COMPARATIVE ASSESSMENT OF MICRO- AND MESOZOOPLANKTON IN DIFFERENT TYPES OF ECOSYSTEMS IN THE DANUBE DELTA (ROMANIA)

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The pattern of species distribution and seasonal dynamics of micro- and mesozooplankton were studied in four different ecosystems from the Danube Delta, during productive seasons between 2011–2013. The differentiation in two size classes of zooplankton is linked to food availability and higher trophic levels. We studied two types of ecosystems, stagnant and flowing ones. Lake Roșu presented the highest species richness. Most of the species found in our study were accidental or accessory. Among the key species, *Diaphanosoma brachyurum* (mesozooplankton) and *Polyarthra minor* (microzooplankton) were the most representative in abundance and frequency. The structural similarity of the zooplankton communities found in our study might be due to the ecosystems connectivity. It was noticed a clear seasonal increasing of larger zooplankton in terms of abundance from spring to autumn. Environmental and biotic factors had significant influences on the both zooplankton groups. Juvenile stages were influenced by temperature and depth and pH and temperature controlled the adults of entire zooplankton.

Keywords: frequency index, dominance index, Diaphanosoma brachyurum, Polyarthra minor.

INTRODUCTION

The Danube Delta is the best-preserved wetland on the continent, being the second largest river delta in Europe. The Danube Delta is one of the most valuable places for biodiversity and it provides a wide range of services for human society (Oosterberg *et al.,* 2000). Thus, to protect its high diversity and natural characteristics very strong protection measures are needed. However, without a scientific background, these measures might provide beneficial effects. Primary consumers at zooplankton level represent an important route for energy and matter from primary producers to higher trophic levels, but also, they are detritus consumers. Therefore, the zooplankton is an important trophic link between the bacterial loop and classical food web (Krevš *et al.,* 2010). Even if there are species from micro and mesozooplankton that access detritus and phytoplankton at the same time, the contribution in nutrient cycling of the two groups differs (Treece, 1995; Harris *et al.,* 2000; Moriarty & O'Brien, 2013; Golz *et al.,* 2015). Zooplankton includes a wide range of organisms ranging from several microns to visible individuals. This led to the necessity of a classification system based on the dimensions of the planktonic components.

The purpose of our study was to perform a comparative analysis of the composition and abundance of the four ecosystems in order to highlight the main factors influencing the development of zooplankton in different hydrological conditions.

MATERIAL AND METHODS

Lake Roșu is a young lake, well supplied with water from the Danube River through different size channels (Zinevici & Parpală, 2007). The lake is among the largest (14.31 km^2) in the Danube Delta. The macrophyte community consists of submerged species and surrounding littoral reed beds. The macrophyte communities are not well developed; instead, the phytoplankton is the representative primary producer (Godeanu & Zinevici, 1983). Lake Mândra is a very shallow lake, in an advanced successional stage, with a small surface (1.47 km^2) almost colmated with developed macrophytic vegetation in detriment of phytoplankton (Parpală & Zinevici, 2002; Zinevici & Parpală, 2007). The Roşu- Puiu channel, makes the connection between Roșu and Puiu lakes. The channel Roșu-Împuțita is partially artificial, created by shortening the channel Împuțita (Iordachi & Assche, 2014) and creating a canal for direct communication of Lake Roșuleț with Sulina branch belonging to the Danube River.

The study was carried out between 2011–2013 in two lakes (Roșu, N 45°03.507', E 029°35.198' and Mândra, N 45°02.082', E 029°31.068') and two channels (Roșu-Împuțita and Roșu-Puiu), located in the proximity of the lakes. These ecosystems belong to Roșu-Puiu lacustrine complex. We established one sampling station for channels and Mândra Lake and five stations for Lake Roșu (Fig. 1). We sampled the ecosystems in the productive seasons (spring, summer and autumn).

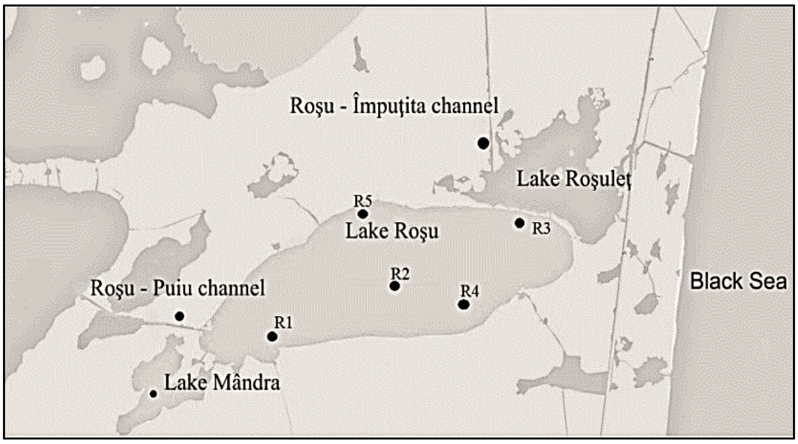


Fig. 1. The study area and sampling stations (modified after GoogleMap).

We measured *in situ* the environmental parameters such as depth, transparency, temperature and pH using a multiparameter WTW 340i.

The phytoplankton samples were collected without filtering, in 0.5 L bottles, preserved with 4% formaldehyde solution. The species composition and abundance were estimated using Utermöhl method and specific taxonomic keys. The abundance was reported as individuals L^{-1}, by counting single cells, colonies and filaments as operating units.

We collected the zooplankton samples on the water column, using a Patalas-Schindler device and standard plankton net (mesh size 50 µm Ø). 50 L of water were filtered and the sample was preserved in formaldehyde (4%). The subsamples were microscopically analyzed for species identification and abundance (individuals L^{-1}) (Edmondson, 1971). The species were identified by following keys: Lamellibranchia (Marsden, 1992); Rotifera (Voight, 1956; Rudescu 1960); Cladocera (Brooks, 1959; Negrea, 1983); Copepoda (Damian-Georgescu, 1963–1970). The zooplankton was split into two groups (micro- and mesozooplankton) based on metric size according to Omori & Ikeda (1984). Microzooplankton includes species ranging in size from 2 to 20 µm. That can be part protozoa, rotifers, cladocerans, copepods nauplii (Elangovan *et al.,* 2012). The mesozooplankton comprises species with sizes between 200 µm – 2 mm such as cladocerans, copepods, larvae, rotifers (Omori & Ikeda, 1984).

There were also assessed the Frequency and Dominance indices. Frequency index represents the proportion of species appearance in total samples. There are four species classes based on their appearance: accidental species (1–25%), accessory species (25.1–50%), constant species (50.1–75%) and euconstant species (75.1–100%) (Botnariuc & Vădineanu, 1982).

The Dominance index (based on Tischler's dominance classes) reflects the role of species in ecosystems.

$D_i = \frac{ni}{N} 100$; where ni = abundance of species, N-total abundance in the sample.

There are five classes of dominance: subrecedent species (<1.1%); recedent species (1.2–2%); subdominant species (2.1–5%); dominant species (5.1–10%); eudominant species (>10%). The species with abundance over 10% are important for the ecosystem (Botnariuc & Vădineanu, 1982).

For statistical analysis, we used XLSTAT software and Past (Hammer *et al.,* 2001).

RESULTS

The environmental parameters varied according to seasons (Table 1). The averages of temperature, in spring (21.57ºC), summer (25.75ºC) and autumn (16.36ºC). Regarding transparency, the channels presented a high transparency in spring and low in summer and autumn. The values of this parameter of the two lakes were high in spring and decreased until autumn, excepting autumn 2011.

Unlike the other two years of study, in autumn of 2011 there were recorded low temperatures (average 13ºC), which reflected in a high value of transparency.

Table 1

Values of environmental parameters in studied ecosystems
(avg – average, min – minimum; max – maximum)

		Mândra	Roșu	Roșu-Puiu	Roșu-Împuțita
Depth (m)	avg	1.50	2.33	3.21	1.66
	min	1.00	2.12	2.00	1.20
	max	2.20	2.94	5.00	2.50
Temperature (ºC)	avg	20.81	21.36	20.72	21.98
	min	13.00	13.70	13.50	14.10
	max	26.80	27.00	27.10	27.80
Transparency (m)	avg	0.84	0.92	0.93	1.10
	min	0.30	0.25	0.30	0.40
	max	2.00	2.90	3.00	1.90
pH	avg	8.41	8.58	8.26	8.04
	min	7.48	7.45	7.25	7.55
	max	9.28	9.20	8.74	8.56

In Lake Roșu, the phytoplankton communities consisted of 168 species belonging to six groups: Cyanobacteria, Euglenophyceae, Pyrrophyceae, Chrysophyceae, Bacillariophyceae, Chlorophyceae, while in Lake Mândra, 123 species were registered. The channels showed the lowest diversity (Table 2).

Table 2

The taxonomic composition of phytoplankton communities of the studied ecosystems

	Mândra	Roșu	Roșu-Puiu	Roșu-Împuțita
Cyanobacteria	33	45	29	32
Euglenophyceae	9	12	6	5
Pyrrophyceae	3	5	1	4
Chrysophyceae	2	1	1	1
Bacillariophyceae	35	57	37	26
Chlorophyceae	41	48	34	32
Total number of species	123	168	108	100

The percentage of morphological forms of phytoplankton in the four studied systems remained almost equal. For example, colonial and cenobial forms registered a percentage between 32.73 and 35% in all ecosystems (Table 3).

Table 3

Species richness of phytoplankton of the studied ecosystems

Morphological forms	Mândra	Roșu	Roșu-Puiu	Roșu-Împuțita
Cenobial	16	16	14	13
Colonial	25	39	23	22
Filamentous	20	26	18	18
Single cell	61	87	45	55
Total number of species	123	168	108	100

In total, 46 rotifer species belonging to microzooplankton were found during the study period. In this category, there were also added the first stage of copepods – nauplii and Lamellibranchia larvae. Mesozooplankton included 90 species (rotifers, cladocerans, and copepods). In addition, the mesozooplankton included the copepodites stages (Cyclopidae, Calanoida, Diaptomida, Harpacticoida).

The lentic ecosystems showed a higher number of species comparing with channels (Table 4). Lake Roșu presented the highest values in both groups.

Table 4

Species richness of the studied ecosystems

	Mândra	Roşu	Roșu-Puiu	Roșu-Împuțita
microzooplankton	30	35	21	31
mesozooplankton	62	71	54	56
Total number of species	92	106	75	87

During the study, the species richness of microzooplankton was much lower than mesozooplankton. In Lake Mândra and channel Roșu-Împuțita a slight increase in species number from spring to autumn was recorded, while in Lake Roșu and Roșu-Puiu an inverse trend was observed (Fig. 2).

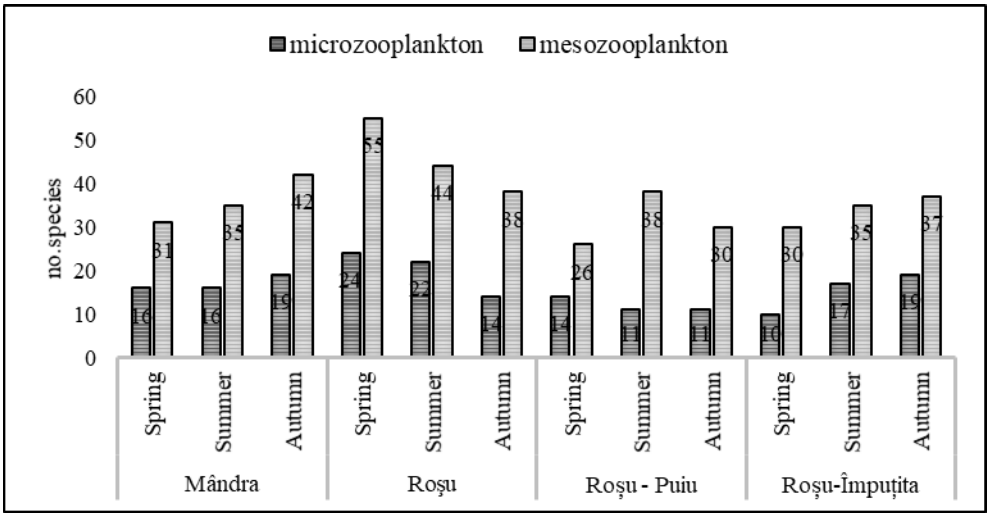


Fig. 2. The seasonal species richness in the four studied ecosystems.

In all four ecosystems, most of the planktonic species were accidental or accessory species. Few species of phytoplankton were constant in comparison with accessories or accidental species. Most of the constant species were identified in Lake Roșu and Mândra, probably due of more stable hydrological conditions (Table 5).

Table 5

The frequency of phytoplankton species in the studied ecosystems

	Mândra	Roșu	Roșu-Puiu	Roșu-Împuțita
Accidental species	80	93	46	73
Accessory species	28	27	33	20
Constant species	14	49	21	15

It can be noticed a higher number of constant species belonging to mesozooplankton comparing with microzooplankton. This might reflect the sensibility of small organisms to the environmental conditions (Table 6).

Table 6

Number of species belonging to micro- and mesozooplankton according to the Frequency Index

	Microzooplankton				Mesozooplankton			
	Mândra	Roșu	Roșu-Puiu	Roșu-Împuțita	Mândra	Roșu	Roșu-Puiu	Roșu-Împuțita
Accidental species	18	23	15	19	41	36	33	27
Accessory species	11	6	1	7	13	11	8	10
Constant species	1	6	5	5	8	24	13	19

Constant species varied from one ecosystem to another. Thus, in Lake Mândra *Lecane cornuta* (Müller, 1786) (microzooplankton) and copepods nauplii presented the highest frequency. Eight constant species belonging to mesozooplankton, 5 rotifers, and 3 cladocerans, were recorded. The cladoceran *Diaphanosoma orghidani* (Negrea, 1983) (89%) had the highest frequency. In Lake Roșu, the rotifer *Polyarthra minor* (Voigt, 1904) presented the highest frequency in microzooplankton, while mesozooplankton presented four times more constant species, to which it was added three juvenile stages of copepods.

In the channel Roșu-Puiu, the following constant species *Hexarthra fennica* (Levander, 1892), *Diaphanosoma brachyurum* (Liévin, 1848) (mesozooplankton) and *Dreissena polymorpha* (Pallas, 1771) and *Polyarthra minor* (microzooplankton) were found. *Polyarthra minor* and *Diaphanosoma brachyurum* were representative in channel Roșu-Împuțita.

The microzooplankton abundance varied depending on season and ecosystem. In Lake Mândra and channel Roșu-Puiu were abundant in spring with decreasing trends in other seasons, while in Lake Roșu and Roșu-Împuțita channel, presented a low abundance in spring with a significant increase in summer. Mesozooplankton abundance increased from spring to summer and slightly decreased in autumn (Table 7).

The mesozooplankton/microzooplankton ratio of seasonal abundances emphasized that mesozooplankton presented a higher increase from spring to autumn comparing microzooplankton, especially in lakes and Roșu-Puiu channel, while Roșu-Împuțita presented a different pattern. The spring was the favorable period for microzooplankton development while the peak of mesozooplankton registered in summer (Fig. 3).

Table 7

The seasonal averages of micro and mesozooplankton abundance (ind L^{-1}) during the study period

		Mândra	Roșu	Roșu-Puiu	Roșu-Împuțita
spring	microzooplankton	186.94	96.09	143.78	17.95
	mesozooplankton	336.09	185.03	302.00	149.45
summer	microzooplankton	163.59	138.26	89.80	128.08
	mesozooplankton	457.64	995.15	747.69	823.90
autumn	microzooplankton	180.88	40.14	56.08	149.15
	mesozooplankton	642.02	454.93	563.45	584.93

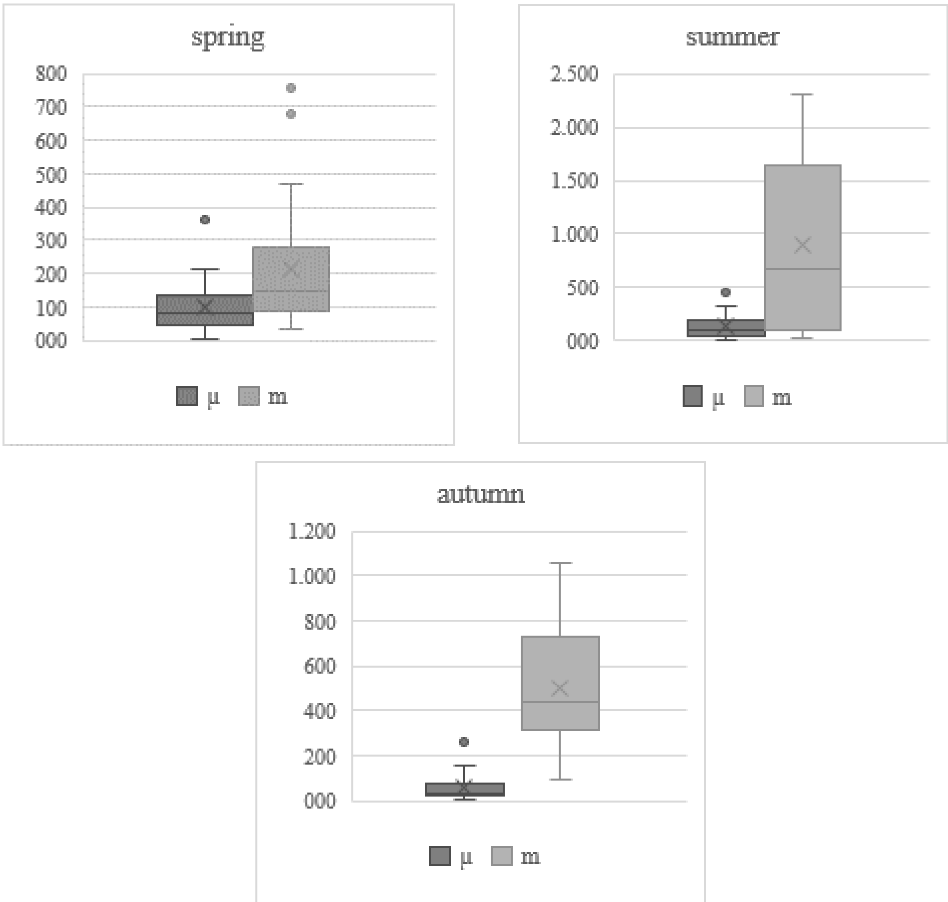


Fig. 3. The seasonal boxplots of μ-microzooplankton and m-mesozooplankton (x-mean markers, mean line and median).

An important contribution in abundance of mesozooplankton belonged to the juvenile stages of copepods, especially in summer as maximum reproduction period. In all sampling seasons, based on ANOVA test between the two groups of zooplankton, there were significant differences: spring $F_{(1, 51)} = 8.35$, $p < 0.005$; summer $F_{(1, 52)} = 25.35$, $p < 0.0001$; autumn $F_{(1, 52)} = 68.67$, $p < 0.0001$.

The dominant species, based on Dominance index of microzooplankton group, represented less than 20% of total species. The lakes presented a higher dominant species than channels, among them *Lecane cornuta, Polyarthra minor* and *Lecane copeis* (Harring & Myers, 1926) dominated in all four ecosystems. However, during the nine seasons of sampling, there were also other dominant species although they did not meet the constant species condition. The environmental conditions favored the development of certain species and subsequently went into a decline. The mesozooplankton eudominant species like *Diaphanosoma brachiurum, Chydorus sphaericus* (O.F. Müller, 1785), *Hexarthra fennica* (Levander, 1892), *Brachionus diversicornis* (Daday, 1883), *Brachionus angularis* (Gosse, 1851) were constant species. However, the number of species with a high abundance was dominant in lakes comparing with channels (Fig. 4).

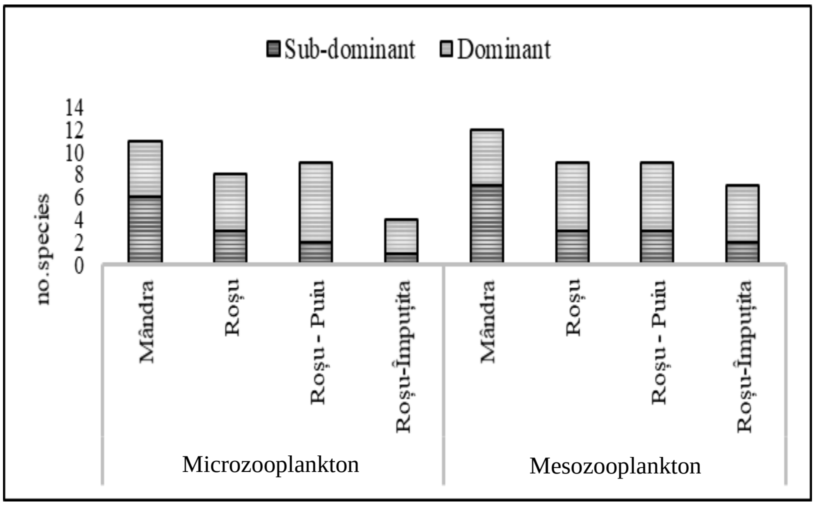
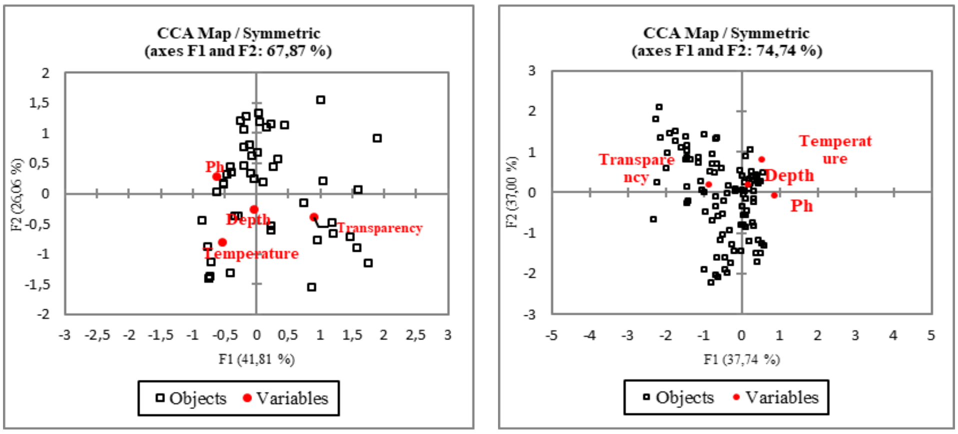


Fig. 4. Number of dominant and sub-dominant species recorded during the study.

The physical-chemical parameters influence the development, functionality, and distribution of zooplankton. The interactions of the environmental parameters are complex, with synergic influences on biological components, thus in understanding the processes that take place in systems it is also necessary an overall analysis. The CCA with microzooplankton species emphasis that majority of species were influenced by pH and few species have responded to the depth and temperature. Mesozooplankton presented stronger response to the conditions; most of the species were associated with depth and transparency (Fig. 5).



microzooplankton mesozooplankton

Fig. 5. Canonical Correspondence Analysis (CCA) of the relationship between zooplankton taxa and environmental variables (squares – represent zooplankton species).

Using PCA analysis it can be evidenced the grouping based on the correlation coefficient of the morphological forms of phytoplankton and the two categories of zooplankton (Fig. 6). The axis 1 and axis 2 explained together 81.74% of the micro and mesozooplankton variance.

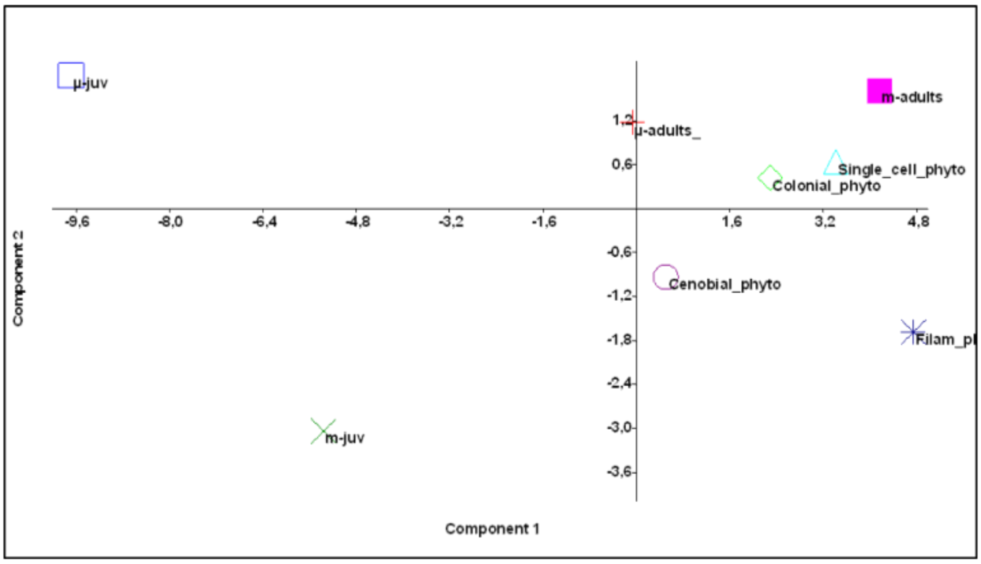


Fig. 6. Principal Component Analysis (PCA) of the relationship between zooplankton taxa and morphological forms of phytoplankton.

DISCUSSION

The hydrographic network of the Danube Delta strongly influences the composition of biological communities in aquatic ecosystems. Thus, high connectivity of the lakes through channels makes certain species be found both in lotic and in lentic systems. Zooplankton consists of tiny organisms, unable of migrations like fish; therefore, the species composition resemblance of the four ecosystems can be explained by passing them once with the water currents. In any delta, the water flow along the channels might be bidirectional depending on water regime (Tejedor *et al.,* 2015). In spring, the water flow is high, flooding the hydrographic system of the Delta channels and lakes, while in dry periods, in some areas, the water circulation sense can change. In addition, the residence time of the Roșu-Puiu complex is low but increases for the small lakes (Oosterberg *et al.,* 2000) and channels showed rich macrophyte vegetation (Godeanu & Zinevici, 1983). These conditions did not shape the channels as authentic lotic ecosystems. However, two-way ANOVA test indicated statistically significant differences both between systems ($F=4.92$, $p<0.05$) and between the four morphological types of phytoplankton ($F=56.37$, $p<0.0001$). This fact suggests that hydrogeomorphological traits of systems were reflected in the phytoplankton composition.

The structural features of an ecosystem are determined by key species the most numerous as abundance and constant frequency. The constant species reflects the continuity of the species in existing conditions and the dominant species have an important role in the matter and energy transfer in the ecosystem.

The key species of our study like following rotifers *Polyarthra minor, Hexarthra fennica, Brachionus diversicornis, B. angularis* and cladocerans *Diaphanosoma brachyurum, Chydorus sphaericus* are very common in the Danube Delta (Zinevici & Parpală, 2007). *D. brachyurum* might be found as dominant species in oligo-mesotrophic conditions and prefers warm and alkaline waters (Yousuf & Qadri, 1981; Haberman, 2000). *C. sphaericus* has a wide distribution in different types of reservoirs, being tolerant to a wide range of temperature and pH (Basińska *et al.*, 2014).

Rotifers are extremely adaptable, highly abundant than other taxonomic components (Bērziņš & Pejler, 1987, 1989). However, taxonomic structure and abundance of communities reflect the environmental conditions. Species of Brachionus are able to accommodate in environmental high changes conditions (Scott, 1983; Bennett & Boraas, 1988). In addition, in other studies, it reported being occasionally associated with macrophytes (Duggan *et al.,* 2001, 2002; Bozkurt & Guven, 2009).

Principal Component Analysis showed the accessibility of primary producers to trophic needs of the two-dimensional components of zooplankton. The plot suggested that the larger forms of zooplankton (mainly adults) could eat most of the morphological forms of algae. Instead, the juvenile forms of zooplankton and the microzooplankton probably feed on detritobacterial aggregates. These kinds of trophic relations are characteristic of eutrophic conditions in lakes. The multiple strategies of zooplankton feeding in freshwater make difficult to assess the exact model (Colina *et al.,* 2016).

The spatial distribution of micro and mesozooplankton did not show significant differences, but it was found a high seasonal variability. Temperature and pH are among of the most important factors that influence the development of the zooplankton (Galkovskaja, 1987). Temperature is the first factor those life cycles from spring to autumn (McLaren, 1963; Heinle, 1969). On the other hand, the life span determined the seasonal differences of micro and mezooplankton. Larger species, like copepods and cladocerans, present a larger life span. Thereby, in spring the mesozooplankton species are in smaller proportions comparing with summer.

CONCLUSIONS

The comparative investigation of micro and mesozooplankton pointed out seasonal differences in their dynamics. Microzooplankton shows sensitivity to variations in environmental conditions, reflected by reduced number of constant and dominant species compared with mesozooplankton.

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