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11. Zooplankton Communities as Bioindicators in Zaghen Restored Wetland, Danube Delta Biosphere Reserve

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Abstract: Eutrophication is a process characterized by an increase in the aquatic system productivity, which causes profound changes in the structure of its communities. Owing to the high environmental sensitivity of planktonic species, the study of their communities can indicate the deterioration of the environment. Zaghen was the most clogged sector of the Danube meadow, North of Tulcea Hills, which included many fluvial levees, former riverbeds, reedbeds , swamps, lakes, and channels. Since the beginning of the century, the Zaghen area has been mentioned as a marsh not connected with the Danube, flooded only at high water levels, over the time has been affected by several forms of continuous human interference. Here we analyze some properties of the zooplankton community as bioindicators of eutrophication and water quality change. Water was collected for analysis and quantitative zooplankton samples were taken at four sites during June to December 2017. Species were identified and their numerical abundances and biomasses were determined and used to estimate some biological indices like *Brachionus* species diversity, the ratio large Cladocera to total numbers of Cladocera , the ratio of crustacean zooplankton biomass to phytoplankton biomass - chlorophyll "a", and the Calanoida/Cyclopoida ratio.
Keywords: *zooplankton, trophic state, bioindicators species*

INTRODUCTION

Wetlands are among the most productive life support systems in the world and are of immense socio-economic and ecological importance to mankind. They play a tremendous role in the flood control, improvement of water quality, recycling of natural groundwater, buffer against natural disasters, controlling of erosion, sediment's trapping, maintenance of biodiversity, wastewater treatment, and nutrients recycling (Schuyt and Brander, 2004).

The man-made changes in the inside delta, are mainly consequences of different land-use policies promoted in the past, thus during the 1960-1970 period the so-called ‘reed period’, a network of canals and earth platforms was created. Between, 1970-1980 the ‘fish period’, many areas were embanked and leveled to be used for commercial fishing. The decade 1980-1989 the ‘agriculture period’ marks an explosive extension of agricultural polders, all these human interventions considerably modified the local landscape and influenced the functioning of Danube Delta ecosystems. (Gâștescu et al, 1998)

The Zaghen wetland was also under the influence of anthropogenic interventions aimed at expanding Romania's agricultural area to the detriment of natural ecosystems.

In the flooded area, recovered by drainage and embankment work, called polder, the main activities were grazing, agriculture and fishing, for these reasons ecosystems, have been rapidly degraded and lost.

During the years 2012-2015, it was implemented the project "Ecological Reconstruction in the Zaghen Polder of the Danube Delta Cross-Border Biosphere Reserve Romania / Ukraine SMIS-CNSR 36276", which aimed at enabling the water input from the Danube into lake Zaghen, to establish the adequate hydrological regime typical for flooded areas, restoration of natural habitats, conservation of biological diversity, and providing ecosystem services in line with local community needs.

The zooplankton study in Zaghen wetland was carried out after the hydrotechnical works, within a monitoring program, as a part of ecological reconstruction project with the aim to the description of the ecosystems of this recently restored wetland and analysis of their evolution.
In the present study has been investigated the zooplankton community of Zaghen wetland, during June and December 2017, with the aim to relate the zooplankton community structure through to analysis of abundance and biomass and the proportion of bioindicator groups.

Zooplankton reacts rapidly to ecological changes and is viewed as excellent indicators of water quality and trophic conditions due to their short time and rapid rate of reproduction.

Zooplankton may be present in an extensive variety of ecological conditions, and they are assumed to be a vital part in indicating water quality, eutrophication, and production of a freshwater body. (Parmar et. al, 2016)

MATERIALS AND METHODS

Study site
The Zaghen wetland is located in the Eastern part of Tulcea, at West of the Danube Delta, the general aspect is a dammed enclosure that falls within the Danube river basin, on the lower course under the direct influence of the Tulcea arm, upstream of St. George's arm, and is identified as part of the floodplain of the Danube River.
Situated in the economic area of Danube Delta Biosphere Reserve, Zaghen wetland is under the administration of the Danube Delta Biosphere Reserve Authority, as part of the Natura 2000 sites: ROSCI0065 Danube Delta and ROSPA0031 Danube Delta and Razim-Sinoe Complex.
Lake Zaghen is a floodplain lake with an area of 180 ha and a volume of 937.000 mc. (Dimitriu et al., 2010)

In the studied area, the hydrological regime is a controlled one, there are pumps that bring water from the Danube into the enclosure with an adjustable flow, and the outflow of water in the enclosure takes place with the same pumps, in the opposite direction, thus the level can be kept constant regardless of the Danube waters and rainfall.

Sample collection and processing
For the present research investigation, four sampling stations have been selected based on contrasting characteristics, as follows: station 1 (S1) is located at the smallest distance from the urban area; station 2 (S2), located in the center of the lake; station 3 (S3), near decanter basin and station 4 (S4) located near pumping station. Sampling points were showed in Figure 1.

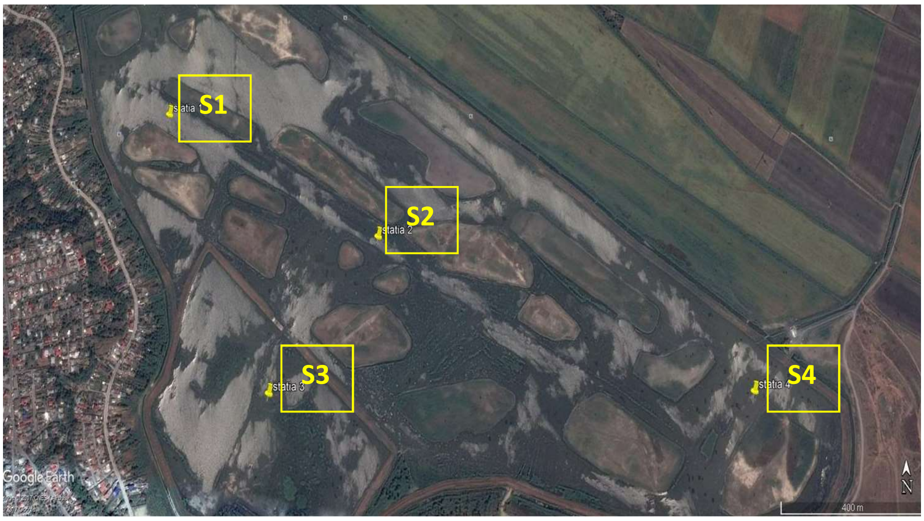

Figure 1. Study area with sampling stations in Zaghen weetland (acc. Map Google 2017; amended)

Sampling activities were made in 2017, (June, July, August, September, and December), totally were collected a number of 19 samples of zooplankton. Due to the low depth at station 3 (S3), in September, sampling was not possible.

Collecting and processing the zooplankton samples followed established standards (Tudor et al., 2015; APHA, 1989).

Zooplankton, samples were collected by filtering 30 L of water at each sampling site using a Hydro-Bios plankton net with mesh size of 55 µm, zooplankton concentrate obtained was transferred into 100 ml bottles and preserved with 96° alcohol, before being transported into the laboratory.
Into laboratory samples were again concentrated by slow sedimentation method for at least two weeks, the supernatant water was siphoned out in order to obtain 30-50 ml concentrate sample and the settled planktons has been counted under Zeiss Axio Lab A1 microscope by extracting subsamples of 1 mL, and transferring them to a Sedgewick – Rafter, counting cell, five subsamples for 1 mL, for each sample were determined and counted.

Zooplankton samples were identified up to species level as much as possible, following systematic keys of Rudescu, 1960 for Rotifera, Damian Georgescu, 1963, Dussart, 1969, Negrea,1983, and Leszek, 2016 for Crustacea.
Zooplankton density was expressed as the number of organisms per liter and biomass as mg ww.

RESULTS AND DISCUSSION

The zooplankton community of Zaghen wetland is composed of mainly Rotifera and zooplanktonic crustaceans, Copepoda and Cladocera.

A total of 88 species of zooplankton were recorded, Rotifera was dominant with 59 species followed by 15 species of Copepoda, and Cladocera with 14 species, a list of zooplankton organisms present in the samples is provided in Table 1.

Table 1. List of zooplankton (Cladocera, Copepoda, Rotifera) species occurring at sampling stations
Key: (+) presence (-) absence (*) pollution indicator species (Sladecek, 1983)

Species	Stations	S1	S2	S3	S4
Acanthocyclops sp.		-	+	-	-
Acanthocyclops vernalis		+	-	-	-
Alona quadrangularis		+	-	-	-
Alona rectangula		+	-	-	-
*Anuraeopsis fissa**		+	+	+	+
Ascomorpha ovalis		+	+	+	+
Asplanchna girodi		-	+	-	+
Asplanchna priodonta		+	+	+	+
Bosmina longirostris		-	-	+	-
*Brachionus angularis**		+	+	+	+
*Brachionus angularis bidens**		+	+	+	+
*Brachionus budapestinensis**		+	+	+	+
*Brachionus calyciflorus amphiceros**		-	-	+	-
*Brachionus calyciflorus dorcas**		+	-	-	+
*Brachionus calyciflorus pala**		+	+	+	+
*Brachionus diversicornis**		+	-	-	-
Brachionus forficula		+	+	+	+
*Brachionus leydigi**		+	+	+	+
Brachionus plicatilis		+	+	+	+

Brachionus quadridentatus	+	-	-	-
Brachionus rubens	+	+	+	-
Brachionus urceolaris	+	+	+	+
Calanipeda aquaedulcis	+	-	-	-
Cephalodella derbyi	+	+	-	-
Cephalodella gibba	+	-	+	-
Ceriodaphnia reticulata	+	-	-	-
Chydorus sphaericus	+	+	+	-
Colurella obtusa	-	+	-	-
Colurella uncinata	+	+	-	-
Cyclops strenuus	-	-	+	+
Cyclops vicinus	-	+	-	+
Diaphanosoma brachiurum	+	+	-	+
Ectocyclops phaleratus	-	-	+	+
Eosphora najas	-	-	+	-
Epiphanes macroura	+	-	-	-
Euchlanis deflexa	+	+	+	-
Euchlanis dilatata	+	+	+	+
Eucyclops serrulatus	-	-	+	+
Eudiaptomus gracilis	+	-	+	-
*Filinia longiseta**	+	+	+	+
*Keratella cochlearis**	+	+	+	+
Keratella quadrata	+	+	-	+
Keratella serrulata	+	+	-	-
Keratella tecta	+	+	+	+
Keratella ticinensis	+	-	+	-
Keratella tropica	+	-	-	-
Keratella valga	+	+	+	+
Lecane flexilis	-	+	-	-
Lecane luna	+	+	+	+
Lecane quadridentata	-	+	-	-
Lecane ungulata	+	-	-	-
Lepadella ovalis	+	-	+	+
Lepadella patella	+	+	+	-
Lepadella rhomboides	+	-	-	-
Lepadella triptera	+	+	-	-
Macrocyclops albidus	+	+	+	+
Macrocyclops fuscus	+	+	+	+
Macrothrix laticornis	+	-	-	-
Megacyclops viridis	+	+	+	+
Mesocyclops leuckarti	-	-	-	+
Moina brachiata	+	-	+	+
Monommata sp.	-	-	-	+
Mytilina mucronata	+	-	-	-

Mytilina ventralis	+	+	-	-
Paracyclops fimbriatus	+	+	+	-
Philodina sp.	+	-	-	-
Plationus patulus	+	-	-	-
Pleuroxus aduncus	+	-	-	+
Pleuroxus trigonellus	+	-	-	-
Polyarthra vulgaris	+	+	+	+
Pompholyx sulcata	+	+	-	+
Rotaria sp.	+	-	-	+
Scapholeberis mucronata	+	+	-	-
Scaridium longicaudatum	-	+	-	-
Simocephalus expinosus	+	+	-	-
Simocephalus serrulatus	+	+	-	-
Simocephalus vetulus	+	-	-	-
Synchaeta oblonga	+	+	-	+
Synchaeta pectinata	+	+	+	+
Testudinella parva	+	-	+	+
Testudinella patina	+	+	-	+
Thermocyclops crassus	+	+	+	+
Thermocyclops oithonoides	+	+	+	+
Trichocerca capucina	+	-	-	-
Trichocerca cylindrica	+	+	+	+
Trichocerca longiseta	+	+	+	+
Trichotria tetractis	-	-	-	+
Wolga spinifera	+	-	-	-

Looking at annual average densities of zooplankton, it results that Copepoda was higher with 22.65 ind/L followed by Rotifera (22.04 ind/L) and Cladocera (1.69 ind/L).
The percentage compositions of the number of taxa of zooplankton show that the small-sized zooplankton dominated the community: Rotifera was higher (67 %), followed by Copepoda (17 %) and Cladocera (16 %).

The highest number of taxa were found in summer at station 1 (30 species), the lowest number of zooplankton were observed in winter at station 4 (9 species).

Copepoda was the dominant group in summer and autumn, due to high densities of nauplii and copepodites stages, and Rotifera in the winter season, as compared to rotifers and copepods, the population density of Cladocera was very low in all seasons.

Densities and biomass of each group of zooplankton are shown in Figure.2.

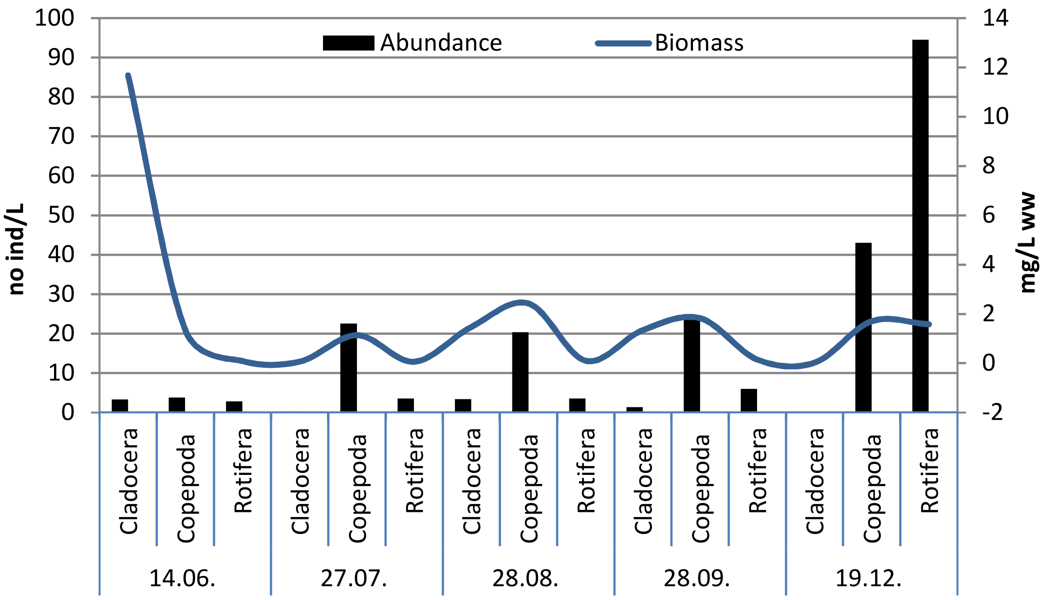

Figure 2. Average values of density (no ind/L) and total biomass (mg/L ww) of the main components of the zooplankton community groups in the study area in 2017

Rotifera

Rotifers play a vital role in the trophic tiers of freshwaters, are the connecting link between primary producers and consumers in aquatic food webs as grazers, suspension feeders and predators within the zooplankton community. (Kulkrani and Zade, 2018).
In the present study rotifers dominated zooplankton community with 59 species, as compared to other groups of zooplankton, previous studies have shown that taxonomic dominance of rotifers is a common pattern in freshwater ecosystems.
The rotifers density varied from 2.78 ind/L, in June to 94.44 ind/L in December, biomass values were highest in December 1.57 (mg/L ww) and lowest in July (0.06 mg/L ww).

Thanks to their short life cycles, rotifers react rapidly to changes in environmental conditions and so may be useful for biological monitoring and in assessing the trophic status and the level of lakes pollution (Gutkovska et al, 2013).
Several studies have provided lists of rotifer species that are indicative of different trophic states of aquatic ecosystems (Sládeček 1983; Berzins and Pejler 1989; Matveeva 1991; Duggan et al. 2001).
Throughout the study period, 13 different species of *Brachionus* were recorded: *Brachionus angularis*, *Brachionus angularis bidens*, *Brachionus budapestinensis*, *Brachionus calyciflorus pala*, *Brachionus forficula*, *Brachionus leydigi*, *Brachionus urceolaris*, *Brachionus plicatilis,* are present at all the stations.

They were dominated by *Brachionus angularis bidens* and *Brachionus calyciflorus pala* with an annual average density which formed 24% respectively 23% of the total Brachionus counts (Figure 3).
Zannatul and Muktadir in 2009, shown that the vicinity of three types of Brachionus indicates that the lake is being eutrophicated and is naturally contaminated.

Mageed (2008) and Uzma (2009) have stated that the presence of more than five species of Brachionus refers to the eutrophication of water bodies. (El-Damhogy et al., 2016)

Maemets (1983) and Nogueira (2001) showed in their studies, that a high abundance of *Brachionus* can be considered as a biological indicator of eutrophic waters, also Attayde and Bozelli in 1998 proved that these rotifers can be considered a target taxon for monitoring of water quality and conservation planning on aquatic environments.

Various authors (Tasevska et al. 2012; Radwan 1976; Gannon and Stemberger 1978; Dadhich et al.1999), was reported in their studies that generally, good indicators of eutrophic conditions are *Brachionus spp.*, *Anuraeopsis fissa*, *Pompholyx sulcata*, *Pompholyx complanata*, *Trichocerca* *cylindrica*, *Trichocerca pusilla*, *Filinia longiseta*, *Keratella cochlearis*, *Keratella quadrata* and *Polyarthra euryptera.*

Most of this species were recorded in the present study: *Anuraeopsis fissa*, *Filinia longiseta*, *Pompholyx sulcata*, *Keratella cochlearis*, *Trichocerca cylindrica,* *Keratella quadrata*.

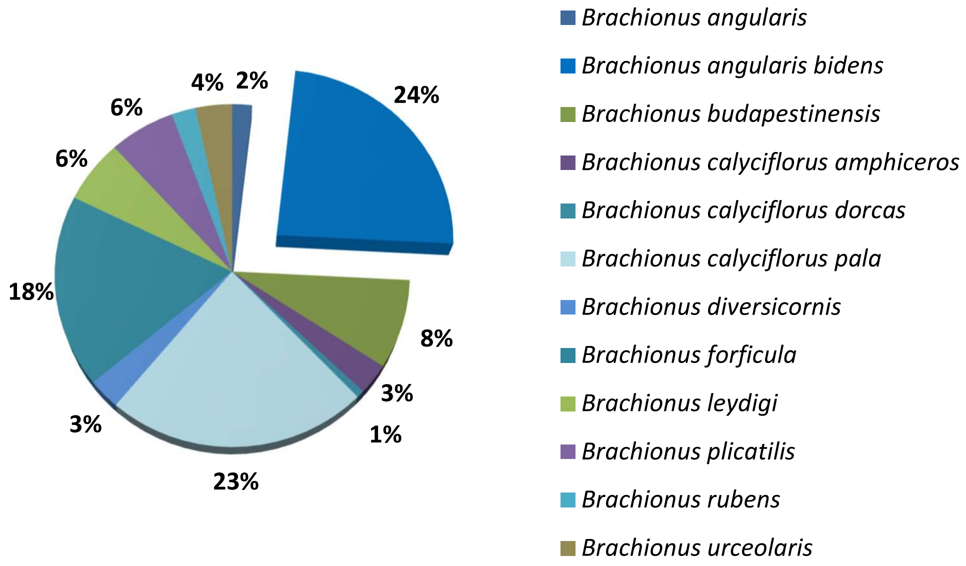

Figure 3. The percentage of total *Brachionus sp.* in the study site, from June 2017 to December 2017

Cladocera

Cladocera, as one of the major zooplankton groups, play a central role in the flow of energy in pelagic food webs, their ecological position in the middle of the food web makes them very suitable to track short- and long-term environmental changes and top-down or bottom-up processes (Davidson et al. 2011; Kattel and Sirocko, 2011).

They form a link between primary producers and higher trophic levels, with a combination of benthic, pelagic, littoral, and plant-associated taxa showing sensitivity to rapid environmental changes.

Regarding the values of density, it was found that this ranged from 0.22 ind/L in December to 3.36 ind/L in August, in terms of biomass the highest values were recorded in June 11.67 (mg/L ww) may be related to larger-bodied species such as: *Simocephalus vetulus*, *Simocephalus expinosus*, *Simocephalus serrulatus*, and lowest in December (0.02 mg/L ww).

Mareover Cladocera group was represented largely by small-bodied cladocerans like *Chydorus sphaericus*, *Bosmina longirostris*, *Alona quadrangularis*, *Alona rectangula*, *Moina brachiata, Pleuroxus aduncus.*

Like rotifers, the cladocerans can be used as ecological indicators of water quality, in lakes at high ecological status, there is a larger proportion of large Cladocera, which find refuges from fish predation among the plant communities. (Moss et al., 2003)
In order to asses the ecological status of water quality from the point of view of the zooplanktonic communities, ratios have proved more useful than absolute measures of zooplankton communities and two were used, according to the ECOFRAME classification system.
The first is the ratio of numbers of large species of Cladocera to total numbers of Cladocera, the second is the ratio of crustacean zooplankton biomass (mg/L ww) to phytoplankton biomass - chlorophyll "a" (µg/L).
The ratio of zooplankton biomass to phytoplankton or chlorophyll a biomass gives an independent measure of the influence of the zooplankton and also includes copepod zooplankton as well as

cladoceran zooplankton, the inclusion of rotifers did not increase the usefulness of the ratio, due to the small biomass of rotifers. In the case of Zaghen Lake, these ratios are equivalent to good quality lakes and shown in Table 2.

Table 2. Ecological status of Zaghen Lake, according to ECOFRAME classification of Moss et al., 2003.

Quality class	chl a (µg/L) /zpk (mg/L ww)	Cladocera (no. large/total no.)	Zaghen Lake
High	>50	>0.3	**Chlorophyll a (µg/L) /zoopl (mg/L ww) 98.239**
Good	>50	>0.3	
Moderate	20-50	0.1-0.3	
Poor	<20	<0.1	**Cladocera (no. large/total no.) 0.507**
Bad	<20	<0.1	

Copepoda

Freshwater copepods constitute one of the major zooplankton communities, make up a major portion of the biomass and productivity of aquatic ecosystems, they also occupy an important intermediate position in the food chains as predators having substantial impact on their prey population and as filtrators and selective feeders playing the fundamentally different role than large Cladocera (Reid and Williamson 2010).

Oligotrophic waters are usually dominated by copepods of the order Calanoida, whereas smaller copepods of the order Cyclopoida, predominate in eutrophic waters (Paturej et al., 2012)

Studies performed by Gannon and Stemberger, 1978 have associated lower proportions of calanoid to cyclopoid copepods and cladocerans with eutrophic environments. One generalization usually made in relation to zooplankton size structure and trophic state of water bodies is that species with larger bodies such as Calanoida (predominantly herbivores), occur primarily in oligotrophic environments, where there is a predominance of nanophytoplankton (Hillbritch-Ilkowska, 1997; Echevarria et al, 1990), whereas the Cyclopoida occur at higher density in meso-eutrophic environments, owing to their ability to handle larger food particles (Pace, 1986; Santos-Wisniewski and Rocha,2007).

We used Calanoid:Cyclopoida (CA/CY) ratio (Gazonato Neto et al., 2014), between the numerical densities of populations belonging to the sub-orders Calanoida and Cyclopoida which was assessed as a possible bioindicator of the trophic state

Copepods of the order Cyclopoida were dominant at most sampling points. To calculate Calanoid:Cyclopoid copepods density ratio (CA/CY), we took into account months June and September 2017, we observe that in June at sampling point S3, dominate Cyclopoida copepods with a value of ratio 0.12, at S1 and S4 it could be observed dominance of Calanoida copepods, where the value of CA/CY was 1.90 and 2. In December, the numerical densities of both groups were similar resulting values of the ratio close to 1.0 Table 3.

Table 3. Calanoid:cyclopoid copepods density ratio (CA/CY) in zooplankton communities sampled in June 2017 and December 2017 in sampling sites

CA/CY RATIO	S1	S2	S3	S4
June 2017	1.90		0.12	2
December 2017		1	1	

CONCLUSION

Many studies were carried on zooplankton in Danube Delta, research about zooplankton of Zaghen wetland are lesser-known, no further studies were previously carried out on the zooplankton fauna of the study area, consequently it is not possible to follow the changes of zooplankton fauna, we hope that the present study, will be a base for future studies in this area.

The presence of organic pollution indicator species along with clean water indicator species like cladocerans, *cyclopids*, and calanoids indicates a good water quality of the lake.

In conclusion, it can be seen that biological indices can be used to assess the water quality in a different way.

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