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Seasonal Variation of Epipelic Diatom Assemblages and Their Relationships with Abiotic Variables in the Utility and Drinking Water Source of İstanbul: Büyükçekmece Reservoir

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Abstract

In this study, the distribution and composition of epipelic diatoms in Büyükçekmece Reservoir, which is one of the most important and main freshwater resources for İstanbul, Türkiye were investigated seasonally. Epipelic diatom samples were collected from five different stations each season (February, May, August, and November) in 2019. A total number of eighty-seven taxa belonging to thirty-seven genera were identified, six of which were centric and eighty-one were pennate; five of the epipelic species were new record diatoms for the Büyükçekmece Reservoir. The highest number of epipelic diatom species were encountered in February while the lowest was in November. Considering the frustule counts, it was observed that the species that increased their relative abundance changed in each sampling period. Consequently, it was observed that *Gomphonella olivacea* was the most abundant in February, *Navicula reichardtiana* in May, *Achnanthidium* sp. in August, and *Cocconeis placentula* in November. Shannon-Weaver Diversity Index showed that the highest diversity of diatom species was determined in February (station 4), and the lowest was observed in August (station 3). According to the results of redundancy analysis, it was observed that silicate-Si, conductivity, and temperature were the most efficient variables in the distribution of the epipelic diatom species.

Introduction

Diatoms, microscopic single-celled algae distributed generally in freshwater and marine ecosystems, are characterized by having a unique silica cell wall and are considered strongly tolerant species against ecological changes (Sládeček, 1986; Round et al., 1990). Diatom classification is primarily based on the shape and structure of their siliceous frustules, which can be observed under a light microscope. This helps identify the genera and species (Snyder et al., 2013). Their extensive presence in nearly all aquatic ecosystems and shorter life cycles compared to macroinvertebrates (fish, macroalgae, macrophyte) make them useful indicators for biomonitoring (Van De Vijver et al., 2003; Bennion et al., 2010; Stevenson et al., 2010). Moreover, particular diatom species and/or communities can lead us to conclude about the past, present, and future of any freshwater resource (Meriläinen et al., 1982). One of the ecological attributes of benthic diatoms is that they can exist in various substrates. Epipelic diatoms are essential primary producers and key food sources for benthic life in aquatic ecosystems (Daume et al., 1999; Beltrones &

Romero, 2001) and they also effectively contribute to carbon cycling (Hecky & Hesslein, 1995; Barranguet, 1997). The composition and diversity of the epipelic diatoms are affected differently by changes in the physico-chemical variables such as temperature, salinity, dissolved oxygen (DO), conductivity, and concentration of nutrients in the water (Ács et al., 2004). Büyükçekmece Reservoir, which used to be connected to the Sea of Marmara, is very crucial in terms of providing drinking and utility water to İstanbul, Türkiye, and studies on benthic diatoms in this freshwater resource date back decades. Studies on epipelic diatoms in Türkiye have been conducted, focusing on various aspects such as taxonomy (Sahin, 2003), ecological observations, water quality indices, and bio-monitoring (Sivaci et al., 2007; Mustafa Kargıoğlu et al., 2012; Kivrak & Uygun, 2012; Tokatlı et al., 2020), as well as the cellular composition of selected diatoms (Tokatlı et al., 2011). A significant amount of diatom research has also been conducted in the Büyükçekmece Reservoir (Balkıs, 2003; Gülecal & Temel, 2014; Yılmaz, 2019; Aykut et al., 2021; Yılmaz et al., 2022; Aykut et al., 2024). The sampling and analysis of epipelic diatoms for this study were conducted concurrently with those of epiphytic (Aykut et al., 2019) and epilithic (Aykut et al., 2024) diatoms to compare species richness across these different substrates. The primary objective of this study was to determine the recent composition, variations, and distribution of epipelic diatom species in the Büyükçekmece Reservoir. Additionally, the study aimed to explore the relationships between epipelic diatom assemblages and environmental factors.

Materials and Methods

Study Area

Büyükçekmece Reservoir, located at approximately 41.0466° N latitude and 28.4720° E longitude on the European side of Istanbul, is the city's second largest drinking water reservoir, serving as a critical source of drinking water and a recreational area. However, the lake faces significant challenges due to anthropogenic pollution stemming from multiple sources. Agricultural activities around the basin, including the use of pesticides and fertilisers, contribute substantial nutrient loads to the lake through runoff, particularly via influent streams such as Karasu, İzzettin, Eskice, Ahlat, Beylikçayı, Çekmece, Çakmaklı and Tahtaköprü (Yılmaz, 2019; Yılmaz et al., 2022). In addition, the pollution load is expected to increase further with the urbanisation and industrial developments in the basin (Yılmaz et al., 2022). Despite efforts to monitor and manage water quality, including projects to divert and treat wastewater (Tezcanli-Guyer & Ilhan, 2011), the lake exhibits signs of eutrophication, driven by elevated nutrient concentrations and periodic algal blooms (Yılmaz, 2019; Yılmaz et al., 2022) (Figure 1).

Collecting Epipelic Diatom Samples

The epipelic diatom samples were collected from five stations in 2019 seasonally to determine the seasonal variations of epipelic diatom species in Büyükçekmece Reservoir. To collect the epipelic diatom species, the samples were taken from shores with the help of a plastic spoon into 100 ml sampling containers (Marcoglise et al., 2015). The samples brought to the laboratory immediately after the fieldwork and were stored in a refrigerator set at +4°C. The epipelic diatom assemblages were collected from five distinct shoreline stations around the Büyükçekmece Reservoir, with these locations selected for their varying characteristics. Station 1 is located near the confluence of Menekşe Stream and close to the Büyükçekmece Biological Treatment Plant, while Station 2 is situated to the south of the reservoir, near the dam, where it is separated from the Sea of Marmara. Station 3 is located near the Büyükçekmece Water Treatment Plant, where water is captured for human consumption. Station 4 is situated in an area most impacted by agricultural activities. While there is a small agricultural area around Station 5, the region is primarily influenced by animal husbandry. Additionally, Station 5 is located near Karasu Creek, which provides the majority of nutrient input to the reservoir.

Pre-treatment of Diatom Samples, Preparations of the Permanent Slides, and Identification

To create a permanent diatom slide and ensure clear identification, it is necessary to remove the internal organelles of the cell. Combinations of hydrochloric acid (HCl) and hydrogen peroxide (H_2O_2) were used to achieve this. At the end of the process, diatom frustules were suitable for determination (Hendey, 1974; Battarbee et al., 2001). Naphrax diatom mountant was used for the preparation of the permanent slides and these slides were examined under an "Olympus BX51" light microscope at 100× magnification. Hustedt (1930), Patrick & Reimer (1966), Patrick & Reimer (1975), Krammer & Lange-Bertalot (1988), Round et al. (1990), and Krammer & Lange-Bertalot (1991a, b) were used for identification of the species. Three hundred diatom frustules were randomly counted under the microscope to reveal the relative abundance of the species per sample.

Statistical Analysis of the Biotic and Abiotic Variables

Spearman's rank correlation method was used to show the associations of epipelic diatom species with abiotic parameters (Siegel, 1956). Shannon-Weaver Diversity Index (Shannon & Weaver, 1949) was applied to determine the species diversity at stations and seasons, Pielou's Evenness Index (Pielou, 1964) was used to determine whether these species are regularly distributed, and Simpson's Dominance Index (Magurran,

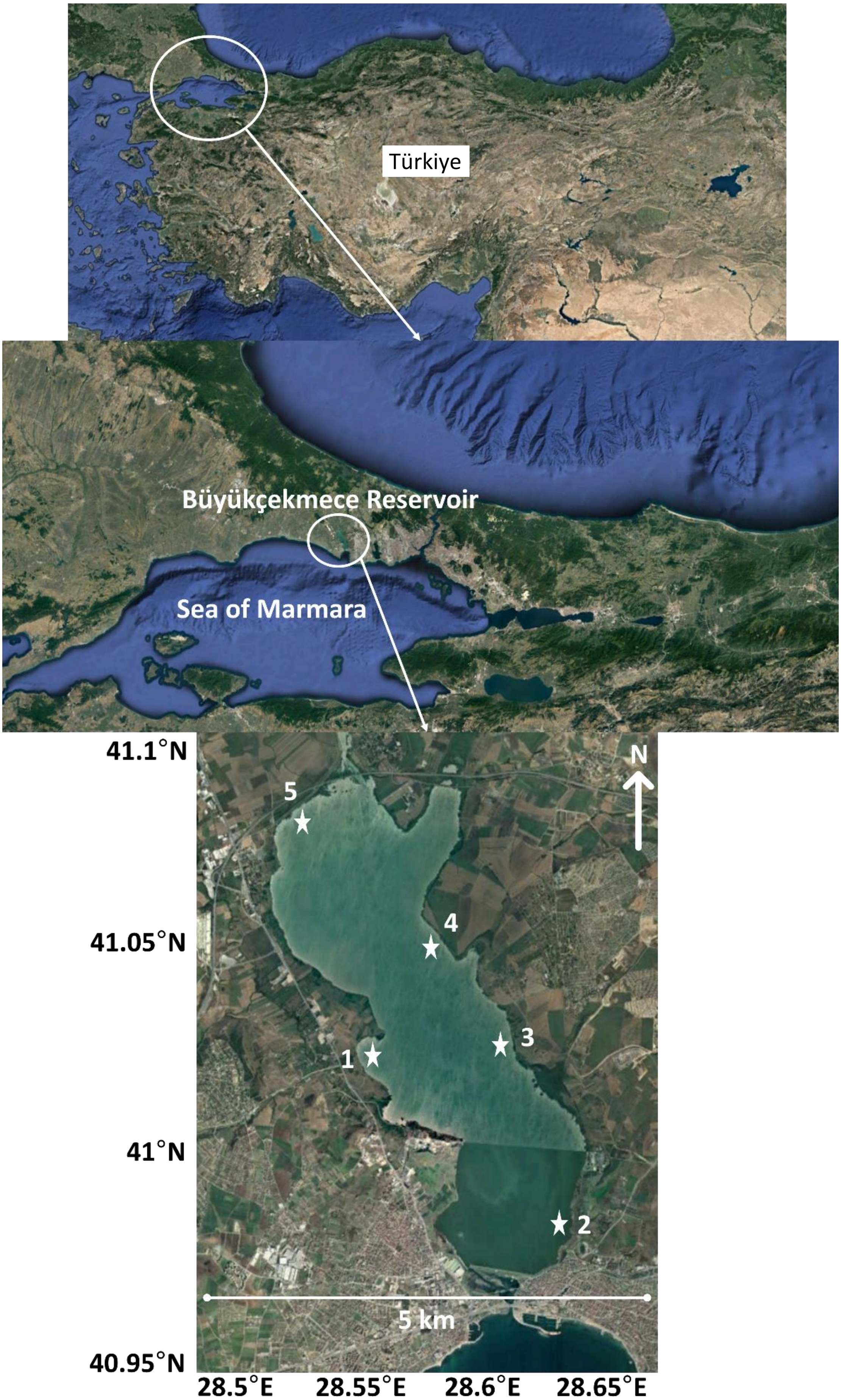

Figure 1. Research area and the coordinates in the Büyükçekmece Reservoir.

1988) was used both to both confirm Shannon-Weaver Diversity Index and to determine the dominant species. Bray-Curtis Cluster analysis was applied to see the similarity of stations based on biotic structure (epipelic diatom species) and their relative abundance. In terms of ecological variables, Euclidean Distance (ED) was applied to analyze the differences of seasons (Clarke & Warwick, 2001). Frequency analysis was used during the study to declare the percentage of the presence of epipelic diatoms in the stations. According to this analysis, the groups defined as follows: Rarely found species (between 1 and 20%), Seldomly found species (between 21 and 40%), Commonly found species (between 41 and 60%), Frequently found species (between 61 and 80%), and Continuously found species (between 81 and 100%) (Kocataş, 1996). Shannon-Weaver Diversity, Pielou's Evenness Index, Simpson's Dominance, Bray-Curtis Cluster analysis, and Euclidean Distance were completed in Primer v.6 software program and Spearman's rank correlation method was analyzed in SPSS v.21 software. The ordination analysis was used to determine the relationship webs between abiotic parameters and the relative abundance data of epipelic diatom species. Before this method was preferred, firstly, the detrended corresponding analysis (DCA) specified by Lepš & Šmilauer (2003) was tested using the PC-ORD 6.0 program (McCune & Meffort, 2011). While choosing the ordination analysis, as a result of DCA, the highest gradient length in the axes was taken into account, and the linear ordination method, Redundancy Analysis (RDA), was approved accordingly. The relative abundance data of diatoms were analyzed by including species with an incidence between "10% and 90%" at all sampling periods and stations (Lepš & Šmilauer, 2003). Monte Carlo permutation test was used to observe which analytical aspects necessarily drive the distribution of epipelic diatom composition and the ordination diagram was drawn in the PAST v.4.03 program (Hammer et al., 2001).

Results

Abiotic Parameters

Some of the physico-chemical parameters used in this study, such as water temperature, salinity, dissolved oxygen (DO), pH, and conductivity, were taken from Aykut et al. (2021), while others, including BOD_5 (biological oxygen demand), TDS (total dissolved solids), TSS (total suspended solids), NO_3+NO_2-N, NH_4-N, TN (total nitrogen), PO_4-P, SiO_4-Si, and TP (total phosphate), were sourced from Aykut et al. (2024). All of these environmental factors were measured simultaneously during the collection of epipelic diatoms. According to Aykut et al. (2021) and Aykut et al. (2024), water temperature was highest in August and lowest in February, although these values varied across different stations. Salinity was highest in November at all stations. Additionally, salinity values at station 3 were the highest in all seasons, except for November, when station 5 recorded the highest values. DO values were highest in February, particularly at station 5, which recorded the lowest DO values in November and August. A peak in DO values was observed at station 2 in May. pH values were generally lowest in November. However, both the minimum (station 3) and maximum (station 5) pH values were recorded in February. Conductivity values showed a marked contrast, with the lowest recorded in February and the highest in November. BOD5 values exhibited considerable variation across both seasons and stations, with the highest values typically recorded in February. TDS and TSS values also showed great variation but the highest values were calculated in November for both. Conversely, the lowest TDS values were observed in August, while the lowest TSS values were recorded in May. For NO_3+NO_2-N, PO_4 -P, TN, and TP, the highest values were typically observed in February and May, while the lowest were recorded in August and November. NH_4-N values showed considerable variation, with the highest recorded in May at Station 2. Lastly, SiO_4-Si values were highest in February and gradually decreased throughout the year.

Composition and Disturbution of Epipelic Diatoms, and Relative Abundance Rates

In this study conducted in Büyükçekmece Reservoir seasonally, 87 epipelic diatom taxa were identified, eight of which were genus level (Figure 2a-2c). Six of the species (6.90%) were centric while 81 (93.10%) taxa were pennate diatoms (Table 1). Five of these species are new records (*) for the reservoir. Most of the taxa in the study belonged to *Navicula* (7 taxa) and *Nitzschia* (7 taxa) followed by *Fragilaria* (6 taxa), and *Gomphonema* (6 taxa). The highest number of species was found in February (52 species) while the lowest number of species was obtained in both May and August (47 taxa) equally. In addition, there were no centric diatoms detected in May and the highest number of centric diatoms identified in February (5 taxa) while there were only one in August and two in November. Moreover, there were 50 epipelic diatoms observed in November. In February, the highest number of species was observed in station 3 (32 taxa) and the lowest number of species in station 2 (19 taxa) and in this sampling period *Achnanthidium* sp., *Cocconeis placentula*, *Cymbella affinis*, *Encyonopsis minuta*, *Gomphonella olivacea*, and *Gyrosigma kuetzingii* were observed in all stations (100%). When evaluated in terms of relative abundance, *G. olivacea* (>24.5%) at stations 1, 2, and 3, *Fragilaria* sp.2 (9.0%) at station 4, and *G. wormleyi* (15.3%) at station 5 were noted as dominant. *Pantocsekiella ocellata*, *Stephanodiscus balatonis*, *Stephanodiscus minutulus*, *Stephanodiscus neoastraea*, *Cocconeis scutellum*, *Cymbella cantonatii*, *Cymbella neocistula*, *Frustulia vulgaris*, *Hantzschia amphioxys*, *Nitzschia* cf. *frustulum*, *Nitzschia intermedia*, *Surirella angusta*, *Surirella minuta*, *Tryblionella angustata*, and

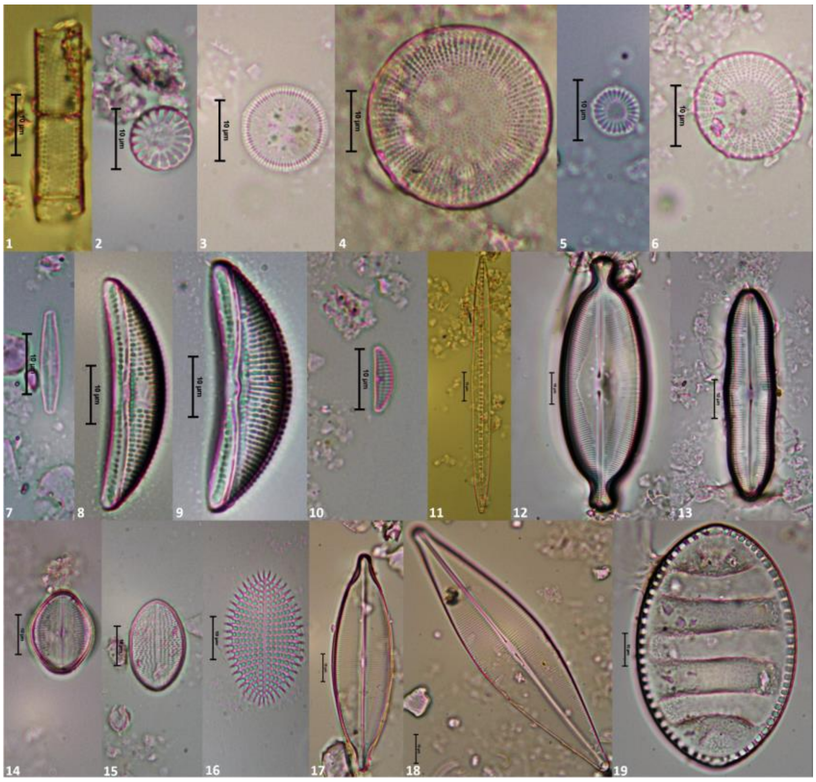

Figure 2a. Epipelic diatoms in Büyükçekmece Reservoir: 1) *Aulacoseira granulata*, 2) *Cyclotella meneghiniana*a, 3) *Pantocsekiella ocellata*, 4) Stephanodiscus balatonis, 5*) S. minutulus*, 6) *S. neoastraea*, 7) *Achnanthidium* sp., 8) *Amphora copulata*, 9) *A. ovalis*, 10) *A. pediculus*, 11) *Bacillaria paxillifera*, 12) *Caloneis amphisbaena*, 13) *C. silicula*, 14) *Cocconeis pediculus*, 15) *C. placentula*, 16) *C. scutellum*, 17) *Craticula* cf. *ambigua*, 18) *Craticula cuspidata*, 19) *Cymatopleura elliptica*.

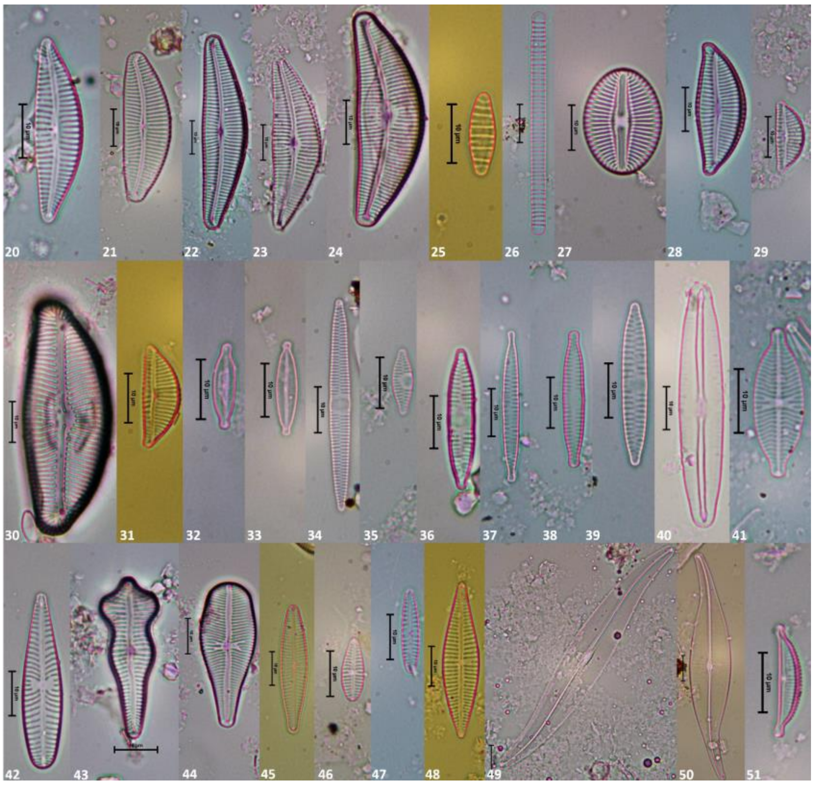

Figure 2b. Epipelic diatoms in Büyükçekmece Reservoir: 20) Cymbella affinis, 21) *C. cantonatii*, 22) *C. lange-bertalotii*, 23) *C. neocistula*, 24) *C. tumida*, 25) *Diatoma mesodon*, 26) *D. tenuis*, 27) *Diploneis elliptica*, 28) *Encyonema auerswaldii*, 29) E. minutum, 30) *E. prostratum*, 31) *E. ventricosum*, 32) *Encyonopsis minuta*, 33) *E. subminuta*, 34) *Fragilaria* cf. *capucina*, 35) *F. perminuta*, 36) *F. vaucheriae*, 37) *Fragilaria* sp.1, 38) *Fragilaria* sp.2, 39) *Fragilaria* sp.3, 40) *Frustulia vulgaris*, 41) *Geissleria decussis*, 42) *Gomphonella olivacea*, 43) *Gomphonema acuminatum*, 44) *G. italicum*, 45) *G. micropus*, 46) *G. minuta*, 47) *G. pumilum*, 48) *Gomphonema* sp., 49) *Gyrosigma kuetzingii*, 50) *G. wormleyi*, 51) *Halamphora* sp.

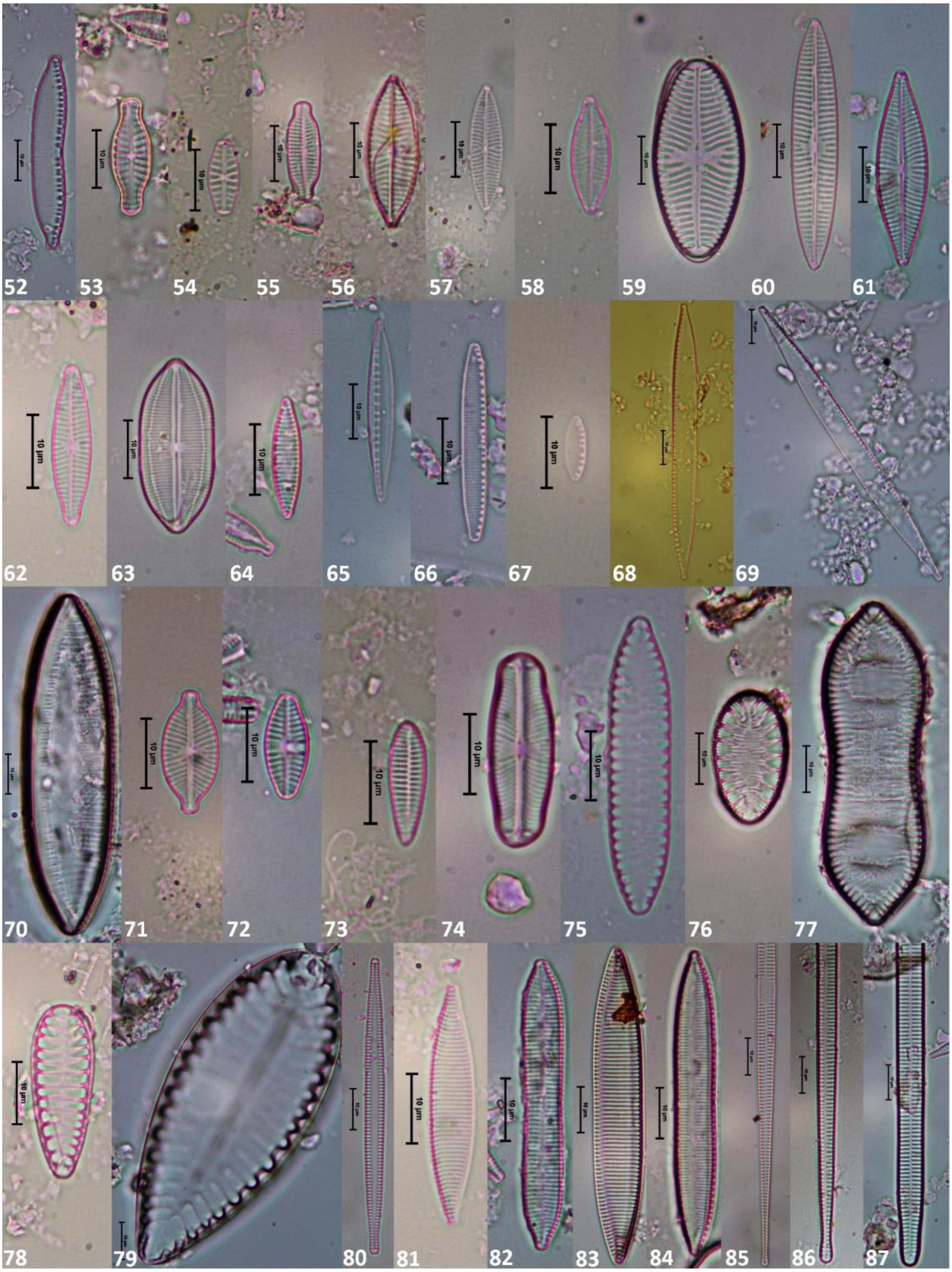

Figure 2c. Epipelic diatoms in Büyükçekmece Reservoir: 52) *Hantzschia amphioxys*, 53) *Hippodonta capitata*, 54) *H. hungarica*, 55) *Karayevia* cf. *ploenensis*, 56) *Navicula cryptotenella*, 57) *N. cryptotenelloides*, 58) *N. reichardtiana*, 59) N. reinhardtii, 60) *N. tripunctata*, 61) *N. trivialis*, 62) *N. veneta*, 63) *Neidium dubium*, 64) *Nitzschia amphibia*, 65) *N. dissipata*, 66) *N.* cf. *frustulum*, 67) *N. inconspicua*, 68) *N. intermedia*, 69) *N. recta*, 70) *N. tryblionella*, 71) *Placoneis* sp., 72) Planothidium frequentissimum, 73) *Rhoicosphenia abbreviata*, 74) *Sellaphora pupula*, 75) *Surirella angusta*, 76) *S. brebissonii*, 77) *S. librile*, 78) *S. minuta*, 79) *Surirella* sp. 80) *Tabularia fasciculata*, 81) *Tryblionella angustata*, 82) *T. apiculata*, 83) *T. brunoi*, 84) *T. hungarica*, 85) *Ulnaria acus*, 86) *U. biceps*, 87) *U. ulna*.

Table 1. The list of epipelic diatoms in Büyükçekmece Reservoir

RDA No	Taxa	February	May	August	November
	Centric diatoms				
1	*Aulacoseira granulata* (Ehrenberg) Simonsen, 1979			+	+
2	*Cyclotella meneghiniana* Kützing, 1844	+			+
	Pantocsekiella ocellata (Pantocsek) K.T.Kiss&Ács, 2016	+			
	**Stephanodiscus balatonis* Pantocsek, 1901	+			
	**Stephanodiscus minutulus* (Kützing) Cleve&Möller, 1882	+			
	**Stephanodiscus neoastraea* Håkansson&Hickel, 1986	+			
	Pennate diatoms				
	Achnanthidium sp.	+	+	+	+
3	*Amphora copulata* (Kützing) Schoeman&R.E.M.Archibald, 1986	+	+	+	+
4	*Amphora ovalis* (Kützing) Kützing, 1844	+		+	+
5	*Amphora pediculus* (Kützing) Grunow, 1875	+	+	+	+
	Bacillaria paxillifera (O.F.Müller) T.Marsson, 1901			+	+
	Caloneis amphisbaena (Bory) Cleve, 1894		+		
	Caloneis silicula (Ehrenberg) Cleve, 1894			+	
6	*Cocconeis pediculus* Ehrenberg, 1838	+	+		+
	Cocconeis placentula Ehrenberg, 1838	+	+	+	+
	Cocconeis scutellum Ehrenberg, 1838	+			
	Craticula cf. *ambigua* (Ehrenberg) D.G.Mann, 1990				+
	Craticula cuspidata (Kützing) D.G.Mann, 1990			+	+
7	*Cymatopleura elliptica* (Brébisson) W.Smith, 1851	+	+	+	+
	Cymbella affinis Kützing, 1844	+	+	+	+
	Cymbella cantonatii Lange-Bertalot, 2002	+			
8	*Cymbella lange-bertalotii* Krammer, 2002	+	+	+	+
	Cymbella neocistula Krammer, 2002	+			
	Cymbella tumida (Brébisson) Van Heurck, 1880	+		+	
	Diatoma mesodon (Ehrenberg) Kützing, 1844			+	+
	Diatoma tenuis C.Agardh, 1812		+		
9	*Diploneis elliptica* (Kützing) Cleve, 1894	+	+	+	+
10	*Encyonema auerswaldii* Rabenhorst, 1853	+	+	+	+
	Encyonema minutum (Hilse) D.G.Mann, 1990		+		
	Encyonema prostratum (Berkeley) Kützing, 1844			+	
11	*Encyonema ventricosum* (C.Agardh) Grunow, 1875		+	+	+
	Encyonopsis minuta Krammer&E.Reichardt, 1997	+	+	+	+
12	*Encyonopsis subminuta* Krammer&E.Reichardt, 1997	+	+	+	+
	Fragilaria cf. *capucina* Desmaziéres, 1830		+		
13	*Fragilaria perminuta* (Grunow) Lange-Bertalot, 2000	+	+	+	+
14	*Fragilaria vaucheriae* (Kützing) J.B.Petersen, 1938	+	+	+	+
	Fragilaria sp.1		+		
15	*Fragilaria* sp.2		+		
16	*Fragilaria* sp.3	+	+		+
	Frustulia vulgaris (Thwaites) De Toni, 1891	+			
17	*Geissleria decussis* (Østrup) Lange-Bertalot&Metzeltin, 1996		+	+	+
18	*Gomphonella olivacea* (Hornemann) Rabenhorst, 1853	+	+	+	+
	Gomphonema acuminatum Ehrenberg, 1832			+	
	Gomphonema italicum Kützing, 1844			+	
19	*Gomphonema micropus* Kützing, 1844		+	+	+
	Gomphonema minuta P.Fusey, 1953				+
20	*Gomphonema pumilum* (Grunow) E.Reichardt&Lange-Bertalot, 1991	+	+	+	+
	Gomphonema sp.				+
21	*Gyrosigma kuetzingii* (Grunow) Cleve, 1894	+	+	+	+
22	*Gyrosigma wormleyi* (M.B. Harrison) Elmore, 1921	+			+
	Halamphora sp.		+		
	Hantzschia amphioxys (Ehrenberg) Grunow, 1880	+			
	Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin&Witkowski, 1996		+		
23	*Hippodonta hungarica* (Grunow) Lange-Bertalot, Metzeltin&Witkowski, 1996		+		+
	**Karayevia* cf. *ploenensis* (Hustedt) Bukhtiyarova, 1999				+
24	*Navicula cryptotenella* Lange-Bertalot, 1985		+	+	+
25	*Navicula cryptotenelloides* Lange-Bertalot, 1993		+	+	+
	Navicula reichardtiana Lange-Bertalot, 1989	+	+	+	+
26	*Navicula reinhardtii* (Grunow) Grunow, 1880		+		+
27	*Navicula tripunctata* (O.F.Müller) Bory, 1822	+	+	+	+
28	*Navicula trivialis* Lange-Bertalot, 1980	+	+	+	+
29	*Navicula veneta* Kützing, 1844	+	+	+	+
30	*Neidium dubium* (Ehrenberg) Cleve, 1894	+	+		
31	*Nitzschia amphibia* Grunow, 1862	+	+	+	+

Table 1. ***Continued***

RDA No	Taxa Pennate diatoms	February	May	August	November
32	*Nitzschia dissipata* (Kützing) Rabenhorst, 1860	+	+	+	+
	Nitzschia cf. *frustulum* (Kützing) Grunow, 1880	+			
33	*Nitzschia inconspicua* Grunow, 1862	+			+
34	*Nitzschia intermedia* Hantzsch *in* Cleve&Grunow, 1880	+			
35	*Nitzschia recta* Hantzsch *in* Rabenhorst, 1862	+	+	+	+
	**Nitzschia tryblionella Hantzsch, 1860*			+	
36	*Placoneis* sp.				+
	Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot, 1999			+	+
	Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot, 1980			+	
	Sellaphora pupula (Kützing) Mereschkovsky, 1902			+	
37	*Surirella angusta* Kützing, 1844	+			
38	*Surirella brebissonii* Krammer&Lange-Bertalot, 1987	+	+	+	+
39	*Surirella librile* (Ehrenberg) Ehrenberg, 1845	+	+	+	+
	Surirella minuta Brébisson *in* Kützing, 1849	+			
	Surirella sp.				+
	Tabularia fasciculata (C.Agardh) D.M.Williams&Round, 1986		+		
	Tryblionella angustata W.Smith, 1853	+			
40	*Tryblionella apiculata* W.Gregory, 1857	+	+		+
	Tryblionella brunoi (Lange-Bertalot) Cantonati&Lange-Bertalot, 2017	+			
41	*Tryblionella hungarica* (Grunow) Frenguelli, 1942	+		+	
	Ulnaria acus (Kützing) Aboal, 2003		+	+	
42	*Ulnaria biceps* (Kützing) Compère, 2001	+			
43	*Ulnaria ulna* (Nitzsch) Compère, 2001	+	+	+	
	Total Number of Species in Seasons	**52**	**47**	**47**	**50**
	Total Number of Species	**87**			

Ulnaria biceps were identified only in this sampling period in all study and all of their relative abundances were determined to be low (Figure 3a). When the number of species obtained at stations was evaluated, the highest number of species was obtained from station 3 (28 taxa), and the lowest number of species was obtained from station 1 (21 taxa) in the May sampling. *Achnanthidium* sp., *Cymbella affinis*, *Cymbella langebertalotii*, *Encyonema ventricosum*, *Fragilaria* sp.2, *Gomphonella olivacea*, *Navicula reichardtiana*, *Navicula tripunctata*, and *Nitzschia dissipata* were identified in every station. When evaluated in terms of relative abundance, *N*. *reichardtiana* (>21.0%) at stations 1,2,3 and 5, *Achnanthidium* sp. (22.7%) at station 4 were noted as dominant. *Caloneis amphisbaena*, *Diatoma tenuis*, *Encyonema minutum*, *Fragilaria* cf. *capucina*, *Fragilaria* sp.1, *Halamphora* sp., *Hippodonta capitata*, and *Tabularia fasciculata* were only encountered in this sampling period throughout the whole study (Figure 3b). In August, the highest number of species was obtained from station 4 (30 taxa) and the lowest number of species from station 1 (10 taxa). *Achnanthidium* sp., *Cocconeis placentula*, *Cymbella affinis*, *Encyonopsis minuta*, *Encyonopsis subminuta*, and *Navicula reichardtiana* were detected in every station. In terms of the relative abundance of the diatom species, *Achnanthidium* sp. (>20.0%) at stations 1, 2, 3, and 5, *C*. *placetula* (31.3%) at station 4 were dominant. The relative abundance of *C*. *placentula* was also high in the other stations, but not in station 4. *Caloneis silicula*, *Encyonema prostratum*, *Gomphonema acuminatum*, *Gomphonema italicum*, *Nitzschia tryblionella*, *Rhaphoneis abbreviata*, and *Sellaphora pupula* were identified only in this sampling period (Figure 3c). In November, the highest number of species was obtained from station 3 (32 taxa) and the lowest number of species from station 1 (25 taxa). *Achnanthidium* sp., *Cocconeis placentula*, *Encyonema auerswaldii*, *Encyonopsis minuta*, *Fragilaria perminuta*, *Gyrosigma wormleyi*, *Nitzschia amphibia*, and *Nitzschia dissipata* were observed in every stations during this period. When we assessed species in terms of relative abundance, *Cocconeis placentula* (>37.0%) at stations 1,2,3, and 4, *Achnanthidium* sp. (40.0%) at station 5 were identified as dominant species. *Craticula* cf. *ambigua*, *Fragilaria* sp.3, *Gomphonema minuta*, *Gomphonema* sp.1, *Karayevia* cf. *ploenensis*, *Placoneis* sp., and *Surirella* sp. were only obtained in this season (Figure 3d). During the whole study, a total of 37 genera were identified, of which 4 belong to the Centrales and 33 belong to the Pennales. In Centrales, *Stephanodiscus* had the largest number of species (3), while only one species was reported from *Aulacoseira*, *Cyclotella*, and *Pantocsekiella*. In Pennales, the highest number of species (7) was determined as belonging to *Navicula, Nitzschia*. In addition, *Fragilaria* and *Gomphonema* were also found to have a high number of (6) species (Figure 4).

Statistical Assessment of Data

To reveal the relationship between the epipelic diatoms in the reservoir and ecological parameters, Spearman's rank correlation was performed (Table 2),

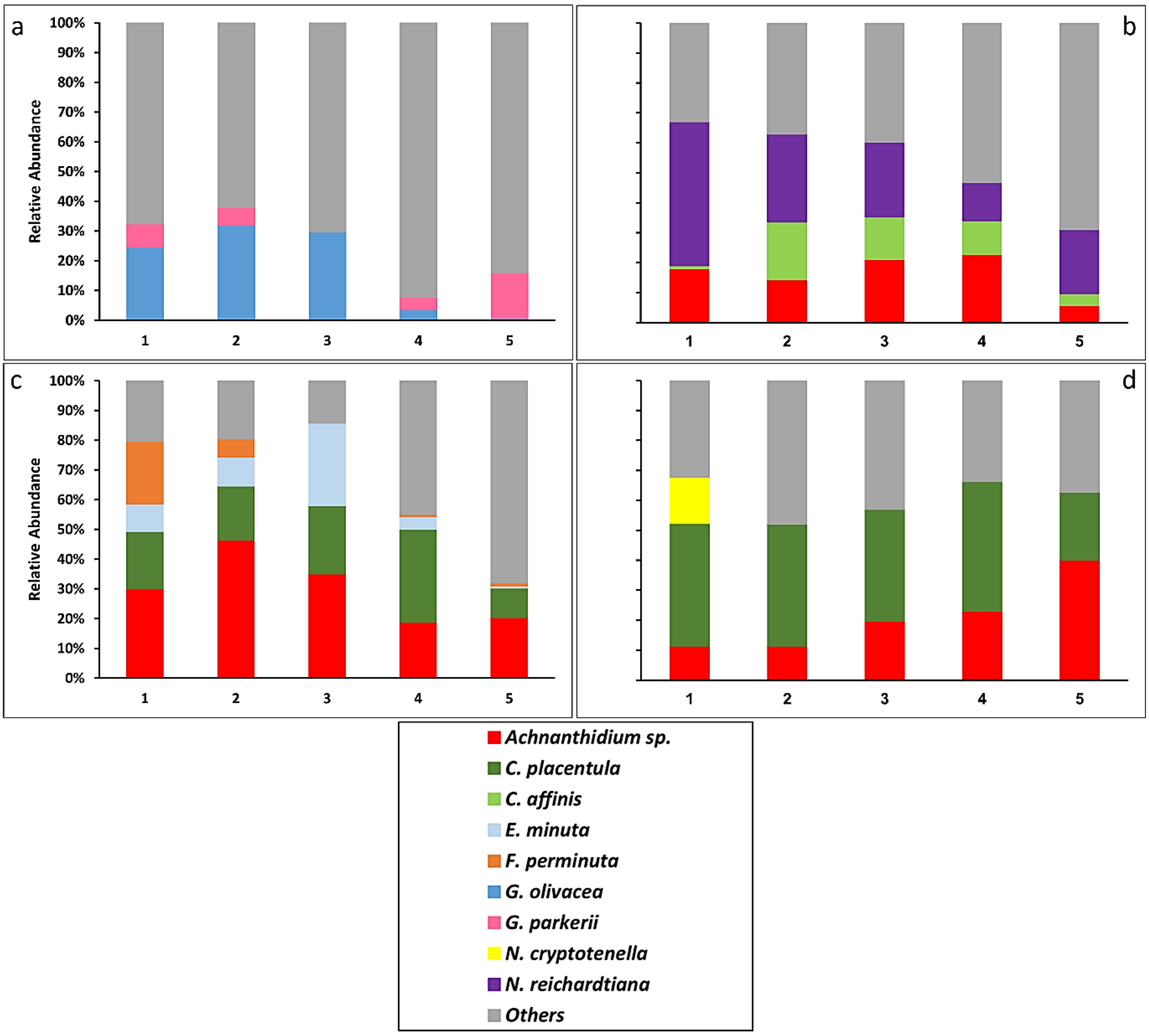

Figure 3. Distribution of species with relative abundance greater than "> 15" at stations according to sampling seasons. a) February, b) May, c) August, d) November.

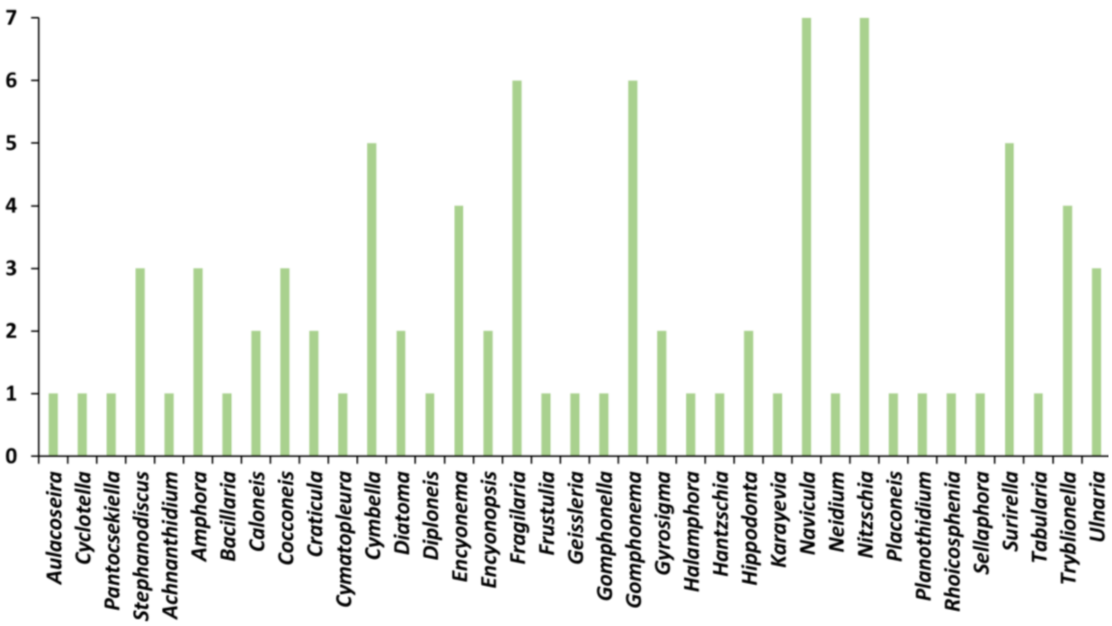

Figure 4. Number of species in every diatom genera collected from Büyükçekmece Reservoir (C: Centrales, P: Pennales).

but it was observed there was not any significant correlation between them. However, it was determined that epipelic diatoms had a slightly positive correlation with salinity, conductivity, and NO_3+NO_2-N while there were negative correlations with temperature, pH, NH_4-N, PO_4-P, SiO_4-Si, and TP. Moreover, neither a negative nor a positive correlation could be detected with DO and TN (Table 2). Frequency analysis was carried out to determine which species were present in what percentage of the stations. According to this, it was determined that the most species (50 taxa) belonged to "Rarely Found Species" (57%) and the least species (5 taxa) belonged to "Continuously Found Species" (6%). In addition, it was observed that there were six species (7%) belonged to "Frequently Found Species" while 13 species (15%) to both "Commonly Found Species" and "Seldomly Found Species". Additionally, every newly-recorded species belonged to "Rarely found species" (Figure 5). In order to determine the diversity of epipelic diatoms, the Shannon-Weaver *(H')* Diversity Index was applied based on the number of species and individuals in all sampling periods (Figure 6). According to this, it was determined that the index varied between 2.42 (August, station 3) and 4.51 (February, station 4). According to the Pielou's Evenness Index (*J'*), it was observed during the whole study, the lowest value (0.56) was determined at station 3 in August, and the highest value (0.91) was determined at station 4 in February. This was expected because these two indices must correspond to each other. Simpson's Dominance Index (*D'*) was applied to diagnose dominance and diversity in Büyükçekmece Reservoir. It was observed that the lowest value was determined at station 4 in February (0.04), and the highest value was determined at station 1 in May (0.27) (Figure 6). Bray-Curtis Cluster analysis was applied to obtain the similarities of the stations in terms of biotic variables studied in Büyükçekmece Reservoir. It was found that the highest similarity was detected between stations 4 and 5 (62.94%) and the lowest similarity was observed between stations 1 and 5 (54.61%). By using Euclidean Distance (ED) to determine the similarity between seasons based on ecological parameters, May and August were found to be the closest ones (ED=9.37). Also, it was observed that February separated from the other seasons (Figure 7). Ordination analyses were used to reveal the relationship between epipelic diatom species and ecological variables (Figure 8). Firstly, detrended correspondence analysis (DCA) was applied to the relative abundance of epipelic diatoms and environmental parameters. In the study, 43 epipelic diatom taxa were included in the ordination analyses with an observation rate between "10% and 90%". When DCA results were evaluated, it was decided to

Table 2. Spearman's rank correlation that shows relations between ecological parameters and epipelic diatoms (EPP: Epipelic diatoms, DO: Dissolved oxygen, NO_3+NO_2-N: Nitrite+Nitrate-N, NH_4-N: Ammonium-N, PO_4-P: Phosphate-P, SiO_4-Si: Silicate-Si, TN: Total nitrogen, TP: Total phosphorus).

Epipelic Diatoms	**Temperature**	**Salinity**	**Conductivity**	**Dissolved Oxygen**	**pH**
	-0.4	0.4	0.3	0	-0.3
	NO_3+NO_2-N	**NH_4-N**	**PO_4-P**	**SiO_4-Si**	**TN / TP**
	0.1	-0.3	-0.1	-0.1	0 / -0.1

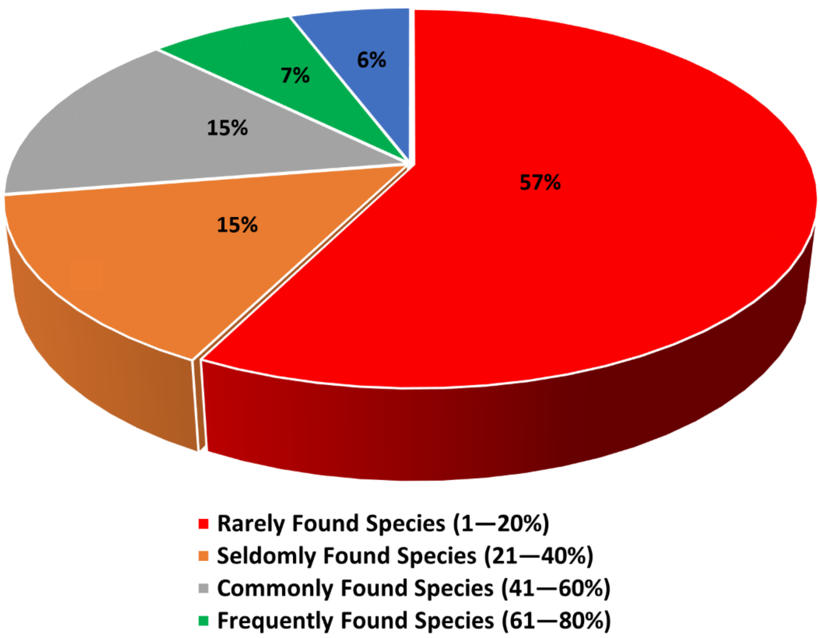

Figure 5. Percentage distribution of epipelic diatoms in Büyükçekmece Reservoir according to their frequency.

apply linear ordination analysis as the redundancy analysis (RDA), taking into account the longest gradient (Axis 1=2.983; Eigenvalue: 0.52214). A total of 47.7% of the cumulative variance in the species data was explained by the first two RDA axes, and the correlation between epipelic diatoms and ecological parameters was determined as 94.2%. The most effective explanatory factors were SiO4-Si (λ=-0.956), temperature (λ=0.745), and NO_3+NO_2-N (λ=-0.567), respectively, and these variables played an important role in the distribution of species ($P<0.05$). According to RDA, it was observed that the water temperature was negatively correlated with DO, pH, NO_3+NO_2-N, SiO_4-Si, and PO_4-P while it is positively correlated with conductivity to Axis 2 ($P<0.05$). While conductivity was negatively correlated with pH, DO, NO_3+NO_2-N, SiO_4-Si, and PO_4-P, it had a positive correlation between TDS and NH_4-N to axis 1 ($P<0.05$). Additionally, according to Axis 2, DO, pH, NO_3+NO_2-N, SiO_4-Si, and PO_4-P were correlated positively with each other ($P<0.05$). According to the RDA, some species in the study were not affected by ecological variables but, *Encyonema ventricosum* and *Encyonopsis subminuta* were found to be significantly affected by water temperature. It was observed that *Cymatopelura elliptica* and *Navicula veneta* increased their numbers, especially in the presence of NO_3+NO_2-N and their dominance decreased with the increase in water temperature. It was also determined that *Cocconeis pediculus* associated positively with SiO_4-Si, *Cyclotella meneghiniana* and *Nitzschia intermedia* with DO, *Nitzschia recta* with NH_4-N, *Amphora pediculus*, *Hippodonta hungarica* and *Navicula reinhardtii* with TDS, and lastly *Placoneis* sp. with conductivity. In addition, pH, DO, NO_3+NO_2-N, SiO_4-Si, and PO_4-P showed a positive correlation with each other, they showed a negative correlation only with temperature. TDS, conductivity, and NH_4-N had positive relations with each other (Figure 8).

Discussion

From past to present, there is only one study on the epipelic diatom flora of Büyükçekmece Reservoir. In this study (Temel, 2002), all epipelic diatom species were investigated and it was determined that diatoms (=Bacillariophyceae) were the most dominant group. According to Temel (2002), 44 of the 61 epipelic species identified belong to diatoms, and three of them were centric while 41 were pennate. The reason why there were almost twice as many species found in our study was the number of sampling stations, collecting frequency, and changes in the habitat over the years. A total of 11 identical species (*Amphora ovalis*, *Caloneis amphisbaena*, *Cocconeis placentula*, *Cyclotella*

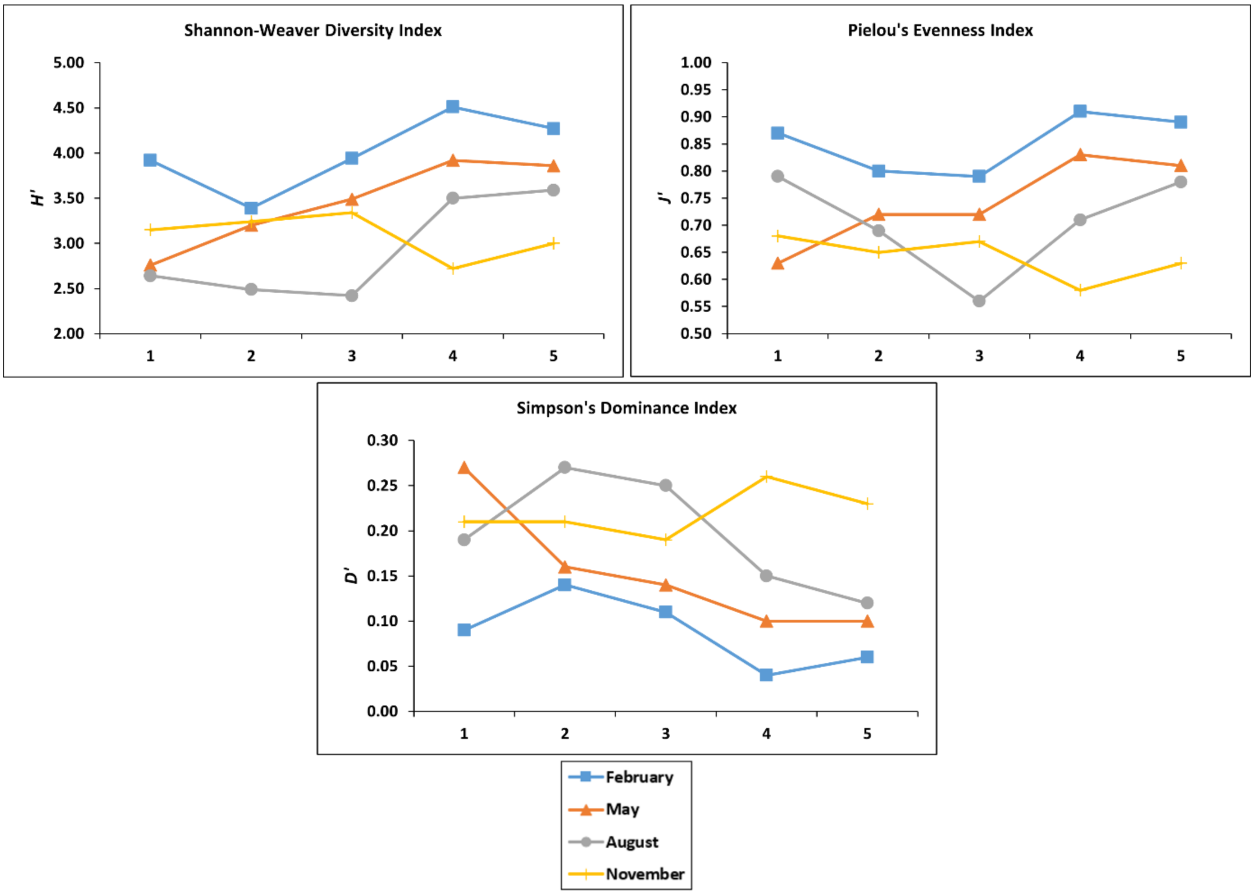

Figure 6. a) Shannon–Weaver Diversity, b) Pielou's Evenness and c) Simpson's Dominance indices in the sampling stations and periods.

meneghiniana, *Cymbella affinis*, *C. tumida*, *Frustulia vulgaris*, *Gomphonella olivacea*, *Hantzschia amphioxys*, *Surirella angusta*, and *S. librile*) were identified in both studies. Temel (2002) indicated that the *Nitzschia*, *Navicula*, and *Cymbella* as dominant, respectively. Similarly, in our study, this order was determined as *Navicula*, *Nitzschia*, and *Fragilaria*. In his study, Temel (2002) reported the habitat with the fewest species diversity as epipelic. On the contrary, according to the simultaneous epiphytic (Aykut et al., 2021) and epilithic (Aykut et al., 2024) diatom studies in the same region and the data of this study, the habitat with the highest species diversity found as epipelic with 87 species, while there were 66 species in the epiphytic and 76 species in the epilithic habitat. In addition, it is known that there are a total of 176 diatom species in the region with the

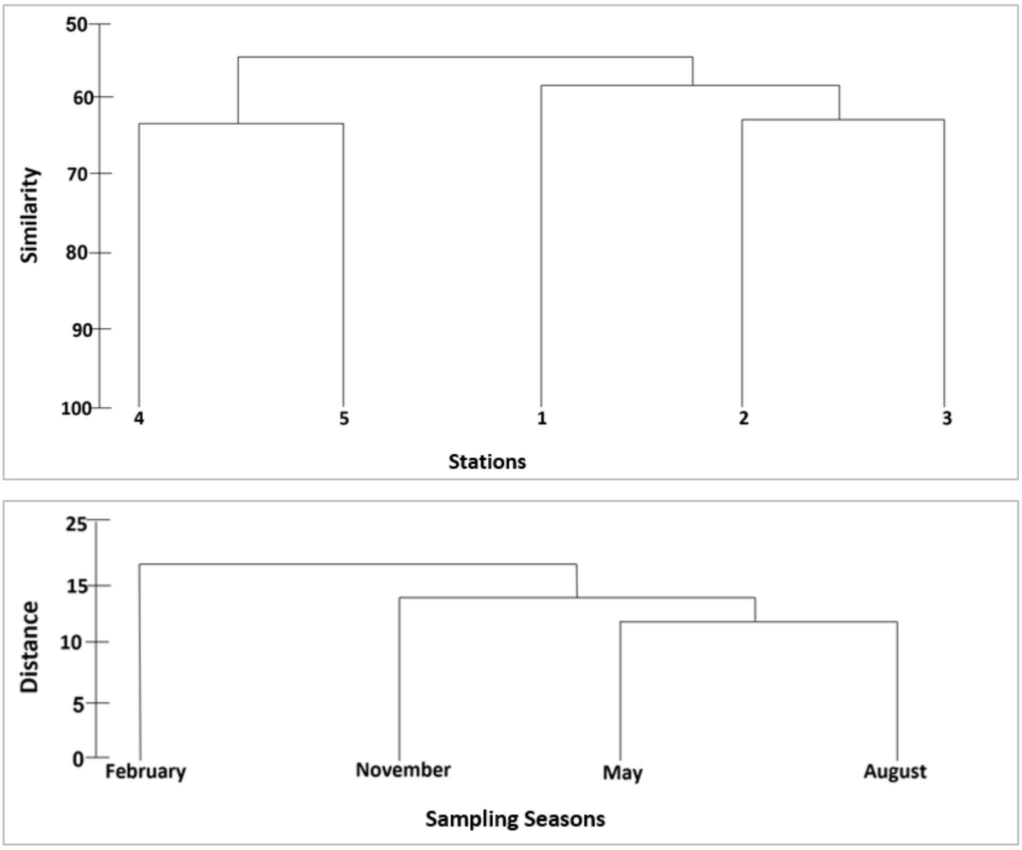

Figure 6. a) Shannon–Weaver Diversity, b) Pielou's Evenness and c) Simpson's Dominance indices in the sampling stations and periods.

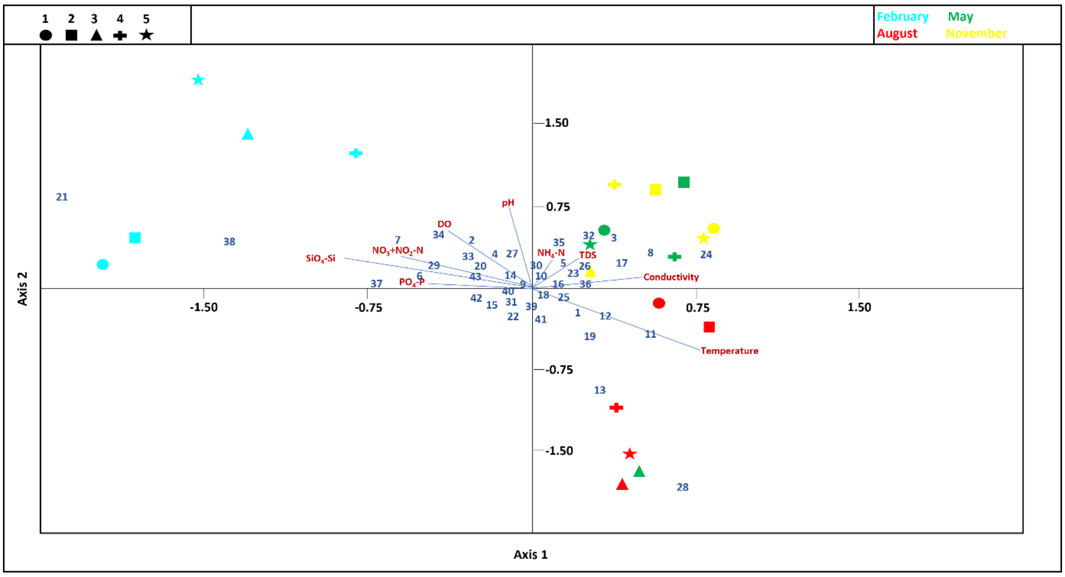

Figure 8. RDA ordination of epipelic diatom distribution along environmental, temporal, and spatial gradients: Species-Environment biplot (DO: Dissolved oxygen, NO_3+NO_2-N: Nitrite+Nitrate-N, NH_4-N: Ammonium-N, PO_4-P: Phosphate-P, SiO_4-Si: Silicate-Si, DO: Dissolved oxygen, TDS: Total dissolved solids, the numbers (RDA no) represent the species in Table 1).

studies on plankton (Balkis-Özdelice et al., 2020; Aykut et al., 2021; Aykut et al., 2024) in Büyükçekmece Reservoir. With this study, the total number of diatom taxa in the reservoir increased to 181, with five epipelic diatom species given as a new record for the reservoir. Additionally, according to studies (Aykut et al., 2021; Aykut et al., 2024), a strong positive correlation was determined between epiphytic and epilithic diatoms and conductivity. However, although this relationship is positive with epipelic diatoms, it wasn't considered as strong according to Spearman's rank correlation. Every station in Temel (2002)'s study, also corresponds to the stations where we collected our samples. Accordingly, in his study, he stated that he found *Nitszchia palea* (Kützing) W.Smith 1856 as a dominant species at station 1. However, thist species were not encountered in our study. In our study, *Achnanthidium* sp., *Cocconeis placentula*, and *Navicula reichardtana* were determined as the most dominant species, respectively. Temel (2002), on the other hand, identified only *C. placentula* among these species in his study. According to Spearman's rank correlation, the epipelic diatom species was positively correlated with salinity, conductivity, and NO_3+NO_2-N, while it had negative correlations with temperature, pH, NH_4-N, PO_4-P, SiO_4-Si, and TP. Also, no association was observed with DO and TN. However, it was noted that none of these relationships were effective and strong. In the study conducted in the Büyükçekmece reservoir (Aykut et al., 2021), it was found that epiphytic diatoms also showed a positive relationship with conductivity and a negative relationship with pH. However, epiphytic and epipelic diatoms showed different responses to other ecological parameters. In another study investigated in the same region (Aykut et al., 2024), it was determined that epilithic diatoms show the same reactions to salinity, conductivity, pH, NH_4-N, PO_4-P, SiO_4-Si, and TP variables as epipelic diatoms. However, they showed opposite responses to the rest of the parameters (temperature, DO, NO_3+NO_2-N, and TN). Shannon-Weaver Diversity Index (*H'*), applied to identify the structure of the communities, was varied between 2.42 and 4.51 in the study. The lowest value in the study was obtained in August (station 3). The reason for this low diversity was determined as the over-dominance of *Achnanthidium* sp. (35.0%), *Cocconeis placentula* (23.0%), and *Encyonopsis minuta* (27.7%) species. The second lowest value in the examination was observed in August, station 2 and it was only because of the dominance of *Achnanthidium* sp. (46.3%). Simpson's Dominance Index (*D'*) was used to understand both the dominance and diversity of the species. It was calculated during the complete study and varied between 0.04 and 0.27 in the study. The lowest value, proportional to *H'*, was detected in February (station 4). In addition, Pielou's Evenness Index (*J'*) was measured to see how equally the relative abundance of epipelic diatom species is distributed and the values varied between 0.56 and 0.91 in the whole study. The lowest value in the study was obtained in August (station 3) when the *Achnanthidium* sp., *Cocconeis placentula*, and *Encyonopsis minuta* had domination. The highest value was detected only in February (station 4) because no species was overly dominant. According to several studies performed in USA (Stockner, 1967; Hein & Koppen, 1979), *H'* is determined at high levels during cold sampling periods. On the same side of this, in our study, the highest *H'* value on average was found in February and the lowest in August. Additionally, as observed in studies conducted in European freshwaters for the same purpose, the H' value ranged from 0.93 to 4.65. Based on this, we can assume that the *H'* value and thus the diversity is high in the epipelic habitat of the Büyükçekmece Reservoir (Facca & Sfriso, 2007; Kamberović et al., 2017). Frequency analysis was applied during the study to understand the percentage of the presence of the species in the stations. In the study, it was conducted that most of the species (57%) belonged to "Rarely found species" and the least species (6%) belonged to "Continuously found species". In addition, it has been determined that all new record species (*Stephanodiscus balatonis*, *S. minutulus*, *S. neoastraea*, *Karayevia* cf. *ploenensis*, and *Nitzschia tryblionella*) in the reservoir also belonged to "Rarely found species". According to Temel (2002), *Amphora ovalis*, *Caloneis amphisbaena*, *Cocconeis placentula*, *Cymbella ventricosa*, *Gyrosigma acuminatum*, *Navicula cuspidata*, *N. exigua*, *N. radiosa*, *Nitzschia palea*, *N. sigmoidea*, *N. solea*, *Pinnularia brebissoni*, *Surirella librile*, *S. ovata*, *Synedra acus*, and *S. ulna* were frequent during the whole study and has been identified from all sampling stations. However, in our study, only *C. placentula* was frequent, and even though *A. ovalis*, *C. amphisbaena*, and *S. librile* were encountered and their percentage of presence was low. Other mentioned species were not found in any station. When the evaluation was considered based on stations, it was determined that stations 3 and 5 were represented with higher species while stations 2 and 4 were lower in terms of the diversity of diatom species in the reservoir In addition, it was determined that centric diatoms were generally detected in the stations 3 and 5, but they were not encountered in the 4th station. From the clustering analyses applied it was detected that station 1 was separated from the others according to the Bray-Curtis Cluster analysis. The reason for this separation could be that while more or less epipelic diatom species were detected in other grouped stations (stations 4 and 5, stations 2 and 3), moderate-level epipelic diatom species were found in this particular station. According to Euclidean Distance (ED), it is observed that February is separated from the other sampling periods. According to the redundancy analysis (RDA), it was determined that most of the species were not directly affected by ecological parameters. However, it was determined that *Encyonema ventricosum* and *Encyonopsis subminuta* were affected by temprature, *Cymatopleura elliptica* and *Navicula veneta* by NO_3+NO_2-N, *Cocconeis pediculus* by SiO_4-Si, *Cyclotella meneghiniana*, and *Nitzschia intermedia* by DO, *Nitzschia recta* by NH_4-N, *Amphora pediculus*, *Hippodonta hungarica*, and *Navicula reinhardtii* by TDS and lastly *Placoneis* sp. by conductivity. Additionally, the temperature was inversely proportional to DO and NO_3+NO_2-N. While pH, DO, NO_3+NO_2-N, SiO_4-Si, and PO_4-P showed a positive correlation with each other, they showed a negative association with temperature. TDS, conductivity, and NH_4-N also showed a positive relation with each other. In a study about the diatoms of USA(Reimer et al., 2001), it was reported that *Cocconeis placentula* is a cosmopolitan species due to its high level of adaptation to environmental changes. In our study, this *C. placentula* was the species with the highest relative abundance after *Achnanthidium* sp. and it was found at every station except the station 1 of May sampling. In another study performed in Central Poland (Szczepocka & Szulc, 2009), it was stated that this species is sensitive to organic pollution. However, in the epipelic habitats of Büyükçekmece Reservoir, no significant relationship could be determined between organic pollution and *C. placentula*. According to a study performed by Potapova et al. (2004) in Northern Piedmont, they claimed that *C. placentula* and *Rhoicosphenia abbreviata* are indicators of eutrophication. Although *R. abbreviata* was observed in extremely low relative abundance only in August, the density of *C. placentula* in the region may serve as a warning for eutrophication. According to a study carried out in Yorkshire (Round, 1953), they reported that *Fragilaria*, *Cocconeis*, *Gyrosigma*, *Caloneis*, *Navicula*, *Amphora*, *Cymbella*, and *Nitzschia* are very common in calcareous waters, while *Pinnularia* and *Neidium* are in acidic waters in his study. In addition, according to another study conducted in several parts in USA (Round, 1957a, 1957b), *Cymatopleura elliptica* multiplied in neutral and slightly alkaline waters, and *Amphora ovalis* in alkaline waters. Similarly, species common in calcareous waters were dominant in our study. *Pinnularia*, representing acidic environments, was never found, and *Neidium* was encountered with very low relative abundance only in February and May. The fact that the pH value, which is one of the ecological variables, is between 7.49 and 8.28 also supports this situation. In a study (Ha et al., 2003), it was determined that *Stephanodiscus* increase their relative abundance in cold sampling seasons in Nakdong River. Similarly, in our study, although only three cells from three species (*S. balatonis*, *S. minutulus*, and *S. neoastraea*) were detected, all of these species were found in February, when the temperature was the lowest. In another study implemented in Australia (Potter et al., 1975), it was reported that the relative abundance of *Navicula* was observed the most in February and *Nitzschia* in July. In our study, on the contrary, while the relative abundance of *Navicula* was determined as the lowest in February, it was determined as the highest in the May sampling. Although *Nitzschia* reached the highest relative abundance in February, as Potter et al. (1975) stated, it was higher in May compared to other sampling periods. In a study on epilithic diatom species of rivers of USA (Winter & Duthie, 2000), the researcher observed that *Nitzschia recta* prefers epiphytic habitats. However, in our study, this species significantly increased its relative abundance, especially in November. Compared to other studies (Aykut et al., 2021; Aykut et al., 2024) conducted at the same time in Büyükçekmece Reservoir, it was determined that *N. recta* preferred epipelic habitat over epiphytic and epilithic ones. In a study on epiphytic and epilithic algae performed in Topçu Göleti, Eastern Türkiye (Akgöz & Güler, 2004), *Nitzschia* was found in small numbers at each station. Similarly, in our study, this particular genus was found frequently but in low relative abundance in epipelic habitats. In previous studies, *Navicula cryptotenella*, *N. veneta*, and *Surirella angusta* were given as indicators for eutrophic freshwaters (Potapova & Charles, 2002; Soininen et al., 2004). However, our study does not support this claim. Because in our study, in the February sampling, where the highest organic pollution was detected, none of the *Navicula* species and *S. angusta* increased their numbers significantly. When we look at the stations in more detail, no significant increase in individuals was observed in station 4 as well, where the possibility of pollution and potential eutrophication is higher. The species *S. angusta* was detected in the lowest abundance. Although the same species were determined as indicators of high pH and conductivity (Virtanen & Soininen, 2012), our study does not support this either. At station 5 of November, where the highest pH value was measured in the epipelic habitat, there was no increase or decrease in the number of species. However, in another study (Aykut et al., 2024) conducted in the epilithic habitat of the same study area, increasing numbers *N. cryptotenella* support this theory, accounting for nearly half of the detected species. In some different studies (Martínez de Fabricius et al., 2003; Soininen et al., 2004), *Cymbella minuta* and *Gomphonema pumilum* were claimed as important indicator species for high silica freshwaters. Our study on the other hand partially supports this. Although *C. minuta* was never encountered in the entire study, *G. pumilum* was found to be of higher density in the February sampling, where silica was the highest, compared to other sampling periods. In addition, other studies state *Navicula veneta* is highly tolerant of organic pollution and able to increase its numbers where areas are close to agricultural areas (Fawzi et al., 2002; Soininen, 2002; John, 2004; Rakowska, 2004). In support of this, in our study, this species significantly increased its number, especially at station 4 of the February sampling, which is close to farms. In a study carried out to determine indicator species in the Mediterranean streams (Tornés et al., 2007), it was reported that *Gomphonema pumilum* was found in waters with high silica values. Although this species could not increase its relative abundance in Büyükçekmece Reservoir, it increased it to the maximum level in February, when silica values were the highest. Likewise, the relative abundance of the species was determined to be higher in May, when silica was the second highest, compared to other sampling periods. According to a study conducted in the Netherlands (Van Dam et al., 1994), it was determined that *Cymbella* and *Eunotia* increased their numbers in regions with high DO concentrations. In our study, the highest average DO values were determined in February and May. In support of the past study, the number of *Cymbella* detected during these sampling periods is higher than in August and November. However, no species of *Eunotia* could be detected. In addition, in the study on epiphytic diatoms in Büyükçekmece Reservoir, it was determined that *Eunotia* was detected in May when DO was high. According to Van Dam et al. (1994), it was also claimed that *Neidium* was an indicator for high DO and low organic nitrogen concentrations. In our study, only one species (*Neidium dubium*) belonging to this genus was identified and although the species was found in the May sampling when the DO was high, the NO_3+NO_2-N concentration was also determined at high levels in this sampling period. Lu et al. (2020) claimed that *Achnanthidium*, *Encyonema*, and *Gomphonem*a were dominant in areas with high nutrient input, in their study performed in Northeastern China on the indicator properties of diatoms. Contrary to this conclusion in our study, the highest concentrations of TN and TP were determined in February and May, when *Achnanthidium* was at the lowest level compared to other sampling periods. However, in our study, similarly, *Encyonema* had a relatively high relative abundance in May and *Gomphonema* in February. In another study (Cox, 1995), it was determined that *Navicula capitatoradiata* and *N*. *veneta* increased their relative dominance in regions with high conductivity. According to our study, no *Navicula capitatoradiata* was found in the epipelic habitat of Büyükçekmece Reservoir, and contrary to the claim, the lowest number of *N*. *veneta* was detected in November, when conductivity was highest. Rovira et al. (2012) claimed there are some species (*Amphora pediculus*, *Cocconeis placentula*, *Navicula cryptotenella*, *N*. *cryptotenelloides*, and *Nitzschia amphibia*) can increase their abundance in areas where marine effect is decreased. In our study in Büyükçekmece Reservoir, all mentioned species were identified in the epipelic habitat, and station 2 is the closest area to the sea connection that used to exist in the region and was converted into a dam. The fact that *A*. *pediculus* formed almost half of the abundance (46.3%) in station 2 in August supports this. In addition, the species was detected in relatively higher numbers at station 2 during the other sampling periods as well. C. *placentula* was one of the most abundant species throughout the entire study, but there was no appreciable individual increase at station two. While the situation was the same as *C*. *placentula* for the mentioned *Navicula* species, *N*. *amphibia* were determined relatively more in station 2. In a study conducted in UK (Bennion et al., 2014), they reported that *Cymbella affinis* is specified as a high-quality indicator for moderately alkaline lakes. Similarly, in our study, the highest number of this particular species was detected in the May sampling, which is the closest period to the medium alkalinity pH. Another study reported (Delgado et al., 2018) that the relative abundance of *Encyonopsis minuta* and *E*. *subminuta* increased in the same regions. In our study, which supports this claim, the stations where these two species are dominant were determined to coincide with each other.

Conclusion

As a conclusion of this study carried out seasonally in Büyükçekmece Reservoir, epipelic diatom species and their relative abundance in the region were determined and new record species were reported. Considering the cell numbers and relative abundance, the most dominant species were determined as *Achnanthidium* sp., *Cocconeis placentula*, and *Navicula reichardtana*, respectively. *Cyclotella meneghiniana*, on the other hand, was the most abundant centric diatom in the region, even though it was considerably lower than pennate diatoms. Shannon-Weaver Diversity Index showed that the highest diversity was in February, station 4. Although no significant relationship was observed between species and ecological parameters according to Spearman's rank correlation, redundancy analysis (RDA) showed that the most important ecological parameters affecting the distribution and abundance of epipelic diatom species were SiO_4-Si, temperature, and NO_3+NO_2-N, respectively. *Achnanthidium* sp. was identified as the most common species since it was detected at all stations and during sampling periods. In addition, the density of cosmopolitan and eutrophication indicator species such as *Cocconeis placentula* and *Gomphonella olivacea* drew attention in the region.

Ethical Statement

I hereby ensure all research studies submitted papers have been conducted ethically and responsibly, and are in full compliance with all relevant codes of experimentation and legalization.

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Author Contribution

Tümer Orhun Aykut: Data analysis and interpretation, performed experiments and data collection, primary author.

Neslihan Balkıs-Özdelice: Principal investigator, design of the research, provided revisions to the scientific content of the manuscript.

Turgay Durmuş: Data analysis and interpretation, design of the research, performed experiments and data collection, provided revisions to the scientific content of the manuscript.

Cüneyt Nadir Solak: Data analysis and interpretation, improvement on the methodology.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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