptive statistics, mean, variance, and standard deviation of the measured water environmental parameters

| **Statistic** | **Min** | **Max** | **Mean** | **Standard deviation (n-1)** |
|---|---|---|---|---|
| | **November** | | | |
| Depth (m) | 1.00 | 2.00 | 1.48 | 0.50 |
| Transparency (m) | 1.00 | 1.70 | 1.21 | 0.31 |
| Turbidity (FNU) | 0.71 | 1.93 | 1.26 | 0.48 |
| Temperature (°C) | 9.45 | 10.44 | 9.85 | 0.36 |
| pH | 7.45 | 9.54 | 8.37 | 0.71 |
| $O_2$ sat % | 78.70 | 110.80 | 95.18 | 12.99 |

| Statistic | Min | Max | Mean | Standard deviation (n-1) |
|---|---|---|---|---|
| $DO_2$ (ppm) | 8.82 | 12.38 | 10.60 | 1.48 |
| Cond. (µS $cm^{-1}$) | 797.00 | 823.00 | 815.83 | 9.85 |
| TDS (ppm) | 398.00 | 412.00 | 407.83 | 5.23 |
| Salinity | 0.39 | 0.41 | 0.41 | 0.01 |
| ORP | 29.20 | 54.30 | 38.78 | 9.73 |
| | February | | | |
| Depth (m) | 1.55 | 2.35 | 2.03 | 0.27 |
| Transparency (m) | 1.50 | 2.00 | 1.77 | 0.21 |
| Turbidity (FNU) | 1.38 | 2.38 | 1.80 | 0.38 |
| Temperature (°C) | 6.00 | 6.38 | 6.26 | 0.13 |
| pH | 8.58 | 9.45 | 9.21 | 0.32 |
| $O_2$ sat % | 59.10 | 122.00 | 80.52 | 25.01 |
| $DO_2$ (ppm) | 7.06 | 15.63 | 11.01 | 3.63 |
| Cond. (µS $cm^{-1}$) | 864.00 | 891.00 | 879.83 | 9.28 |
| TDS (ppm) | 439.00 | 446.00 | 441.83 | 2.64 |
| Salinity | 0.43 | 0.44 | 0.44 | 0.01 |
| ORP | -12.40 | 49.30 | 20.53 | 26.21 |

### *Phytoplankton community structure*

A total of 43 phytoplankton species were recorded, including *Cyanobacteria* (2 species), *Dinophyceae* (1 species), *Xanthophyceae* (1 species), *Bacillariophyceae* (29 species), and *Chlorophyceae* (10 species). While the number of species remained constant during the winter (Table 2), their taxonomic composition and abundance changed. For instance, 2 species of *Cyanobacteria* were present in November (*Chroococcus turgidus* Nägeli, 1849, and *Gomphosphaeria aponina* Kützing, 1836) and no longer found in February. By February, new green algae and diatom species appeared alongside *Dinophyceae* (*Peridinium* sp.) and *Xanthophyceae* (*Tribonema affine* (Kütz.) G.S.West, 1904). The variability in the taxonomic composition and abundance of species like *Chroococcus turgidus* and *Gomphosphaeria aponina* during winter is influenced by several factors. Winter temperatures can reduce the growth of *Cyanobacteria*, which prefer warmer conditions. Reduced daylight limits photosynthesis, impacting phytoplankton. Seasonal nutrient changes and cooler temperatures can favor the growth of species like *Peridinium* and *Tribonema affine*, which appear in February.

In November, the *Cyanobacteria species* reached the algal bloom ($1.04 \times 10^7$ cells $L^{-1}$) threshold, followed by diatoms (9.67Ex$10^5$ cells $L^{-1}$). During the winter, *Bacillariophyceae* and *Chlorophyceae* dominated in species richness and abundance, showing significant increases in density until February (Table 3).

Table 3. The species richness and density of the phytoplankton groups

| | Species richness | | Density (cells $L^{-1}$) | |
|---|---|---|---|---|
| | Nov. | Feb. | Nov. | Feb. |
| Total phytoplankton | 27.0 | 27.0 | 1.15 x$10^7$ | 6.97 x$10^6$ |
| *Cyanobacteria* | 2.0 | 0.0 | 1.04 x$10^7$ | 0 |
| *Dinophyceae* | 0.0 | 1.0 | 0 | 9.10 x$10^3$ |
| *Xanthophyceae* | 0.0 | 1.0 | 0 | 2.19 x$10^6$ |
| *Bacillariophyceae* | 19.0 | 19.0 | 9.67 x$10^5$ | 3.85 x$10^6$ |
| *Chlorophyceae* | 6.0 | 6.0 | 1.19 x$10^5$ | 9.20 x$10^5$ |

Based on IndVal analysis, February generally exhibits a stronger association and higher indicator values for species, suggesting distinct ecological conditions compared to November. In November, *Epithemia zebra* and *Cocconeis placentula* had high IndVal values (72.97 and 70.23, respectively), indicating the species were strongly associated with the period ecological conditions.

In February, a higher number of species presented elevated IndVal values, most of them diatoms thus highlighting the capacity to exhibit several specific adaptations that enable them to thrive in cold periods (Table 4). These adaptations are crucial for their survival and competitive success during winter months when temperatures drop and light conditions change.

Table 4. Significant results of Indicator Species analysis (IndVal) of phytoplankton assemblage

| | Group | IndVal% | p |
|---|---|---|---|
| | November | | |
| *Epithemia zebra* (Ehr.) Kütz. | *Bacillariophyceae* | 72.97 | 0.03 |
| *Cocconeis placentula* Ehrenberg, 1838 | *Bacillariophyceae* | 70.23 | 0.04 |
| *Gomphosphaeria aponina* Kützing 1836 | *Cyanobacteria* | 66.67 | 0.03 |
| | February | | |
| *Synedra ulna* (Nitzsch.) Ehrenberg, 1832 | *Bacillariophyceae* | 100.00 | 0.00 |
| *Gomphonema ventricosum* W.Gregory, 1856 | *Bacillariophyceae* | 96.67 | 0.00 |
| *Fragillaria intermedia* (Grunow) Grunow, 1881 | *Bacillariophyceae* | 89.92 | 0.00 |
| *Synedra affinis* Kützing, 1844 | *Bacillariophyceae* | 83.33 | 0.01 |
| Mougeotia sp. | *Chlorophyceae* | 76.34 | 0.00 |
| *Gomphonema constrictum* Ehrenberg, 1832 | *Bacillariophyceae* | 66.67 | 0.03 |
| *Navicula pupula* Kützing, 1844 | *Bacillariophyceae* | 66.67 | 0.03 |
| *Synedra acus* Kützing, 1844 | *Bacillariophyceae* | 66.67 | 0.03 |
| *Neidium dubium* (Ehr.) Cleve, 1894 | *Bacillariophyceae* | 66.67 | 0.03 |
| *Navicula longirostris* Hustedt, 1930 | *Bacillariophyceae* | 66.67 | 0.03 |

***Zooplankton community structure***

The zooplankton assemblage consisted of 40 taxa, with *Rotifera* (20 species) and *Cladocera* (14 species) being the most representative, both showing an increase from November to February (Table 5).

*Ciliata* (1 species) and *Testacea* (2 species) were less representative, while *Copepoda* (comprising three groups: *Calanoida*, *Harpacticoida*, and *Cyclopoida*) was present with a high proportion of juvenile stages. In terms of abundance, the zooplankton in November was characterized by few species with high abundance. Total Zooplankton species richness and densities indicate an important increase from November (521.32 ind. $L^{-1}$) to February (1025.65 ind. $L^{-1}$), reflecting cold-tolerant species development. *Ciliata* and *Testacea* showed low densities, with *Ciliata* absent in February and *Testacea* exhibited a significant increase during the winter (from 0.10 to 9.97 ind. $L^{-1}$). *Rotifera* was the dominant group, with an important growth from November (207.20 ind. $L^{-1}$) to February (764.20 ind. $L^{-1}$), indicating favorable conditions for their proliferation during this period. *Cladocera* densities increased slightly from November (64.35 ind. $L^{-1}$) to February (89.17 ind. $L^{-1}$), while *Copepoda* densities saw a decrease from November (249.43 ind. $L^{-1}$) to February (162.31 ind. $L^{-1}$), suggesting varying ecological responses among different taxa (Table 5).

Table 5. Zooplankton composition in Văcărești Natural Park Lake

| | Nov. | Feb. | Nov. | Feb. |
|---|---|---|---|---|
| | **Species richness** | | **Density (ind. $L^{-1}$)** | |
| Total zooplankton | 23.0 | 32.0 | 521.32 | 1025.65 |
| *Ciliata* | 1.0 | 0.0 | 0.25 | 0.00 |
| *Testacea* | 1.0 | 2.0 | 0.10 | 9.97 |
| *Rotifera* | 10.0 | 18.0 | 207.20 | 764.20 |
| *Cladocera* | 8.0 | 11.0 | 64.35 | 89.17 |
| *Copepoda* | 3.0 | 1.0 | 249.43 | 162.31 |

Among the species that formed zooplankton assemblages, few exhibited significant specificity ($p < 0.05$) for a particular study period. In November, the highest IndVal index percentages (Table 6) were observed for the cladocerans *Daphnia* sp. and *Moina* sp., as well as the *Calanoida* copepod group. In contrast, only rotifers were found in February. During this month, environmental conditions favored the proliferation of *Synchaeta oblonga* Ehrenberg, 1832, which attained the highest IndVal index percentage (98.90%), followed by *Ascomorpha* sp. (66.67%).

***Plankton diversity***

The Diversity t-test highlights significant changes in both the phytoplankton and zooplankton communities in terms of diversity during the winter. Of both indices (Shannon and Simpson), t-values and p-values indicate significant differences between the two periods (Table 7).

Table 6. Significant results of Indicator Species analysis (IndVal) of zooplankton assemblage

| | Group | IndVal% | p(raw) |
|---|---|---|---|
| **November** | | | |
| *Moina* spp. | *Cladocera* | 62.09 | 0.03 |
| *Calanoida* | *Copepoda* | 91.18 | 0.00 |
| *Daphnia* spp. | *Cladocera* | 100.00 | 0.00 |
| **February** | | | |
| *Synchaeta oblonga* Ehrenberg, 1832 | *Rotifera* | 98.90 | 0.00 |
| *Ascomorpha* sp. | *Rotifera* | 66.67 | 0.03 |

Table 7. Diversity t-test ($p < 0.05$) for phytoplankton and zooplankton communities during the study period

| | **Phytoplankton** | | **Zooplankton** | |
|---|---|---|---|---|
| | **Nov.** | **Feb.** | **Nov.** | **Feb.** |
| **Shannon index** | 0.56 | 1.80 | 1.69 | 2.17 |
| Variance | $1.81 \times 10^{-7}$ | | 0.0023 | |
| t | -1845.30 | | -7.83 | |
| df | $1.53 \times 10^{7}$ | | 1142.50 | |
| p | 0.00 | $2.71 \times 10^{-7}$ | 0.00 | 0.0014 |
| **Simpson index** | 0.82 | 0.27 | 0.25 | 0.18 |
| Variance | $2.40 \times 10^{-8}$ | | 0.0001 | |
| t | $2.83 \times 10^{3}$ | | 5.72 | |
| df | $1.85 \times 10^{7}$ | | 956.32 | |
| p | 0.00 | $1.35 \times 10^{-8}$ | 0.00 | $5.08 \times 10^{-5}$ |

The phytoplankton Shannon Index showed a significant increase from November (0.56) to February (1.80), indicating a rise in diversity. Conversely, the Simpson Index decreased from November (0.82) to February (0.27), further supporting the observed increase in diversity. On the other hand, the zooplankton showed a higher diversity, compared with phytoplankton, with moderate changes regarding the species composition.

The results of the Diversity t-test indicate that there have been changes in the structure and diversity of the two planktonic components. However, to obtain a complete overview, it is necessary to identify the response of the communities that form them. Diversity indices, such as Shannon - H; Evenness - E, and Dominance - D are interconnected and provide a robust framework for assessing the health of aquatic ecosystems. These indices not only reflect the state of biological communities but also indicate their trends. Plankton diversity was assessed based on component groups, characterized by various traits related to species richness and abundance.

Shannon index showed that the most diverse were *Bacillariophyceae* (H = 2.26) and *Chlorophyceae* (H = 0.94), while *Cyanobacteria* showed very low diversity (H = 0.01). Until February, all mentioned groups showed a decline (Table 8).

Table 8. Diversity indices Shannon - H; Evenness – E and Dominance – D of phytoplankton and zooplankton groups

| | November | | | February | | |
|---|---|---|---|---|---|---|
| | H | E | D | H | E | D |
| **Phytoplankton** | | | | | | |
| *Bacillariophyceae* | 2.26 | 0.50 | 0.15 | 1.27 | 0.19 | 0.54 |
| *Chlorophyceae* | 1.17 | 0.54 | 0.44 | 0.97 | 0.44 | 0.43 |
| *Cyanobacteria* | 0.01 | 0.51 | 1.00 | NA | NA | NA |
| *Dinophyceae* | NA | NA | NA | ND | ND | ND |
| *Xanthophyceae* | NA | NA | NA | ND | ND | ND |
| **Zooplankton** | | | | | | |
| *Ciliata* | ND | ND | ND | NA | NA | NA |
| *Testacea* | 0.00 | 1.00 | 1.00 | 0.55 | 0.87 | 0.64 |
| *Rotifera* | 0.36 | 0.14 | 0.88 | 1.57 | 0.27 | 0.29 |
| *Cladocera* | 0.94 | 0.32 | 0.61 | 1.11 | 0.28 | 0.44 |
| *Copepoda* | 0.35 | 0.48 | 0.83 | | | |

NA - Not applicable - no species found; ND - not determined – insufficient data to perform the analysis.

At the zooplankton level, the taxonomic groups were characterized by different traits in diversity. *Protozoa* were poorly represented. Notably, *Testacea* shows an interesting trend where its Shannon value increases from near zero to moderate levels (from H = 0 to H = 0.55), while its dominance decreases significantly from D = 1 to D = 0.64, indicating an increase in diversity. Instead, *Rotifera* demonstrates a development highlighted by the increase of the Shannon Index from November (H = 0.36) to February (H = 1.57), with a corresponding decrease in dominance (from D = 0.88 to D = 0.29). *Cladocera* shows slight improvement in both months but remains relatively low in Evenness and high in Dominance. *Copepoda* indicates a decline during the period (Table 8). The Evenness index varies significantly across categories and periods.

Species groups with low diversity showed high evenness values (Table 8), whereas groups with high Shannon values exhibited low evenness values. This indicates that communities with higher diversity were dominated by one or a few species.

### *The response of plankton communities to environmental conditions*

The environmental conditions during the two periods functioned as ecological stressors on plankton communities. The relationships between environmental variables and plankton communities were established through RDA analysis.

Axes F1 (56.67%) and F2 (21.97%) together explain 78.63% of the total variation in the data. In Redundancy Analysis (RDA), F1 (56.67%) and F2 (21.97%) represent the first two *constrained canonical axes* that capture most of the variation in the biological response variables (species communities) explained by the environmental predictors in the model. Phytoplankton groups, including *Cyanobacteria*, *Chlorophyceae*, *Bacillariophyceae*, and *Xanthophyceae*, exhibited a dispersed distribution in the plot, contrasting with the more cohesive grouping of zooplankton (such as *Cladocera*, *Copepoda*, and *Rotifera*). This difference in dispersion may suggest that phytoplankton and zooplankton employ distinct ecological strategies in response to environmental pressures.

Phytoplankton were more responsive to variations in parameters such as TDS, conductivity, turbidity, and pH, reflecting nutrient availability. Zooplankton (*Cladocera*, *Copepoda*, and *Ciliata*) were closely correlated with oxygen and temperature conditions, indicating a preference for well-oxygenated waters and higher temperatures.

In the RDA biplot, the distribution of physico-chemical parameters highlights an inverse relationship between turbidity and dissolved oxygen (DO%), suggesting that higher turbidity coincides with lower oxygen availability. Conversely, these conditions were unfavorable for *Ciliata*, *Cladocera*, and *Copepoda*. Increases in conductivity, turbidity, and TDS were tolerated by diatoms, green algae, *Dinophyceae* (dinoflagellates), and *Xanthophyceae* (yellow-green algae). The association of *Rotifera* and *Testacea* (testate amoebae) with these parameters reflects the availability of food resources (phytoplankton and detritus) and a negative response to cyanobacterial blooms. Organisms such as *Cladocera* and *Copepoda* could indicate well-oxygenated and good-quality water, while the presence of *Cyanobacteria* may indicate water quality degradation.

*Environmental controlling factors of plankton*

Winter conditions significantly affect phytoplankton and zooplankton communities. Seasonal changes, driven by temperature, light, and nutrient fluctuations, also impact water quality parameters. The evaluated physicochemical parameters (Table 2) indicated active organic matter decomposition. Temperature declines during winter significantly alter environmental conditions, affecting biotic components through interconnected chemical processes. In this period, correlations of TDS, conductivity, turbidity, transparency, and depth with plankton reflected nutrient access for primary producers.

Simultaneously, under these conditions, the zooplanktonic components that demonstrated tolerance, such as rotifers and testate amoebae, were observed (Figure 2). On the other hand, the decrease in oxygen saturation showed negative effects on both phytoplankton and zooplankton communities.

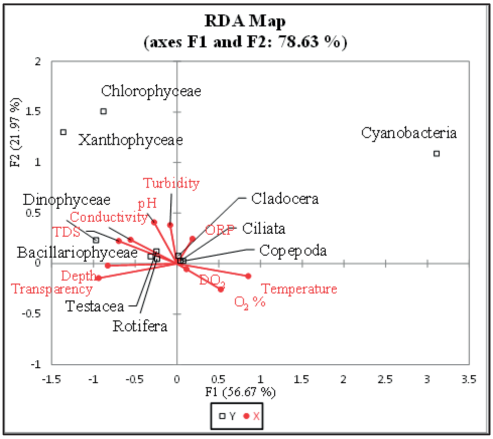

Figure 2. Redundancy analysis (RDA)
for the relationship among the phytoplankton
and zooplankton groups' abundance and environmental
variables in Văcărești Lake

***Plankton diversity***

The dynamics of plankton communities can exhibit distinct patterns throughout the seasons, often characterized by shifts in species composition and abundance. Previous studies in the Văcărești Natural Park, during the growing warm period, have reported the presence of 80 species in phytoplankton and 89 species in zooplankton structure. Shannon's diversity index for phytoplankton was 1.78 and for zooplankton - 3.48 (Florescu et al., 2022). Thus, our results reveal a decline in diversity; species richness, and abundance of planktonic communities (Table 3; Table 5; Table 7; Table 8) during the winter reflecting the different conditions compared with the vegetation period.

Other studies confirm the critical role of these factors during colder months with a negative impact on both phytoplankton and zooplankton communities, leading to reduced biodiversity and altered community structures (Dokulil & Herzig, 2009). Contrary to expected trends, certain species within the planktonic assemblage exhibited tolerance to winter conditions. As a result, with the shift in community composition during the winter, an increase in diversity was observed (Table 7). The increasing species diversity can be due to the reduction in competition from less tolerant species may allow more resilient species to thrive (Geng et al., 2022).

In urban environments like Văcărești, anthropogenic pressures have led to eutrophication and algal blooms. Previous studies emphasized that the phytoplankton in Văcărești Natural Park exhibited seasonal variations with eutrophic and hiper-eutrophic conditions, especially during the autumn The composition of the algal blooms primarily consisted of *Cyanobacteria* and *Chlorophyceae* species (Florescu et al., 2022). The decline of *Cyanobacteria* during the winter period showcased the ecosystem's resilience. This decline could allow other groups like *Dinophyceae* and *Xanthophyceae* to develop, thus maintaining the overall biodiversity and functional stability of the ecosystem. Furthermore, the reduction in cyanobacterial blooms can prevent potential negative effects common during cyanobacteria-dominated periods. The shift in community composition during the winter underscores the ecosystem's ability to self-regulate and support a diverse range of species, despite the adverse conditions.

*The shift of the phytoplankton species during the cold season*

Because phytoplankton assemblages play a crucial role in aquatic ecosystems, species that are strongly associated with particular conditions, make them indicators of an ecological state. Identifying key indicator species and their associations with environmental conditions offers a better understanding of ecological dynamics (Sidding et al., 2016). Given these practical applications, the general utility of IndVal for the analysis of phytoplankton assemblages becomes apparent and highly valuable. Conversely, the presence or absence of a particular single species of organism might be used to indicate chemical or physical attributes of the biotope. The data reflects the shifts in phytoplankton assemblages, with different species dominating. The distinction between November and February groups was determined by driven factors such as temperature, light availability, or nutrient dynamics (Figure 2). Thereby, *Epithemia zebra* (IndVal 72.97, p = 0.030), *Cocconeis placentula* preferred, nutrient-rich conditions typical of November (Table 4). The IndVal results of *Gomphosphaeria aponina* (IndVal 66.67, p = 0.03) confirm their role in cyanobacterial blooms from November (Table 4). Instead, species like *Synedra ulna* (IndVal 100.00, p = 0.00) and *Gomphonema ventricosum* (IndVal 96.67, p = 0.00), were highly indicative of February, pointing to the ecological relevance as strong indicators of winter-specific ecological conditions. The higher number of significant species in the IndVal analysis in February compared to November suggests a distinct phytoplankton community that defines the end of the winter.

The dominance of *Bacillariophyceae* in winter is a complex interplay of ecological adaptations, nutrient dynamics, and climatic influences. The group is generally well-adapted to lower temperatures, allowing the species to thrive even in the cold winter months. Diatoms' contribution to the plankton assemblage was also reflected in the highest species diversity in response to environmental changes during the winter season (Table 8). Their ability to develop in cold conditions allowed them to show their ecological importance as most numerous as significant IndVal species in both periods of investigation (Tabel 4) (Zepernick et al., 2024; Zang et al, 2018).

The *Chlorophyceae* was the second group after *Bacillariophyceae* present in phytoplankton during the entire study. Green algae are often abundant in urban lakes during the cold season. This abundance can be attributed to several factors, including reduced competition from other phytoplankton groups, lower grazing rates, and their ability to thrive (Tapolczai et al., 2015). Similar to other studies, the filamentous green algae *Mougeotia* spp. showed a proliferation. *Mougeotia* spp. tends to bloom earlier in the year when conditions are favorable, such as stable water columns and sufficient nutrient availability. The species is adapted to access the light by the water's surface utilizing its buoyant structures. Consequently, its growth can outcompete other phytoplankton species, leading to its dominance in urban lakes during the cold season and impacting the overall plankton community structure (Tapolczai et al., 2015).

During the study, the temperature dropped along with oxygen levels and redox potential (ORP) inhibited cyanobacteria, leading to their decline (Figure 2; Table 3). The cumulative effect of these factors significantly impacts cyanobacterial populations during the winter months, changing the overall composition and dynamics of the aquatic ecosystem. Most cyanobacterial taxa become less active or enter dormancy during winter, producing specialized cells (akinetes) that can survive adverse conditions until warmer temperatures return (Cottingham et al., 2021; Mânica & de Lima Isaac, 2023).

*The shift of zooplankton species during the cold season*

The changes in structure and composition in the main zooplankton groups depended on the adaptation strategies to the pressures of the winter conditions, their life cycle patterns, and physiological traits. Also, the quality and accessibility of food were important factors (Varpe, 2012). During the study, zooplankton abundance and species richness increased, indicating favorable conditions and opportunities for some species development. Late warm autumn, without ice cover, has significant hints in aquatic organisms with dormant stages, as temperature stimulates production or release from the overwintering stages. The thermal responses of different species vary and are correlated with their seasonal abundance and distribution. Thus, periods of prolonged warm periods have different effects depending on the thermal tolerance limits and could influence survival and wintering strategies to the advantage of some species and the disadvantages of others (Chen & Folt, 1996).

The structural changes observed in the zooplankton communities in urban lakes during winter, leading to improved diversity, can be attributed to the prevalent environmental conditions. In February, the species richness of *Rotifera* and *Cladocera* showed an increase compared cu November. Thus, this period was opportune for other species to grow and distribute more evenly. This phenomenon underscores the intricate relationship between the ecological factors and zooplankton community dynamics. In addition, the capacity for rapid responses to environmental pressures makes the zooplankton a valuable indicator for evaluating the consequences of anthropogenic impacts (Umi et al., 2024). According to Haberman & Haldna (2017), water temperature in temperate shallow lakes has a significant impact on the abundance and composition of zooplankton. The communities have high dynamics of abundance, with a seasonal trend, such as, in spring with higher values than autumn. In addition, the approximately 10°C water temperature was considered "*a critical time window*" for generating a shift in species composition and abundance.

In the Văcărești Lake, the recorded abundance of rotifers during the winter can be explained by the resilience capacity of these communities to the pressure of unfavorable conditions. Rotifers are versatile organisms characterized by a great diversity of survival strategies, that include tolerance to eutrophication and pollutants, a fast reproductive cycle, and a change in feeding regime depending on the trophic resources. The responses of rotifers can be attributed to various factors (Figure 2) that highlight the species' food availability and cosmopolitan features. Rotifers have shown sensitivity to decreased oxygen saturation and ORP but tolerate fluctuations in water turbidity, pH, conductivity, or depth.

The cladoceran life history strategy is more complex and dependent on selective food availability. Also, cladocerans, are generally more sensitive to lower temperatures (Figure 2). Their metabolic rates slow down, and their reproductive rates decrease in colder conditions, leading to lower overall abundance (May & Wallace, 2019; Jensen, 2019). Also, most copepod species exhibit seasonal diapause in winter to avoid unfavorable conditions. This behavior can contribute to our study's observed decrease in adult copepod abundance. However, water temperature (ranged 6 and 10°C) maintained the presence of juvenile stages in Văcărești. Under these conditions, copepods may exhibit a range of responses to changes in temperature and food availability. Some species might adjust the timing of their life cycle to better align with the new environmental conditions.

The response of copepod assemblages to environmental changes was significantly influenced by temperature. Similar to *Cladocera*, copepods' life-history traits, such as reproductive strategy and generation length, are crucial for understanding how these microcrustaceans adjust their life cycles to align with environmental conditions, including temperature shifts. Research indicates that life-history traits, such as reproductive strategy and generation length, are critical for understanding how copepods adjust their life cycles in response to environmental conditions, including temperature shifts. Studies show that temperature variations and food availability interact to influence copepod life stages, which can contribute to observed changes in adult abundance in specific environments such as urban temperate shallow lakes. These factors differentially affect the naupliar and copepodite stages. For instance, nauplii showed reduced survival at elevated temperatures unless provided with phosphorus-replete food, emphasizing the importance of nutrient quality alongside temperature in shaping copepod life stage dynamics. In our study, after autumn phytoplankton growth, nauplii benefit longer from an abundant food source, favoring high development. Thus, they were able to develop in the following stages as well. The interaction of these factors may explain the dynamics of the young stages of the copepods in Văcărești Lake. These findings suggest that climate-driven alterations in microplankton communities could affect copepod survival, particularly during periods of food scarcity, thus influencing overall abundance (Banas et al, 2016; Haberman & Haldna, 2017; Mathews et al, 2018; Halsband-Lenk et al., 2002; El-Sherbiny & Al-Aidaroos, 2021).

Regarding the decline of ciliata, low temperatures significantly decreased their growth rates, with effects on their abundance. The development of heterotrophic protists is affected by a decrease in temperature. Numerous studies have claimed that low temperatures have a strong negative impact on herbivorous protozoa; for example, ciliates prefer higher temperatures (20-25°C) (Rose & Caron, 2007). From this point of view, in our study, it was noted that the low representation of ciliates and testate amoeboids may indicate changes in environmental conditions or population dynamics in the ecosystem.

Thereby, the winter compositional patterns in zooplankton were driven by the fitness of rotifers and copepods juveniles to colder temperatures and their ability to exploit available food resources, whereas other communities as cladocerans, adult copepods, and protozoa were less able to cope with the harsh conditions.

The diversity increases were associated with a change in the key species in the ecosystem. Of the species that showed a significant response in abundance during winter (Table 6), some were advantaged by environmental conditions, such as *Ascomorpha* sp. (November – 0; February – 315.44 indv. $L^{-1}$); *Synchaeta oblonga* (November – 0.42; February – 37.48 indv. $L^{-1}$); while others such as *Calanoida* adults (November – 29.80; February– 2.88); *Daphnia* spp. (November – 50.25; February – 0.00 indv. $L^{-1}$) have gone through a decline. The zooplankton the taxonomic composition and diversity traits during the cold period in our study reflected mainly the known responses to a moderate winter. According to these conditions, Goździejewska & Kruk (2023) emphasized a weak interspecific relationship between rotifers, cladocera, and protozoa during moderate winters. Also, the authors mention that *Ascomorpha* spp., and *Synchaeta* spp. were the representatives that outlined the typical features of the period. Stressors such as temperature and phytoplankton availability significantly influence these dynamics. The interplay between the warming effects, nutrient supply, and grazing varies with seasonality and the ecosystems' long-term history. Concerning temperature and grazing, the decrease in temperature is due to modifications in species composition, and the predominance of smaller species (Goździejewska & Kruk, 2023). Also, the research by paleolimnological analyses based on sediment cores indicated a significant negative correlation between *Daphnia* abundance and winter temperature series (Nevalainen et al., 2013). The mild temperatures and the absence of ice on the surface of the lakes during the winter period would be advantageous for some species and allow them to increase.

In Lake Văcărești during winter, limited food sources shape zooplankton structure by quality and quantity. For copepods, size and abundance were linked, with juveniles driving population numbers and adults contributing to biomass despite lower abundance. As part of zooplankton communities, there are taxonomic groups like *Protozoa*, *Rotifera* and *Crustacea* with distinct features that, through their availability through biomass, can decisively influence the feeding behavior of higher consumers. The seasonal succession of the plankton community described by Sommer et al., 1986 as the PEG model, is found in numerous freshwater ecology studies, focused especially on the growth periods, and less on the winter season. Because winter for many species of zooplankton is a period of dormancy, the season was considered less important. Because of the interest in climate change, this period has gained crucial importance for the seasonal dynamics of plankton communities. Thus, is necessary to understand the dynamics of zooplankton under winter conditions (Jensen, 2019).

## CONCLUSIONS

Winter conditions led to significant changes in environmental parameters, indicating organic matter decomposition and eutrophic conditions. The phytoplankton responded to the environmental changes: green algae and diatoms became more prominent, while cyanobacteria declined. Zooplankton responded with increased diversity and abundance of *Rotifera* and *Cladocera*, while *Ciliata* and *Copepoda* decreased. Phytoplankton showed responsiveness to TDS, conductivity, turbidity, and pH, while zooplankton correlated with oxygen and temperature. There were significant changes in diversity for both phytoplankton and zooplankton, with zooplankton showing higher diversity and moderate species composition changes. Winter conditions acted as ecological stressors, leading to structural and functional changes in plankton communities.

The development of cold-tolerant species and moderate species composition changes reflected the ecosystem's resilience and adaptability to winter conditions.

The urban lake environment appears to mitigate some of the harsh effects of winter, supporting higher abundance and species richness of zooplankton, while the phytoplankton assemblage showed a decline in abundance but with recovery effects after autumn algal blooms.

These findings underscore the importance of long-term monitoring and comparisons with other urban lakes to gain broader insights into urban aquatic ecosystems.

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