

## A SPATIAL APPROACH OF THE ENVIRONMENTAL FACTORS CONTROLLING PLANKTON COMMUNITIES IN THE DANUBE DELTA

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**Abstract.** The Danube Delta represents an open socio-ecological system of international importance. The services provided by this environmental complex to human society are endangered. The aim of this study was to understand the driving mechanisms triggering harmful algal blooms, in the context of environmental changes and their consequences for food webs. The cyanobacterial blooms are triggered by a combination of abiotic factors: nutrient content, increasing temperatures, light intensity. The bloom risk increases by removing natural competitors (green algae, macrophytes), by affecting the natural grazers which control the algal growth. The results of our investigations carried out in 26 ecosystems belonging to the 4 lake complexes (Roșu-Puiu, Matița-Merhei, Gorgova-Uzlina, Șontea-Fortuna) indicated a higher frequency of cyanobacterial blooms in late summer or early fall. The quantification of algal community performed *in situ* with a field fluorometer (Fluoroprobe III, bbe Moldaenke, DE) indicated that 4 major algal groups were present in all the lakes: Chlorophyta, Bacillariophyta, Cryptophyta, Cyanobacteria. The structural parameters fluctuated in a wide range. The complex most affected by the algal blooms was Roșu – Puiu. The results of multiple regression analysis evidenced a strong relationship between Cyanobacteria and TP ( $p < 0.001$ ) and marginally with  $\text{NO}_3$  and  $\text{PO}_4$ . The light and TP were significant factors that influenced the Chlorophyceae development. The phytoplankton biomass, expressed as chlorophyll *a* content, was explained by the TP (89%,  $p < 0.001$ ). The zooplankton analysis of the samples collected in spring evidenced 95 taxa, Rotifera (44 sp.), Ciliata (21 gen), Cladocera (18 sp.) and Copepoda (6 sp.). The minimum value of zooplankton abundance was in Gorgostel ( $1.67 \text{ ind L}^{-1}$ ) and maximum values in Roșu ( $29.93 \text{ ind L}^{-1}$ ). An analysis of the similarity degree highlighted a 50% resemblance among the following lakes that belongs to Matița-Merhei complex: Dracului, Lung, Babina, Rădăcinoasele. Future analyses will focus on the cyanobacteria effects on food web.

**Keywords:** HABs, nutrients, Danube Delta, eutrophication, multiple regressions.

### Rezumat. O abordare spațială a influenței factorilor de control ai comunităților planctonice din Delta Dunării.

Delta Dunării reprezintă un sistem socio-ecologic deschis de importanță internațională. Serviciile oferite de acest complex societății umane sunt alterate. Scopul acestui studiu a fost de a înțelege mecanismele care determină dezvoltarea algelor dăunătoare, în contextul schimbărilor de mediu și consecințele acestora pentru rețeaua trofică. Înfloririle cu cianobacterii sunt declanșate de o combinație de factori abiotici: conținutul de nutrienți, creșterea temperaturii, intensitatea luminii. Riscul de înflorire crește prin eliminarea concurenților naturali (alge verzi, macrofite), prin afectarea populațiilor de organisme ierbivore care controlează creșterea algelor. Cercetările noastre, efectuate în 26 de ecosisteme aparținând la 4 complexe lacustre (Roșu - Puiu, Matița - Merhei, Gorgova - Uzlina, Șontea-Fortuna), au indicat o frecvență mai mare a înfloririlor cianobacteriene vara târziu sau la începutul toamnei. Cuantificarea comunităților fitoplanctonice, efectuată *in situ* cu un fluorometru submersibil (Fluoroprobe III, bbe Moldaenke, DE) a indicat prezența a 4 grupe principale în toate lacurile: Chlorophyta, Bacillariophyta, Cryptophyta, Cyanobacteria. Parametrii structurali au fluctuat într-un interval larg. Complexul cele mai afectat de înfloririle algale a fost Roșu - Puiu. Rezultatele regresiei multiple au evidențiat o relație puternică între cianobacterii și TP ( $p < 0,001$ ) și marginal cu  $\text{NO}_3$  și  $\text{PO}_4$ . Lumina și TP au fost factorii care au influențat semnificativ dezvoltarea cloroficeelor. Biomasa fitoplanctonului ( $\mu\text{g chl a/L}^{-1}$ ), a fost explicată prin TP (89 %,  $p < 0,001$ ). Analiza zooplanctonului din primăvară a pus în evidență 95 de taxoni, Rotifera (44 sp.), Ciliata (21 genuri), Cladocera (18 sp.) și Copepoda (6 sp.). Valoarea minimă a abundenței zooplanctonului a fost în Gorgostel ( $1,67 \text{ ind L}^{-1}$ ) iar maxima în Roșu ( $29,93 \text{ ind L}^{-1}$ ). O analiză a gradului de similaritate, bazată pe abundența zooplanctonului a arătat o asemănare de 50 % între următoarele lacuri: Dracului, Lung, Babina, Rădăcinoasele (complexul Matița-Merhei). Analizele viitoare se vor concentra pe efectele dezvoltării cianobacteriilor asupra rețelei trofice.

**Cuvinte cheie:** înfloriri toxice, nutrienți, Delta Dunării, eutrofizare, regresie multiplă.

## INTRODUCTION

The importance of monitoring of continental water quality is well known. Especially, the Danube Delta Biosphere Reserve is a complex of ecosystem whose structural and functional integrity must be preserved. The assessment of the health of ecosystems is the main goal for conservation policy. Ecosystem conservation efforts should go in the sense of keeping their structural and functional features, so that they can provide a qualitative flow of services to socio-economic systems (VĂDINEANU, 2004; RÎȘNOVEANU et al., 2008; MOLDOVEANU et al., 2010).

The ecological conditions of the deltaic lakes depend on the quality of input water. The dynamics of eutrophication in the Danube River has been correlated with the eutrophication level inside of the delta (TÖRÖK, 2009). As a major component of primary producers, phytoplankton provides the “life support” system for the whole food web in the aquatic ecosystems. The composition of the algal community and the quality of the food provided for the higher trophic levels have a tremendous importance on shaping the development of the other aquatic communities.

Cyanobacteria provide in general low nutritional value; even more, in specific conditions, the toxins they release may pose health hazard on aquatic biota and human health. Species composition and abundance of phytoplankton groups in a community are the main determinants of aquatic ecosystem structure and function.

Alterations in the phytoplankton community can lead to negative impacts on entire ecosystems, harmful and nuisance algal blooms (PAERL, 1988), bottom water hypoxia/anoxia (BIANCHI et al., 2000), nutrient availability (PINCKNEY et al., 1999), eutrophication (NIXON, 1995), and quality of food sources for higher trophic levels (KLEPPEL, 1992).

The competition for light and nutrients with other algal groups and macrophytes, grazing and some environmental parameters may enhance or, on the contrary, may hinder their development.

One of the first attempts to use *in situ* fluorescence method in the Danube Delta was made in 2008, but only in the summer season, in some branches of the Danube and channel network (TÖRÖK, 2009)

The ability to monitor phytoplankton community structure *in situ* and in real-time can provide useful scientific data for policy managers when attempting to observe how short-term changes in environmental conditions in freshwaters are translated into phytoplankton community responses (BEUTLER et al., 2002; SEE et al., 2005).

It is simplistic to support the hypothesis that all plankton species in the ecosystem are limited by the same factor. Different phytoplankton species in the same community may have different needs and may be limited by different factors. It has been shown that multiple factors limit phytoplankton development (MEDINA-SÁNCHEZ et al., 1999). In our study, multiple regressions between plankton variables and key factors were performed in order to prove which are the drivers determining phytoplankton and zooplankton communities in the four main ecological complexes of the Danube Delta.

## MATERIAL AND METHODS

### Study site and sampling:

The Danube Delta Biosphere Reserve is located at 45°0'N latitude, 29°0'E longitude in the eastern part of Romania. The sampling campaigns were carried out in 2013, in an extensive way, seasonally (May, July and September), on the water column, from 26 different deltaic ecosystems belonging to 4 complexes (Roșu-Puiu, Matița-Merhei, Gorgova-Uzlina, Șontea-Fortuna) (Fig. 1).

### Methods

Water samples have been taken for the physical-chemical determinations of variables. The transparency was established with Secchi disk and the depth with Humimbird 260 sonar. The temperature, pH, dissolved oxygen content were measured in the field with a multiparameter WTW 340i (Germany). Samples for chemical analyses were frozen for further analyses in the lab. Nutrients were determined spectrophotometrically:  $\text{NH}_4^+$  - as blue-green compound due to the interaction between active chlorine and ammonia in aqueous solutions chloramines are formed, which react with phenols in alkaline solution and with the help of a catalyzer,  $\text{NO}_2^-$  - nitrite in acidic solution forms diazonium salts with primary aromatic amines who then form an azo dye with aromatic amines (Standard Operating Procedure from EAWAG, 2001).  $\text{NO}_3^-$  - as yellow compound with sodium salicylate (TARTARI & MOSELLO, 1997). Ortho-Phosphate - reacts with molybdenum reagent to a blue colour. (TP) - by oxidation with potassium peroxodisulphate (TARTARI & MOSELLO, 1997). Total organic matter and total nitrogen were determined by multi N/C 3100 TOC/TN<sub>b</sub> analyzer; the digestion is performed by thermocatalytic high-temperature oxidation in the presence of special catalysts.

The total phytoplankton biomass and the gravimetric abundance of different algal groups (expressed as chlorophyll *a* content) were assessed by a submersible fluorometer. The Fluoroprobe III, bbe Moldaenke, DE, is a highly sensitive measuring instrument for the analysis of chlorophyll with algae class determination.

The fluorescence of algae due to excitation by visible light mainly depends on the presence of chlorophyll *a*, a common pigment in the plant world. The occurrence of other pigments is typical for different algae classes. Interactions between these different pigment systems with chlorophyll result in a special excitation spectrum for the taxonomical algae classes (470 nm LED for green algae; 610 nm LED for Cyanobacteria; 525 nm LED for diatoms; 570 nm LED for cryptophyceae) and other fluorescing matter (for example, yellow substances) to enhance the accuracy of the measurements. Measurements are made every second, allowing for vertical and horizontal profiles in aquatic systems. Results are provided in terms of chlorophyll *a* per liter of water ( $\mu\text{g L}^{-1}$ ).

The zooplankton samples were collected by filtering 50 liters of water using a Patalas-Schindler device (5 L) on water column through a 65  $\mu\text{m}$   $\emptyset$  mesh network, and preserved with 4% formaldehyde solution.

The zooplankton species were identified by specific keys for each taxonomic group. The abundance ( $\text{ind L}^{-1}$ ) was assessed by microscopic methods, using a Zeiss inverted microscope type, by direct counting into a Kolkwitz chamber (UTERMÖHL, 1958).

### Data analysis

Statistical analyses were performed using SPSS 15.0 Windows Evaluation Version, available for download at <http://www.spss.com>.

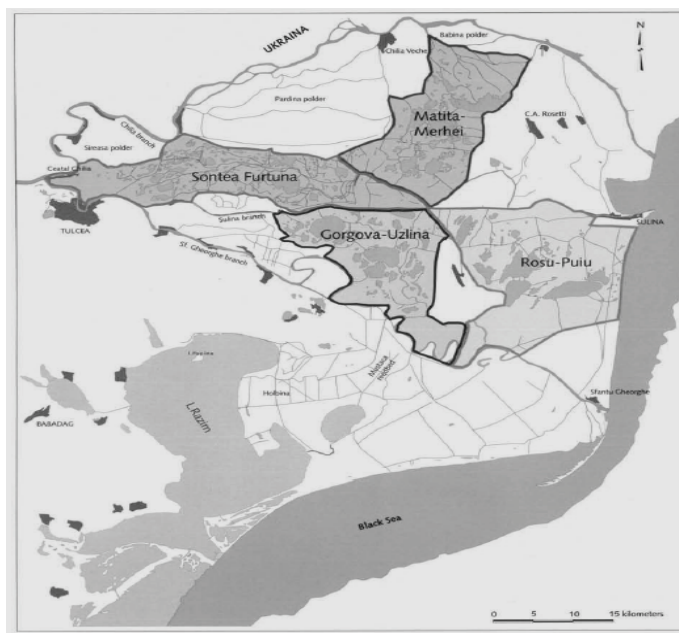


Figure 1. The map of the Danube Delta with the sampling ecosystems (adapted after OOSTERBERG et al., 2000).

### RESULTS AND DISCUSSIONS

The determination and quantification of algal community performed *in situ* with a field fluorometer (Fluoroprobe III, bbe Moldaenke, DE) indicated that 4 major algal groups were present in all the lakes, in all the investigated seasons: Chlorophyta, Bacillariophyta, Cryptophyta and Cyanobacteria. However, the structural parameters fluctuated in a wide range.

In May, the highest values were found in Merhei, Erenciuc and Cuibul cu lebede ( $6.39\text{--}7.63 \mu\text{g L}^{-1}$ ). Low values were recorded in 50% of the lakes, ranging from  $0.90\text{--}2.48 \mu\text{g L}^{-1}$  (Fig. 2). This result was to be expected because in spring, when the temperature is low, phytoplankton does not record a remarkable development but special circumstances. An analysis of the group contribution of the studied lakes revealed as dominant groups Chlorophyta (37%), followed by Bacillariophyta and Cryptophyta, both with 22%. Cyanobacteria group had a contribution of 20% in the phytoplankton community.

In spring, the diversity (in terms of species richness) showed high values, characteristic of typical lacustrine systems of the Delta. The number of phytoplankton species reached a maximum in Merheiu Mic (42 sp.), many other lakes varying in the range of 22-42 sp. This parameter was in inverse correlation with the reduced biomass recorded in this period ( $3.12 \mu\text{g L}^{-1}$ ).

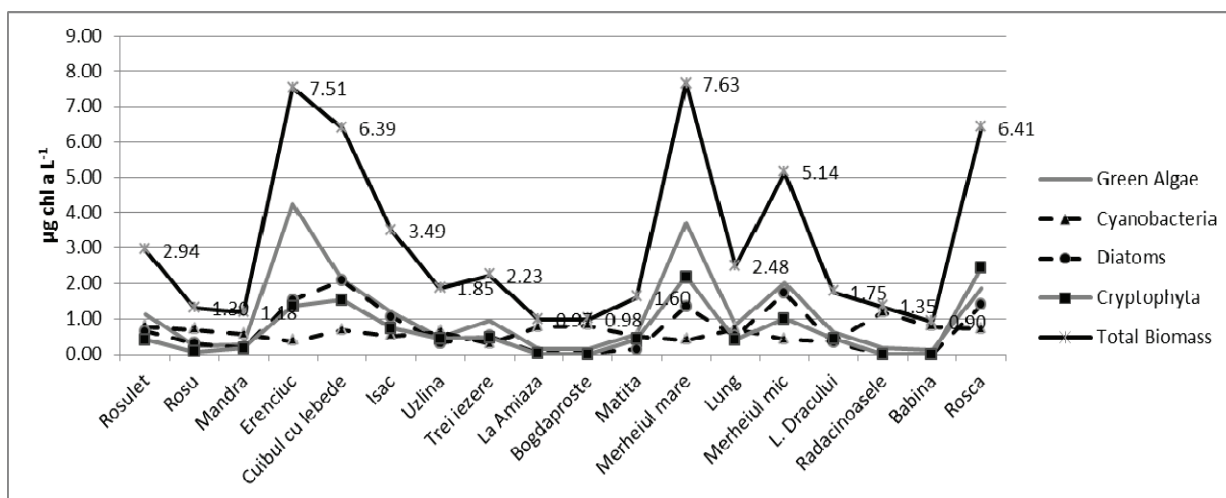


Figure 2. The variation of the phytoplankton biomass in May in the investigated ecological complexes of the Danube Delta.

The same four groups were present in July, but the biomass values changed significantly.

The lakes average of the total phytoplankton biomass doubled ( $6.78 \mu\text{g L}^{-1}$ ), compared to May. The highest values were recorded in Lake Puiu and Roşuleţ  $28.07$  and  $25.76 \mu\text{g L}^{-1}$  respectively. Over 50% of the sampled lakes (25 lakes) showed low levels of biomass, between  $0.97$  and  $2.78 \mu\text{g L}^{-1}$  (Fig. 3). The dominant group belonged to Chlorophyta (37%), followed by Cyanobacteria, 26%.

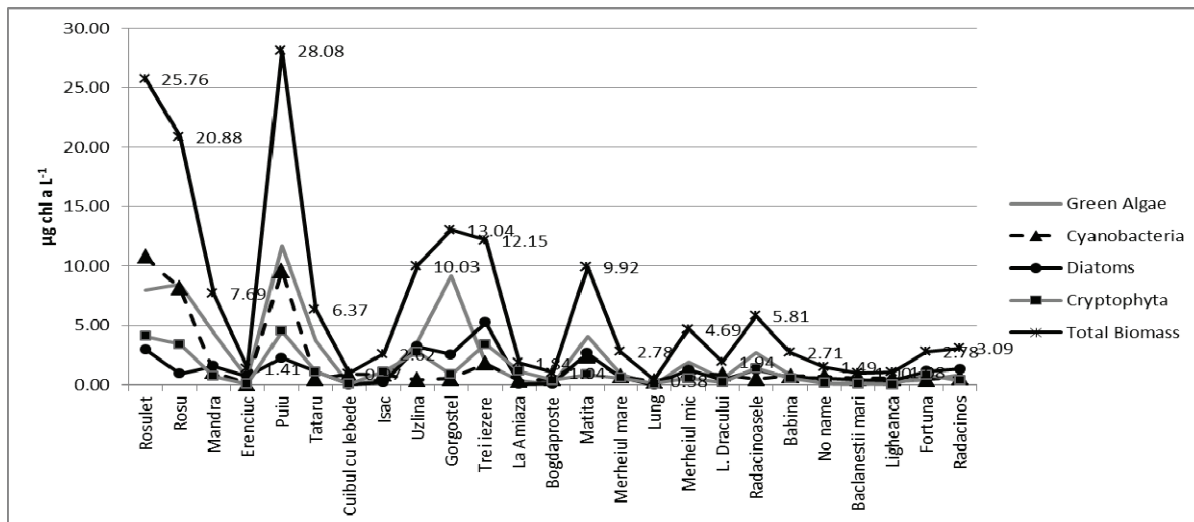


Figure 3. The variation of the phytoplankton biomass in July in the investigated ecological complexes of the Danube Delta.

In Lake Roşu, in July, a total of 30 species was present. The dominant species belonged to *Anabaena*, *Lyngbya* and *Oscillatoria* genus. In Lake Mândra, a very shallow lake, a number of 32 species was recorded.

Due to long periods of high temperatures during the summer, in September a peak of phytoplankton biomass was recorded ( $27.44 \mu\text{g L}^{-1}$ ). The minimum of the period was recorded in Lake Bogdaproste  $1.37 \mu\text{g L}^{-1}$  and the maximum in Lake Dracului and Puiu, very high values ( $81.30$ , or  $57.41 \mu\text{g L}^{-1}$ ). The range of high values increased, thus in 12 of the 20 investigated lakes, fluctuated from  $21.05$  to  $81.3 \mu\text{g L}^{-1}$  (Fig. 4). The dominant group belonged to Chlorophyta (47%), followed by Cyanobacteria, 22%.

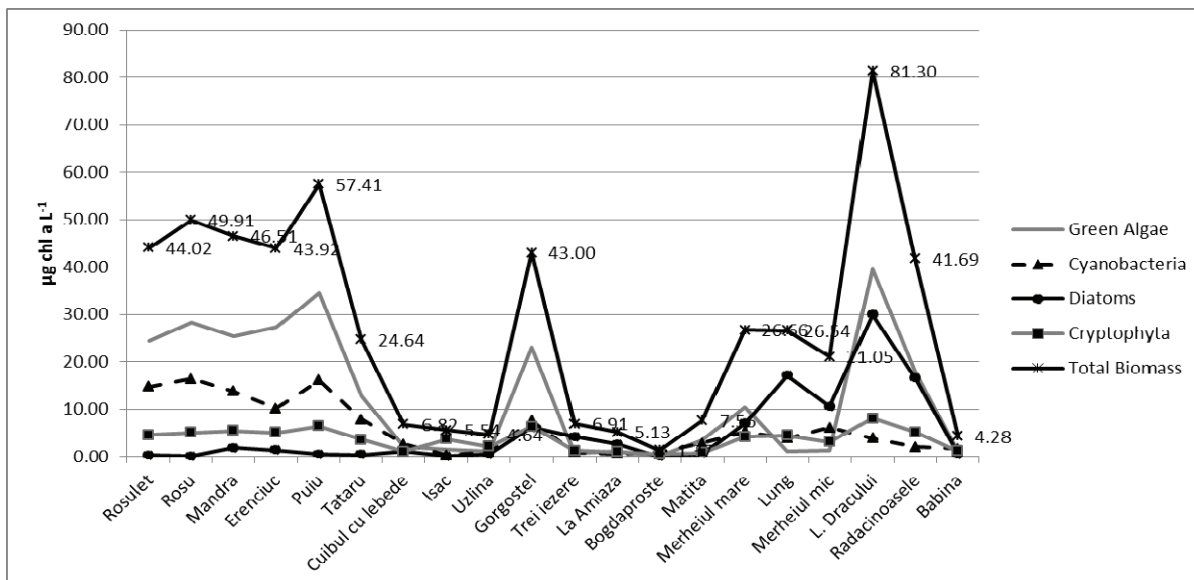


Figure 4. The variation of the phytoplankton biomass in September in the investigated ecological complexes of the Danube Delta.

In Lake Roşu, in September, a total of 37 species were present. The dominant species belonged to *Microcystis*, *Oscillatoria* and *Gonatozygon* genus. In Lake Mândra, a number of 39 species recorded.

During 2013, Chlorophyta was the dominant group, Cyanobacteria becoming the second as importance in summer and autumn, as expected. In September, several lakes were dominated by cyanobacteria (Cuibul cu lebede, Bogdaproste, Babina), while others were dominated by diatoms (Lung, Merheiu Mic, Trei Iezere). The relationship

between diatoms and Cyanobacteria was antagonistic, probably because of their suppression by cyanobacteria that produce toxic substances (GREGOR & MARŠÁLEK, 2004).

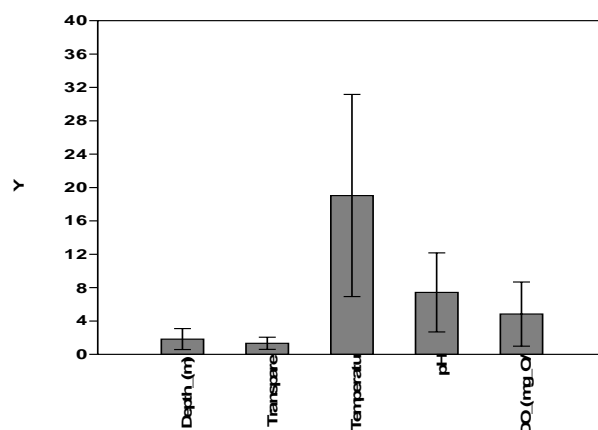


Figure 5. The boxplots of the field parameters (with standard deviations).

In table 1 and figure 5, it is presented the descriptive statistics of the physical-chemical parameters recorded during 2013 in the four complexes (annual averages).

Table 1. The descriptive statistics of the main physical-chemical parameters.

	NH <sub>4</sub>	NO <sub>2</sub>	NO <sub>3</sub>	PO <sub>4</sub> <sup>3</sup>	Depth	Transp.	Temp.	pH	DO
Mean	0.0921	0.0120	0.1431	0.0082	1.8342	1.3312	20.9400	8.1678	5.3155
Std Error	0.0103	0.0035	0.0146	0.0018	0.1360	0.0797	0.1176	0.0638	0.2796
Median	0.0946	0.0039	0.1214	0.0050	1.6250	1.2583	20.9167	8.1900	5.7150
Mode	0.0448	0.0014	#N/A	0.0015	#N/A	1.1000	20.9000	8.3700	#N/A
St Dev	0.0448	0.0151	0.0651	0.0079	0.6381	0.3737	0.5260	0.2853	1.2506
Variance	0.0020	0.0002	0.0042	0.0001	0.4071	0.1397	0.2767	0.0814	1.5639
Kurtosis	-0.7199	3.5611	-0.5737	2.0161	-0.1294	0.5518	0.1875	0.2834	1.0278
Skewness	0.4358	1.8997	0.6827	1.5977	0.9090	1.0384	0.0138	-0.5942	-1.3052
Range	0.1540	0.0553	0.2222	0.0285	2.1500	1.3667	2.0667	1.1700	4.4767
Minim	0.0346	0.0008	0.0556	0.0015	1.0500	0.8333	19.8333	7.4867	2.1867
Maxim	0.18	0.05	0.27	0.03	3.20	2.20	21.90	8.65	6.66
Sum	1.75	0.22	2.86	0.16	40.35	29.28	418.8	163.35	106.31
Count	19	19	20	20	22	22	20	20	20

Kurtosis and Skewness indices show that the data are not normally distributed; therefore statistical processing was performed on transformed data by logarithms.

Depth and transparency values fall in the range of typical values for the delta shallow lakes, the maximum depth 3.20 m, respectively 2.20 m for transparency and minimum 1 m and 0.83 m for transparency, in very shallow lakes such as La Amiază. The average of the annual temperature did not exceed 22 °C, the maximum being recorded in summer, 26 °C, in Isac and Gorgostel. The pH values indicated slightly alkaline waters, usual found in the Delta (maximum of 8.65) (POSTOLACHE, 2006).

Nutrients registered a wide range of variation. DIN (dissolved inorganic nitrogen) was found in the range 0.09 - 0.5 mg L<sup>-1</sup>, while PO<sub>4</sub> reached a maximum value of 0.03 mg L<sup>-1</sup>, located at the eutrophication threshold (WETZEL, 1983). The mean ratio of DIN/TRP, derived from the two maximum nutrient concentrations was 16. This is a ratio > 10, indicating that phosphorous tends to become a potentially limiting factor for phytoplankton development. However, the very low ratio, close to 10, should indicate a tendency for the cyanobacteria toxic blooms (REYNOLDS, 1998; 2000).

Given the complexity of the ecological systems, the dynamics of the biological communities are not responses to the action of a single factor. A combination of factors, which act synergistically, describes most accurate this dynamics. To describe the changes of the dependent variable (phytoplankton and zooplankton), based on the fluctuations of several factors as independent variables, a multiple regression was applied. A strong relationship

between Cyanobacteria and TP ( $p < 0.001$ ) and marginally with  $\text{NO}_3$  and  $\text{PO}_4$  was evidenced. The light and TP were significant factors that influenced the Chlorophyceae development. The phytoplankton biomass, expressed as chlorophyll *a* content, was explained by the TP (89%,  $p < 0.001$ ) (Table 2).

The zooplankton group plays a key role in aquatic ecosystems, being a link between primary pelagic producers and superior trophic levels, its taxonomic and functional diversity indicating the ecosystem health. Within this project, the major zooplankton groups were analysed: Ciliata, Testacea, Rotifera, Cladocera and Copepoda.

A correlation matrix for the dependent parameter of interest (zooplankton community sampled in May) and independent variables (control factors) was made. The results emphasized the importance of light ( $r = 0.50$ ) and temperature ( $r = 0.48$ ) at the level of significance 0.05. Also, Lamellibranchia was positively correlated with  $\text{NO}_2$  ( $r = 0.44$ ), Testacea with the total phytoplankton biomass and  $\text{NH}_4$  ( $r = 0.53$ ) at the same significance level.

An analysis of the Bray Curtis similarity degree highlighted a 50% resemblance among the following lakes that belong to Matița-Merhei lacustrine complex: Dracului, Lung, Babina and Rădăcinoasele.

Table 2. The multiple regression of phytoplankton groups and environmental parameters.

Independent/Dependent variables	Cyanobacteria	Chlorophyceae	Diatoms	Total biomass
	<i>P-value</i>			
TP	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>
$\text{NH}_4$	0.89	0.29	0.13	0.62
$\text{NO}_2$	0.11	0.41	0.18	0.14
$\text{NO}_3$	<b>0.05</b>	0.59	0.78	0.35
$\text{PO}_4$	<b>0.05</b>	0.47	1.00	0.44
Temp.	0.77	0.41	0.26	0.48
Light intensity	0.19	<b>0.01</b>	0.21	0.32

Based on chlorophyll *a* values an ANOSIM analysis on each season was applied, to identify whether there are differences between the four complexes. The test revealed the following patterns of phytoplankton development: in spring, there was no statistically significant differences between the three complexes studied ( $p > 0.05$ ). This result could be explained because the chlorophyll *a* values in this season showed large fluctuations, due to environmental conditions.

In summer, the complex Roșu-Puiu differed significantly from Matița-Merhei and Șontea-Fortuna ( $p < 0.05$ ). During autumn, the complex Roșu-Puiu highlighted differences from Gorgostel-Uzlina ( $p < 0.05$ ), the algal blooms manifesting mainly in September in the complexes Roșu-Puiu and Matița-Merhei ( $p > 0.05$ ).

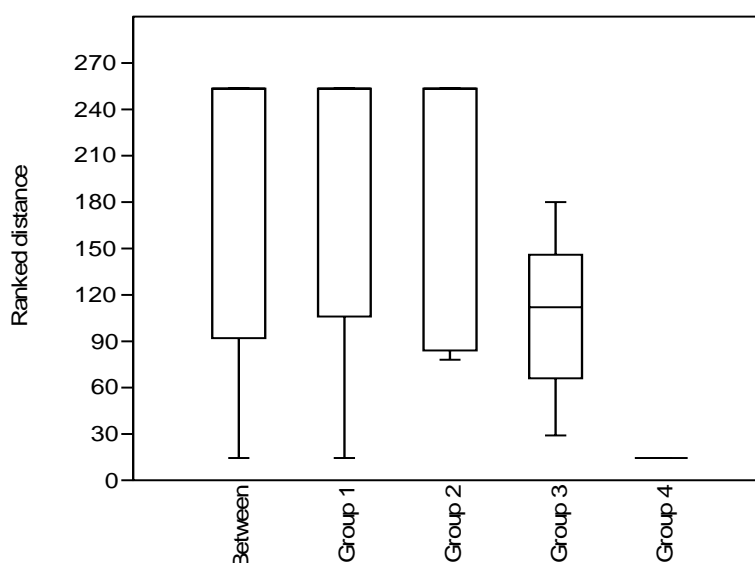


Figure 6. The ANOSIM analysis of the phytoplankton biomass ( $\mu\text{g L}^{-1}$ ) in spring (Group 1 – Roșu-Puiu, Group 2 – Gorgova-Uzlina, Group 3 – Matița-Merhei).

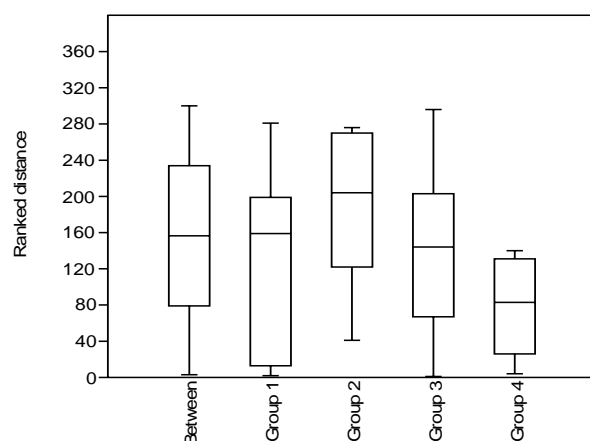


Figure 7. The ANOSIM analysis of the phytoplankton biomass ( $\mu\text{gL}^{-1}$ ) in summer (Group 1 – Roșu-Puiu, Group 2 – Gorgova-Uzlina, Group 3 – Matia-Merhei, Group 4 – Șontea-Fortuna).

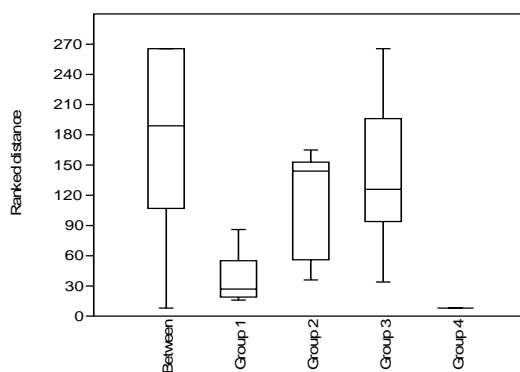


Figure 8. The ANOSIM analysis of the phytoplankton biomass ( $\mu\text{gL}^{-1}$ ) in autumn (Group 1 – Roșu-Puiu, Group 2 – Gorgova-Uzlina, Group 3 – Matia-Merhei).

## CONCLUSIONS

The development of phyto- and zooplankton communities in the Danube Delta is correlated to different environmental characteristics, such as nutrients content (mainly total phosphorous for Cyanobacteria and total phytoplankton), light intensity and TP for Chlorophyceae, or light and temperature for total zooplankton.

Future analyses will focus on the Cyanobacteria effects on food web.

These findings are especially useful for the administrative and policy makers in order to implement the suitable measures for the conservation of the Danube Delta, an inestimable complex of ecosystems.

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