THE INFLUENCE OF ENVIRONMENTAL FACTORS ON PHYTOPLANKTON VARIETY - A CASE STUDY OF THREE PONDS OF WESTERN POLAND

Abstract: The structure of phytoplankton assemblages in three small water bodies was compared and abiotic factors were described. It indicated considerable differences in the species abundance and biomass of the phytoplankton as well as the chemical composition of water between artificial pond (No. 2) and others. A total of 455 phytoplankton taxa were recorded. All ponds were characterized by greatest species richness of Chlorophyta. The highest biomass was noted in August-September 2015, and it was true for each pond. The CCA models showed dependences between the variables under study and phytoplankton groups. They indicated which environmental variables had the greatest influence on the biomass of phytoplankton in the waterbodies under analysis. The biomass of most of the taxonomic group in the phytoplankton (except Miozoa) depended on the presence of nitrogen not only in form of nitrates but also in the form of mineral nitrogen. The research findings suggest the trend of future studies on the phytoplankton in these ponds. The analysis of its variability should also include the influence of light and the influence of consumers on the food chain in the ecosystem.

Keywords: small waterbody, agricultural landscape, phytoplankton structure, model CCA

Introduction

Small waterbodies are characterised by high variability of environmental conditions, mainly due to their low volume, frequent water level fluctuations and the negative influence of the surroundings [1]. Various suspensions and substances from surrounding areas become dissolved and accumulated in water. They provide good conditions for the development of different groups of water organisms, including phytoplankton [2]. Access to light, the temperature of water and concentration of biogenic compounds are key parameters affecting the development of phytoplankton [3, 4]. The concentration of nutrients, especially in the smallest waterbodies, is strictly related to the type, rate, period and manner of pollution of the water environment [5-7]. On the other hand, phytoplankton affect a large number of physical and chemical parameters of water. In consequence, this affects the possibility of using water for economic purposes. Intensive development of the phytoplankton causes an increase in the following parameters of water quality: pH, colour, turbidity, the content of total organic carbon [8]. The seasonal dynamics of phytoplankton groups and the abundance of species have been better investigated in large waterbodies, i.e. lakes. Small waterbodies located in fields and forests are hardly ever objects of detailed phycological studies [9-11]. Usually researchers tend to pay more attention to water
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However, these waterbodies are characterised by high diversity due to numerous environmental issues related to their drainage basins. It is substantial to identify key factors managing the structure of populations in these waters. Identification of the species structure in the bioseston and the factors regulating its abundance (density) gives a possibility to improve these waterbodies by selection of adequate economic (restoration) activities [15].

The aim of this study was to identify the dynamics of variation in the phytoplankton of selected waterbodies by analysing physical and chemical water parameters and to determine relations between these parameters. Three small and shallow waterbodies of anthropogenic nature were selected for analyses. Due to the common origin and morphometric similarities between the waterbodies we assumed that there would not be significant differences in the research parameters referring to the phytoplankton dynamics.

**Materials and methods**

The three waterbodies under study are located in the north of the Commune of Dopiewo. The commune is located in the macroregion of Greater Poland Lake District, mesoregion of Poznan Lake District, in the drainage basin of the Samica Steszewska and Wirynka Rivers, which are left-bank tributaries of the Warta River.

Pond No. 1 is located in the village of Drwesa. It is of anthropogenic origin and was formed in the late 1980s by deepening a small depression where water accumulated after heavy rain. The pond is oval with a poorly diversified shoreline. It occupies an area of about 0.03 ha. Its maximum depth is about 1.9 m. There is an agritourist building, residential buildings and farm buildings in the drainage basin of the pond.

Pond No. 2 is located in the village of Wieckowice. It is an artificial pond occupying an area of about 0.2 ha. Its maximum depth is about 1.7 m. The shoreline is not much diversified. It is used by anglers and local inhabitants for recreation. There are farmlands, a park established in the 19th century, single-family houses and farm buildings in the drainage basin of the pond. In June 2013 the pond underwent restoration. Bottom sediments were removed, the pond was surrounded by fascines and grass was sown on the surrounding escarpments.

Pond No. 3 is located in Dopiewo. In terms of the surface of the water table (0.58 ha), the basin (about 111 ha) and the maximum depth (2.0 m) is the largest, among others. It is a flow pond. Pond No. 3, like pond No. 2, performs fire, fishing and recreational functions. The building of the Volunteer Fire Brigade is located at the south-east coast. Every year, in the autumn period, reclamation works are carried out at the edge of the pond consisting in the removal of hydromacrophytes from the pond.

**Fieldwork**

Once a month during three years at one station samples of water were collected from the surface layer of water (0.2 m) for analysis. The water for phytoplankton determination was collected into 0.5 dm$^3$ plastic containers and fixed with Lugol’s iodine. The water for nutrients analysis was sampled into 0.7 dm$^3$ PC containers and fixed with chloroform. Samples for measurements of the concentration of chlorophyll $a$, total suspension (seston) and organic carbon were collected into 0.5 dm$^3$ bottles and they were not fixed. The temperature of water, its pH, the concentration of dissolved oxygen and electrolytic conductivity were measured in situ by means of a multi-parameter probe (YSI 556 MPS).
Laboratory work

Phytoplankton was analysed for species composition, abundance and biomass using cylindrical 9 or 14 cm³ planktonic chambers and MOD-2 reverse microscopy (PZO) and CKX41 (Olympus). A CKX-41 microscope with image analysis was used for precise measurements and photographic documentation. The concentration of chlorophyll a, was measured by means of spectrophotometry having filtered the water through Whatman filters grade GF/F (pore diameter about 0.6-0.8 µm), according to the Polish standard PN-C-05560-02:1986 [16]. The seston was measured with the weight method.

The chemical parameters: total phosphorus, Pₜₒₜ, dissolved phosphates, PO₄³⁻, ammonium nitrogen, NH₄⁺, nitrate nitrogen, NO₃⁻, nitrite nitrogen, NO₂⁻, Total Kjeldahl Nitrogen, TKN, organic nitrogen, Nₜₒᵦ, total nitrogen, Nₗₒᵦ, were measured using the methods reported by Baird et al. [17]. Total and dissolved carbon were measured with the infrared method by means of a TOC-V CPN analyser (SHIMADZU).

Statistical analysis

In order to assess the difference between parameters in examined waters one-way analysis of Anova Variance was performed (with significant level of \( p < 0.05 \)).

The synthesis of all the variables resulted in the formation of CCA model. Canonical variate analysis (CVA), which is the canonical variety of Fisher’s linear discriminant analysis, was used to construct the model. Stepwise analysis was used to construct the best prediction models. It enabled selection of the variables which explained the model best. First, all the variables were included in the model. Next variables not statistically significant were eliminated.

The discriminant analysis gave only the variables which were significant to the model. The reverse step analysis was based on \( F \) and \( p \) values. Outliers were eliminated from the model, because they might have had negative influence on dependences between the data and environmental variables. The Monte Carlo permutation test was conducted to determine the significance limit (individually for each variable and for the entire model - 999 repetitions). The Canoco 4.5 for Windows program was used for calculations and the CanoDraw for Windows program was used for their graphic presentation.

Results

Environmental variables

The pH of water in all ponds was alkaline (Table 1). The maximum nitrate nitrogen concentration in ponds No. 2 and No. 3 was greater than 16 mg N · dm⁻³, which might indicate high eutrophication of those water bodies. Organic carbon occurred mainly in the dissolved form, DOC. The mean values of total organic carbon, TOC, were similar in examined ponds. There were considerable fluctuations in the concentration of chlorophyll a in all ponds. The concentration of chlorophyll a varied in a wide range from 2.9 in pond No. 2 to 865.1 µg · dm⁻³ in pond No. 3 during the tests, as in the case of chlorophyll a, the seston dry mass content in the pond No. 2 and No. 3 were higher (Table 1). The high average value of the nitrogen-phosphorus ratio shows that phosphorus seemed to limit the development of the phytoplankton to a greater extent, but it was not statistically proved. On the basis of one-factor analysis of Anova variance it was indicated, that examined ponds significantly differed (\( p < 0.05 \)) comparing pH, conductivity, dissolved oxygen, N-NO₃,
N-NO₂, TKN, P-PO₄, Pₜₒᵗ, DOC, seston, abundance. The difference for others parameters was not statistically significant.

Table 1

| Variable                     | Pond No. 1       | Pond No. 2       | Pond No. 3       | ANOVA          |
|------------------------------|------------------|------------------|------------------|----------------|
| Temperature [°C]             | (13.0) 0.5-22.4ᵃ | (14.9) 1.2-27.1ᵇ | (15.4) 2.3-29.0ᵃ | 0.620 0.481 |
| pH value [-]                 | (7.6) 7.1-9.6ᵃ   | (8.5) 7.5-10.3ᵇ  | (8.4) 7.4-10.9ᵇ  | 0.003 6.391 |
| Conductivity [µS · cm⁻¹]     | (457) 330-564ᵃ   | (928) 662-1297ᵇ  | (787.5) 509-1068ᵃ| < 0.001 90.747|
| Dissolved oxygen [mg O₂ · dm⁻³] | (5.7) 1.5-12.4ᵃ  | (9.1) 5.7-24.3ᵇ  | (7.9) 5.2-17.0ᵃ  | < 0.001 13.156|
| N-NH₄ [mg N · dm⁻³]         | (1.1) 0.59-7.07ᵃ | (1.31) 0.82-2.33ᵇ| (1.3) 0.53-3.14ᵃ | 0.701 0.356 |
| N-NO₂ [mg N · dm⁻³]         | (0.007) 0.002-0.017ᵃ | (0.068) 0.008-8.76ᵇ | (0.055) 0.007-0.154ᵇ | < 0.001 19.899 |
| N-NO₃ [mg N · dm⁻³]         | (0.76) 0.03-2.69ᵃ | (6.42) 0.03-19.9ᵇ | (2.73) 0.06-16.63ᵃ| < 0.001 10.566|
| Nₐₒᵣ [mg N · dm⁻³]         | (1.24) 0.04-7.84ᵃ | (1.63) 0.36-6.44ᵃ | (1.76) 0.77-8.06ᵃ | 0.267 1.343 |
| TKN [mg N · dm⁻³]           | (1.99) 0.63-9.01ᵃ | (3.14) 1.52-8.73ᵇ | (3.22) 1.50-11.20ᵇ | 0.016 4.329 |
| P-PO₄ [mg P · dm⁻³]         | (0.171) 0.042-0.462ᵃ | (1.024) 0.249-4.153ᵇ | (0.554) 0.118-1.928ᵇ | 0.010 4.880 |
| Pₜₒᵗ [mg P · dm⁻³]         | (0.198) 0.038-0.287ᵇ | (0.421) 0.138-1.361ᵇ | (0.398) 0.079-2.527ᵇ | 0.010 4.880 |
| Nₙₒₜ:Pₜₒᵗ                  | (27.0) 2.4-288.8ᵃ | (33.3) 1.9-120.4ᵃ | (31.0) 3.7-171.0ᵃ | 0.080 0.923 |
| TOC [mg C · dm⁻³]           | (9.8) 5.0-15.0ᵃ   | (11.6) 7.1-23.0ᵇ  | (13.9) 7.2-32.0ᵇ  | 0.005 5.525 |
| DOC [mg C · dm⁻³]           | (8.3) 6.3-12.0ᵃ   | (9.8) 6.9-17.0ᵇ  | (10.2) 5.8-31.0ᵇ  | 0.017 4.303 |
| Chlorophyll a [µg · dm⁻³]   | (79.6) 5.4-379.0ᵃ | (125.7) 2.9-678.1ᵇ | (112.1) 4.3-865.1ᵃ | 0.189 1.700 |
| Seston dry mass [mg · dm⁻³] | (11.6) 2.8-27.0ᵃ   | (26.9) 4.0-61.6ᵇ  | (26.5) 6.0-114.0ᵇ | < 0.001 13.057|
| Abundance of phytoplankton [cells · cm⁻³] | (4251.8) 738.3-9650ᵃ | (69119.3) 3871.1-1032299ᵇ | (283474.4) 606.4-3295242.0ᵇ | 0.001 8.526 |
| Biomass of phytoplankton [ng · dm⁻³] | (15.1) 0.56-156.3ᵃ | (19.7) 1.1-77.8ᵃ | (27.4) 0.76-123.2ᵃ | 0.166 1.831 |

Biological variables

In total 455 taxa were identified in the phytoplankton. There were 163 taxa identified in pond No. 1, 232 taxa in pond No. 2 and 311 taxa in pond No. 3. All ponds were characterised by the greatest species richness of Chlorophyta - 58, 103 and 130 taxa, respectively (Table 2). Phytoplankton taxonomic groups were presented in Appendix². In the pond No. 1 Chlorophyta were represented by Mychonaster jurisii, Chlamydomonas sp., Spermatozopsis exultans and Cryptophytes by Cryptomonas massonii i C. curvata, whereas in pond No. 2, particularly among spring-summer season phytoplankton abundance was

² Appendix is available at the paper page
dominated by Chlorophytes (*Monoraphidium circinale*, *Chloroidium ellipsoideum*, *Tetrastrum staurogeniaeforme* and *Siderocelis granulate*).
Fig. 2. Fluctuation of phytoplankton biomass in examined ponds in 2013-2015: a) pond No. 1, b) pond No. 2, c) pond No. 3
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Table 2

| Phytoplankton Group | Pond No. 1 | Pond No. 2 | Pond No. 3 |
|---------------------|------------|------------|------------|
| Cyanobacteria       | 9          | 12         | 37         |
| Euglenozoa          | 20         | 17         | 23         |
| Cryptophyta         | 12         | 17         | 18         |
| Mioza               | 7          | 4          | 10         |
| Ochrophyta          | 24         | 21         | 25         |
| Bacillariophyta     | 26         | 53         | 52         |
| Chlorophyta         | 58         | 103        | 130        |
| Charophyta          | 7          | 5          | 16         |
| Total               | 163        | 232        | 311        |

In pond No. 3 dominated Chlorophyta (*Chlorella oocystoides*, *Kirchneriella contorta* and *Coelastrum microporum*) and Cyanobacteria (*Pseudanabaena limnetica*, *Dolichospermum flosaqua*, *Lyngbya* sp.). Analysing seasonal changes in examined ponds during three years of study it was indicated the highest amounts of biomass in August and September. Comparing biomass (Fig. 1) and its fluctuations in examined years, the highest biomass was found in 2015 (Fig. 2).

**Statistical analysis of results**

The canonical correspondence analysis (CCA) showed which variables had the greatest influence on the amount of the biomass of various taxonomic groups in the phytoplankton. The analysis was preceded by a progressive stepwise analysis, in which each environmental variable was tested for its statistical significance. Next, the decision was made whether it should be included in the model. Among the 23 variables under analysis the ones that were visible in the model proved to be the most significant (Total Kjeldahl Nitrogen, orthophosphates, temperature, conductivity, pH, chlorophyll, the total nitrogen/phosphorus ratio, nitrates and mineral nitrogen). The model used in the study also revealed interesting dependencies between the environmental variables under analysis - indicating synergistic effect of such variables as pH and orthophosphates. There was a similar correlation between electrolytic conductivity and the forms of nitrogen: mineral and nitrate. The water with higher concentrations of $N_{\text{min}}$ and $\text{NO}_3^-$ was characterised by high electrolytic conductivity (Table 3).

Table 3

| Variable   | p-value | F-value | [%] Expl. |
|------------|---------|---------|-----------|
| TKN        | 0.001   | 14.007  | 14.51     |
| Conductivity| 0.001  | 12.269  | 13.02     |
| P-PO$_4^-$ | 0.001   | 11.227  | 12.85     |
| Temperature| 0.002   | 10.895  | 11.66     |
| pH         | 0.002   | 9.796   | 10.23     |
| Chlorophyll| 0.003   | 9.456   | 8.77      |
| N$_{\text{tot}}$ | 0.005 | 8.110   | 7.16      |
| N-NO$_3^-$ | 0.013   | 6.541   | 6.21      |
| $N_{\text{min}}$ | 0.027 | 6.374   | 5.11      |
As the analysis below shows, the biomass of most of the taxonomic groups in the phytoplankton (except Miozoa) depended on the presence of nitrogen not only in the form of nitrates but also in the form of mineral nitrogen. Apart from that, the ratio between total nitrogen and phosphorus (Fig. 3) proved to be an important factor limiting the growth of phytoplankton groups. The analysis also revealed similar values of the variables under study in waterbodies 1 and 2. The most important difference between waterbody 3 and the other waterbodies was its higher biomass of Miozoa, which altogether in the following years amount to: 82.92, 54.74, 163.32 mg · dm\(^{-3}\).

**Discussion**

There is high taxonomic and quantitative diversification in the phytoplankton even in waterbodies of the same trophic state and identical origin. After a short time anthropogenic waterbodies begin to function similarly to natural waterbodies. Although the ponds under study were of identical origin and they were located near each other, there were significant differences in the phytoplankton density between them. Pond No 3 was the most abundant in phytoplankton than the other. Chlorophyta had the highest share in the total count of phytoplankton in all examined ponds. The study of shallow artificial pond in Turkey also indicated that the most dominant group in this pond was Chlorophyta (21 of 39 taxa) [18]. This study results agree with the findings in agricultural ponds reported by various authors [19]. Species with small cells and colonies were predominant in this group. Most of the species were commonly found in a large number of eutrophic and hypertrophic waterbodies. The comparison of the phytoplankton composition revealed considerable differences between the species in examined waterbodies. The pond No. 1 was less abundant in species than the other ponds. Pond No. 2 was previously examined by
Pajchrowska and Szpakowska [20]. In 2011 they identified 98 taxa in the phytoplankton, i.e. only three taxa more than in this study, whereas in 2014 106 taxa were identified. It is most likely that differences in the floristic list were caused by analysis of different numbers of samples for phytoplankton investigations. There have been no data published about the abundance of phytoplankton species in the pond No. 2 and pond No. 3. During the investigations the superficial layer of sediment was removed and escarpments were formed again. These procedures were conducted between the third and fourth collection of the water samples. They caused a decrease in the phytoplankton density and an increase in the TOC concentration. The studied water bodies were characterised by some increases of biomass in warm months (August 19.8 °C and September 22.1 °C). Moreover, the dynamics changes in the abundance of the phytoplankton were noted in the investigated ponds. Also Celekli et al. [18] who studied relationship between phytoplankton composition and environmental variables in an artificial pond indicated that the number of phytoplankton species increased during the fall season. Kozak et al. [21] found that some phytoplankton species can adapt to lower temperature. They revealed that the highest participation in the abundance and biomass of phytoplankton in spring was noted in case of Diatoms, Cryptophytes and Chlorophytes. These groups prefer lower temperature.

Changes in the physicochemical properties of water usually result in qualitative and quantitative changes in the phytoplankton [22]. The interaction of physical, chemical and biological parameters of water controls the production of phytoplankton. These parameters differently affect these densities [23]. In ponds No. 2 and 3 the largest number of representatives of individual species in the phytoplankton was noted in the water with high concentration of oxygen, which was produced in the photosynthetic process.

The investigations showed that the occurrences of some phytoplankton species was influenced by phosphorus compound (P$_{tot}$, P-PO$_4$). The CCA results provided by Meng et al. [24] revealed that P$_{tot}$ and N$_{tot}$ followed by pH exhibited a close relation with dominant species appeared in examined pond. Borics et al. [25] who studied the variables that can be considered as main drivers of phytoplankton biomass indicated significant relationship between N$_{tot}$ and ammonium concentration and phytoplankton biomass which agrees well with our previous findings. Also Zebek and Szymanska [26] who studied abundance, biomass and community structure of pond phytoplankton related to the catchment characteristics using CCA analysis indicated that N-NH$_4$ and N$_{tot}$ enhanced phytoplankton growth in the mid-meadow and mid-field ponds, whereas P-PO$_4$ and pH influenced growth in the mid-forest pond. The study by Kozak et al. [21] also showed a strong correlation between the biomass of cyanobacteria and P-PO$_4$. Some studies indicated that the growth of the biomass of cyanobacteria may be influenced by nutrients excreted by zooplankton organisms, as was observed in Swarzedzkie Lake. The study by Kozak et al. [21] indicated that the biomass of various phytoplankton groups was positively correlated with N-NO$_2$, pH, oxygen, saturation and biochemical oxygen demand (BOD$_5$), but it was negatively correlated with conductivity.

Moreover the size of the cyanobacteria biomass largely depends on factors such as temperature, orthophosphate content and water pH. A high content of orthophosphates, high temperature of water and its alkaline pH are optimal conditions for the growth of cyanobacteria in small waterbodies. The analysis revealed a positive correlation between temperature and the chlorophyll content. Elevated temperature may cause an increase in the chlorophyll content in phytoplankton cells.
The dependences shown on the aforementioned models prove that the nutrients contained in the water of the ponds may have different (not necessarily positive) influence on the phytoplankton biomass. For example there was positive correlations between cyanobacteria and P-PO$_4$, pH, temperature and Total Kjeldahl Nitrogen, whereas there was no significant correlation between this phytoplankton group and conductivity. Experiments conducted on several ponds in North-East Poland using CCA analysis also showed that orthophosphates influenced phytoplankton growth in mid-field and mid-forest ponds [26].

While some authors [11] reported clear associations between algal assemblages and a variety of environmental factors such as nutrient loading and catchment character, this study determined differences in ponds algal biomass and abundance based on surface area. The relationships established in this study between phytoplankton and environmental conditions can influence future directions in small water-body protection.

**Conclusion**

The ponds differed significantly in their maximum and average phytoplankton density. In all ponds representatives of Chlorophyta had the greatest share in the total count of phytoplankton. Most of these species were commonly found in a large number of small eutrophic and hypertrophic waterbodies.

Fluctuations of particular groups of phytoplankton for each examined water-bodies were different, in pond No. 2 species from Chlorophyta groups predominated, whereas in pond No. 3 Miozoa. In July-September in ponds No. 1 and 2 also Cyanobacteria was observed. The highest biomass was noted in August-September 2015, and this was true for each ponds.

The canonical correspondence analysis (CCA) shows the biomass of most of the taxonomic groups in the phytoplankton (except Miozoa) depended on the presence of nitrogen not only in the form of nitrates but also in the form of mineral nitrogen. Apart from that, the ratio between total nitrogen and phosphorus proved to be an important factor limiting the growth of phytoplankton groups.

The analysis also revealed similar values of the variables under study (Total Kjeldahl Nitrogen, orthophosphates, temperature, conductivity, pH, chlorophyll, the total nitrogen/phosphorus ratio, nitrates and mineral nitrogen) in waterbodies 1 and 2. The most important difference between waterbody 3 and the other waterbodies was its higher biomass of Miozoa.

The model showed that environmental variables could selectively affect the count and distribution of individual phytoplankton species in the ecosystem of shallow waterbodies.

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