RESEARCH ARTICLE

BioRisk

Influence of some environmental factors on the distribution of zooplankton complexes in Mandra Reservoir, in Southeastern Bulgaria

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Abstract

The aim of the present study was to trace the influence of some environmental factors (w.temperature, wind, transparency, depth) on the distribution of zooplankton communities in the system Reservoir Mandra and the ecotone zones formed at the confluence of rivers Fakiyska, Sredetska, Izvorska and Rusokastrenska. Four samplings were performed at seven sites between February 2020 and January 2021. After determining the species composition and abundance, the results were subjected to structural analysis and Canonical Correspondence Analysis (CCA). A total of 67 taxa were identified, constituting about 48% of the Rotifera group, 27% of Cladocera and 19% of the Copepoda and only 6% from Protozoa. The Shannon-Weaver index for individual species diversity was between 2.37 and 0.62. The positive and negative correlation of zooplankton distribution in CCA shows that the relative abundance of any species depends on specific environmental variables. Analysis showed that temperature and wind had the strongest impact on the distribution of zooplankton.

Keywords

Canonical Correspondence Analysis, community structural analysis, Mandra Reservoir, zooplankton

Introduction

The distribution of aquatic organisms in the environment is the result of influences of biotic and abiotic factors as well as of the interactions between the organisms in the different parts of the food webs (Menge and Sutherland 1976; Arnott and Vanni 1993; Harley 2003; Abdul et al. 2016; Carter et al. 2017). Many authors have discussed the influence of wind and other abiotic factors on holo-polymictic water basins (George and Edwards 1976; Karabin et al. 1997; Naidenov 1998; Pehlivanov et al. 2004; Güher et al. 2011; Traykov and Vladimirova 2015; Güher 2016; Ismail and Adnan 2016, Tyor et al. 2018; Hayee et al. 2021).

Shallow and deep lakes are affected differently by weather conditions and shallow polymictic fresh water ecosystems are particularly vulnerable to climate warming (Mooij et al. 2005, 2007; Tuvikene et al. 2011; Jeppesen et al. 2014; Haberman and Haldna 2017).

Zooplankton is not included in the European Union Water Framework Directive (Directive 2000/60/EC) as obligatory biological quality elements, despite it being considered a key component of pelagic food webs. Many authors such as Stemberger and Lazorchak (1994), Dodson et al. (2000, 2009), Pehlivanov et al. (2006), Imoobe and Adeyinka (2009), Caroni and Irvine (2010), Tisheva and Kozuharov (2013), Haberman and Haldna (2014) report that zooplankton can be used as a good indicator in assessing the trophic status of lakes.

Zooplankton is an integral part of aquatic ecosystems, playing a crucial role in connecting primary producers and higher trophic levels, such as fish. Zooplankton communities, on the other hand, are sensitive to changes in their resources and their predators and therefore reflect the balance of food web processes through body size distribution and taxonomic composition (Mills and Schiavone 1982; Carpenter et al. 1985; Hansson et al. 2007; Braun et al. 2021).

Mihailova-Neikova (1961) studies the food spectrum of fish in Lake Mandra. On the basis of this study is clear that the food of all fish species contain species from Copepoda, Cladocera, Rotifera groups and some chironomid larvae. It can be concluded that the zooplankton in Mandra Reservoir is a major trophic resource for both small and large fish.

Reservoir Mandra, situated in Southeastern Bulgaria, is part of the Mandra-Poda complex, which is a protected area under the two main environmental directives of the European Union – Directive 92/43 / EEC on the protection of natural habitats and of wild flora and fauna and Directive 2009/147 / EU Wildlife Conservation. The Via Pontica bird migration route passes over Mandra.

Earlier studies that were conducted on Mandra Reservoir (Kozuharov et al. 2021) have shown the high indicative ability of zooplankton to reflect the state of the ecosystem and water quality. The article traces the changes in zooplankton complexes due to the reconstruction of the coastal lake to the reservoir and the interrupted connection with the sea. Results indicate an acceleration of the eutrophication process in Mandra Reservoir. Some previous data that concern plankton in the reservoir have been given

by Michev and Stoyneva (2007). In the previous research about zooplankton in this reservoir (Kozuharov et al. 2021), we suggest that there might be a direct link between the distribution of zooplankton and certain environmental factors, in particular wind. To test this hypothesis, several field studies of Mandra Reservoir were conducted over a one-year period.

Materials and methods

Mandra Reservoir covers an area of 33 km^2 and the maximum depth reaches 7 m. The strong winds common to coastal lakes and reservoirs define Mandra as a holo-polymictic basin. The four sampling sessions (Feb 20, June 20, Sep 20, Jan 21) were performed between 1 February 2020 and 1 January 2021, during which qualitative and quantitative zooplankton samples were collected, as well as data on environmental factors. Our study is focused more on the dynamics in overlapping seasons when plankton comes under strong environmental pressure. The geographical coordinates of the sampling points (Fig. 1) were determined by using a Garmin Striker 5DV sonar with highly sensitive GPS. It was also used to measure the depth of the water body at the various stations, as well as the temperature. Transparency was measured by Secchi disc. The values for the wind speed for the period under investigation were taken from the information page of the National Institute of Meteorology and Hydrology in Bulgaria for the strength of the winds for the region on the respective day.

24 quantitative and 24 qualitative samples were collected by using an Apstein plankton net 55 μm mesh size and via filtering of 100 dm^3 of water through the net. As the reservoir is shallow, in places between 1 and 2 meters (Table 2), it was not possible to use a Juday net for quantitative samples. Because of this reason, zooplankton samples, each of 100 dm3 of water, were collected from various spots around each station by means of a bucket and filtered through an Apstein plankton net. This method of directly filtering a certain amount of water through Apstein plankton net is widely used in the study of shallow holo-polymictic standing water bodies such as the studied reservoir and in ecotone river-reservoir zones (EN–15110: 2006; Kozuharov et al. 2007; Yakimov et al. 2016; Protasov et al. 2019). Samples, fixed in 4% formalin, were counted by using the method of V. Hensen modified by Dimoff (1959) and Naidenow (1981). This method includes the following operations, applied to each sample:

• Samples are brought to volume of 100 ml and mixed intensively until all organisms were distributed randomly in the sample volume.
• 5 or 10 ml of sample (depending upon zooplankton density) are taken and poured in the counting chamber of Dimov for count.
• All the organisms in this sample are counted through the use of stereomicroscope Leyca GZ6.
• The data obtained are then expressed in terms of cubic meters.
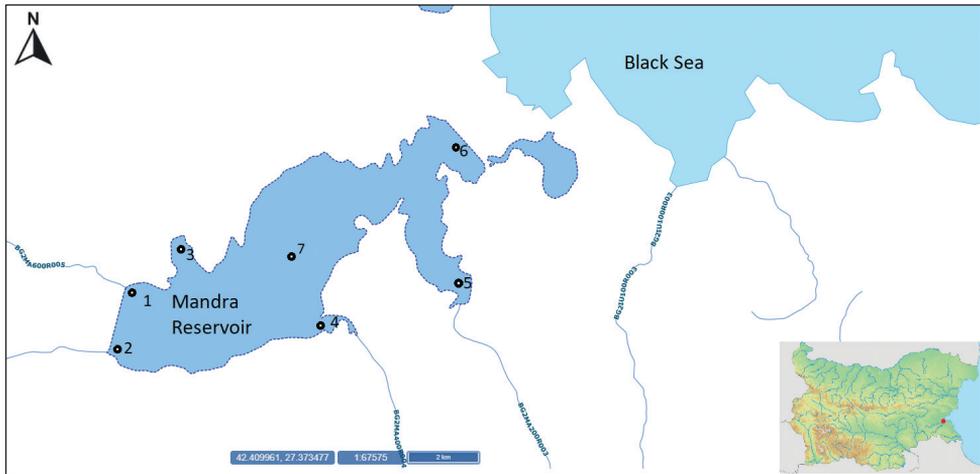
Figure 1. Location of the sampling points on Mandra Reservoir. 1. 42°24.14'N, 27°19.26'E – the mouth of the Rusokastrenska River; 2. 42°23.19'N, 27°18.84'E – the mouth of the Sredetska River; 3. 42°24.68'N, 27°20.41'E – northern dike; 4. 42°23.57'N, 27°22.57'E – the mouth of the Fakiyska River; 5. 42°24.15'N, 27°26.06'E – the mouth of the Izvorska River; 6. 42°26.28'N, 27°26.11'E – dam; 7. 42°24.70'N, 27°22.65'E – central part.

We used three indicators that generally characterize the biological completeness of water through the parameters of the species structure of communities. These indicators are the Shannon-Weaver index for individual species diversity (H), Simpson's index of dominance (c) and the Pielou's evenness index (e) after Shannon and Weaver (1949), Pielou (1975). Margalef richness index was also used to express the degree of uniformity in the distribution of individuals among taxa in the study area. De Vries (1937) frequency of occurrence (pF), was calculated in %. A species with an encounter frequency pF ≥ 70% is considered permanent. Canonical Correspondence Analysis (CCA) was used in order to determine the influence of environmental variables on the abundance and distribution of zooplankton (Czerniawski et al. 2013; Abdul et al. 2016). In this analysis we used the species which are dominant in the abundance of zooplankton.

Results

A total of 67 taxa were identified during the laboratory processing of zooplankton samples. 10 of them were found in very low quantities only in qualitative samples. The list of taxa and their frequency of occurrence (pF) for the studied period are presented in Table 1.

The abundance observed in February and June is relatively low, compared to the other months (Fig. 2).

In February 2020, the highest numbers had Nauplius with 32 500 ind/m³, measured at sampling point 5. With a slightly lower number, but close in value, are Copepodites-Copepoda and *Asplanchna priodonta*. The maximum number of Copepodites-

Table 1. List of zooplankton species found in Reservoir Mandra and their values of pF – frequency of occurrence for the studied period.

Taxa	pF	Taxa	pF
Testacea		*Keratella hiemalis* Carlin, 1943	75.00
Difflugia sp. Leclerc, 1815	4.17	*Notholca squamula* (Müller, 1786)	8.33
Arcella catinus Penard, 1890	12.50	*Lepadella patella* (O. F. Müller, 1773)	4.17
Ciliatea		*Lepadella ovalis* (O.F. Müller, 1786)	8.33
Stentor polymorphus	4.17	*Asplanchna sieboldi* (Leydig, 1854)	50.00
Stentor roeseli Oken, 1815	4.17	*Asplanchna priodonta* Gosse, 1850	50.00
Rotifera		*Trichocerca* sp.	4.17
Pompholyx complanata Gosse, 1851	79.17	*Trichocerca similis* (Wierzejski, 1893)	33.33
Testudinella sp.	20.83	*Trichocerca cylindrica* (Imhof, 1891)	4.17
Testudinella truncata (Gosse, 1886)	12.50	*Trichocerca capucina* (Wierzejski & Zacharias, 1893)	25.00
Filinia longiseta/ Triarthra longiseta (Ehrenberg, 1834)	12.50	*Trichocerca pusilla* (Jennings, 1903)	4.17
Filinia terminalis (Plate, 1886)	8.33	*Synchaeta* sp. Ehrenberg, 1832	12.50
Lecane sp.	12.50	*Polyarthra* sp.	20.83
Lecane monostila (Harring & Myers, 1926)	4.17	*Polyarthra remata* Skorikov, 1896	62.50
Lecane luna (Müller, 1776)	4.17	*Polyarthra dolichoptera* Idelson, 1925	62.50
Epiphanes sp.	4.17	*Polyarthra vulgaris* Carlin, 1943	62.50
Euchlanis sp.	4.17	*Polyarthra minor* Voigt, 1904	16.67
Brachionus angularis Gosse, 1851	20.83	*Polyarthra major* Burckhardt, 1900	8.33
Brachionus calyciflorus Pallas, 1776	8.33	**Cladocera**	
Keratella cochlearis (Gosse, 1851)	100.00	*Diaphanosoma lacustris* Korjinek, 1981	33.33
Keratella tecta (Gosse, 1851)	75.00	*Bosmina longirostris* (O. F. Müller, 1776)	12.50
Keratella quadrata (Müller, 1786)	54.17	*Bosmina kessleri* Uljanin, 1874	54.17
Bosmina coregoni Baird, 1857	83.33	*Harpacticoida genus* sp. G. O. Sars, 1903	4.17
Daphnia cucullata G.O. Sars, 1862	58.33	*Cyclops* sp.	4.17
Daphnia galeata G. O. Sars, 1864	37.50	*Cyclops* c.f. *insignis*	8.33
Daphnia pulex (O.F. Müller, 1785)	4.17	*Tropocyclops prasinus* (Fischer, 1860)	12.50
Daphnia sp. *Juv.*	12.50	Copepodites-Copepoda	100.00
Ceriodaphnia quadrangula (O.F. Müller, 1785)	4.17	Nauplius	100.00
Simocephalus vetulus (O.F. Müller, 1776)	4.17		
Alona guttata Sars, 1862	8.33		
Alonella nana (Baird, 1850)	4.17		
Chydorus sp.	4.17		
Chydorus sphaericus (O.F. Müller, 1776)	79.17		
Chydorus latus G.O.Sars, 1862	4.17		
Chydorus sp. *Juv.*	4.17		
Pleuroxus sp. Baird, 1843	4.17		
Leptodora kindti (Focke, 1844)	8.33		
Copepoda			
Eudiaptomus gracilis (Sars, 1862)	50.00		
Cyclops strenuus Fischer, 1851	12.50		
Cyclops vicinus Uljanin, 1875	29.17		
Thermocyclops crassus (Fischer, 1853)	37.50		
Acanthocyclops sp.	4.17		
Acanthocyclops americanus (Marsh, 1893)	16.67		

Copepoda is 23 200 ind/m^3, also measured at sampling point 5, and for *A. priodonta*, respectively, 20 400 ind/m^3, measured at sampling point 7.

Dominant in number in June 2020 are three taxa, with maximum numbers as follows – Nauplius – 172 800 ind/m^3, at sampling point 3, *Chydorus sphaericus* – 102

Table 2. Hydrological values measured in Mandra Dam in the period 02.2020–01.2021.

date-sampling point	depth (m)	transparency Secchi (cm)	wind (m/s)	t (°C)
Feb 20-S4	1.10	50	6	7.7
Feb 20-S5	1.70	150	6	8.4
Feb 20-S6	3.00	130	6	7.5
Feb 20-S7	2.30	65	6	6.2
June 20-S1	1.50	40	0	26
June 20-S2	1.50	40	0	25
June 20-S3	1.80	45	0	22
June 20-S4	1.20	50	0	22
June 20-S5	1.50	60	0	22
June 20-S6	3.80	60	0	26
Sep 20-S1	1.50	30	4	20.38
Sep 20-S2	1.50	30	4	18.7
Sep 20-S3	1.80	35	4	19.8
Sep 20-S4	1.20	30	4	20.14
Sep 20-S5	1.50	35	4	20.17
Sep 20-S6	3.80	35	4	20.5
Sep 20-S7	3.20	30	4	20.35
Jan 21-S1	2.00	70	8	10.2
Jan 21-S2	1.50	80	8	10
Jan 21-S3	2.00	45	8	10.4
Jan 21-S4	2.60	65	8	10.15
Jan 21-S5	1.30	90	8	10.6
Jan 21-S6	3.70	70	8	9
Jan 21-S7	4.00	75	8	9.9
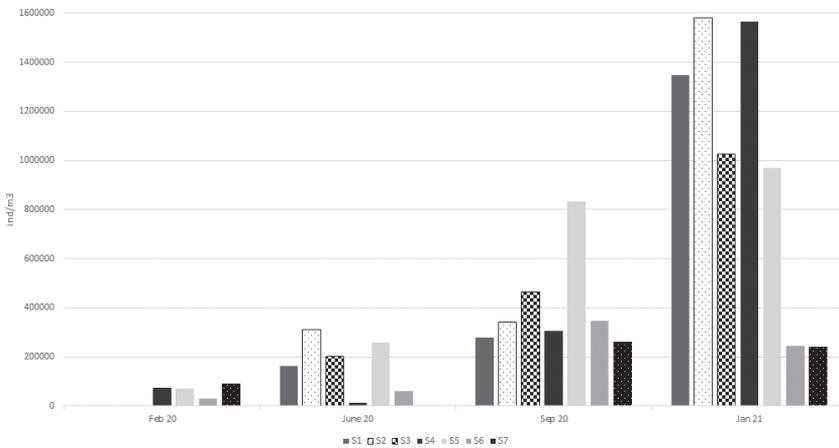
Figure 2. General zooplankton abundance in Mandra Reservoir for the studied period.

813 ind/m³, at sampling point 2, *Polyarthra vulgaris* – 72 500 ind/m³, measured at sampling point 5.

In September 2020, the highest numbers had *Keratella cochlearis* – 339 000 ind/m³, measured at sampling point 5, *Polyarthra vulgaris* – 156 000 ind/m³, at sampling point 5, Nauplius – 136 000 ind/m³, measured sampling point 3.
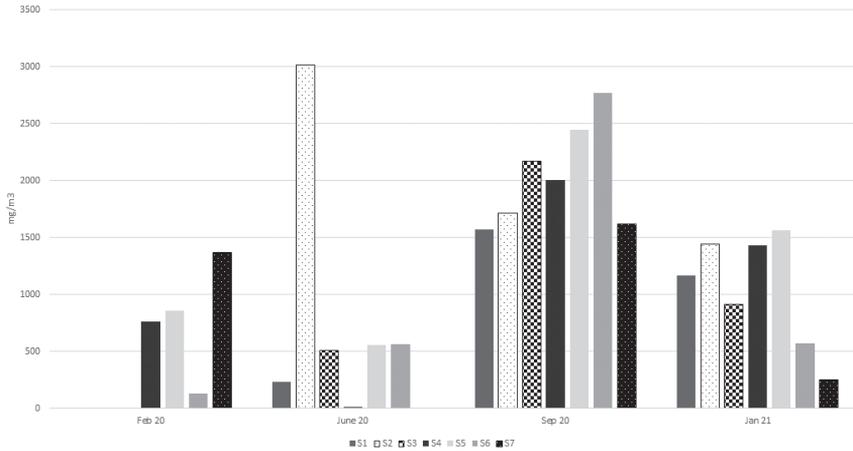
Figure 3. General zooplankton biomass in Mandra Reservoir for the studied period.

While in the other seasons the dominants are followed by other species with a slightly smaller value, in January the absolute dominant for the Mandra Reservoir is *K. cochlearis*. The maximum number of 1 282 000 ind/m³ was measured at S2.

The highest and the lowest biomass within the four samplings were measured in June (Fig. 3), respectively, at sampling point 2 with 3013 mg/m³ and at sampling point 4 with 8.4 mg/m³. The high biomass of station 2 is due to the high number of relatively large Cladocera *C. sphaericus*. This is euribiont, a species with a cosmopolitan distribution.

The ratio between the species composition of the different zooplankton groups during the four periods is shown in Fig. 4.
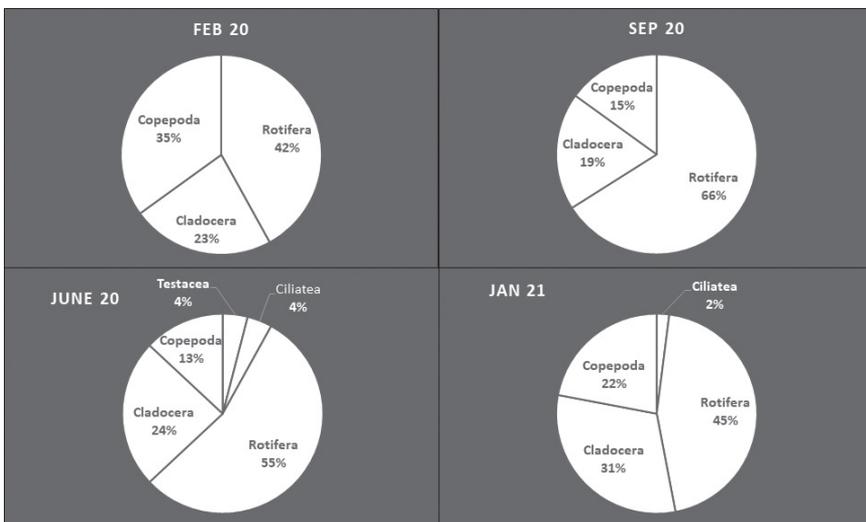
Figure 4. Percent species composition of different plankton groups (February 2020, June 2020, September 2020, January 2021).

Results of Shannon-Weaver diversity index (H), Simpson's index of dominance (c), Pielou's evenness index (e) and Margalef richness index are shown in Fig. 5. It can be seen that the trends of all indices are relatively constant during the different periods except in June, when the values vary a lot.

For the study period, the Shannon-Weaver diversity index ranged between 0.52 at station 3 in June and 2.37 at station 6 in September. These are comparatively low values of the index. The degree of dominance index was always inversely proportional to the individual species diversity index. Its value was lowest at station 6 in September (0.13) and highest at station 3 in June (0.75). This was the period of higher abundance in the larval stages – Nauplius and Copepodites-Copepoda.

The Margalef richness index varies between 1.23 at station 4 in June and 4.09 at station 7 in January. In general, there is a relatively constant trend between stations for different periods, except for the June series. Then the index varies between 1.23 (station 4) and 3.26 (station 1). This trend is also observed in Pielou's evenness index. The maximum and minimum values were reported at the same time – June, at station 5 (0.78) and at station 3 (0.25). High values of Pielou's index are registered when and where abiotic factors often change and a species or group of species cannot be dominant.

The CCA (Fig. 6) of the samples and dominant zooplankton taxa abundance revealed that temperature, depth, transparency and wind correlated best with the first axis 1, which accounted for a total variance of 91.45%. It was positively correlated with depth, transparency and wind, but negatively correlated with temperature. Axis 2, showed 7.22% variation, and it was positively correlated with temperature, and negatively correlated with the other factors.
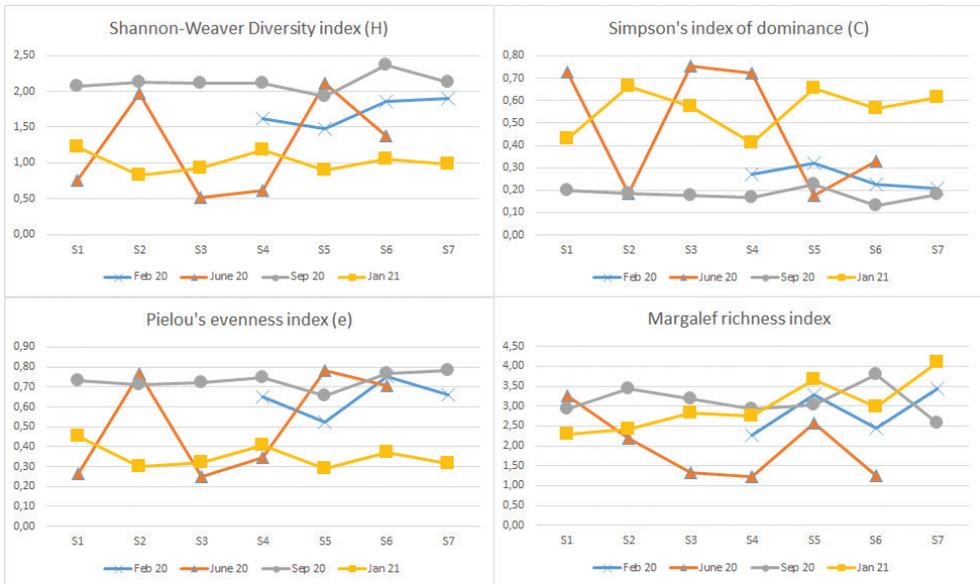
Figure 5. Shannon-Wiener diversity index (H), Simpson's index of dominance (c), Pielou's evenness index (e) and Margalef richness index after Shannon and Weaver (1949), Pielou (1975), (S1, S2, S3, S4, S5, S6, S7 – sampling points).
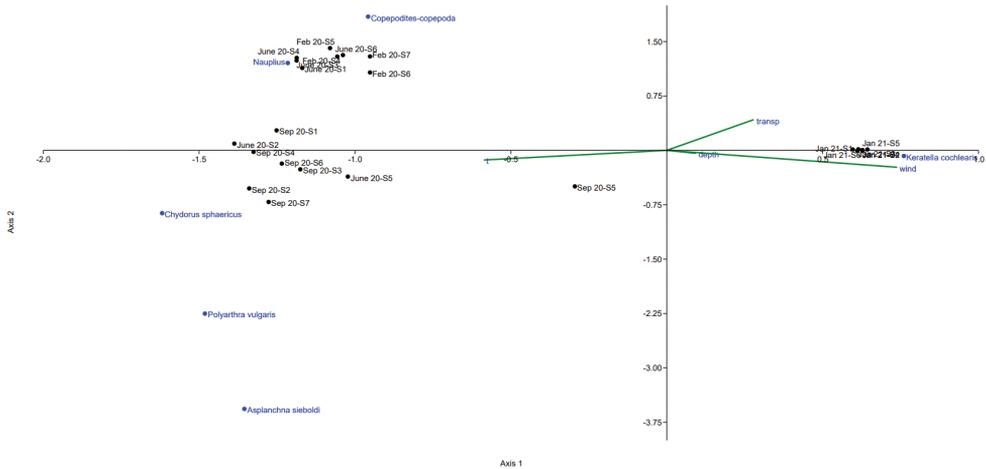
Figure 6. Canonical correspondence analysis (CCA) triplot for the ecological correlations between dominant zooplankton taxa in Mandra Reservoir and some environmental variables. (S1, S2, S3, S4, S5, S6, S7 –sampling points).

Discussion

Some species like *Keretella quadrata*, *Brachionus angularis*, *Trichocerca pusilla*, *Filinia longiseta* are considered indicative of advance processes of eutrophication (Imoobe and Adeyinka 2009). These 4 species were recorded in the species composition of Mandra Reservoir during our study.

The ratio between the groups of zooplankton taxa in different seasons and the predominance of the species diversity of organisms from the Rotifera type confirm the observations in the dominant complexes from a previous study (Kozuharov et al. 2021), which indicate the effects of eutrophication. Light rotifers *K. cochlearis* showed strong correlation (0.76) with wind in January. Nauplius and Copepodites of *Copepoda* are strongly influenced by the summer temperature when they are also the dominant group. The same tendency can be seen from the structural analysis. *Ch. sphaericus*, which is indicatory species of eutrophic waters, showed strong correlation with warm water in September.

The analysis also shows that depth is not essential for the distribution of zooplankton in this shallow polymictic basin. It showed weak positive correlation (0.09) with Axis 1.

According to one of the biocoenotic principles formulated by Thienemann (1931), for aquatic communities, as early as 1920, the more variable the abiotic conditions of a place, the richer in the species is the local community (Sladecek 1973).

The rare zooplankton taxa established only in qualitative samples could be called casual components. Their quantities are lower than the range of quantitative parameters of the samples. That means very rare components.

Probably the main reason for the comparatively low values of the Shannon – Weavers index is the stabile dominant species and complexes that have high quantitative values in the reservoir of zooplankton. The obtained high values of the index of dominance confirm that conclusion.

The significant differences in the values of the Simpson's index of dominance show that different conditions were observed in various parts of this comaratively large (in surface) reservoir during different samplings and seasons. Environmental factors have a great influence, but, on the other hand, the low diversity and richness values might be result of fish predation on site 3 and 4, moreover the observed data corresponded with lowest zooplankton biomass at the seasons (Fig. 3). The presence of small rotifers and the lack of large cladoceras and copepods might be an indirect sign of planktivorous fish pressure (Mihailova-Neikova 1961; Carpenter et al. 1985; Stemberger and Lazorchak 1994; Imoobe and Adeyinka 2009) and coincides right after the breeding period of the fish species. The results reveal the fish spawning and feeding key zones and should be used to increase the efficiency of the conservation measures in a protected area, the part of the European ecological network Natura 2000 and site of the "Via Pontica" bird migration route.

Winter and summer conditions show characteristics of two different water basins. Water basins in which Rotifera predominate go from mesotrophic to eutrophic. Large zooplankton organisms from the group Copepoda like *Cyclops strenuus* and *Eudiaptomus gracilis*, which have the highest biomass in winter, are typical indicators for mesotrophic conditions in the reservoir. As a whole, the conditions in the studied shallow artificial water body are very dynamic during different seasons, which determines the dynamics in the structure and the distribution of zooplankton complexes of the zooplankton in Mandra Reservoir.

Conclusions

Based on the results of our study and taking into account relevant data from numerous zooplankton studies, we can conclude that the zooplankton can be used as key indicator in the monitoring of shallow holo-polymictic water bodies such as Mandra Reservoir.

The results obtained for the calculated structural indices are normal for mesotrophic and eutrophic water basins. The obtained high values of the diversity index are determined by the more diverse habitat conditions along the reservoir and ecotone zones of the inflowing rivers. However, biotic interactions may have adverse impact on the formation of a community structure and should be the next step in our investigation.

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