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# Driving factors affecting the phytoplankton functional groups in a deep alkaline lake

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Abstract: This study evaluated the phytoplankton communities based on functional groups to obtain information about the water quality of Lake İznik, Turkey. The phytoplankton consisted of 103 taxa, classified in 12 phytoplankton functional groups (PFGs), with dominancy of 5 species, Chrysosporum ovalisporum, Dolichospermum mendotae, Planktothrix rubescens, Fragilaria capucina, and Mougeotia sp. The Shannon-Wiener diversity index (H') was calculated and results ranged between 0.41 and 2.47. The redundancy analysis (RDA) and Spearman's correlation analysis were used to assess the relationships between the PFGs and environmental variables. According to the multiple comparisons of the data, the main efficient factors that determined the seasonal distribution of the PFGs were TP, DO, SiO,, SD, and pH. The ecological requirements of the dominant PFGs (C, D, F, J, H., Lo, S., N, P, R, T, and X.) indicated mainly meso-eutrophic waters. Similarly, Carlson's trophic state index (TSI) stated mesotrophy conditions. As a result, the approach of PFGs can be successfully applied in a deep, alkaline lake to understand the water quality and trophic status.

Key words : Phytoplankton functional groups, Lake İznik, Turkey, trophic status

#### 1. Introduction

Phytoplankton are primary producers of aquatic ecosystems, and the composition, abundance, and structure of the phytoplankton community can be quickly affected by the physical and chemical changes. The diversity and density of the phytoplankton provide information about the trophic level of an aquatic ecosystem (Çelekli and Öztürk, 2014). Moreover, the temporal distribution of algal communities is affected by environmental disturbances (Reynolds et al., 2002).

The phytoplankton have been accepted as one of the biological elements used to assess the ecological status of surface waters based on the EU Water Framework Directive (EC Parliament and Council, 2000; Padisák et al., 2006). The parameters which are used to monitor the ecological quality are the species composition and biomass of phytoplankton, bloom frequency, and intensity (Pasztaleniec and Poniewozik, 2010). Therefore, monitoring of phytoplankton biomass and composition provides important information on the ecological quality of lakes and drinking water reservoirs.

Since phytoplankton community structure could be affected by physical, chemical, and biological properties in lakes, the variety and quantity of phytoplankton species could be used for bio-monitoring of aquatic ecosystems (Reynolds et al., 2002). To this respect, many studies on phytoplankton distribution have been conducted in the past for water quality assessment (Albay and Akçaalan, 2003; Padisák et al., 2006; Pasztaleniec and Poniewozik, 2010; Atıcı and Alaş, 2012; Petar et al., 2014; Salmaso et al., 2015; Di Maggio et al. 2016; Varol, 2019). Generally, phytoplankton abundance or phytoplankton community structure, including dominant and indicator species, were evaluated in these studies to determine the ecological status of the aquatic ecosystems.

Phytoplankton species with different morphological forms (single-celled, filamentous, or large multicelled) can be classified into a single group taxonomically, although they have different ecological requirements and survival strategies. To have a better understanding of how the ecosystems function, it's important to define the morphological and physiological structural features, life strategy, and also distribution characteristics of organisms (Körner, 1994; Salmaso and Padisak, 2007). The phytoplankton functional groups were first described by Reynolds (1988) based on the studies of Grime (1979) who used morphological and physiological characteristics of terrestrial vegetation. Reynolds et al. (2002) defined 33 functional groups according to lake types, and each symbolized by alphanumeric codon. Further, Padisák et al. (2003; 2009) updated these functional groups and then Salmaso et al. (2015) evaluated which codon was related to which trophic status.

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Few studies had also been conducted to use PFGs in determination of the water quality of Turkish reservoirs and lakes in recent years (Soylu and Gönülol, 2010; Çelekli and Öztürk, 2014; Demir et al., 2014; Maraşlıoğlu and Gönülol, 2014; Çelik and Sevindik, 2015; Sevindik et al., 2017; Varol, 2019).

Lake İznik, which is the 5th biggest lake in Turkey, is an important aquatic environment. However, there are few studies related to biodiversity, especially algal community structure of the lake including benthic diatoms, periphyton communities (Albay, 1996; Aktan and Aykulu, 2001) or cyanotoxins linked to cyanobacteria populations (Akçaalan et al., 2014a; Köker et al., 2017). To our knowledge, this is the first study to determine the phytoplankton distribution and community structure in the lake.

This study aimed to determine the water quality of Lake İznik using the phytoplankton community data. The specific objectives of the paper include i) determination of the quantitative distribution of the phytoplankton groups ii) assessment of ecological quality based on PFGs iii) evaluation of the relationship between phytoplankton assemblages and physicochemical parameters.

# 2. Materials and methods

# 2.1. Study area and sampling

Lake İznik located in the southeast of the Marmara region is the 5th biggest lake in Turkey (32 km and 12 km in length and width, respectively) with a 313 km<sup>2</sup> surface area. It is located at an altitude of 85 m above sea level. It is a deep (max. depth is 80 m), stratified, and alkaline lake with high conductivity. Water samples of phytoplankton were collected from the waters' surface (0 to 50 cm deep) at monthly intervals between January 2013 and December 2014 from four sites; on these samples, physical and chemical analyses were carried out. Three stations out of four were chosen, close to the littoral area, with a minimum of 10 m depth to avoid the coastal effect, and the 4th station (50–55 m deep) was located in the middle of the lake (Figure 1). Ocean data view program was used for sampling site map (Schlitzer, 2020)<sup>1</sup>.

# 2.2. Physical and chemical analysis

Water temperature (WT), dissolved oxygen (DO), electrical conductivity (EC) and the pH value were measured in situ using a portable digital multiparametric analyzer (YSI 650 MDS), and water transparency was measured with a Secchi disc (SD). Samples were collected for nutrient analysis and kept in dark and cool conditions. Nutrients; Nitrate + Nitrite (NO<sub>3</sub> + NO<sub>2</sub>), silicate (SiO<sub>2</sub>), soluble reactive phosphorus (SRP), total phosphorus (TP) were analyzed according to APHA-AWWA WPCF (1989). Chlorophyll-*a* (chl-*a*) was determined according to the ISO standard method (ISO 10260,1992).

# 2.3. Phytoplankton analysis

Phytoplankton samples were fixed by Lugol's iodine solution, and the phytoplankton enumeration and identification were performed using a Zeiss Axiovert (Carl Zeiss Microscopy GmbH, Oberkochen, Germany) inverted microscope according to Utermöhl (1958). For

<sup>1</sup> Schlitzer R (2020). Ocean Data View. ODV 5.2.1 (Online). Website https://odv.awi.de (accessed 30 January 2020).



Figure 1. Study area and the sampling sites.

taxa identification, the most well-known studies in the literature, such as the ones of John (2005), Komarek and Anagnostidis (2007), Krammer and Lange-Bertalot (1986), were used. The current names of taxa were also checked using Algaebase website footnote reference to website (Guiry and Guiry, 2020)<sup>2</sup>. The phytoplankton biovolume was calculated according to Hillebrand et al. (1999) and the conversion of biovolume to biomass was achieved by assuming a specific density of phytoplankton cells of 1 g/cm<sup>3</sup>. For the filamentous species, such as *Planktothrix* spp., *Aphanizomenon* spp., *Dolichospermum* spp., 100 μm length was assumed to be equal to 1 filament.

# 2.4. PFGs, trophic state and diversity indices

During the two-years survey, species that account for more than 5% of the monthly phytoplankton biomass at any station in any month were evaluated under PFGs according to Reynolds et al. (2002) and Padisák et al. (2009) (Table 4). The trophic state of these codons was determined according to Salmaso et al. (2015).

Biological diversity (H') and evenness (J) were used to analyze the community structure (Shannon and Weaver, 1949; Pielou, 1966). Based on Shannon–Wiener diversity index, results could be classified as oligotrophic (>3),  $\beta$ -mesotrophic (2–3),  $\alpha$ -mesotrophic (1–2), and eutrophic (0–1) (Zhang and Zang, 2015).

Trophic state indices (TSIs) were calculated using the equations described by Carlson (1977). Deviations between TSIs were calculated based on  $\text{TSI}_{\text{SD}}$ ,  $\text{TSI}_{\text{Chl-}a}$ , and  $\text{TSI}_{\text{TP}}$  according to Carlson (1991) and Havens (2000).

#### 2.6. Statistical analyses

For the statistical analyses, the data (limnological and biological variables) were mainly tested for normal

distribution (Shapiro-Wilk test) and homogeneity (homogeneity of variance test). Since the data did not show normal distribution, nonparametric tests were preferred. To determine the correlation between biological (PFGs and chl-*a*) and environmental (WT, DO, EC, pH, SRP, TP,  $NO_2+NO_3$ , SiO<sub>2</sub>, and SD) variables, Spearman's rho was used. Kruskal–Wallis test was used to state differences of physicochemical and biological variables among seasons and stations. Nonparametric tests were applied by using the IBM SPSS Statistics 19.0 (SPSS (IBM Corp., Armonk, NY, USA) for Windows.

For the multiple comparison of PFGs, chl-a, and limnological variables, the canonical ordination method was applied. All the variables, except the pH value, were converted to  $\log (x + 1)$  for clustering analysis. The linear model of redundancy analysis (RDA) was preferred to determine the association of all of the variables due to the short gradient length (SD < 2) on the first two axes of the biological data (ter Braak and Šmilauer, 2002). Because of the multicollinearity between the environmental variables, the data was controlled based on the variance inflation factor (VIF < 10) (ter Braak and Šmilauer, 1998). A type of stepwise regression - forward selection (FS) and the Monte Carlo permutation test (999 unrestricted permutations) were used to determine the environmental variables, which were significantly correlated (P < 0.01). The computer program CANOCO 4.5 was performed for the DCA and RDA analyses for Windows. Limnological variables (Table 1) were used in the RDA at the beginning of the clustering analysis; however, due to the high inflation factors (VIF > 10) of the EC, TDS, and SRP, they were eliminated from the data set.

<sup>2</sup> Guiry MD, Guiry GM (2015). AlgaeBase. World electronic publication. National University of Ireland. (Online) Website http://www.algaebase.org (accessed 08 June 2020).

Environmental variables	2013		2014		
	Mean ± S.D.	(Min. –Max.)	Mean ± S.D.	(Min. –Max.)	
Water temp. (°C)	17.1 ± 7.0	8.4-27.8	18.3 ± 6.3	9.6–29.7	
DO (mg L <sup>-1</sup> )	$9.5 \pm 0.9$	8.1-10.9	9.1 ± 1.0	7.4–11.9	
рН	$9.1 \pm 0.5$	8.1-10.4	$9.2 \pm 0.4$	8.4-10.3	
EC (µS cm <sup>-1</sup> )	831 ± 134	671-1050	893 ± 130	702–1165	
TDS (mg L <sup>-1</sup> )	641 ± 12	615-682	$664 \pm 18$	637-701	
SD (m)	$4.4 \pm 1.9$	1.1-8.9	$4.7 \pm 2.1$	2.2-10.4	
$NO_{2}+NO_{3}$ (µg L <sup>-1</sup> )	185 ± 139	33-628	$126 \pm 60$	35-348	
SRP (µg L <sup>-1</sup> )	$11.7 \pm 8.6$	0.8-29.0	$10.4 \pm 8.3$	0.85-34.1	
TP (μg L <sup>-1</sup> )	$33.5 \pm 21.7$	8.0-96.7	$26.9 \pm 12.8$	10.9-55.4	
SiO <sub>2</sub> (mg L <sup>-1</sup> )	$1.1 \pm 0.8$	0.1-2.3	$1.1 \pm 0.4$	0.3-2.1	
Chl-a (µg L <sup>-1</sup> )	9.5 ± 7.8	1.2-30.8	$4.7 \pm 5.1$	0.1–19.5	

Table 1. Environmental variables and Chl-a values in Lake İznik in 2013–2014.

# 3. Results

#### 3.1. Physicochemical variables

The Lake İznik was thermally stratified from May to October and a full circulation period began in November and ended up in April during the study period<sup>3</sup>. Since the physical and chemical variables did not show any significant differences between stations (P > 0.05), data were evaluated according to the mean values of all four stations. Lake İznik has an alkaline character; the pH value ranged between 8.10 and 10.4 during the study period. The mean pH value was determined to be 9.1 and 9.2 and mean EC value was 831 and 893  $\mu$ S cm<sup>-1</sup> in 2013 and 2014, respectively. The DO values were found to be high in surface waters of Lake İznik. The SD was significantly correlated with the density of phytoplankton; the lowest value (1.1 m) was measured in August 2013 and the highest value (10.4 m) in June 2014 (Table 1).

Concentrations of SRP were low during the stratified period in the epilimnion and high values were measured during complete water circulation. The mean SRP concentration was determined as 11.7 µg L<sup>-1</sup> and 10.4 µg L<sup>-1</sup> in 2013 and 2014, respectively. TP values were generally in parallel with SRP values and the mean TP values were measured as 33.5  $\mu$ g L<sup>-1</sup> and 26.9  $\mu$ g L<sup>-1</sup> in 2013 and 2014, respectively. Relatively high concentrations of NO<sub>2</sub> +  $NO_3$  were detected in Lake İznik. While the mean  $NO_3 +$ NO<sub>3</sub> value was 185.2 µg L<sup>-1</sup> in 2013, it decreased to 125.6  $\mu$ g L<sup>-1</sup> in 2014. Chlorophyll-*a* did not show a significant variation from January to June of the years 2013 and 2014, but showed a significant fluctuation between July and December. The highest chlorophyll-a values were measured in August and October 2013 as 24.8 and 30.8 µg L<sup>-1</sup>, respectively.

#### 3.2. Phytoplankton composition

A total of 103 taxa were found to have an abundance of Bacillariophyta (36 taxa) and Chlorophyta (42 taxa). The other groups; Charophyta, Cryptophyta, Cyanobacteria, Miozoa, Euglenozoa contributed to the phytoplankton community with low species numbers (Table 2). According to frequencies of species, 17 taxa were 'abundant' (frequency: 5), 16 taxa were 'very common' (frequency: 4), 14 taxa were 'common' (frequency: 3), 16 taxa were 'occasional' (frequency: 2), 41 taxa were 'rare' (frequency: 1).

Temporal changes of total phytoplankton biomass followed a similar trend in all sites and the highest biomass values were measured in August, while the lowest biomass values were recorded in May (Figure 2) in both years, indicating that phytoplankton biomass showed a significant increase from summer to autumn.

Cyanobacteria was the dominant group (49%), Charophyta (20%), and Bacillariophyta (22%) were subdominant groups in the total phytoplankton biomass in 2013 (Table 3). On the other hand, Eugleneozoa was the least frequent group in both years. Unlike the previous year, Charophyta became the dominant group (48%) and Cyanobacteria was the subdominant group (28%) contributing to total phytoplankton biomass in 2014.

Although the seasonal variation of phytoplankton composition was similar to each other in both years, significant changes were observed in the dominant species and their quantities. Cyanobacteria, as a result of *Chrysosporum ovalisporum* bloom, reached to the highest biomass value in August 2013. Chlorophyta was observed in all months, but the highest biomass values were found in July both in 2013 and 2014. The members of Bacillariophyta group increased in all sites in June and October 2013, while the highest biomass values were recorded in station 1 in both years (Figure 3).

#### 3.3. Phytoplankton functional groups

A total of 25 species were identified which accounted for more than 5% of the monthly phytoplankton biomass at any station at any month in Lake İznik. These species were classified in 12 different codons according to Reynolds et al. (2002) and Padisák et al. (2009) and defined as dominant PFGs. A total of 11 codons (C, D, F, J, H<sub>1</sub>, Lo, S<sub>N</sub>, N, P, R, and T) were determined in 2013 and one more codon (X<sub>2</sub>) was added in 2014 (Table 4).

According to the statistical analysis, the percentage of dominant PFGs and the temporal changes were similar between the stations (P > 0.05). Therefore, only the results of station 4, which was located in the middle of the lake, were shown in Figure 4.

Codon R, represented by Planktothrix rubescens, was the dominant group between January and March in both years. It accounted for more than 90% of the total biomass in the lake in January 2013. However, Codon C, consisting of diatom species, contributed to the total biomass as the subdominant group in the same period. Codon H, reached a high biomass value in May 2013 (D. mendotae), and showed a bloom in August 2013 (C. ovalisporum). However, the situation changed in 2014, while the same species still existed in the lake, they did not reach the biomass values of the previous year. Similarly, Codon P, which reached high biomass values in June and October 2013, showed lower biomass values in 2014. Codon T (Mougeotia sp.) group was another functional group that reached high biomass values from August until the end of the year in both years.

# 3.4 Relationships between PFGs and environmental variables

The limnological variables, some PFGs (H1, N, R, and T), and chl-*a* concentrations are statistically different

<sup>&</sup>lt;sup>3</sup> Akçaalan R. A, Gürevin C, Oğuz A, Albay M (2015). Iznik Gölü Siyanobakteri (mavi-yeşil alg) artışı, siyanotoksin üretimi ve su kalitesi ile olan etkileşiminin incelenmesi. Website https://app.trdizin.gov.tr/publication/project/detail/TVRRek1qYzM (accessed date March 2015)

# OĞUZ et al. / Turk J Bot

**Table 2.** Phytoplankton list in the stations of the Lake İznik from the January 2013 to December 2014.(1: rare, 2: common, 3: abundant, 4: very abundant, 5: continuous species).

Phylum	Species	Frequency	Phylum	Species	Frequency
	Achnanthes sp.	1		Fragilaria sp.	1
	Amphora ovalis (Kützing) Kützing	1		<i>Gomphonema</i> sp.	4
	A. proteus W.Gregory	1		Gyrosigma sp.	1
	Amphora sp.	3		Melosira sp.	2
	Asterionella formosa Hassall	4		Navicula rhynchocephala Kützing	2
	Cocconeis pediculus Ehrenberg	1		Navicula sp.	4
	<i>C. placentula</i> Ehrenberg	3		N. trivialis Lange-Bertalot	1
	Cyclotella atomus Hustedt	4		Nitzschia acicularis (Kützing) W.Smith	2
	C. meneghiniana Kützing	5		Nitzschia sp.	3
	<i>Cymbella</i> sp.	4		Nitzschia spp.	1
	C. tumida (Brébisson) Van Heurck	3		Rhoicosphenia curvata (Kützing) Grunow	1
	Denticula tenuis Kützing	1		Rhopalodia sp.	1
	Diatoma sp.	1		Sellaphora sp.	2
	D. vulgaris var. brevis (Grunow)	3		Stephanodiscus astraea (Ehrenberg) Grunow	4
hyta	Fragilaria capucina Desmazières	4	nyta	Surirella robusta Ehrenberg	1
rioph	F. crotonensis Kitton	1	iopl	Synedra sp.	1
cillar	E tanara yan manana Langa Portalat & S. Illrich	2	cillar	Ulnaria acus (Kützing) Aboal in Aboal et al.	5
Bac	r. tenera var. nanana Lange-Bertalot & S.Offich	2	Bac	U. ulna (Nitzsch) Compère	3
	Ankistrodesmus sp.	1		M. minutum (Nägeli) Komárková-Legnerová	1
	Ankyra ancora (G.M.Smith) Fott	1		M. pusillum (Printz) Komárková-Legnorová	3
	A. judayi (G.M.Smith) Fott	1		M. tortile Komárková-Legnerová	3
	Carteria sp.	1		Netrium sp.	3
	Chlamydomonas sp.	3		Oocystis borgei J.W.Snow	4
	Chlorella sp.	1		<i>O. naegelii</i> A.Braun	2
	Chlorogonium sp.	1		O. natans Lemmermann	1
	Chroomonas sp.	1		O. parva West & G.S.West	1
	Closteriopsis acicularis J.H.Belcher & Swale	3		Penium sp.	2
	<i>Coelastrum astroideum</i> De Notaris	1		Pinnularia sp.	1
	C. microporum Nägeli in A.Braun	5		Pseudopediastrum boryanum E.Hegewald.	5
	Desmodesmus bicaudatus P.M.Tsarenko	1		Scenedesmus acutiformis Schröder	1
	Elakatothrix genevensis (Reverdin) Hindák	4		S. arcuatus (Lemmermann) Lemmermann	1
	Franceia sp.	5		S. quadricauda (Turpin) Brébisson	5
	<i>Golenkinia radiata</i> Chodat	5		Scenedesmus sp.	2
	Lagerheimia ciliata (Lagerheim) Chodat	neimia ciliata (Lagerheim) Chodat 5   iformis (J.W.Snow) Collins 4		Schroederia setigera Lemmermann	3
	L. citriformis (J.W.Snow) Collins			Tetraëdron caudatum (Corda) Hansgirg	1
ta	L. longiseta (Lemmermann)	4	ta	T. minimum (A.Braun) Hansgirg	5
phy	Monoraphidium contortum Komárková-Legnerová	2	phy	<i>T. triangulare</i> Korshikov	5
loro	M. dybowskii Hindák & Komárkova Legnerová	5	loro	Tetraëdron sp.	2
Ch	<i>M. litorale</i> Hindák	1	Ch	Tetradesmus lagerheimii M.J.Wynne & Guiry	1

Table 2. (Continued).

rta	Cryptomonas marssonii Skuja	2		<i>Cosmarium neodepressum</i> G.J.P. Ramos & C.W.N. Moura	4
tophy	<i>C. ovata</i> Ehrenberg	5	]	C. laeve Rabenhorst	3
Cryp	Plagioselmis nannoplanctica (H.Skuja) G.Novarino	5		<i>C. turpinii</i> Brébisson	4
	<i>Chrysosporum ovalisporum</i> (Forti) E.Zapomelová, O.Skácelová, P.Pumann, R.Kopp & E.Janecek	1		Mougeotia sp.	5
acteria	Cyanodictyon planctonicum B.A.Mayer	1		Closterium acutum Brébisson in Ralfs	4
	Dolichospermum mendotae (W.Trelease) Wacklin, L.Hoffmann & Komárek1D. smithii (Komárek) Wacklin, L.Hoffmann & Komárek1		hyta	Staurastrum cingulum G.M.Smith	5
			aroț	S. polymorphum Brébisson	1
	Limnothrix redekei (Goor) Meffert	1	CP	Staurastrum sp.	5
	Oscillatoria sp.	1		<i>Ceratium</i> sp.	2
	Planktolyngbya sp.	1	pzoa	Gymnodinium paradoxum A.J.Schilling	5
	Planktothrix rubescens Anagnostidis & Komárek	4	Mid	Peridinium sp.	4
	Raphidiopsis raciborskii (Woloszynska) Aguilera, Berrendero Gómez, Kastovsky, Echenique & Salerno	2	ozoa	Euglena sp.	2
Cyanoł	Sphaerospermopsis aphanizomenoides (Forti) Zapomelová, Jezberová, Hrouzek, Hisem, Reháková & Komárková	2	Euglen	Trachelomonas sp.	2



Figure 2. Monthly variations of the total phytoplankton biomass in Lake İznik.

between seasons (Kruskal-Wallis, P < 0.05, N = 32), on the contrary, no significant differences were found between the stations (Kruskal–Wallis, P > 0.05, N = 32). According to the multiple comparisons, the first two axes show 84.1% of total variation. The first axis was related with TP (0.8061), DO (0.7813), and SiO<sub>2</sub> (0.7447), whereas the second axis correlated with SD (0.3401) and pH value (–0.4596), mainly. The limnological variables and PFGs showed seasonal distribution as shown on the RDA graph.

Environmental variables gained importance in each season concerning different PFGs (Figure 5).

In the RDA analysis, codon R became important in winter and the codons  $H_1$ ,  $L_0$ , F, and J were the main groups in the summer period. Besides, codon C, D and Codon T, P, S<sub>N</sub> were identified as important groups in spring and autumn, respectively. Significant negative correlations were observed between WT and codon R, and positive correlation with codon  $H_1$ ,  $L_0$ , and F (P < 0.01). SD and

Dhutonlankton groups	Biomass (%)			
Phytoplankton groups	2013	2014		
Cyanobacteria	49	28		
Bacillariophyta	22	10		
Charophyta	20	48		
Chlorophyta	7	9		
Cryptophyta	1	2		
Miozoa	1	2		
Euglenozoa	0	1		

**Table 3.** Composition of the phytoplankton taxonomic groups in Lake İznik in 2013–2014.

codons C, F, H<sub>1</sub>, N, P, S<sub>N</sub>, T showed significant negative correlations (P < 0.01); however, positive correlations were determined between nutrients and some PFGs. TP had a significant positive correlation with codons R and had significant negative correlations with codons L<sub>0</sub>, T, and F. NO<sub>2</sub> + NO<sub>3</sub> were correlated positively with H<sub>1</sub> (P < 0.05) and negatively with codon T and codon L<sub>0</sub> (P < 0.01). SiO<sub>2</sub> had significant positive correlations with codons R and SN, while it had a significant negative correlation with codon F (P < 0.01). Besides, only codon F and codon L<sub>0</sub> showed a significant positive correlation with pH value, while there was no significant correlation between pH value and other groups.

#### 3.5. Trophic state, and diversity indices

Since any differences were not observed in  $\text{TSI}_{\text{Chl-a}}$ ,  $\text{TSI}_{\text{TP}}$ and  $\text{TSI}_{\text{SD}}$  values among the stations, the trophic status was calculated based on the average of all stations.  $\text{TSI}_{\text{SD}}$  and  $\text{TSI}_{\text{Chl-a}}$  ranged from oligotrophy to eutrophy throughout the sampling period and the lake was in low mesotrophy conditions according to the mean  $\text{TSI}_{\text{SD}}$  and  $\text{TSI}_{\text{Chl-a}}$  (39 and 44, respectively). However,  $\text{TSI}_{\text{TP}}$  ranged from mesotrophy to eutrophy and the mean values indicated eutrophy. As a result, all variables were evaluated together, and the lake was found to be in mesotrophy condition according to Carlson TSI (Figure 6).

Shannon–Wiener Diversity (H') and evenness (J) indexes did not differ significantly among stations. The lowest H' and J values were calculated as 0.41 and 0.16, respectively in August 2013. The mean H' values were measured as 1.53 and 1.71, and J values were 0.51 and 0.59 in 2013 and 2014, respectively.

#### 4. Discussion

#### 4.1. Trophic state and ecological status

The perspective of the definition of water quality has changed markedly in recent decades. Together with physicochemical parameters, biological integrity becomes important. Therefore, to evaluate the water quality of a lake, we need to consider ecological quality in which phytoplankton community has a significant role as primary producers.

Trophic state index, based on the relationships between TP, Chl-a, and SD, could be used to define certain conditions associated with factors that limit the growth of phytoplankton in lakes and reservoirs (Havens, 2000). The lowest TSI<sub>Chla</sub> values were observed in the spring periods in both years. According to Carlson (1991), this might suggest that the algae growth was probably limited by zooplankton grazing instead of nutrients such as phosphorus. High zooplankton biomass, especially Cladoceran species, was observed in May for both years, and it was correlated with TSI results (unpublished data). On the other hand, TSI<sub>Cbla</sub> values were higher in summer and autumn. Havens (2000) stated that if there are conditions where  $\mathrm{TSI}_{\mathrm{Chl}\text{-}a}$  $> TSI_{TP}$  in a lake, there is a phosphorus limitation, and also large phytoplankton taxons such as Aphanizomenon sp. are dominant when  $TSI_{Chl-a} > TSI_{SD}$ . It is known that phosphorus could be a limiting factor for phytoplankton in the epilimnion of deep lakes during the summer and bloom periods (Lenard and Solis, 2009). The dominance of large filamentous species such as C. ovalisporum and Mougeotia sp. in late summer and early autumn periods was consistent with the results pointing out  $TSI_{Chl-a} > TSI_{SD}$ at the same time period (Figure 6).

Relatively high water transparency despite the high annual mean  $\text{TSI}_{\text{TP}}$  values in Lake İznik was a general phenomenon of mesotrophic lakes (OECD, 1980). This may be related to the high resistance of deep and large lakes to eutrophication (Lenard and Solis, 2009).

Phytoplankton diversity index has also been used to understand the variation of phytoplankton species throughout the year and the Shannon–Wiener Diversity Index (H') values of all stations were found between 1 and 2 in Lake İznik. Zhang and Zang (2015) indicated that values between 1–2 refer to  $\alpha$ -mesotrophic conditions. H' and J indexes were lower in 2013 as a result of the more frequent bloom episodes. When *Chrysosporum ovalisporum* bloom occurred in August 2013, the diversity index (H') was 0.41 and the evenness index (J) was 0.16 which was the lowest value during the study period. When H' values indicated eutrophic status (H' <1), also TSI<sub>Mean</sub> values specified eutrophic conditions (Figure 6).

Reynolds et al. (2002) and Padisák et al. (2009) identified PFGs based on the assumption that certain groups could be classified according to similarities of their biological and ecological characteristics. This view also provides important information to understand the selection dynamics of communities in different regions (Soylu and Gönülol, 2010). Then, the PFGs approach was



OĞUZ et al. / Turk J Bot

Figure 3. Monthly variations of phytoplankton taxonomic groups in Lake İznik.

640

Dominant		2013		2014		
PFGs	Trophic state	Dominant species	Annual average (%)	Dominant species	Annual average (%)	
	Mesotrophy	A. formosa		C. atomus S. astraea		
С		C. atomus	5		7	
		C. meneghiniana				
D	Hypertrophy	U acus	1	U. acus	2	
F	Mesotrophy	O. borgei	3	O. borgei O. naegelii L. ciliata	6	
J	Eutrophy	T. minimum	2	T. minimum P. boryanum	2	
H	Eutrophy	D. mendotae D. smithii	32	A. mendotae	12	
		C. ovalisporum		C. ovalisporum		
L <sub>o</sub>	Mesotrophy	Peridinium sp.	1	Peridinium sp.	2	
N	Mesotrophy	C. depressum		C. depressum		
		C. turpunii	4	C. turpunii	11	
		S. cingulum		S. cingulum		
Р	Eutrophy	F. capucina	15	F. capucina Melosira sp.	2	
R	Eutrophy	P. rubescens	21	P. rubescens	11	
Т	Mesotrophy	<i>Mougeotia</i> sp.	15	<i>Mougeotia</i> sp.	38	
X <sub>2</sub>	Mesotrophy	-	0	P. nannoplanctica	1	
S <sub>N</sub>	Eutrophy	R. raciborskii	1	R. raciborskii	6	

**Table 4.** The phytoplankton species that represented the PFGs (Reynolds et al., 2002; Padisák et al., 2009) and trophic state (Salmaso et al., 2015)



Figure 4. Monthly variations of PFGs biomass in Lake İznik.

further developed by Salmaso et al. (2015) to evaluate which codons were related to which trophic status. Among the 12 PFGs (C, D, F, J,  $H_1$ ,  $L_0$ , N, P, R, T,  $X_2$ , and  $S_N$ ),

which were dominant in Lake İznik, codon D represents hypertrophic conditions, codons C,  $H_1$ , P,  $S_N$  refer to eutrophic water bodies, and codons R, F,  $L_0$ , N, T, and  $X_2$ 



**Figure 5.** The relations between phytoplankton groups (PFGs; R,  $S_N$ , C, D, J, F,  $L_o$ ,  $H_1$ , T, N, and P) and environmental variables (SRP, TP, DO, SD,  $S_1O_2$ , EC,  $NO_2$ + $NO_3$ , pH, and WT) at different sampling periods and stations in Lake İznik. Stations; circle: St.1, square: St.2, star: St:3, uptriangle: St.4. Sampling periods; 1: Winter 2013, 2: Spring 2013, 3: Summer 2013, 4: Autumn 2013, 5: Winter 2014, 6: Spring 2014, 7: Summer 2014, 8: Autumn 2014.



**Figure 6.** Trophic state indices derived from SD, TP and Chl-*a*, and Shannon–Wiener diversity (H') and evenness index(J) for Lake İznik (E: Eutrophy, M : Mesotrophy, O: Oligotrophy)

are generally found in mesotrophic lakes and reservoirs. In comparison to the annual percentage biomass of total PFGs presented in the lake, their distributions were 1% hypertrophic, 71% eutrophic, 28% mesotrophic in 2013. These ratios changed to 2%, 33%, and 65% in 2014, respectively. The phytoplankton functional groups, which represent eutrophic status prevail in 2013, were replaced by the mesotrophic groups in 2014 indicating that the lake is in a transition period from mesotrophy to eutrophy.

#### 4.2. PFGs and their ecological preferences

The seasonal succession of phytoplankton functional groups showed very close similarity among stations (Kruskal–Wallis, P > 0.05, N = 32); however, a significant quantitative difference was detected between the years. Functional groups such as H<sub>1</sub>, P, R, and T reached high biomass in 2013, while the distribution of these groups was found to be more balanced in 2014.

The temporal variations, water circulation, light and nutrients availability are the most important variables on the distribution of phytoplankton (Varol, 2019). Even, the genera, which classified in the same group, respond in different ways to these variables. Cyanobacterial genera P. rubescens (Codon R), R. raciborskii (Codon S<sub>N</sub>), D. Mendotae, and C. ovalisporum, (Codon H.), which are detected in high numbers in Lake İznik, were placed in different functional groups according to their ecological requirements. The dominance and seasonal distribution of these species were found to be remarkable in Lake İznik. While codon R was abundant in surface waters during the winter period, Codon  $S_N$  and Codon  $H_1$  reached high numbers at the end of the thermal stratification (late summer) in which the epilimnion of the lake had more limited conditions in terms of nutrient. Codon R is generally found in the metalimnion or upper hypolimnion of deep oligo-mesotrophic lakes in summer months since it prefers low temperature and light intensity (Reynolds et al., 2002). However, in some favorable conditions, a surface bloom of P. rubescens could occur in winter months (Akcaalan et al., 2014b). P. rubescens reached high biomass at all stations of Lake İznik between January and March in both years, but it did not form a bloom. On the other hand, codon H, which is found in stratified or shallow lakes and generally eutrophic and have low nitrogen content (Reynolds et al., 2002), caused a bloom in two different periods of a year. These blooms were caused by D. mendotae in May 2013 and C. ovalisporum in August 2013. It is stated that D. mendotae is present especially in the meso-eutrophic lakes in the world and the distribution area is quite large (Akcaalan et al., 2014a). Chrysosporum ovalisporum form blooms in stagnant waters with high water temperature (Forti, 1911). We found a very strong relationship (r = 0.524; P < 0.01) between Codon H, and water temperature in Lake İznik. When the water temperature was 27 °C, the bloom was observed in all stations and continued until September. Another functional group including cyanobacteria is codon  $\boldsymbol{S}_{_{\!N}}$  which is usually found warm and in mixed layers, and the group tolerates the deficiency of light and nitrogen (Reynolds et al., 2002). Codon S<sub>N</sub> was always accompanied by codon H, for both years. Padisák et

al. (2003) reported that the species, belonging to codon  $H_1$  and  $S_N$ , are generally in competition and their successions continue as  $H_1 S_N$ . Although *R. raciborskii* originates from tropical regions, it is considered an invasive species that is frequently encountered in European water bodies (Padisák 1997; Akcaalan et al., 2014a).

Cyanobacteria blooms are known to be closely related to eutrophication (Padisák, 1997). However, the dominance of cyanobacteria can be supported by certain environmental conditions such as high temperature, stagnant waters (Hadas et al., 2002), nutrient richness, and high alkalinity (Carvalho et al., 2011). Lake İznik is a highly alkaline lake (Albay and Aykulu, 2002) and has a long thermal stratification period, which can support cyanobacterial blooms, especially during the late summer when thermal stratification was more stable. Studies in Kinneret Lake, which has high alkalinity and long thermal stratification properties, showed that the proliferation of cyanobacteria was supported by high alkalinity since they can absorb carbonate together with CO<sub>2</sub> (Hadas et al., 2012). On the other hand, long thermal stratification periods cause the limitation of nitrogen and phosphorus in the epilimnion in late summer. However, members of Nostocales, which can fix atmospheric nitrogen, are more advantageous than ... are more advantageous than other species in stratification periods when nitrogen concentration was low (Briand et al., 2004). Species such as R. raciborskii and C. ovalisporum, which form blooms in late summer, were tolerant in light-limited and low nutrients conditions (Padisák, 1997).

Apart from the cyanobacteria, another group that reached high biomass values in the lake during the summer-autumn period is the Charophyta. Mougeotia sp. represented by Codon T showed bloom especially between October and November 2014 in the lake. Mougeotia is a successful competitor in a stratified condition and becomes dominant in oligo-mesotrophic lakes, especially mean total phosphorus concentrations were below 20 µg L<sup>-1</sup> in the epilimnion (Polli and Simona, 1992, Tapolczai et al., 2015). A negative correlation was found between codon T and TP in Lake İznik (r = -0.487; P < 0.05). Mougeotia was detected in the lake from August to the end of the year when total phosphorus was measured below 20 μg L<sup>-1</sup> in both years. Especially Especially in October and November 2014, it constituted 80% of the total biomass at Station 4 (Figure 3). This situation could be considered as a sign of an eutrophication process. The codon T was also reported from Batman dam reservoir (Varol, 2019), Çaygören Reservoir (Çelik and Sevindik, 2015), and Lake Garda (Salmaso, 2002)

Codon C (*C. atomus* and *C. meneghiniana*), Codon D (*U. acus*) and Codon P (*F. capucina*) functional groups are diatoms which were detected in Lake İznik at different

periods. Di Maggio et al. (2016) reported three annual peaks for diatoms: the codon P is in summer, the codon C is in early winter, and the codon D is between late winter and early spring. Generally, the codon P is found in the epilimnion of eutrophic lakes and can reach high biomass values depending on the nutrient concentrations in the lake (Grime, 1979; Reynolds et al., 2002). F. capucina reached high biomass in June2013 and October 2013. In normal conditions, the inedible colonial chlorophytes become predominant in the early summer and are replaced by large diatoms (Codon P) (Sommer et al., 1986). However, as a result of low TP (10.1  $\mu$ g L<sup>-1</sup>) concentrations in the early summer of 2013, Chlorophyta, which is sensitive to nutrient deficiency, was replaced by diatoms and the bloom of the F. capucina formed in the lake. On the other hand, the increased TP concentrations in 2014 resulted in a more balanced distribution of Chlorophyta groups (codon J and F) and could not allow F. capucina to bloom. Another diatom, C. meneghiniana (codon C) increased in spring periods and had a significant positive correlation with SiO<sub>2</sub> and negative correlation with WT (P < 0.01). Previous studies have also reported that centric diatoms are typically related to low water temperature and high SiO, concentrations (Schlegel and Scheffler, 1999; Varol, 2019). Moreover, nonfloating species such as Cyclotella prefers high turbulence to avoid rapid sinking in stagnant waters (Hoyer et al., 2009).

Codon F and  $L_0$  represent mainly the colonial chlorophytes and dinoflagellates and prefer stable conditions in summer epilimnia of mesotrophic lakes (Reynolds et al., 2002). Codon F is also known to increase at higher pH values (Lopez-Archilla et al. 2004). Both codons were present in Lake İznik during the summer period and showed a significant positive correlation with the pH values (r = 0.445; P < 0.01 and r = 0.659; P < 0.05, respectively). The codon  $L_0$ , which is known to regulate its buoyancy and tolerate low nutrient concentrations (Petar et al., 2014), showed a negative correlation with NO<sub>2</sub> + NO<sub>3</sub> (r = -0.621; P < 0.05) and TP (r = -588; P < 0.05) in the lake. Similarly, Sevindik et al. (2017) detected the codon  $L_0$  and F in the mesotrophic İkizcetepeler reservoir during the summer period.

Codon  $X_2$  represented by *Plagioselmis nannoplanctica* in Lake İznik is another functional group found in mesotrophic waters. The members of this group are the smallest nanoplanktonic flagellates, thus forming an important food source for zooplankton. During the

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Albay M (1996). The investigation of pollution levels from the point of view of biology of Lake İznik. PhD, İstanbul University, Istanbul,Turkey. research period, this species was identified as a "continuous species" in the lake (Table 2). *P. nannoplanctica* reached to high abundance in the mixing period between December and April of both years; however, because of its small biovolume (usually < 20  $\mu$ m), its biomass was low compared to other organisms. Codon X<sub>2</sub> showed a significantly positive correlation with the codon C (P < 0.05) since their ecological requirements are very similar.

#### 5. Conclusion

The distribution and ecological requirements of phytoplankton groups may differ from each other even though they are classified in the same group taxonomically. Since phytoplankton functional groups are based on common ecological requirements of different species, they could be used to determine water quality. The percentage of dominant functional groups represented eutrophic lakes in 2013 and changed in favor of mesotrophic groups in 2014 in Lake İznik. However, when considered the overall diversity of functional groups throughout the sampling period, the lake could be characterized as a mesotrophic lake. Similarly, TSI values also indicated mesotrophic conditions. Besides, Lake İznik had relatively high transparency despite the high annual TSI<sub>TP</sub> values. High phosphorus and good transparency values generally indicate mesotrophy due to the resistance of deep lakes to eutrophication. (Lenard and Solis, 2009). On the other hand, a heavy cyanobacterial bloom (C. ovalisporum) was detected in the lake and some Cyanobacteria species, D. mendotae, S. aphanizomenoides, R. raciborskii and P. rubescens, reached high biomass showing the signals of eutrophication.

Considering the ecological requirements of the dominant PFGs detected in the lake, they coincide with the trophic status of Lake İznik. Therefore, this study confirmed that phytoplankton functional groups could be applied to understand the trophic status of a deep alkaline lake based on phytoplankton community structure.

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