ENVIRONMENTAL FACTORS CONTROLLING CILIATES IN SHALLOW LAKES OF THE DANUBE DELTA – SPATIAL AND TEMPORAL VARIABILITY

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ABSTRACT

The Danube Delta Biosphere Reserve encompasses a complex of aquatic ecosystems with a mosaic of structural and functional features, that influence significantly the biological communities. Ciliata group plays an important role as part of the zooplankton communities, being a key factor in nutrient cycling within the microbial loop. The microbial food web gained lately a wide recognition, especially in eutrophic and hypertrophic systems (such as the Danube Delta) as circulation pathway of organic matter within the planktonic trophic networks and reintroduction of inorganic forms of nutrients in the water, rendering them available again to primary producers. In 2013, an intensive study was carried out, covering a seasonal sampling program (spring, summer, autumn) in four large deltaic lake complexes. This paper focuses on the characterization of ciliate community, highlighting also the biotic and abiotic factors that control this community in the investigated lakes. A number of 30 genera and species were identified, the highest diversity being reached in the marine part of the delta (23 in complex Roşu – Puiu, and 20 in Matiţa – Merhei), and the lowest in the fluvial complex Şontea – Fortuna (7). The abundance varied in a large spatial and temporal range, in all the four lake complexes: in Roşu – Puiu, it fluctuated between 38 ind L^{-1}in summer and a minimum of 0.28 ind L^{-1} in Mândra, in spring; in Gorgova - Isac, it varied between 27.60 ind L^{-1} in Gorgostel, in October, and 0.22 ind L^{-1} in Uzlina, in July; in Matiţa – Merhei, it fluctuated between 48 ind L^{-1}in Merheiul Mic, in October, and 0.30 ind L^{-1} in Matiţa, in May, while in Şontea-Fortuna complex it ranged between 1.60 in Ligheanca, and 0.24 in Lake No name, both in summer. In terms of spatial distribution of Ciliata abundance, the maximum averages were recorded in Roşu - Puiu complex (7.61 ind L^{-1}) and the minimum in Şontea- Fortuna (0.81). Anova single factor analysis reveals no statistically significant differences in terms of abundance in the 4 lake complexes ($p>0.05$, Fcrit>F). The corelation of ciliate development according to the season has been tested with single factor ANOVA and revealed that seasons affected statistically significant this community ($p<0.05$, $F_{3,\ 128} = 4.591$). An important biotic factor controlling ciliates development was phytoplankton biomass (estimated as total chlorophyll *a* concentration ($r=0.46$, $p<0.0001$). In particular, two phytoplankton groups had a significant influence on the ciliates: Cyanobacteria ($r= 0.269$, $p<0.05$) and Chlorophyceae (0.38, $p<0.01$). Among the physical-chemical factors that had a significant influence on the ciliate community were: pH ($r=0.29$, $p<0.05$), conductivity ($r=0.31$, $p<0.01$), turbidity ($r=0.293$, $p<0.01$), the redox potential ($r=0.304$, $p<0.05$), DO ($r=0.35$, $p<0.05$) and TP concentration ($r=0.245$, $p<0.05$).

Keywords: ciliates, control factors, Danube Delta, microbial loop

1. Introduction

The ciliates of the Danube Delta lakes have been less studied, although ecological studies of these ecosystems were carried out for over 50 years. Most of the references include only taxonomic lists and abundances, but not interpretations of the effects of physical and chemical factors on these populations (Zinevici and Parpală, 2006). Planktonic ciliates are found in a variety of habitats with significant differences in physical and chemical conditions of water (Zhang *et al.*, 2015), representing one of the most important components in the microbial web where they are major consumers of nano- and picoplankton (Azam *et al.*, 1983). They are efficiently transferring matter and energy from the primary production level to higher trophic levels. The Danube Delta encompasses a mosaic of ecosystems, mainly shallow lakes, depending on hydrological and climate regime. In the Delta, the energy from primary producers flows on detrital pathways to the bottom of the lakes, mediated by microbial loop. Ciliates are involved in the reintroduction of inorganic forms of nutrients in the water, rendering them available again to primary producers. The aims of this study were (1) to characterize the species composition, abundance, and diversity of the ciliate communities from the deltaic complexes, (2) to reveal the spatial and temporal distribution pattern of the ciliate communities, and (3) to determine the environmental and biological factors that control the ciliate communities in the investigated lakes.

2. Material and methods

The Danube Delta Biosphere Reserve is located at 45°0′N latitude, 29°0′E longitude in the eastern part of Romania. Previous studies showed that lakes of the Danube Delta can be grouped in several complexes according to their hydromorphological features (Roşu-Puiu, Matiţa-Merhei, Gorgova-Uzlina, Şontea-Fortuna) (Cristofor *et al.*, 1993). Our study was conducted in representative lakes of the four deltaic complexes. The sampling campaigns were carried out in 2013, seasonally (May, July and September), on the water column, from 26 different lakes (Figure 1).

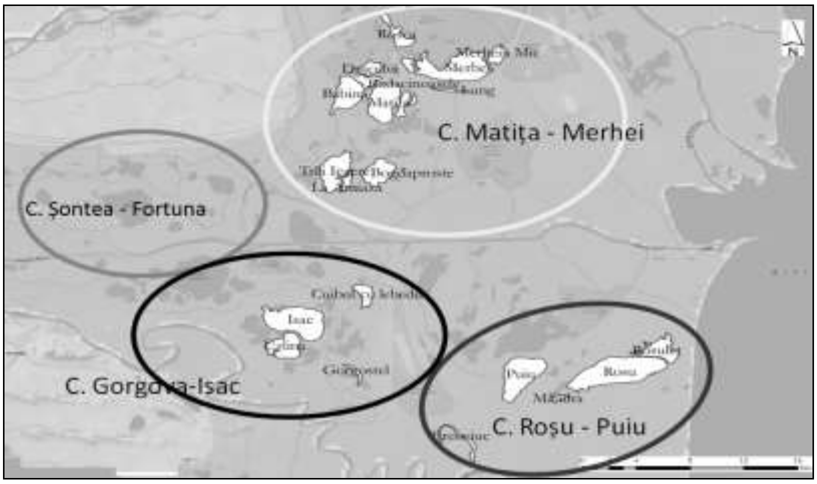

Figure 1: The four main lake complexes of the Danube Delta in which the study was conducted in 2013.

The redox potential, pH, conductivity, dissolved oxygen content were measured in the field with a multiparameter WTW 340i. Samples for chemical analyses were filtered through GF/F Whatman 65 µm Ø and frozen for further analyses in the lab. Nutrients were determined spectrophotometrically by Berthelot method for $N\text{-}NH_4$ (Berthelot, 1859), Griess – Ilosvay modified method for $N\text{-}NO_2$ (Keeney and Nelson,1982), Tartari and Mosello, 1997 for $N\text{-}NO_3$, $P\text{-}PO_4$ and TP). The turbidity was measured with a Hanna Instruments turbidimeter. The total phytoplankton biomass and the biomass of different algal groups (expressed as chlorophyll *a* content, µg L^{-1}) were assessed by a submersible fluorometer (Fluoroprobe III, bbe Moldaenke). The ciliate samples were collected by filtering 50 liters of water using a Patalas-Schindler device, on water column, through a 65 µm Ø mesh network, and preserved with 4% formaldehyde solution. The species were identified by specific keys. The abundance (ind L^{-1}) was assessed by direct counting using a Zeiss inverted microscope. Data analysis was performed using the statistical software PAST and XLSTAT.

3. Results and discussion

During the study, 30 genera and species were identified in the four lake complexes of Danube Delta, the highest diversity being reached in the marine part of the delta (23 in complex Roşu – Puiu, and 20 in Matiţa – Merhei), and the lowest in the fluvial complex Şontea – Fortuna (7). Highest values of Shannon index were observed in Roşu – Puiu and Şontea – Fortuna complexes, lower values in Gorgova – Isac and Matiţa – Merhei, while only few lakes showed a diversity index over 2. The values of evenness have not decreased below 0.5, which shows a good distribution of individuals among species (Figure 2). The abundance varied in a large spatial and temporal range, in all the four lake complexes: in Roşu – Puiu, it fluctuated between 38 ind L^{-1} in summer and a minimum of 0.28 ind L^{-1} in Mândra, in spring; in Gorgova-Isac, it varied between 27.60 ind L^{-1} in Gorgostel, in October, and 0.22 ind L^{-1} in Uzlina, in July; in Matiţa – Merhei, it fluctuated between 48 ind L^{-1} in Merheiul Mic, in October, and 0.30 ind L^{-1} in Matiţa, in May, while in Şontea-Fortuna complex it ranged between 1.60 in Ligheanca, and 0.24 in Lake No name, both in summer.

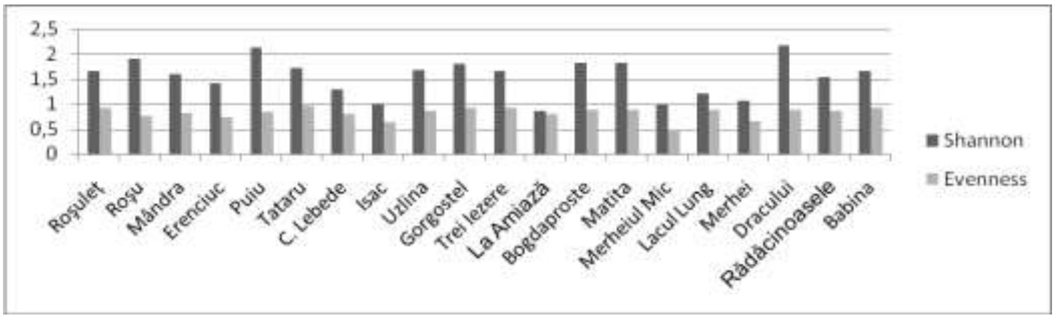

Figure 2: The diversity indices of ciliate communities in Danube Delta complexes in 2013

The highest abundance of ciliates was recorded in autumn in Matiţa-Merhei complex (10.90 ind L^{-1}), followed by Roşu-Puiu complex in summer (10.62 ind L^{-1}) (Figure 3). The minimum values of abundance occurred in the Şontea-Fortuna and Gorgova-Isac, both in summer. It seems these organisms are significantly influenced by local physical-chemical factors, which differed from one complex to another being strongly influenced by hydrological regime which consequently affects the nutrient dynamics. The hydrological connections inside delta provide the complex physical and chemical processes affecting the biocenosis (Postolache, 2006).

The highest values of average abundance were recorded in Roşu-Puiu complex (7.61 ind L^{-1}). This complex was severely disturbed by eutrophication over time, recording high levels N and P concentration and phytoplankton biomass. Due to their rapid growth rate and sensitive response to environmental changes, cilliates are effective bioindicators of water quality and eutrophication (Zhang *et al.*, 2015). In these circumstances, the role of the ciliates in the microbial loop of eutrophic ecosystems is increased compared to other complexes in mesotrophic state. The correlation of ciliate development according to the season has been tested with single factor ANOVA and revealed that seasons affected statistically significant this community ($p<0.05$, $F_{3, 128}=4.591$). The ordination technique Detrended Correspondence Analysis (DCA) was used to distinguish the seasonal and spatial pattern distribution of ciliate communities (Figure 4). The DCA analysis confirmed the grouping of ciliate abundance by seasons, with spring and autumn showing the same trend of ciliate development, while in summer it was different.

An important biotic factor controlling ciliates development was phytoplankton biomass (estimated as total chlorophyll *a* concentration). This statement was mentioned in other similar studies performed in marine waters or freshwater ecotones (Wang *et al.*, 2014, Mieczan *et. al*, 2013). The regression analysis showed the importance of some phytoplankton groups on the development of ciliates in the studied complexes. The group Chlorophyceae represented the main food source of these organisms ($r=0.38$, $p<0.01$), while Cyanobacteria was the secondary source because of its lower nutrient content and possible toxicity ($r=0.269$, $p<0.05$). Among the physical-chemical parameters that had a significant influence on the ciliate community (Tab.1), were: pH, conductivity, turbidity, the redox potential, DO and TP concentration. Similar studies and results indicate the importance of N, P (Wang *et al*., 2014) and other physical-chemical parameters (pH, temperature, turbidity) in ciliate dynamics in aquatic ecosystems (Mieczan *et al*., 2013).

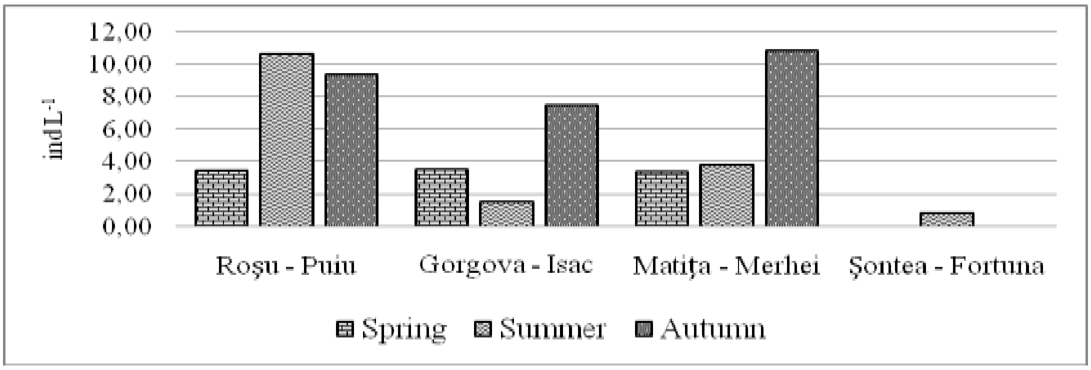

Figure 3: Spatial and temporal variability of ciliate abundance expressed as mean values (ind L^{-1}) of lakes of each complex in 2013.

Table 1: Statistical correlations of cilliate communities with control factors

Correlation statistics	Chlorophyll *a* (µg L^{-1})	pH	Conductivity (µS/cm)	Redox (mV)	Turbidity (NFU)	Dissolved oxygen (mgO_2 L^{-1})	Total phosphorous (µg P L^{-1})
p	<0.0001	<0.05	<0.31	<0.05	<0.01	<0.05	<0.05
r	0.46	0.29	0.31	0.30	0.29	0.35	0.24

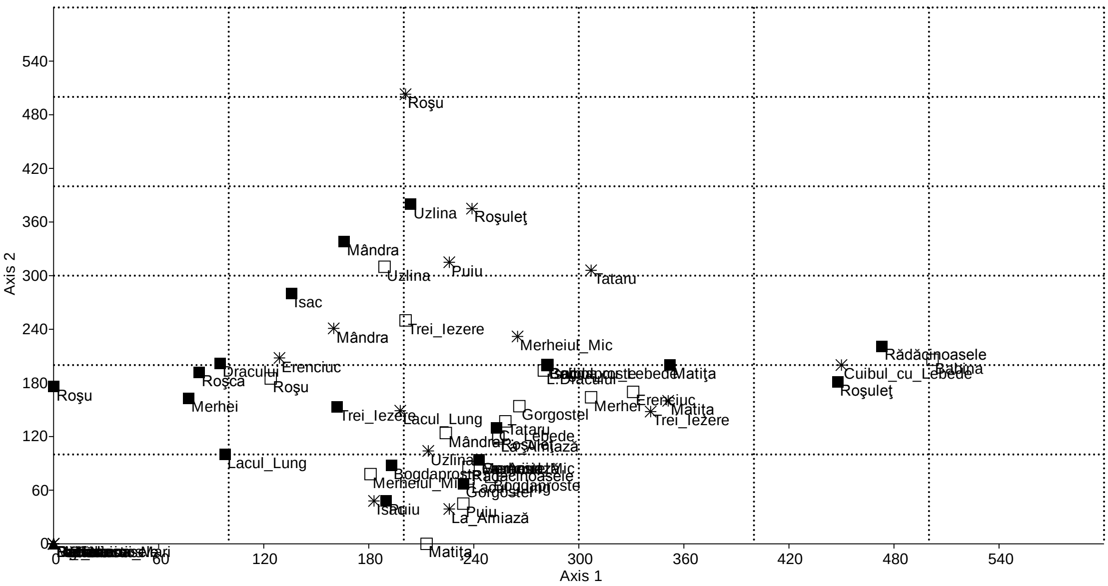

Figure 4: DCA of seasonal and spatial distribution of ciliate abundance in Danube Delta lakes during 2013 (filled square=spring; empty square=summer; stars=autumn)

4. Conclusions

The variation of ciliate communities in terms of diversity and abundance was statistically significant at seasonal level, but was not at spatial level even if some trends in complex differentiation were observed. The results emphasized the fact that total phosphorus, chlorophyll-*a*, pH, DO, conductivity, redox potential and turbidity were the most significant factors controlling the ciliate communities in Danube Delta lake complexes in 2013. Our work provides reference data for further studies of ciliate community structure in the Danube Delta.

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