

## Article

# Assessing the Effects of Phytoplankton Structure on Zooplankton Communities in Different Types of Urban Lakes

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**Abstract:** Urban lakes play important roles in microclimate regulation such as controlling run-off and groundwater recharge, as well as being a source of water supply and a habitat for a wide variety of flora and fauna. Bucharest has a wide variety of water resources where phytoplankton represent the dominant primary producer, the defining biological factor for zooplankton development. Our hypothesis was that as a result of anthropogenic pressures, phytoplankton in the urban aquatic ecosystems diminish the qualitative and quantitative capacity to maintain a good health condition with effects on the food web. By the structural features of the phytoplankton and zooplankton communities, the objectives were to determine the changes in diversity in different types of urban lakes, to explore the relationships between communities, and to determine the response of phytoplankton and zooplankton functional groups to the environmental factors. The ecological status assessed by Chlorophyll-*a* ( $\mu\text{L}^{-1}$ ) highlights that most of the investigated lakes were eutrophic and hypereutrophic. The phytoplankton were influenced by lake types, seasonal variations and nutrient input. The dominance of the Chlorophyceae, Cyanobacteria and Bacillariophyceae influenced the zooplankton's development. The rotifers were the most represented in both species richness and abundance in zooplankton, followed by Copepoda young stages.

**Keywords:** water quality; trophic status; eutrophication; diversity; ecosystem health



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## 1. Introduction

Blue areas such as lakes, rivers and wetlands as components of urban systems play a crucial role in providing ecosystem services such as flood control, groundwater replenishment, heavy-rainfall water management, climate regulation and air purification. In addition, they contribute significantly to improve quality of life through aesthetic and recreational aspects [1,2]. The aquatic ecosystems in urban areas are among the most affected by anthropogenic impacts. The main driver of ecosystem deterioration is urbanization. As a result of increasing urbanization, many sensitive species, especially those that depend on particular habitats, experience habitat loss, which leads to population declines or extinction [3]. Maintaining these systems in good health conditions is one of the priority actions for long-term exploitation of water resources. The term ecosystem health has appeared in the literature since the 1980s. Constanza [4] defines ecosystem health as a set of concepts that combine homeostasis, disease absence, diversity, stability and balance between ecosystem components. Of these, the most intensely studied is eutrophication, seen as a serious disease of aquatic systems. It is one of the most common water-quality problems in the world.

Eutrophication, mainly caused by human intervention, has severely affected the health of ecosystems and has led to an imbalance between biological components, declining diversity and reduced ecosystem stability [5]. Ecosystem health is based on ecological integrity, and the impact of anthropogenic pressure drastically alters the resilience of ecosystems and ultimately has a negative impact on socioeconomic systems and human health [6].

Studies of aquatic urban systems focusing on phytoplankton as a key component to maintain the good health status for the wellbeing of a social community are scarce and not fully clarified. Phytoplankton assemblages perform important functions in aquatic ecosystems, which can have cascading effects on the food web. If these functions are disturbed, it can lead to a degraded state of health of the entire ecosystem. The relationships established by zooplankton with phytoplankton as the primary producer reflect the ecological conditions of the entire ecosystem. Zooplankton is sensitive to water quality, being a bioindicator of pollution. At the same time, the water quality of lakes could increase with decreasing urbanization pressure, exhibiting a positive correlation with zooplankton's species richness [7].

In the lakes of Bucharest, phytoplankton represent both the dominant primary producer and the defining biological factor in the assessment of ecological status. Thus, any change in the phytoplankton community can affect the ecological conditions of the entire system. The ecological role of phytoplankton as a primary producer is as the main source in the food web. For this reason, it can also influence major ecosystem processes by changing the concentration of oxygen and turbidity reflected in water quality [8].

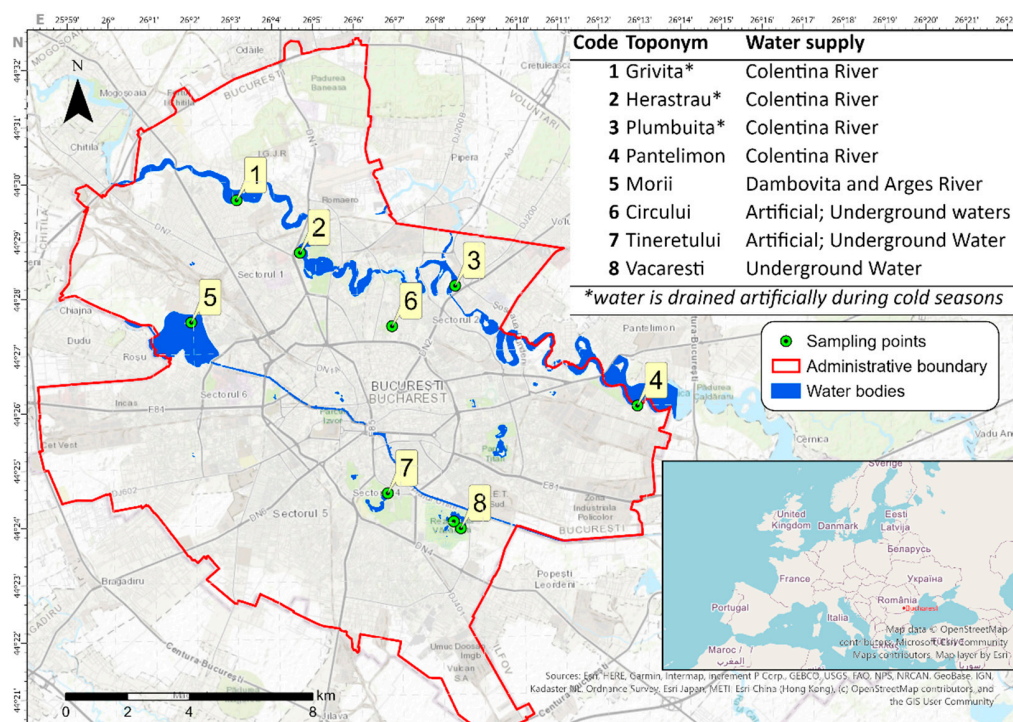
Our hypothesis was that as a result of anthropogenic pressures, phytoplankton in the urban aquatic ecosystems diminish the qualitative and quantitative capacity to maintain a good health condition with effects on food web. The purposes of this study were (1) to determine the changes in phytoplankton and zooplankton species diversity, species richness and community structure in the different types of urban lakes; (2) to explore the relationship between the zooplankton and phytoplankton community; (3) to determine the response of different phytoplankton groups to environmental factors; and (4) to discuss the cascading effects of phytoplankton composition on zooplankton communities in the different conditions of urban lakes and provide a scientific basis for urban-lake environment monitoring and biodiversity protection.

## 2. Materials and Methods

### 2.1. Study Site

Our study was conducted in eight different lakes of Bucharest (Figure 1) as follows: Lakes Grivița, Herăstrău, Plumbuita and Pantelimon belong to the Colentina River chain lakes (101 km length, 37.4 km in Bucharest). During the 1970s, the Colentina River was modified in the aim of water-flow regulation by constructing 15 artificial lakes [9]. The lakes have a controlled seasonal hydrological regime and few of them are emptied before winter and refilled during spring [10]. The above-mentioned lakes are classified as river chain lakes in our study.

The next three lakes are located in different areas of the city. Tineretului and Circului are found in two important parks of Bucharest, and for this reason we consider them as park lakes in our study. Circului is an artificial lake, resulting from excavation during neighborhood constructions, with natural water input from the upper shallow aquifer of the city and natural shores. The subsequent interventions changed the natural hydraulic connection between groundwater and surface, affecting the spring source of the lake, which caused a decrease in water level [11,12]. Tineretului Lake is located in the southern area of Bucharest. It is a seminatural ecosystem, supplied by both natural underground springs and precipitations. Both lakes have a diverse biocenosis, of which we mention European pond turtles, fish, coots and ducks, *Nelumbo nucifera* (only in Circului) and *Phragmites australis*.



**Figure 1.** Location of the studied ecosystems and sampling stations (1—Grivița\*, 2—Herastrău\*, 3—Plumbuita\*, 4—Pantelimon, 5—Morii, 6—Circului, 7—Tineretului, 8—Văcărești). \*—water is drained artificially during cold seasons.

The Morii Lake is the artificial reservoir of Dâmbovița River, the largest lake in Bucharest, with an important role in water-flow regulation and water supply for agricultural and industrial purposes.

The newest ecosystem, Văcărești wetland, located in the south-eastern part of Bucharest, belongs to Văcărești Park and resulted from an unfinished hydrotechnical project with the purpose of preventing flooding of the Dâmbovița River. After a long period of successive ecological transformations, at present it is protected as Văcărești Nature Park Reserve, with a large wetland area with a high biodiversity [13]. The selected lake from the park is considered a natural lake.

## 2.2. Sampling and Laboratory Analysis

The sampling was conducted between 2015 and 2017. During this period, six field campaigns (2/year) were conducted as follows: 2 in spring (2015 and 2017), 1 in summer (2016) and 3 in autumn (2015, 2016 and 2017). For each lake a sampling station was established, except for Văcărești Natural Park, where two sampling points were established. During our study, Herastrău Lake was emptied twice and could not be collected from in 2 campaigns.

In situ monitoring was performed for most of the physicochemical parameters, except for the nutrients.

Light intensity (Lux) was measured with the Lutron LX-1102 Lightmeter. Depth (m) and transparency (m) were measured with the Secchi disk. Temperature (°C), pH, conductivity (mS/cm), DO (mgO<sub>2</sub> L<sup>-1</sup>), DO saturation (%) and ORP (Oxidation-Reduction Potential) were measured with the HI 9828 multiparameter probe, provided by Hanna Instruments.

Chlorophyll-*a* concentration measurements were carried out with Bbe-Moldaenke FluoroProbe, (Kiel, Germany). The device measures the Chlorophyll-*a* (Chl-*a*) of four different phytoplankton spectral groups of Chlorophyceae, Cyanobacteria, Bacillariophyceae, Cryptophyta and yellow substance based on fluorescence principles [14].

### 2.3. Nutrient Samples and Phytoplankton Community

For nutrient analysis, the samples were taken on the water column into plastic bottles, transported in cold boxes and immediately underwent processing. 200 mL of water sample for nutrient content ( $\text{NH}_4$ ,  $\text{NO}_3$  and  $\text{PO}_4$ ) were filtered through GF/F Whatman 65  $\mu\text{m}$   $\varnothing$  and stored at 4 °C for laboratory analysis. Nutrients are determined spectrophotometrically by the Berthelot method (1859) for N- $\text{NH}_4$  and [15] for N- $\text{NO}_3$ , P- $\text{PO}_4$ .

For microscopic analysis, phytoplankton samples were taken on the water column using a Schindler-Patalas device (4 L) and mixed into a 10 L bucket. 500 mL of water were collected into plastic bottles and fixed in buffered formaldehyde (4%) and stored for a period for sedimentation. A Zeiss inverted microscope was used for taxonomical identification according to special keys of Cyanobacteria [16,17], Chlorophyceae [18,19], Bacillariophyceae [20–23], Euglenophyceae [24], Dinophyceae [25] and Chrysophyceae [26]. At the same time, cell enumeration was made according to Ütermohl method [27]. Taxonomic identification of the Cryptophyta was not performed.

### 2.4. Water Quality Parameters

The quality classes and trophic classification of the lakes were assessed based on Chlorophyll-*a*  $\mu\text{g L}^{-1}$  respecting the following limits: oligotrophic 1–2.5; mesotrophic 2.5–8; eutrophic 8–25; hypereutrophic >25; and water quality classes I < 25; II 25–50; III 50–100; IV 100–250; V > 250, according to the national legislation [28], part of the European Water-Framework Directive.

### 2.5. Zooplankton Community

Zooplankton samples were collected on the water column (from near the bottom to the surface) using a Schindler-Patalas trap and filtered through plankton net (50  $\mu$  mesh size). The samples were collected in 50 mL plastic bottles and preserved in a 4% formaldehyde solution. In the laboratory, samples were concentrated by filtration and 3 aliquots were microscopically analyzed for taxonomic identification and abundance assessment. The species composition was carried out for Ciliata [29–32], Testate Amoeba or Testacea [33,34], Rotifera [35], Cladocera [36] and Lamellibranchia (veliger larvae) and Copepoda (development stages, cyclopoids, calanoids and harpacticoids). Abundances were expressed as individuals per Liter [37].

We established the potential indicators used in assessment of the ecosystem health status (Table 1). Instead, the results followed separate parameter analysis.

**Table 1.** Relationships between abiotic and biotic components as potential indicators of health in studied urban ecosystems.

| Ecosystem Features                              | Indicators of Ecosystem Health  |
|---|---|
| Primary production                              | In situ Chlorophyll- <i>a</i>   |
| Water quality                                   | <ul style="list-style-type: none"> <li>• Chlorophyll-<i>a</i> water quality classification</li> <li>• Chlorophyll-<i>a</i> trophic status assessment</li> <li>• Phytoplankton abundances</li> <li>• Algal blooms</li> </ul> |
| Top-down and bottom-up control of phytoplankton | <ul style="list-style-type: none"> <li>• Phytoplankton in nutrient regulation</li> <li>• Phytoplankton in shaping zooplankton abundance and diversity</li> </ul>  |
| Diversity of phytoplankton and zooplankton      | <ul style="list-style-type: none"> <li>• Species richness</li> <li>• Shannon diversity</li> <li>• Evenness</li> </ul>   |



## 2.6. Statistical Analysis

Statistical analysis was performed using XLSTAT pro [38].

The box plot is a widely used technique viewing the statistical summary of the data, including minimum, maximum, median, lower quartile and upper quartile, as well as identifying outliers [38].

The biodiversity indices as species richness (SR—number of taxa) and Shannon's diversity index ( $H$ ) and Evenness ( $e^H/S$ ) were determined for phyto- and zooplankton assemblages. The indices were calculated at the level of total phytoplankton and total zooplankton, on each lake and in each month.

Shannon's index ( $H$ ), taking into account the number of taxa and number of individuals, is as follows:

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

where  $n$  is total number of individuals ( $n$ ) and  $n_i$  is number of individuals of taxon  $i$ .

Evenness measures the uniformity of the individuals among the community's taxa [39].

Both indexes were calculated in PAST statistical software [40].

In order to establish the relationships between the plankton assemblages and specific environmental parameters, multivariate analysis was performed. Prior to their statistical processing, the data were  $\log(n + 1)$ -transformed for normalization, apart from ORP and pH. The multivariate ordination technique Redundancy analysis (RDA) is useful to investigate the relationship of the assessed factors while also taking into account the ecosystem types.

The significance of the ordering axes was tested by means of the Monte Carlo permutation test with 500 permutations. With the help of this test, it is possible to reject the null hypothesis  $H_0$ , which assumes that there is no influence of physicochemical and enzymatic factors in the studied ecosystems on variation in the phytoplankton community. If the Monte Carlo test indicates a  $p < 0.05$ , then the  $H_1$  hypothesis can be adopted, according to which the set of explanatory variables introduced in the analysis has a significant relationship on the response variables. The Monte Carlo permutation test allows us to reject the null hypothesis  $H_0$ , which assumes that there is no linear relationship between the explanatory variables ( $x$ ) and the response variables ( $y$ ) and to accept the alternative hypothesis  $H_a$  ( $p < 0.0001$ ), which assumes a very highly significant relationship \*\*\*). The risk of rejecting the null hypothesis as true is less than 0.01% [41].

## 3. Results

### 3.1. In Situ Physicochemical Parameters

The depths of the studied lakes were not more than 2 m, typical for urban ecosystems, except for the Morii Reservoir (which reached 4.74 m depth). Other parameters measured in the field varied as follows: light (lux) (140–117,000), temperature ( $^{\circ}\text{C}$ ) (6.54–31.50), pH (6.30–10.66), conductivity ( $\text{m Scm}^{-1}$ ) (230.00–1145.00), DO ( $\text{mgO}_2 \text{ L}^{-1}$ ) (1.67–34.81), DO% (saturation) (5.86–158), ORP (mV) (−237–292.80) and transparency (m) (0.15–1.70).

### 3.2. Nutrients

The peaks with a much higher magnitude compared with the average were recorded (Figure 2), which highlighted water deterioration. These outliers of  $\text{NO}_3$  ( $\text{mg N L}^{-1}$ ) were recorded in autumn 2015 in Tineretului (2.23) and Văcărești (0.87).  $\text{NH}_4$  ( $\text{mg N L}^{-1}$ ) showed high concentrations in autumn 2015 in Pantelimon (0.38  $\text{mg N L}^{-1}$ ), Văcărești (0.73  $\text{mg N L}^{-1}$ ) and Plumbuita (0.48  $\text{mg N L}^{-1}$ ). In Văcărești, a maximum of  $\text{NH}_4$  concentrations was registered.  $\text{PO}_4$  ( $\text{mg P L}^{-1}$ ) peaks were reached in spring 2017 in Pantelimon (0.87), Grivița (0.40) and Tineretului (0.28).

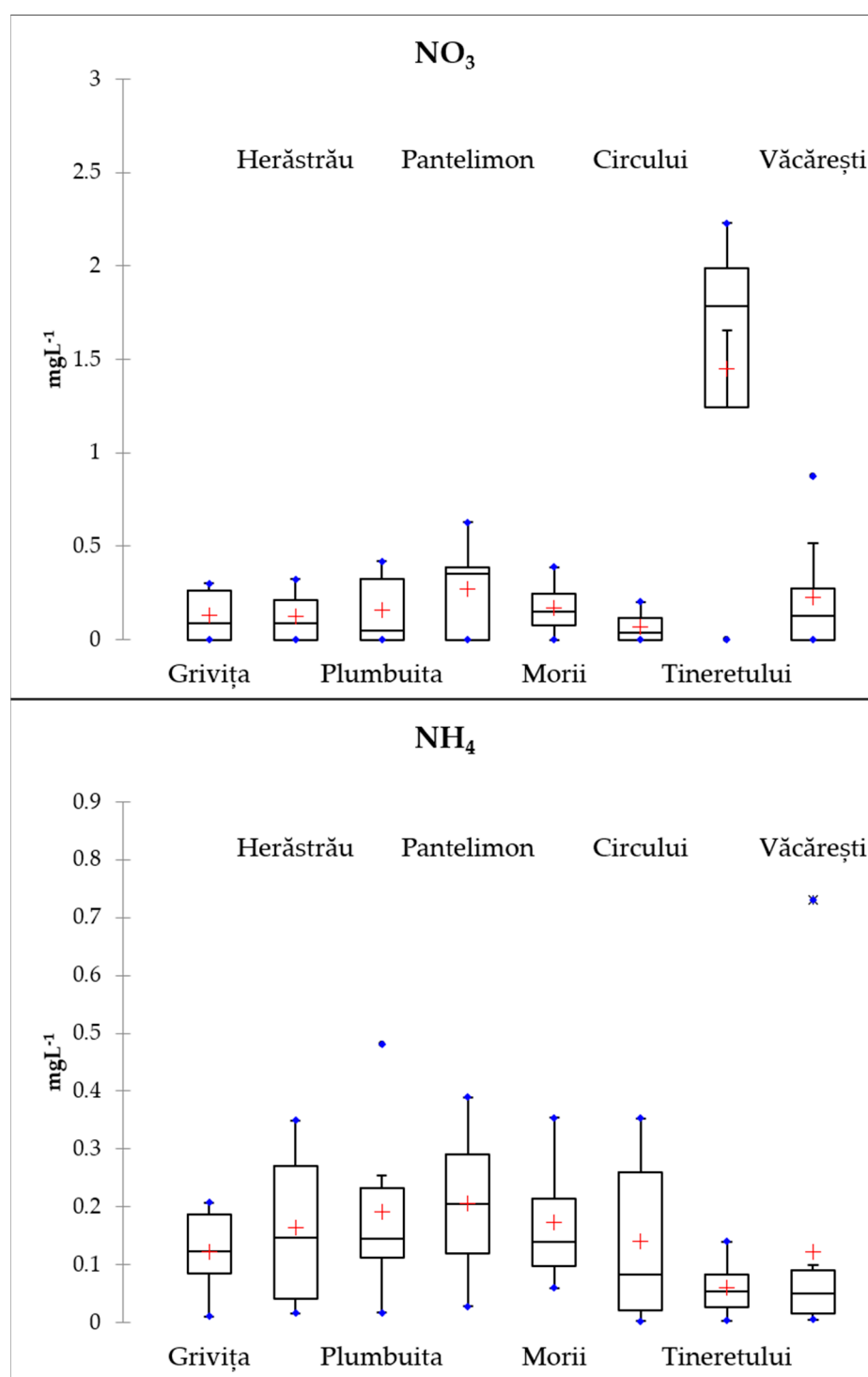
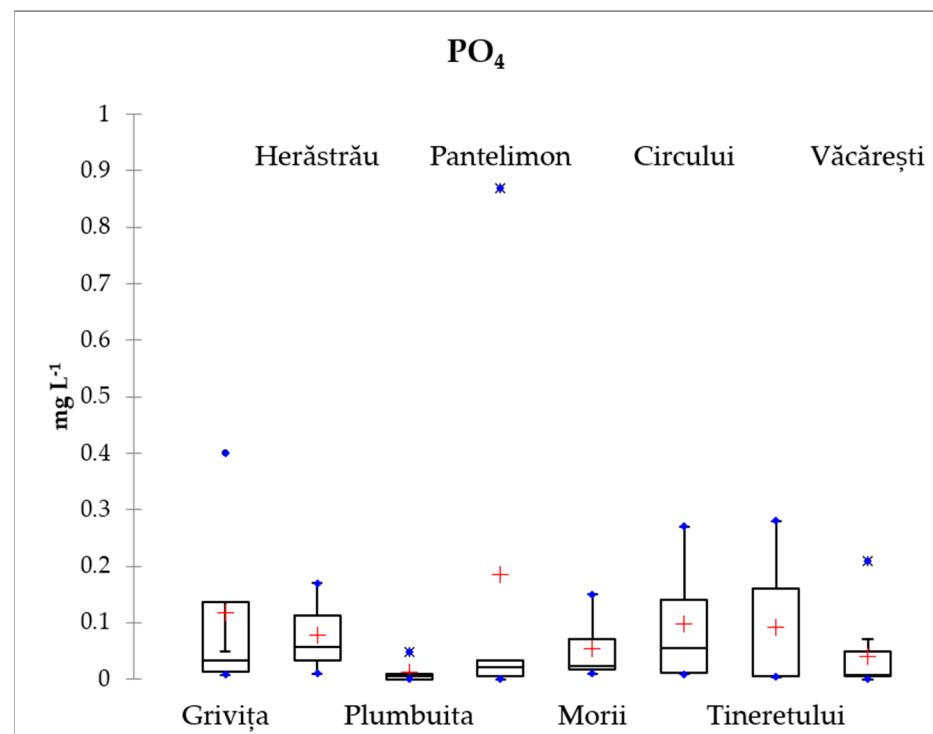


Figure 2. Cont.



**Figure 2.** Boxplots of the assessed nutrients  $\text{NO}_3$  ( $\text{mg N L}^{-1}$ ),  $\text{NH}_4$  ( $\text{mg N L}^{-1}$ ) and  $\text{PO}_4$  ( $\text{mg P L}^{-1}$ ) assessed in eight urban lakes of Bucharest city (Blue points—outliers; red crosses—means; lower and upper limits—first and third quartiles; central horizontal bars—medians).

### 3.3. Chlorophyll-*a*

The in situ total Chlorophyll-*a* ( $\mu\text{g L}^{-1}$ ) measured by fluorometric method ranged from 1.95 to  $68.82 \mu\text{g L}^{-1}$ . The dynamics of this parameter were influenced more by the seasonal variations (one-way ANOVA  $F_{2,27} = 5.18$ ,  $p = 0.009$ ) and less by the type of lakes (one-way ANOVA  $F_{3,47} = 3.55$ ,  $p = 0.02$ ).

According to the national legislation and the Water Framework Directive (WFD), the concentrations of Chlorophyll-*a* above the threshold of  $8 \mu\text{g L}^{-1}$  indicate eutrophy and above the threshold of  $25 \mu\text{g L}^{-1}$  hypereutrophy, conditions which forewarn the occurrence of algal blooms. Complying with the mentioned limits, we noticed that in the studied lakes, the conditions of eutrophic and hypereutrophic status were dominant in 78% of the samples (Table 2).

**Table 2.** Trophic status based on in situ Chlorophyll-*a* ( $\mu\text{g L}^{-1}$ ) concentration.

|                             |             | April 2015 | August 2015 | July 2016 | November 2016 | April 2017 | August 2017 |
|-----------------------------|-------------|------------|-------------|-----------|---------------|------------|-------------|
| Colentina River chain lakes | Grivița     | E          | H           | E         | M             | H          | H           |
|                             | Herăstrău   | H          | H           | E         |               |            | H           |
|                             | Plumbuita   | H          | H           | E         | E             | E          | H           |
|                             | Pantelimon  | M          | H           | H         | E             | E          | H           |
| Reservoir                   | Morii       | O          | H           | H         | M             | M          | E           |
| Park                        | Circului    | E          | H           | H         | H             | H          | H           |
|                             | Tineretului | E          | E           | H         | H             | E          | M           |
| N. Park                     | Văcărești 1 | M          | H           | E         | M             | E          | E           |
|                             | Văcărești 2 | M          | M           | H         |               | M          | H           |

Legend: H—hypereutrophic; E—eutrophic; M—mesotrophic; O—oligotrophic.

Moreover, taking into account Chlorophyll-*a* in assessing the water quality, we identified a few overruns to moderate conditions (Table 3).

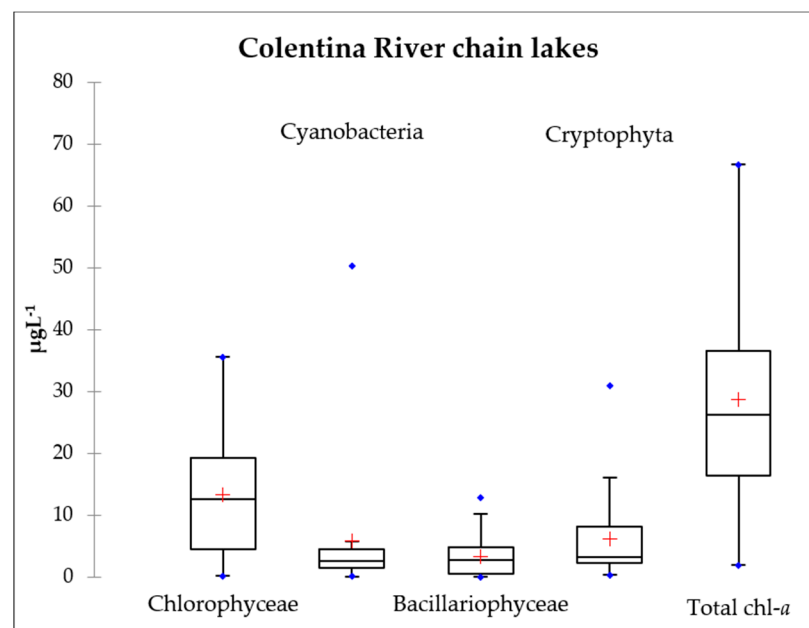
**Table 3.** Water quality classes based on in situ Chlorophyll-*a* ( $\mu\text{g L}^{-1}$ ) concentration.

|                             |             | April 2015 | August 2015 | July 2016 | November 2016 | April 2017 | August 2017 |
|-----------------------------|-------------|------------|-------------|-----------|---------------|------------|-------------|
| Colentina River chain lakes | Grivița     | I          | III         | I         | I             | II         | II          |
|                             | Herăstrău   | II         | II          | I         |               |            | II          |
|                             | Plumbuita   | II         | II          | I         | I             | I          | II          |
|                             | Pantelimon  | I          | III         | II        | I             | I          | II          |
| Reservoir                   | Morii       | I          | II          | II        | I             | I          | I           |
| Park                        | Circului    | I          | II          | II        | III           | II         | II          |
|                             | Tineretului | I          | I           | II        | III           | I          | II          |
| N. Park                     | Văcărești 1 | I          | II          | I         | I             | I          | I           |
|                             | Văcărești 2 | I          | I           | II        |               | I          | II          |

Legend: I—very good conditions, II—good conditions, III—moderate conditions.

By evaluating the total Chlorophyll-*a* concentration, it was highlighted that the lakes in the parks and Colentina River chain lakes were more productive compared to Morii and Văcărești lakes (Figure 3). Total Chlorophyll-*a* reached high peaks of concentrations in different periods of the study in the lakes of the Colentina River (both in IX 2015,  $66.66 \mu\text{g L}^{-1}$  in Pantelimon;  $63.45 \mu\text{g L}^{-1}$  in Grivița) and in park lakes (both in XI 2016,  $68.83 \mu\text{g L}^{-1}$  in Tineretului and  $54.65 \mu\text{g L}^{-1}$  in Circului). In Morii the highest value of Chlorophyll-*a* was recorded in autumn 2015 ( $32.30 \mu\text{g L}^{-1}$ ), and in Văcărești Natural Park the value ( $40.79 \mu\text{g L}^{-1}$  IX 2017) was lower than in the other studied ecosystems (Figure 3).

In addition, the structure of phytoplankton communities and their biomass expressed as the total Chlorophyll-*a* content were determined. Thus, in Chlorophyll-*a* contribution in the lakes of the Colentina River, Chlorophyceae and Cryptophyta were noted as dominant, while in the lakes in the parks Chlorophyceae was followed by Bacillariophyceae. Cyanobacteria and Cryptophyta were present in Morii and Văcărești (Figure 3).



**Figure 3.** Cont.



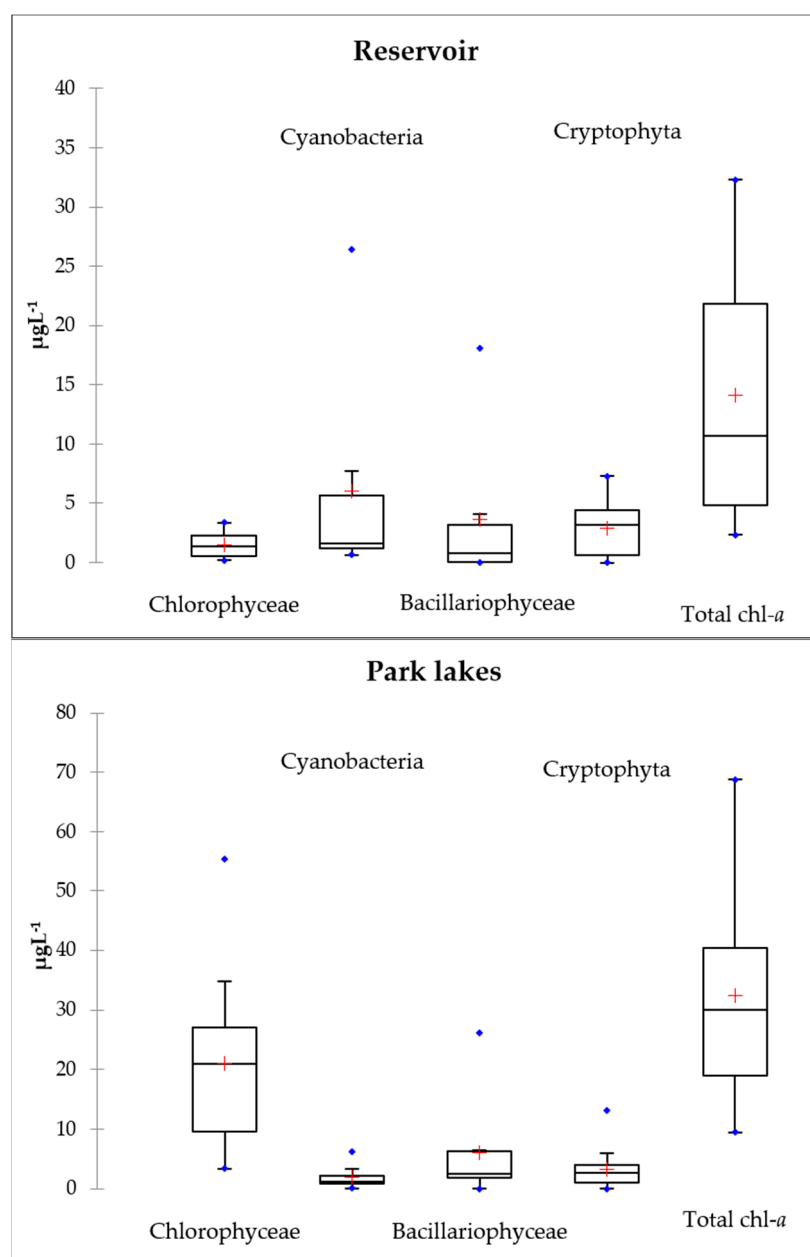
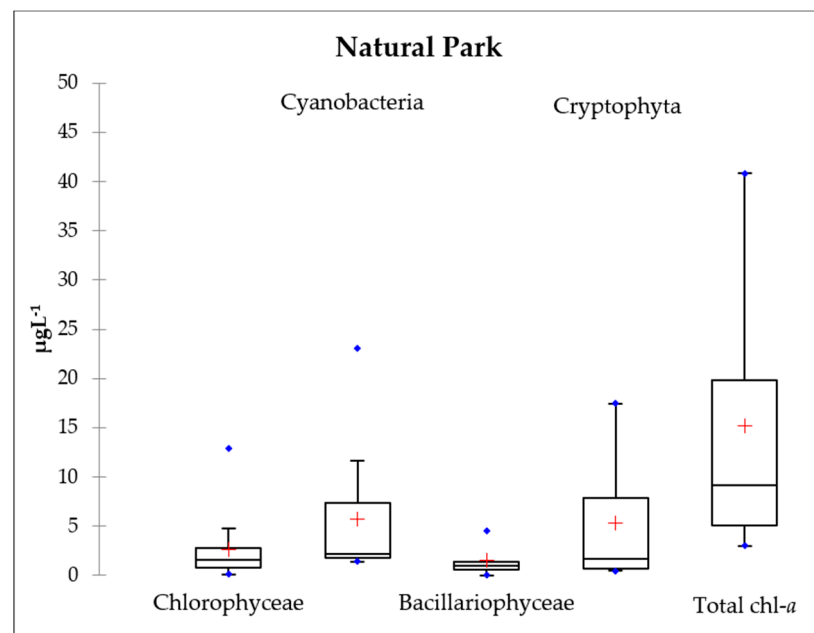


Figure 3. Cont.



**Figure 3.** Boxplot of in situ Chlorophyll-*a* ( $\mu\text{L}^{-1}$ ) for each type of ecosystem over the entire sampling period (Blue points—outliers, red crosses—means; lower and upper limits—first and third quartiles; central horizontal bars—medians).

### 3.4. Phytoplankton Communities

#### 3.4.1. Diversity

Diversity of the phytoplankton communities as a component of the supporting services highlighted the dominants of the previously mentioned taxonomic groups (Table 4). During the entire study, a total of 162 phytoplankton species were recorded: 59 belonging to Chlorophyceae, 47 to Bacillariophyceae, 36 to Cyanobacteria, 14 to Euglenophyceae, 5 to Dinophyceae and 1 to Chrysophyceae. Regarding the species richness, most species were present in Colentina and Văcărești. The Shannon and Evenness diversity indices provided additional structural aspects of the phytoplankton communities. The Shannon diversity was higher in the lakes of the Colentina chain and Circului, while the Evenness was lower in all ecosystems, suggesting an instability of the phytoplankton.

**Table 4.** Phytoplankton species richness and Shannon and Evenness diversity-index averages recorded in studied ecosystems (1—Grivița, 2—Herăstrău, 3—Plumbuita, 4—Pantelimon, 5—Morii, 6—Circului, 7—Tineretului, 8—Văcărești).

|                   | River |      |      |      | Reservoir |      | Park |      | N. Park |
|-------------------|-------|------|------|------|-----------|------|------|------|---------|
|                   | 1     | 2    | 3    | 4    | 5         | 6    | 7    | 8    |         |
| Total species     | 58    | 45   | 63   | 70   | 42        | 51   | 45   | 80   |         |
| Cyanobacteria     | 8     | 5    | 10   | 23   | 8         | 12   | 9    | 18   |         |
| Euglenophyceae    | 6     | 6    | 5    | 0    | 1         | 4    | 1    | 4    |         |
| Dinophyceae       | 0     | 1    | 2    | 1    | 1         | 2    | 1    | 3    |         |
| Chrysophyceae     | 1     |      | 1    | 1    | 1         | 1    | 1    | 1    |         |
| Bacillariophyceae | 17    | 6    | 20   | 12   | 18        | 9    | 15   | 28   |         |
| Chlorophyceae     | 26    | 27   | 25   | 33   | 13        | 23   | 18   | 26   |         |
| Shannon (H)       | 2.93  | 3.06 | 3.12 | 2.62 | 1.59      | 2.81 | 1.87 | 1.78 |         |
| Evenness          | 0.32  | 0.48 | 0.32 | 0.2  | 0.12      | 0.32 | 0.14 | 0.07 |         |

According to the one-way ANOVA ( $F_{3,45} = 11.83, p < 0.0001$ ) and Tukey post hoc test (Table 5), phytoplankton diversity showed significant differences depending on ecosystem types. Colentina chain lakes had diversity peculiarities compared to all the other lakes with regard to the phytoplankton. On the other hand, a one-way ANOVA of the influences of trophic status and seasons on phytoplankton diversity proved to be insignificant.

**Table 5.** Tukey (HSD)/analysis of the differences between the categories with a confidence interval of 95%.

| Contrast                  | Difference | Standardized Difference | Critical Value | Pr > Diff | Significant |
|---------------------------|------------|-------------------------|----------------|-----------|-------------|
| Colentina vs. N.Park      | 0.189      | 5.496                   | 2.667          | <0.0001   | Yes         |
| Colentina vs. Reservoir   | 0.124      | 2.983                   | 2.667          | 0.023     | Yes         |
| Colentina vs. Park        | 0.122      | 3.758                   | 2.667          | 0.003     | Yes         |
| Park vs. N.Park           | 0.067      | 1.755                   | 2.667          | 0.308     | No          |
| Park vs. Reservoir        | 0.002      | 0.042                   | 2.667          | 1.000     | No          |
| Reservoir vs. N.Park      | 0.065      | 1.415                   | 2.667          | 0.497     | No          |
| Tukey's d critical value: |            |                         | 3.772          |           |             |

### 3.4.2. Abundance

In situ measurements of Chlorophyll-*a* provided a preliminary perspective on the phytoplankton composition. In terms of abundance (Table 6), we found that Cyanobacteria, Chlorophyceae and Bacillariophyceae are the major groups that are also considerable in Chlorophyll-*a*.

**Table 6.** Annual averages of the phytoplankton-group abundances (cells L<sup>-1</sup>) in the studied urban lakes (1—Grivița, 2—Herăstrău, 3—Plumbuita, 4—Pantelimon, 5—Morii, 6—Circului, 7—Tineretului, 8—Văcărești).

|                   | River              |                    |                    |                    | Reservoir          | Park               |                    | Natural Park       |
|-------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
|                   | 1                  | 2                  | 3                  | 4                  | 5                  | 6                  | 7                  | 8                  |
| Total             | $1.96 \times 10^7$ | $2.04 \times 10^7$ | $1.17 \times 10^7$ | $3.85 \times 10^7$ | $2.68 \times 10^7$ | $3.46 \times 10^7$ | $4.10 \times 10^7$ | $4.18 \times 10^7$ |
| Cyanobacteria     | $4.53 \times 10^6$ | $6.69 \times 10^6$ | $3.12 \times 10^6$ | $3.30 \times 10^7$ | $2.35 \times 10^7$ | $2.01 \times 10^7$ | $8.86 \times 10^6$ | $3.94 \times 10^7$ |
| Euglenophyceae    | $9.28 \times 10^5$ | $1.07 \times 10^6$ | $5.65 \times 10^4$ | -                  | $4.04 \times 10^3$ | $8.17 \times 10^4$ | $7.77 \times 10^3$ | $1.86 \times 10^4$ |
| Dinophyceae       | -                  | $4.58 \times 10^4$ | $2.02 \times 10^4$ | $1.68 \times 10^4$ | $9.61 \times 10^4$ | $9.19 \times 10^5$ | $2.69 \times 10^5$ | $5.77 \times 10^4$ |
| Chrysophyceae     | $1.01 \times 10^4$ | -                  | $2.68 \times 10^3$ | $1.58 \times 10^5$ | $5.81 \times 10^5$ | $3.37 \times 10^5$ | $7.99 \times 10^6$ | $2.25 \times 10^5$ |
| Bacillariophyceae | $2.25 \times 10^6$ | $1.72 \times 10^6$ | $2.16 \times 10^6$ | $1.27 \times 10^6$ | $1.34 \times 10^6$ | $1.91 \times 10^6$ | $2.24 \times 10^7$ | $3.65 \times 10^5$ |
| Chlorophyceae     | $1.19 \times 10^7$ | $1.09 \times 10^7$ | $6.33 \times 10^6$ | $4.09 \times 10^6$ | $1.32 \times 10^6$ | $1.21 \times 10^7$ | $1.48 \times 10^6$ | $1.71 \times 10^6$ |

The impact of algal blooms has long been debated in the literature, addressing concentration limits of Chlorophyll-*a* or the abundance of cyanobacteria species. In our study, the threshold for algal-bloom occurrence was considered  $2.00 \times 10^7$  cells L<sup>-1</sup> after [42]. During the study, 11 cases of Cyanobacteria bloom were recorded in Văcărești (6 times), Morii (2), Pantelimon (2) and Circului (1), which was also reflected in the annual averages (Table 6). Thus, most of the hypereutrophic and eutrophic moments (Table 2) during our study was determined by a codominance between Chlorophyceae, Cyanobacteria and Bacillariophyceae. In Table 7 there are the dominant species that determined the algal-bloom events. The other groups, Euglenophyceae, Dinophyceae and Chrysophyceae were less-represented. However, in spring 2015, there was an episode of bloom with *Dinobryon sertularia* Ehrenberg 1834.

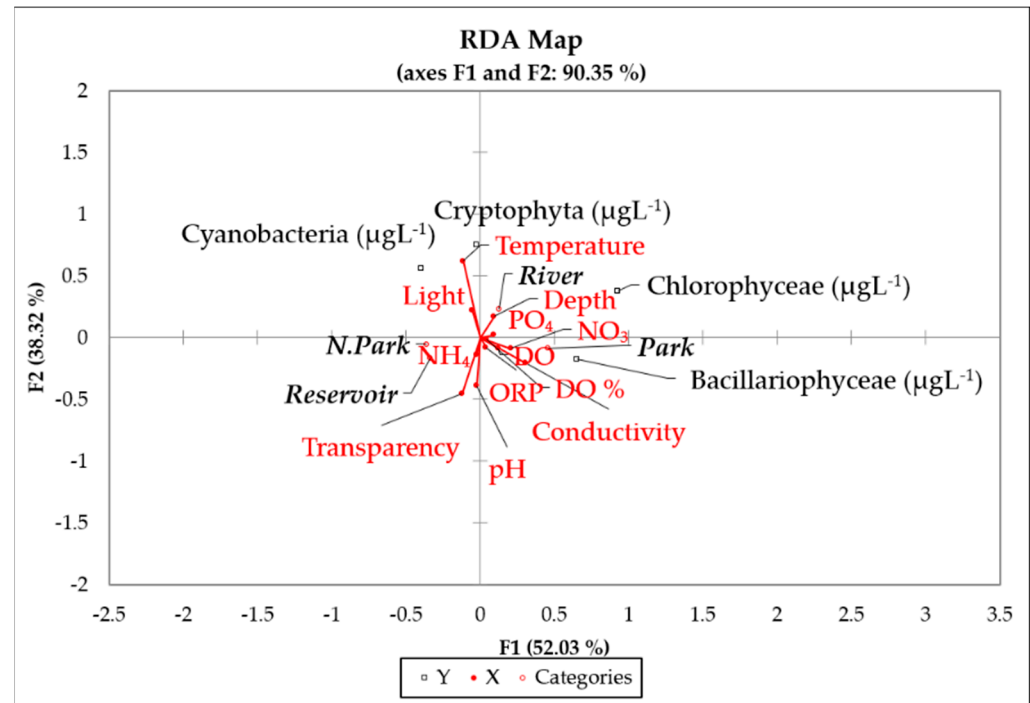
**Table 7.** Dominant phytoplanktonic genus and species recorded during blooming period.

| Genus/Species  | Bloom Event             |
|--|-------------------------|
| Chlorophyceae  |                         |
| <i>Coelastrum microporum</i> Nägeli 1855                                   | Circului autumn 2015    |
| <i>Desmodesmus communis</i> (E. Hegewald) E. Hegewald                      | Plumbuita spring 2015   |
| <i>Kirchneriella lunaris</i> (Kirchner) Möbius 1894                        | Circului autumn 2016    |
| <i>Scenedesmus ecornis</i> (Ehrenberg) Chodat 1926                         | Grivița autumn 2015     |
| <i>Tetradasmus lagerheimii</i> M.J. Wynne & Guiry                          | Herăstrău spring 2015   |
|  | Plumbuita summer 2016   |
|  | Plumbuita spring 2015   |
|  | Circului autumn 2016    |
| Cyanobacteria  |                         |
| <i>Planktolyngbya limnetica</i> (Lemmermann)                               | Circului spring 2015    |
| Komárková-Legnerová&Cronberg   |                         |
| <i>Snowella lacustris</i> (Chodat) Komárek&Hindák                          | Tineretului summer 2016 |
| <i>Wolleea saccata</i> (Wolle) Bornet et Flahault 1886                     | Pantelimon autumn 2015  |
|  | Pantelimon summer 2016  |
| <i>Aphanizomenon flosaquae</i> Ralfs ex Bornet&Flahault 1886               | Morii autumn 2015       |
|  | Morii summer 2016       |
| <i>Chroococcus dispersus</i> (V. Keissler) Lemm. 1904                      | Văcărești autumn 2015   |
| <i>Cylindrospermum</i> sp. F.T. Kützinger ex E. Bornet & C. Flahault, 1886 | Văcărești summer 2016   |
|  | Văcărești autumn 2017   |
|  | Grivița spring 2015     |
|  | Herăstrău spring 2015   |
| <i>Jaaginema minimum</i> (Gicklhom) Anagnostidis&Komárek                   | Herăstrău autumn 2015   |
|  | Pantelimon summer 2016  |
|  | Circului autumn 2016    |
|  | Plumbuita summer 2016   |
| <i>Merismopedia tenuissima</i> Lemmermann 1898                             | Circului autumn 2015    |
|  | Tineretului summer 2016 |
| <i>Merismopedia tranquilla</i> (Ehrenberg) Trevisan                        | Plumbuita summer 2016   |
| <i>Microcystis flosaquae</i> (Wittrock) Kirchner 1898                      | Pantelimon summer 2016  |
| <i>Microcystis</i> sp. Lemmermann, 1907                                    | Morii summer 2016       |
| <i>Oscillatoria limosa</i> C.Agardh ex Gomont 1892                         | Herăstrău autumn 2015   |
|  | Morii autumn 2015       |
| <i>Oscillatoria</i> sp. Vaucher ex Gomont, 1892                            | Circului summer 2016    |
|  | Văcărești autumn 2017   |
|  | Pantelimon autumn 2015  |
| <i>Oscillatoria tenuis</i> C.Agardh ex Gomont 1892                         | Văcărești autumn 2015   |
|  | Văcărești summer 2016   |
| Bacillariophyceae  |                         |
| <i>Nitzschia acicularis</i> (Kützinger) W. Smith 1853                      | Tineretului summer 2016 |
| <i>Ulnaria acus</i> (Kützinger) Aboal                                      | Tineretului autumn 2017 |
| Chrysophyceae  | Tineretului spring 2015 |
| <i>Dinobryon sertularia</i> Ehrenberg 1834                                 | Tineretului spring 2015 |

### 3.5. Phytoplankton and Physicochemical Variables Relationship

The response of the phytoplankton biomass (assessed as Chlorophyll-*a* L<sup>-1</sup>) to the pressure of physicochemical parameters was evaluated by the multivariate RDA analysis (Figure 4). The studied physicochemical factors explain, in a proportion of 90.35% (axis F1 52.03% + axis F2 38.32%), the variation of the phytoplankton biomass in the ecosystems. The significance of the ordering axes was tested by means of the Monte Carlo permutation test with 500 permutations ( $p < 0.0001$ ). The first axis, F1, is related to the distribution of biomass along a gradient of nutrient content (N forms and orthophosphate) and DO. Bacillariophyceae and Chlorophyceae preferred more oxygenated conditions of park ecosystems and those with a higher content of N and P. Furthermore, diatoms preferred colder and

clearer waters, and higher conductivity and low ORP. The F2 axis associated with temperature revealed the preference of Cyanobacteria and Cryptophyta for warmer waters recorded in river ecosystems, characterized by shallow water with low orthophosphate content.



**Figure 4.** Redundancy analysis (RDA) for physicochemical parameters influencing the phytoplankton community biomass ( $\mu\text{gL}^{-1}$ ). Red lines—explanatory variables (X) of the variation of phytoplankton communities.

### 3.6. Zooplankton Communities

#### 3.6.1. Diversity

The zooplankton community as a top-down control factor of phytoplankton throughout the study was represented by Ciliata (21 species), Testate amoebae (11 species), Rotifera (87 species), Gastrotricha (2 species) and Cladocera (33 species). Lamellibranchia larvae and Copepoda (Cyclopida gsp., Harpacticoida gsp; Calanoida g.sp.) and Ostracoda were also present and quantified at group level. Văcărești Natural Park was characterized by the highest species richness (89 species) and Shannon index diversity (3.48), followed by the lakes of the Colentina chain with close values of Shannon diversity. The lowest diversity was observed in Tineretului Lake (1.92) (Table 8).

**Table 8.** Zooplankton species richness and Shannon and Evenness diversity-index averages recorded in studied ecosystems (1—Grivița, 2—Herăstrău, 3—Plumbuita, 4—Pantelimon, 5—Morii, 6—Circului, 7—Tineretului, 8—Văcărești).

|                 | River |    |    |    | Reservoir | Park |    | Natural Park |
|-----------------|-------|----|----|----|-----------|------|----|--------------|
|                 | 1     | 2  | 3  | 4  | 5         | 6    | 7  | 8            |
| Total species   | 57    | 55 | 57 | 64 | 42        | 52   | 54 | 89           |
| Ciliata         | 5     | 9  | 6  | 9  | 5         | 5    | 6  | 4            |
| Testacea        | 6     | 3  | 7  | 6  | 2         | 7    | 7  | 9            |
| Lamellibranchia |       | 1  |    | 1  |           |      |    |              |

**Table 8.** *Cont.*

|              |      | River |      |      | Reservoir | Park |      | Natural Park |
|--------------|------|-------|------|------|-----------|------|------|--------------|
| Rotifera     | 41   | 34    | 36   | 36   | 22        | 33   | 29   | 50           |
| Gastrotricha |      |       |      |      |           | 1    |      | 2            |
| Cladocera    | 5    | 6     | 5    | 9    | 9         | 4    | 9    | 20           |
| Copepoda     | 0    | 2     | 2    | 2    | 3         | 2    | 2    | 3            |
| Ostracoda    |      |       | 1    | 1    | 1         |      | 1    | 1            |
| Shannon (H)  | 2.59 | 2.67  | 2.35 | 2.68 | 2.14      | 2.20 | 1.92 | 3.48         |
| Evenness     | 0.23 | 0.26  | 0.18 | 0.23 | 0.20      | 0.17 | 0.13 | 0.37         |

### 3.6.2. Abundance

The Rotifera group was dominant both in species richness and abundance (Tables 8 and 9). The annual abundances recorded in the lakes of the Colentina River were higher than in the other ecosystems. The highest annual average of zooplankton abundance was recorded in Lake Pantelimon (1240.13 indiv.L<sup>−1</sup>) and the lowest in Lake Morii (381.20 indiv. L<sup>−1</sup>). Besides rotifers, ciliates and copepods were also abundant, especially in the stages of nauplia and copepodites.

**Table 9.** Annual averages of the zooplankton-group abundances (indv.L<sup>−1</sup>) in the study ecosystems (1—Grivița, 2—Herăstrău, 3—Plumbuita, 4—Pantelimon, 5—Morii, 6—Circului, 7—Tineretului, 8—Văcărești).

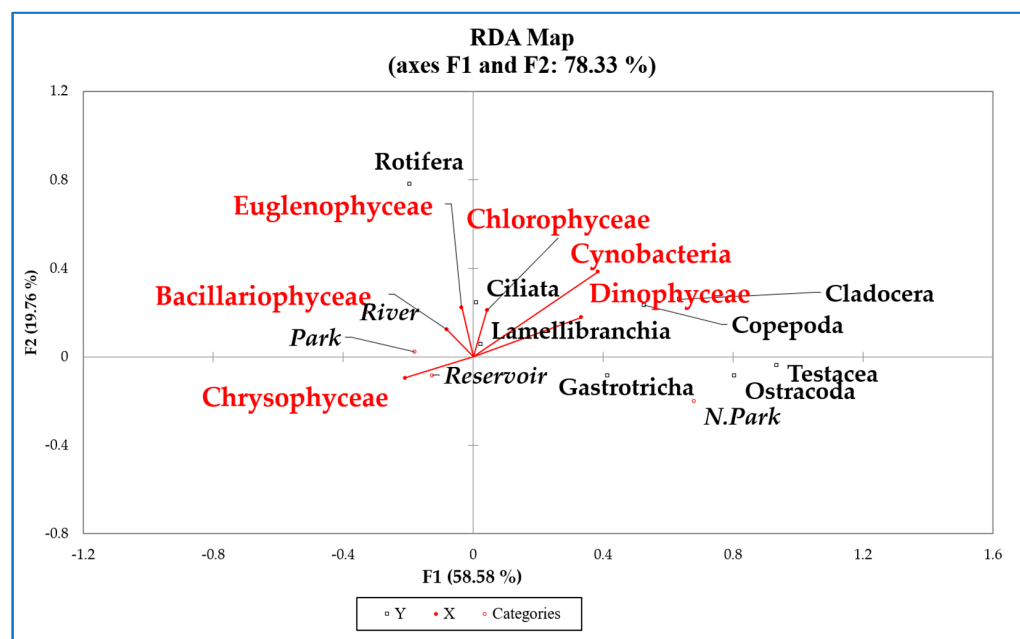
|                 | River  |        |        |         | Reservoir |        | Park   |        | N. Park |
|-----------------|--------|--------|--------|---------|-----------|--------|--------|--------|---------|
|                 | 1      | 2      | 3      | 4       | 5         | 6      | 7      | 8      |         |
| Total           | 576.96 | 921.5  | 939.66 | 1240.13 | 381.2     | 822.61 | 479.8  | 514.91 |         |
| Ciliata         | 9.28   | 50.38  | 4.57   | 138.5   | 15.7      | 12.22  | 7.43   | 15.56  |         |
| Testacea        | 2.79   | 1.69   | 5.64   | 7.16    | 0.83      | 11.96  | 4.68   | 104.24 |         |
| Lamellibranchia | 0      | 0.31   | 0      | 2.11    | 0         | 0      | 0      | 0      |         |
| Rotifera        | 519.01 | 799.69 | 854.82 | 860.86  | 244.18    | 638.98 | 380.19 | 171.82 |         |
| Gastrotricha    | 0      | 0      | 0      | 0       | 0         | 0.28   | 0      | 5.98   |         |
| Cladocera       | 5.38   | 22.5   | 4.4    | 22.24   | 10.14     | 1.75   | 34.16  | 39.2   |         |
| Copepoda        | 40.49  | 46.94  | 69.98  | 210.04  | 108.67    | 157.43 | 52.09  | 155.81 |         |
| Ostracoda       | 0      | 0      | 0.25   | 1.34    | 1.67      | 0      | 1.25   | 22.3   |         |

### 3.7. Phytoplankton and Zooplankton Interactions

The relationship between primary phytoplankton producers and main consumers, assessed by RDA analysis, explains in proportions of 78.33% (F1 axis 58.58% + F2 axis 19.76%) the variation of the zooplankton community in the studied ecosystems (Figure 5). The significance of the ordering axes was tested by means of the Monte Carlo permutation test with 500 permutations ( $p < 0.0001$ ). Chrysophyceae and Bacillariophyceae are associated with the F1 axis, while the other phytoplankton groups are associated with the F2 axis. Thus, the dominance of some phytoplankton groups in the studied ecosystems influenced the structure of zooplankton differently. In the categories of park and river ecosystems, Bacillariophyceae primarily allowed for the development of rotifers. There is a variation in the same direction: if there is an increase in diatom populations, there will be an increase in rotifers. Moreover, due to the development of green algae, cyanobacteria, euglenoids and Dinophyceae, the development of microcrustaceans is favored (Copepoda and Cladocera), as well as ciliates and Lamellibranchia larvae. The existing conditions in



the natural park favor Gastrotricha, Ostracoda and Testacea than the rest of the zooplankton groups. Even if the phytoplankton component developed under the existing conditions in the reservoir, the results of the analysis show that the zooplankton was affected, which led to the diminished support capacity.



**Figure 5.** RDA applied to zooplankton in relationship with phytoplankton. Red lines—explanatory variables (X) of the variation of zooplankton communities.

#### 4. Discussion

The phytoplankton community leads the overall functioning of any aquatic ecosystem with effects in ecological services, highlighted especially by defining its ecological status [43]. Compliance with the quality standards set out in the Water Framework Directive should be an important goal, along with the implementation of strategies for sustainable lake management. Anthropogenic pressure on water bodies in urban areas has become increasingly high as society's living standards rise. For this reason, the extrinsic value of their ecosystem services is closely correlated with their accessibility and how they can be exploited [44].

Bucharest has a wide variety of water resources, which allowed us to select several types of ecosystems in order to evaluate ecosystems in different conditions integrated in the same urban system. In these ecosystems, with the exception of Văcărești wetland, the phytoplankton component represented the main category of primary producers.

By monitoring the water quality, we identified periodic increases in the nutrient values and total Chlorophyll-*a*, indicating eutrophication periods. For most of the study period, the nutrients emphasized acceptable levels of concentration, except for autumn 2015 and spring 2017 where the evaluated nutrients ( $\text{NO}_3$ ,  $\text{NH}_4$  and  $\text{PO}_4$ ) showed increases (Figure 2). According to our results there was a takeover of nutrients by the phytoplankton, but they were characterized especially by the trend to increase production from spring to autumn. As a result, autumn had the most frequent moments of hypereutrophy, and moderate-quality conditions (class III) were most frequent in autumn (Table 3). The eutrophication caused by Chlorophyll-*a* changed communities structurally by influencing diversity through species richness, abundance and evenness [45].

In this regard, diversity and primary production assessment outlines the role of phytoplankton in ecosystems and the basis of the entire food web, influencing the development of higher trophic levels [46]. The primary production, evaluated by the Chlorophyll-*a* content, was based on Chlorophyceae, Cyanobacteria and Bacillariophyceae groups (Figure 3).

Despite these results, we obtained another perspective on the appreciation of the real impact in ecosystems due to Chlorophyceae dominance (Figure 3 and Table 6). It is difficult to separately appreciate the quality of water assessed by nutrient levels and on the other hand by Chlorophyll-*a*. In our case, the level of nutrients indicated a good water quality, but Chlorophyll-*a* indicated numerous eutrophy and hypertrophy episodes. Because the ecological processes are closely related and complex, we consider phytoplankton as a relevant indicator for water quality by the short time response of the population to environmental stressors [8], with effects on the quality of ecosystem services [47].

A rich literature discusses the negative role of cyanobacterial dominance, and the possibility of mass multiplication of potentially toxic species, which, depending on environmental conditions, can eliminate cyanotoxins in water [48]. Green algae provide a quality nutritional food for herbivorous zooplankton, contributing significantly to the production and support service of the food web. Due to the multitude of environmental factors, it is difficult to assess whether the high value of Chlorophyll-*a* due to green-algae development can be a beneficial aspect for the lakes.

Given that the mass development of cyanobacteria was reported in our study (Table 6), we can consider the risk of adverse consequences on the ecosystem, with harmful effects in the cascade, which can lead to the restructuring of the entire food web. Potentially toxic cyanobacteria can cause significant economic losses, affecting aquatic organisms that feed the human population [49]. For example, in the United States, losses in drinking water, recreational, and agricultural water resources have exceeded 2 trillion \$ annually [50]. There is also a prevalence of Cryptophyta, reported under certain conditions [51,52]. Our results are consistent with the studies of other authors [53–55]. There are also patterns of dominance that contradict our research [52,56].

The eutrophication of the urban lakes results from the input of nutrients from various sources and high residence time of water, as well as various other factors in adjacent areas [57]. Temperature and nutrients are known as controlling drivers in algae growth. Rising temperature stimulates phytoplankton productivity, doubling the growth rates with each 10 °C, and above 25 °C Cyanobacteria become prevalent [58,59]. The availability of nutrients for the development of phytoplankton assessed by RDA multivariate analysis highlighted the preference of green algae and diatoms for nitrogen and orthophosphate but in conditions of cold periods, while Cyanobacteria and Cryptophyta have been associated with higher-temperature periods and nitrogen nutrients (Figure 4). Eutrophication conditions by Chlorophyll-*a* were present in all study seasons, with higher frequency in autumn (Table 2), and in many cases were not associated with increases in one of the nutrients. Thereby, the phytoplankton communities consume nutrients in the development processes but were still responsible for the deterioration of water quality to a greater extent than the mentioned chemical parameters.

Even if the phytoplankton represented a diverse food supply, due to the algal blooms, the zooplankton structure was typical of anthropogenic ecosystems, represented especially by rotifers and young stages of copepods (Tables 8 and 9). However, under the existing conditions, zooplankton as primary consumer, selectively accessed the phytoplankton. Likewise, compared to the other components of zooplankton, rotifers found strategies for accessing Bacillariophyceae (Figure 5). Vidussi et al. [59] found significant influences of diatoms on rotifers, proving the possibility of this zooplankton group to consume and develop even in Bacillariophyceae blooms conditions.

On the other hand, zooplankton grazers respond to the environmental changes as primary consumers of phytoplankton. First, as a food source, phytoplankton defines the structural features of zooplankton and conversely controls algal growth, with particular effects on water quality [60]. In addition, these communities, through the fast turnover, capture the ecosystem changes in a very short time. In these terms, rotifers, as the most important group of zooplankton, responded significantly to trophic changes, emphasized by the ANOVA test ( $F_{3,47} = 3.39$ ,  $p = 0.02$ ). The post-hoc Tukey test significances were mesotrophic vs. hypertrophic ( $p = 0.03$ ); mesotrophic vs. eutrophic ( $p = 0.02$ ). The Cladocera

and Copepoda requirements in terms of both environmental conditions and food resources were more restricted, being associated with the presence of Chlorophyceae, Cyanobacteria and Dinophyceae (Figure 5). Both Cyanobacteria blooms and mixed blooms with Bacillariophyceae, Chlorophyceae and Cyanobacteria strongly affect water quality [61] and delay water-purification processes. These aspects were highlighted by the zooplankton response to blooming pressures, by poor representation of adult cladocerans and copepods in exchange for rotifer dominance for most of the study. Zooplankton microcrustaceans are affected by eutrophic states [62].

The relationship between biodiversity and primary production has highlighted the interest in interdependence between them and their role in the functioning and stability of the ecosystems [63,64]. Due to the complexity of the connections established between the communities, diversity plays a key role in determining the primary production and the food-web structure [65]. Thus, the representative groups by species richness and abundance were the defining ones in the primary production.

The studied ecosystems had high species richness and low Evenness of the phytoplankton. These aspects highlight the dominance of some species and pressures on the others. Further statistical analysis on diversity showed that phytoplankton communities were influenced by the ecosystem type. Thus, through the ANOVA test, the lakes from Colentina highlighted peculiar conditions for plankton diversity compared to the other lakes. The hydrotechnical changes for flow control of the Colentina River and the formation of large lakes offer both lentic and lotic features. Maintaining a flow of water allowed the periodic entry of new populations of planktonic organisms. At the same time, the lentic feature acquired by the lakes influenced the planktonic populations through local natural and anthropogenic factors. The introduction of control systems on the hydrological regime of rivers determines not only physicochemical changes but also the structure and composition of the biological component [66].

Shannon's diversity index emphasizes a preference of phytoplankton for relatively large-surfaced lakes and a complex biocenosis, such as with the Colentina River chain lakes (Table 4). Artificial features of the Morii reservoir led to the lowest diversity of the phytoplankton. Contrary to expectations, Văcărești did not reach the highest diversity of phytoplankton. The eutrophic and hypereutrophic periods and cyanobacteria blooms might be the cause for unfavorable conditions for the other phytoplankton groups [62]. At the upper level, the zooplankton (Table 8) expressed higher Shannon index values compared with phytoplankton in Văcărești and Morii. Between phytoplankton and zooplankton, complex relationships are established but were not entirely assessable. The zooplankton communities expressed by the Shannon index were positively influenced by the species richness of the phytoplankton ( $r = 0.329$ ,  $p = 0.02$ ) and negatively by the phytoplankton Evenness ( $r = -0.33$ ,  $p = 0.02$ ). Both phyto- and zooplankton have different types of organisms, with specific ecophysiological features which implicitly respond differently to environmental conditions and to variations in diversity [67]. According to Qian et al. [3], diversity tends to be higher in areas with low-to-moderate levels of human intervention. Moreover, the zooplankton taxonomic groups' response was distinct to the eutrophication regarding grazing and accessing the food web [68,69]. Overall, diversity as a support service has been fostered by features as close as possible to the natural conditions of ecosystems. Diversity is influenced by the health state of the ecosystems, and when conditions are affected by pressure factors, they bring structural and functional changes of the communities [70].

## 5. Conclusions

Urban ecosystems face the highest anthropogenic pressure with effects on ecosystem health. Phytoplankton, as an indicator of ecological status both from the point of view of their structural parameters but also of their interrelations with the zooplankton, represented a valuable approach for the evaluation of the ecological conditions. In this context, our study

used phytoplankton by estimating diversity, primary production and water quality to evaluate the impact on ecosystems as dominant primary producers.

The diversity that the phytoplankton groups share in primary production proved to be closely connected, being determining factors in the nutrient cycling and taxonomic structuring of the direct consumers. On the other hand, the evaluated parameters were also reflected in the impact of algal blooms and water quality. Thus, phytoplankton has been shown by cyanobacterial blooms or mixed blooms to be responsible for the qualitative deterioration of water, especially in hypertrophic conditions. The effects of these phenomena were also highlighted in the direct consumer, zooplankton, whose diversity and abundance were characteristic of eutrophic ecosystems, with possible effects on the food web up to fish production. Overall, however, the features of ecosystems as close as possible to natural conditions offer a higher adaptability to respond to anthropogenic pressures and benefit better ecological services.

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