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Phytoplankton Study in Pomorie Lake, Black Sea (Bulgaria)

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Abstract. Coastal wetlands, such as Pomorie Lake, are among the most dynamic and complex ecosystems in the world and their environmental safety requires special preservation. The aim of the present paper is to study the phytoplankton composition in Pomorie Lake, a protected area in Bulgaria. The development of a total of 49 phytoplankton species distributed in 8 classes was recorded and 6 phytoplankton species were recorded in „bloom" concentrations. The most abundant species were from class Dinophyceae (17 species, 34.69%) and class Bacillariophyceae (9 species, 18.37%). The research found a decrease in the proportion of diatoms (Bacillariophyceae class) and an increase in the number of green and euglena algae. This pilot study contributes to the current characterization of the ecological status of Pomorie Lake. According to the established phytoplankton biomass and part of the ecological assessment criteria for "mesotrophic" lake types the ecological status of Pomorie Lake in 2017 is between "moderate" - "good".

Key words: Pomorie Lake, Black Sea, phytoplankton, Dinophyceae, Bacillariophyceae.

Introduction

Pomorie Lake is part of the Burgas lake complex (Burgas, Atanasovsko, Mandra and Pomorie Lakes), which is one of the three most significant wetland complexes for congregations of waterfowl along the Bulgarian Black Sea Coast.

During recent years the urbanization and building activities around the lake have become more intensive and some of the small wetland habitats on the periphery of the lake are already extinct. Furthermore, a significant pollution of the surrounding land is caused by household and building waste. The natural water regime of the lake is disturbed, because the canal that connects it to the sea was obstructed by sediments.

As it is known phytoplankton is an indicator used for aquatic ecosystem assessments, because it consists of primary producers with extremely short lifecycles, which respond early to any change in the environment (Niemi & McDonald, 2004; Maialen et al., 2014; Pasztaleniec, 2016).

Historical data on the composition, distribution and dynamics of the

phytoplankton in the lake has been described mainly by Vasilev et al. (1998), (documented 39 species (pyrophyta and diatoms)) and Stoyneva (2010) (63 algal taxa of 9 classes, 24 genera, mainly oligo- and mesohaline species).

The values of the quantitative parameters such as biomass and abundance have been described as high, characterized by high degree of eutrophication of the water.

Nowadays, the studies of the phytoplankton in Pomorie Lake as a component of the biota are limited and there`s a lack of systematic quantitative phytoplankton data. Therefore, in this pilot study we set out to investigate the dynamics and composition of phytoplankton in Pomorie Lake during 2017.

Material and Methods

Lake hydrology and hydrochemical characteristics

Pomorie Lake is a hypersaline lagoon area near Pomorie town and has a size of 814 ha. According to Raynova (2010) the freshwater catchment area of the lake is located between the Kamenar and Kableshkovo Villages. The total catchment area is 33.55 km^2. The water surface area is 6 km^2 and the water volume is 6 million km^3 on average (Varbanov, 2002). The average depth of the lake is 1 m and it reaches a maximum of about 1.6 m (Vasilev & Mitrophanova, 1998).

The lake water has mildly to moderately alkaline pH levels with oxygen saturation between 50 and 240% (Hiebaum, 2010). Surprisingly low average salinity levels of 19-19,9 ‰ (Popov, 2015, Burgas, pers. comm), similar to those of the Black Sea, have been measured due to non-functioning southern drainage system. The main reason for that salinity change was the different hydrological regimes and the inflow of fresh water from Kamenarska River. The river was first drained into the protective drainage channel near the lake, but then after the backfilling in the area of the inflow, its fresh water had been entering the lagoon for years.

Phytoplankton sampling and analysis

Six phytoplankton samples were collected with Ruttner bottle (1,8 L) from the surface water layer (0-0.20 m), from 6 different sites (Table 1, Fig. 1) during April, July and October in 2017. Sampling sites were selected so that they can provide a satisfactory geographical coverage of the area according to methodology of Moncheva & Parr (2010). They were fixed with formalin (up to 2% solution) and concentrated by sedimentation (Morozova-Vodianitskaya, 1954). The microalgae were analyzed with an Olympus BX41 light microscope (by light field microscopy and phase contrast; at 100x, 200x, 400x and 800x magnifications) and in Sedgwick Rafter counting chambers (1 ml) and Palmer - Maloney (0.05 ml) by standard methodology (Moncheva & Parr, 2010). Picoplanktonic cells (< 2 μm) were not examined.

The ecological status was assessed according to the evaluation criteria of Surface water characterization (2013), using 5 classes from excellent to very poor environmental condition as defined in Annex 2 of Ordinance № 4. Only a few of the metrics were analyzed due to the pilot character of this study.

A geometric method (Edler, 1979; Olenina et al., 2006) was applied to determine the biomass of microalgae and the individual sizes of several representatives of each species were measured. The cell volume was calculated using geometric formulas (Olenina et al., 2006). We assume that the cell density is 1.

The taxonomic classification of phylum and classes has been made in accordance with WoRMS (2019).

The Phytomar 2.0 (Klisarova, 2008) and Excel 12 (Microsoft Corporation, 2007) software were used for Shanon's index (calculated by abundance) and graph calculations.

Table 1. Sampling sites in Pomorie Lake.

Site №	GPS coordinates
1	42°36'26.2"N 27°37'31.3"E
2	42°33'56.5"N 27°37'57.0"E
3	42°33'58.0"N 27°37'40.4"E
4	42°34'05.5"N 27°37'32.2"E
5	42°34'17.0"N 27°38'07.2"E
6	42°34'04.3"N 27°38'12.7"E

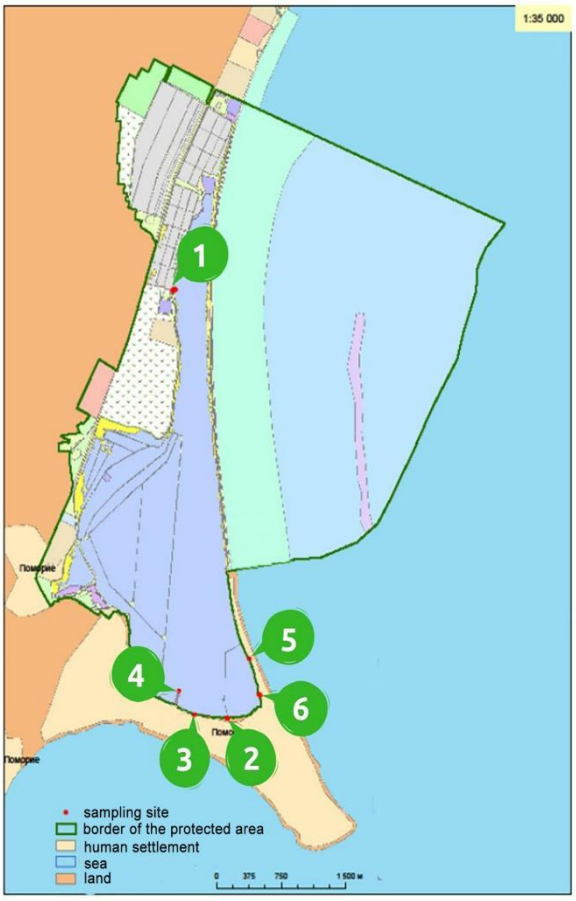

Fig. 1. Map of the study area with indications of the sampling sites.

Results and Discussion

Species composition of phytoplankton

The development of a total of 49 species distributed in 8 classes was recorded in the phytoplankton of Pomorie Lake during 2017. The most abundant species were from class Dinophyceae (17 species, 34.69%) and class Bacillariophyceae (9 species, 18.37%). The classes Chlorophyceae, Cyanophyceae and Euglenophyceae were registered with 6 species or 12.24%, and the rest 3 classes (Cryptophyceae, Trebouxiophyceae, Zygnematophyceae) were respectively registered with 2 and 1 species or 10.20% of the total taxonomic composition (Table 2, Fig. 2).

Compared to the earlier studies of Vasilev et al. (1998), a decrease in the share of diatoms (class Bacillariophyceae) and an increase in the number of green and euglena algae were found. The tendency of development of a large number of typically marine eurythermal and euryhaline microalgae species remains (Vasilev et al., 1998).

In April, a total of 20 species from 6 classes were found in the lake. (Table 2). The maximum annual biodiversity was observed for class Dinophyceae (11 species) and class Trebouxiophyceae (3 species) (Table 2, Fig. 3). No species of the class Bacillariophyceae were registered.

In July, 23 species from 7 classes were found. The number of species varied from 13 to 17 species per sampling site. The classes Dinophyceae (7 species), Bacillariophyceae (6 species) and Cyanophyceae (4 species) were presented with the highest number of species. The remaining groups of microalgae were registered with a small number of species (Table 2, Fig. 3).

Table 2. Taxonomic list of phytoplankton species existing in Pomorie Lake in 2017. *Legend:* 1-6 numbers of sampling points in combination with the month of sampling: A - April, J – July, O - October.

№	Taxa/Sampling site, Month	Site 6, A	Site 4, J	Site 2, J	Site 5, O	Site 1, O	Site 3, O
	BACILLARIOPHYCEAE						
1	*Chaetoceros socialis*				+		+
2	*Chaetoceros sp.*		+		+		

3	*Cocconeis scutellum*		+	+			
4	*Cyclotella caspia*				+	+	+
5	*Diatoma tenuis*		+				
6	*Navicula sp.*		+	+	+	+	+
7	*Nitzschia sp.*		+				
8	*Nitzschia tenuirostris*					+	+
9	*Pleurosigma elongatum*			+			
		DINOPHYCEAE					
10	*Akashiwo sanguinea*	+					
11	*Cochlodinium sp.*	+					
12	*Glenodinium danicum*	+					
13	*Glenodinium sp.*			+			
14	*Gonyaulax sp.*	+					
15	*Gonyaulax spinifera*		+				
16	*Gonyaulax turbynei*	+					
17	*Gymnodinium agiliforme*	+			+		
18	*Gymnodinium albulum*				+	+	+
19	*Gymnodinium sp.*	+	+	+	+	+	+
20	*Gyrodinium spirale*		+				
21	*Oblea rotunda*				+	+	+
22	*Peridinium pusillum*		+				
23	*Peridinium sp.*	+	+		+		+
24	*Polykrikos schwarzii*	+					
25	*Prorocentrum cordatum*	+					
26	*Prorocentrum micans*	+	+	+			
		CHLOROPHYCEAE					
27	*Ankistrodesmus sp.*				+		
28	*Chlamydomonas sp.*						+
29	*Chlorogonium sp.*					+	+
30	*Chlorophyceae indet.*		+		+	+	+
31	*Coelastrum sp.*	+					
32	*Monoraphidium convolutum*	+					
		EUGLENOPHYCEAE					
33	*Euglena sp.*				+		+
34	*Eutreptia lanowii*				+	+	+
35	*Eutreptia sp.*			+			
36	*Eutreptia viridis*				+		
37	*Eutreptiella sp.*					+	
38	*Phacus dangeardii*	+	+				
		CRYPTOPHYCEAE					
39	*Cryptomonas sp.*	+	+	+	+		+
40	*small Flagellates*		+	+	+	+	+
		CYANOPHYCEAE					
41	*Anabaena sp.*	+					
42	*Lyngbya sp.*		+				
43	*Merismopedia sp.*	+		+			
44	*Oscillatoria sp.*			+	+	+	+

45	*Spirulina sp.*				+
46	*Cyanophyceae indet.*	+		+	
		TREBOUXIOPHYCEAE			
47	*Chlorella pyrenoidosa*	+			
48	*Chlorella vulgaris*	+			
		ZYGNEMATOPHYCEAE			
49	*Penium sp.*		+	+	

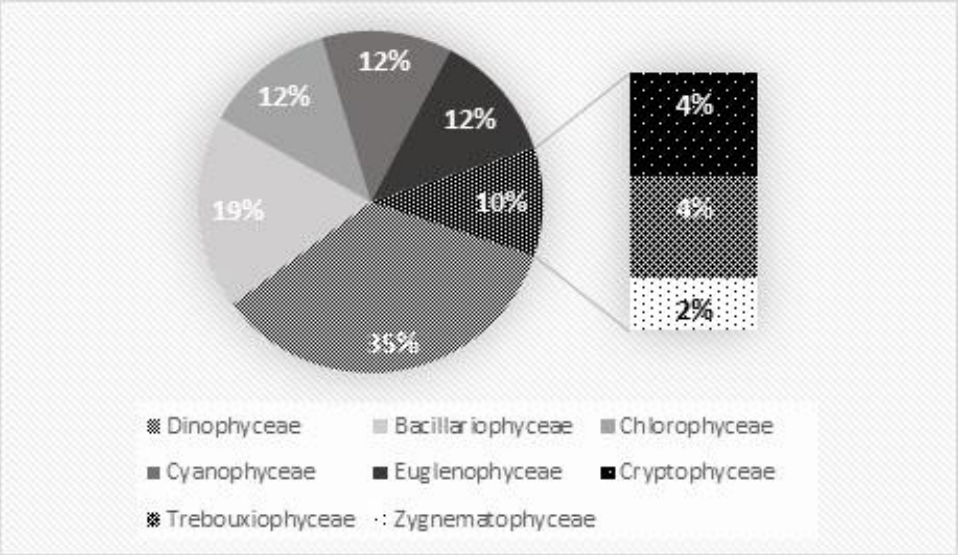

Fig. 2. Taxonomic composition (%) of the phytoplankton in Pomorie Lake by class in 2017.

In October, 22 species from 6 classes were found. We recorded a reduction in the numbers of peridinea and diatom species and an increase in green algae (Table 2, Fig. 3). The number of species in the different sampling sites varied from 13 to 16 species. This shows that the number of species in the study of this basin has slightly increased compared to the numbers (5-13 species) that was reported by Stoyneva (2010).

The Dinophyceae class constitutes the largest share of the taxonomic composition in the spring (55%, April). The share of peridinea species decreases toward the end of the year. In the autumn (October), the proportion of species of class Euglenophyceae increases, probably related to the accumulation of the organic matter in the water (as the Euglenophyceae are indicators of organic pollution according to Petrova & Gerdzhikov (2010). The classes Dinophyceae (7 species), Bacillariophyceae (6 species) and Cyanophyceae (4 species) were presented with the highest number of species during this warm month. The classes Chlorophyceae and Cryptophyceae were also increasing their share. The remaining groups of microalgae were registered with a small number of species (Table 2, Fig. 3). Species of Cyanophyceae are the most abundant in the summer and spring while the diatoms (Bacillariophyceae) - in the summer and autumn 2017 (Fig. 3).The total monthly average number of phytoplankton species had not changed significantly and had been registered between 20 and 23 phytoplankton species.

According to Stoyneva (2010) in 2007 various green and cryptophytic flagellates had a leading role in the composition of phytoplankton. Diatoms were also present.

The established taxonomic structure of phytoplankton in Pomorie Lake is comparable to that in marine coastal waters, characterized

by the highest shares of the classes Dinophyceae and Bacillariophyceae (Petrova & Gerdzhikov, 2015). Perhaps the reason is in the decreasing salinity of the lake - from 52-55‰ (2007) (Hiebaum, 2010) to 19-19.9‰ (Popov, 2015, Burgas, pers.comm.) According to Rozhdestvenski (1986) the average salinity Black Sea coastal water is 16.47‰.

Quantitative dynamics of phytoplankton

A total of 6 phytoplankton species in „bloom" concentrations were recorded in Pomorie Lake. Although there are a lot of uncertainties of what constitutes a bloom (Smayda, 1997) usually concentrations exceeding > 1 x 10^6 cells.l^{-1} for a single species or > 5 x 10^6 cells.l^{-1} for 2-3 species or biomass > 10000 mg.m^{-3} are considered "bloom" (Moncheva & Parr, 2010). In the spring, the green algae *Chlorella pyrenoidosa* bloomed with the highest abundance - 41.06 x 10^6 cells.l^{-1}. High concentration of species from Cyanophyceae (2 µm) - 1.45 x 10^6 cells.l^{-1} was observed in the summer. Most "blooming" species (4) were found during the autumn period.

Phytoplankton "blooming" was recorded at all sites in the autumn, with the highest values occurring at the "Site 3": small Flagellates - 12.79 x 10^6 cells.l^{-1}; *Gymnodinium albulum* - 2.69 x 10^6 cells.l^{-1} and *Gymnodinium sp.* - 1.35 x 10^6 cells.l^{-1}. At the "Site 5", the blue-green *Oscillatoria sp.* 1.56 x 10^6 cells.l^{-1} was also "blooming". The distribution of algal groups by biomass was uneven between sampling sites. In October 2017, within three days and at three different stations phytoplankton values which reflected the current state of the cenosis were measured. These parameters were the result of regular ecological modulations formed under the influence of temporary or permanent factors. According to Uzunov & Kovachev (2002) biomass is always an instantaneous, residual, resultant value.The highest phytoplankton quantities were detected during the spring (Figure 4), then a second peak was recorded during the autumn. In the summer very low values of phytoplankton development were registered.

High values of the Shannon Index in Pomorie Lake - suggesting a "very good" ecological status of the lake water according to BQE (Biological Quality Element) Phytoplankton. The spring and autumn phytoplankton "blooms" present worse ecological status during these seasons. According to the average phytoplankton biomass levels established in the present study (Table 3) and the ecological assessment criteria for "mesotrophic" lake types (Surface water characterization, 2013) the ecological status of Pomorie Lake in 2017 is between "moderate" and "good".

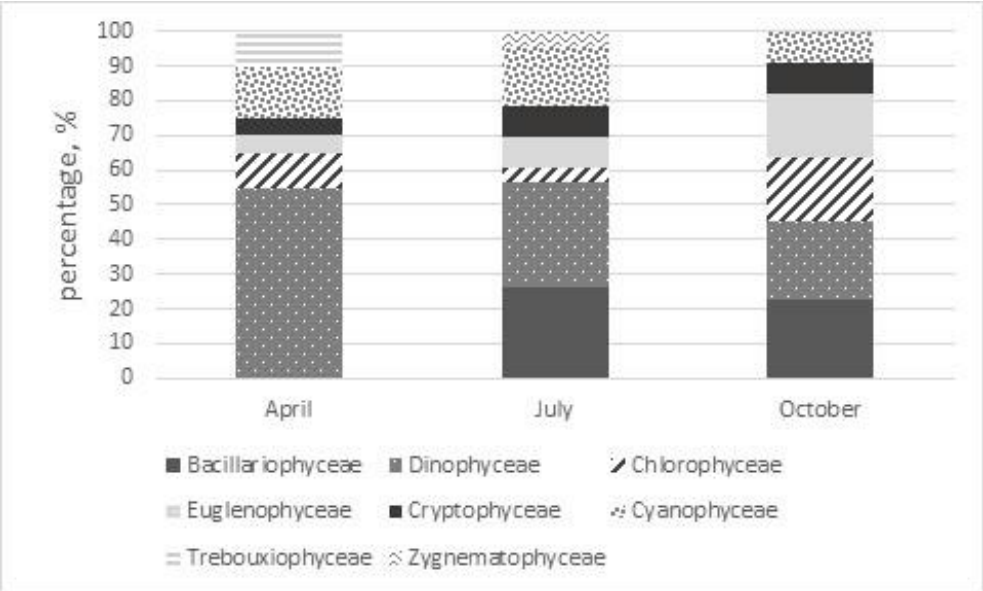

Fig. 3. Seasonal dynamics of the phytoplankton taxonomic composition.

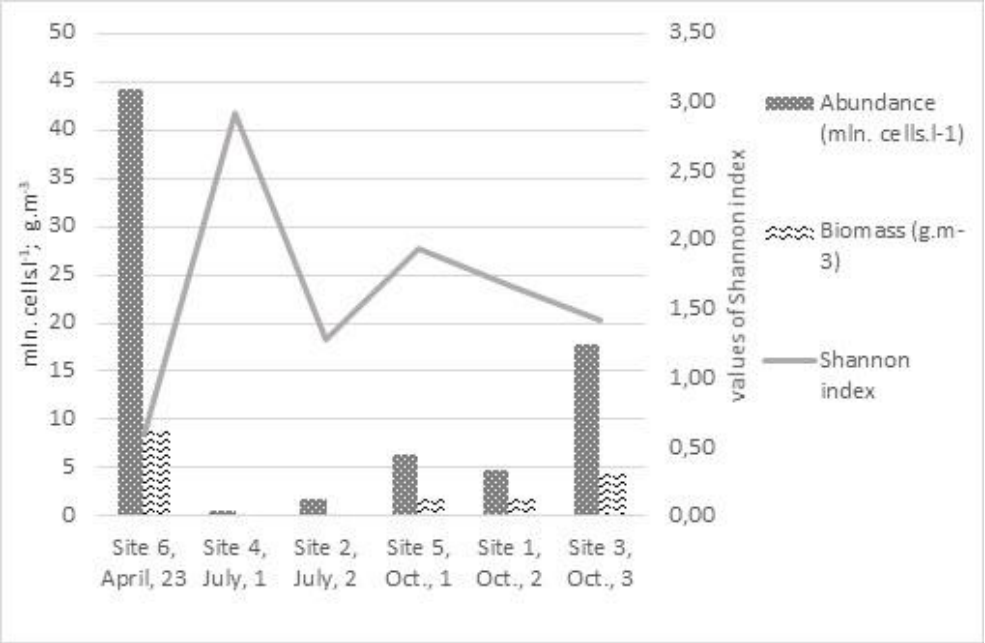

Fig. 4. Phytoplankton abundance (x 10^{-6} cells.l^{-1}), biomass (g.m^{-3}) and Shannon index, Pomorie Lake, values from all sampling sites.

Table 3. Seasonal averages of phytoplankton abundance, biomass and Shannon's diversity index from Pomorie Lake.

Season	Abundance (x 10^6cells.l-1)	Biomass (g.m^{-3})	Shannon index
Spring	44.20	8.94	0.59
Summer	1.18	0.43	2.10
Autumn	9.72	2.86	1.68
Average	18.37	4.07	1.46

Compared to Atanasovsko Lake the number of species is smaller than that of Pomorie Lake (Stoyneva, 2010), but the main groups forming the phytoplankton in the two wetlands are similar. In Black Sea coastal lakes such as Atanasovsko and Vaya (Burgas Lake), the Cyanoprokaryotes have a significantly higher qualitative and quantitative participation in the phytoplankton according to the results of Belkinova et al. (2020) and Dimitrova et al. (2014). The resulted data on the abundance (1.18 x 10^{-6} cells.l^{-1}) during summer is similar to those reported by the authors in Atanasovsko Lake during 2007 (1.15 x 10^{-6} cells.l^{-1}).

These comparisons can only be preliminary since both the studied periods and the number of analyzed samples differ.

Conclusions

The present study was an attempt to supplement the observations on the ecology of Pomorie Lake, as well as to make a qualitative and quantitative assessment of the phytoplankton. This study analyzed the current taxonomic structure and distribution of phytoplankton in 2017.

As a result of analyzed current taxonomic structure and distribution were established 49 species belonging to 8 classes. Dynophyceae is the dominant family, whereas Cyanophycea is species poorer. A probable reason for that state was the entry of Black Sea water into Pomorie Lake.

Were registered six species with "blooming" concentrations. The spring was characterized by the highest development of phytoplankton in the lake, while summer had the lowest values.

According to some of the criteria for ecological assessment and the established biomass of phytoplankton, the ecological condition of Pomorie Lake was between "moderate" and "good".

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