



Seasonal Changes in Phytoplankton Size Classes (PSC) Derived From HPLC Pigment Data along the South-Eastern Black Sea

Ertugrul Agirbas¹, Ahmet Sahin², Ulgen Aytan¹, Serkan Koral⁴, Dilek Fidan³

¹ Recep Tayyip Erdogan University, Faculty of Fisheries, Rize, Turkey.

² Karadeniz Technical University, Faculty of Marine Sciences, Department of Fisheries Technology, Trabzon, Turkey.

³ Central Fisheries Research Institute, Trabzon, Turkey.

⁴ Izmir Katip Celebi University, Faculty of Fisheries, Cigli-İzmir, Turkey.

* Corresponding Author: Tel.: +90.464 223 3385; Fax: +90.464 2234 118;
E-mail: eagirbas@gmail.com

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Abstract

Seasonal distribution of phytoplankton size classes (picoplankton, nanoplankton, microplankton) derived from HPLC pigment analysis was investigated along the south-eastern Black Sea. A large range of *in-situ* Chl-*a* concentrations was observed along the study area, ranged from 0.35 to 4.57 µg L⁻¹ with statistically significant difference. The contribution of phytoplankton size classes to total phytoplankton biomass varied between 1 and 71%; 1 and 92%; 8 and 93% for picoplankton, nanoplankton and microplankton, respectively. The ratio of N to P revealed nutrient limitation shifted from P to N from summer to autumn. An increase in the ratio of Si to N in May and August suggested N limitation in spring and summer. Canonical correspondence analysis revealed that microplankton were found to be positively associated with temperature, Chl-*a* and N/P in autumn and spring, whereas nanoplankton positively correlated with temperature, nitrite+nitrate and N/P, and negatively associated with salinity in spring. Picoplankton were highly correlated with temperature in summer and negatively correlated with key nutrients and temperature in spring. The data indicated that pigment derived community composition in the south eastern Black Sea has seasonal pattern governed by environmental factors.

Keywords: Phytoplankton, pigment, size classes, hplc, Black Sea

Introduction

Phytoplankton constitute almost 50% of the total primary production on Earth (Jeffrey & Veski, 1997; Longhurst, Sathyendranath, Platt, & Caverhill, 1995; Field, Behrenfeld, Randerson, & Falkowski, 1998; Falkowski, Katz, Knoll, Quigg, Raven, Schofield, & Taylor, 2004) and constitute the base of the marine food web that supporting directly or indirectly all the animal populations of the aquatic ecosystems. They also contribute significantly to climatic process (i.e. photosynthetic carbon fixation, downward transport of organic matter; CO₂ concentration and pH of the ocean) and provide nuclei for atmospheric water condensation (Charlson, Lovelock, Andreae, & Warren, 1987; Takahashi *et al.*, 2002). Shifts in phytoplankton size classes (PSC), community structure and biomass affect metabolic rates, growth, nutrients concentrations and energy transfer to higher trophic levels (Chassot, Bonhommeau, Dulvy, Mélin, Watson, Gascuel, & Le Pape, 2010; Chisholm, 1992; Marañón, 2009; Maloney & Field, 1991; Guidi, Stemann, Jackson, Ibanez, Claustre, Legendre, Picheral, & Gorsky,

2009; Laws, Falkowski, Smith Jr, Ducklow, & McCarth, 2000). Hence, phytoplankton biomass and size structure are considered as ecological indicators for the marine environment (Platt & Sathyendranath, 2008). Due to important global role of phytoplankton, monitoring their biomass has high priority in oceanographic research (Jeffrey *et al.*, 1997; Sathyendranath, 1986; Sathyendranath, Watts, Devred, Platt, Caverhill, & Maass, 2004; Nair *et al.*, 2008). To monitor changes in phytoplankton distribution and composition including their influence on global ocean biochemistry, and to identify stable oceanographic province boundaries, requires intensive observations over extensive temporal and spatial scales (Gibb *et al.*, 2000).

Chlorophyll *a* (Chl-*a*) is accepted as a unique molecular marker of phytoplankton biomass. The major role of Chl-*a* is to absorb light for photosynthesis, but there are additional accessory pigments such as the chlorophylls *b* and *c* and various carotenoids, which have a significant role in extending the light-harvesting spectrum in the phytoplankton groups (Barlow, Aiken, Moore, Holligan, & Lavender, 2004). While Chl-*a* is used as

a convenient proxy of phytoplankton biomass, many other phytoplankton pigments (e.g. fucoxanthin, 19'-hexanoyloxyfucoxanthin, and 19'-butanoyloxyfucoxanthin is accepted as biomarkers for diatoms, prymnesiophytes and chrysophytes, respectively) exhibit chemotaxonomic associations which may be exploited to map the oceanographic distribution and composition of phytoplankton assemblages (Gibb *et al.*, 2000; Wright & Jeffrey, 1987; Bjørnland & Liaaen-Jensen, 1989; Barlow, Mantoura, Gough & Fileman, 1993). Traditionally, spectrophotometry and fluorometry have been used to determine Chl-*a* (e.g. Holm-Hansen, Lorenzen, Holmes & Strickland, 1965; Lorenzen, 1967). However, the mentioned methods suffer from inaccuracies associated with spectral interferences from chlorophyll *b*, carotenoids and from Chl-*a* degradation products (e.g. chlorophyllides, phaeophytins and phaeophorbides). The using of HPLC facilitated the separation and quantification of other pigments in marine phytoplankton. The exploitation of pigment data generated from HPLC analysis of phytoplankton extracts has greatly advanced our understanding of the distribution, composition and functionality of phytoplankton in the global ocean (Jeffrey, 1997).

The Black Sea is a unique marine environment which has suffered from severe ecological deteriorations over the last three decades (Oguz, 2005). It is a semi-enclosed and largest anoxic marine ecosystem in the world ocean (Tolmazin, 1985). A considerable amount of chemicals, organic matter and nutrients from surrounded rivers (especially in the western Black Sea from the River Danube) affect the Black Sea ecosystem (Eker-Develi & Kideys, 2003; Yilmaz, Coban-Yildiz, & Tugrul, 2006). Surface salinity is around 17 ‰, and excess precipitation together with run-off from the rivers (e.g. Danube,

Dniester, and Don etc) constitutes a surface with low salinity layer overlying a halocline at about 100 m (Longhurst, 2007). Sea surface temperature (SST) exhibits typical seasonal characteristic with the highest in August and the lowest in February (Agirbas, Feyzioglu, Kopuz, & Llewellyn, 2015). Phytoplankton community composition and group ratios have drastically changed due to dramatic ecological changes occurred in the Black Sea ecosystem. Traditionally, phytoplankton studies in the Black Sea are conducted by microscopic examination (e.g. Bologa, 1986; Cociasu *et al.*, 1997; Moncheva & Krastev, 1997; Ivanov, 1965 etc). Despite significant roles of phytoplankton communities, information about Phytoplankton Size Classes (PSC) derived from pigment composition by using HPLC are limited in the Black Sea (Agirbas *et al.*, 2015; Ediger, Soydemir, & Kideys, 2006; Eker-Develi, Berthon, Canuti, Slabakova, Moncheva, Shtereva, & Dzhurova, 2012). Therefore, in order to establish the changes in the PSC reported from the Black Sea, particular attention has been paid to reveal seasonal changes in *in-situ* Chl-*a* and PSC derived from HPLC pigment analysis in the south eastern (SE) Black Sea; and to investigate the relationship among the indicators (*in-situ* Chl-*a*, PSC and nutrient concentrations) over the same period.

Materials and Methods

Samples for the determination of spatio-temporal distribution of phytoplankton size classes (picoplankton, <0.2-2µm; nanoplankton, 2-10 µm; mikroplankton, >10 µm) at 12 stations were collected seasonally from November 2014 to August 2015 along the south-eastern Black Sea (Figure 1). Seawater samples (1 liter for each depth) for pigment and nutrient analysis were taken from the surface to

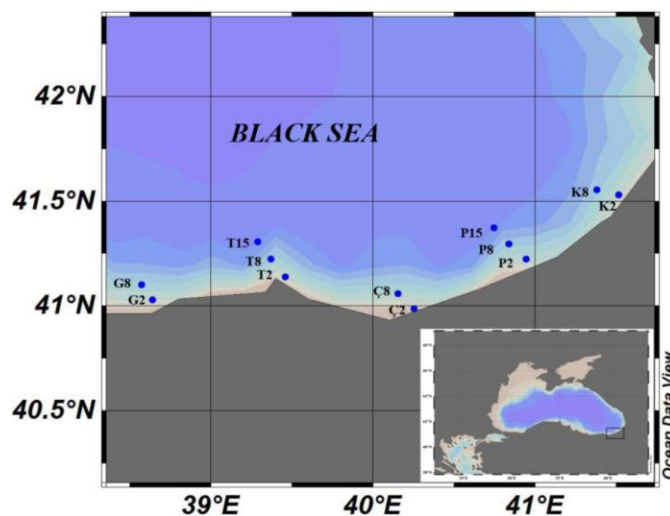


Figure 1. Location of sampling stations in the South-eastern Black Sea (G2: Giresun 2 miles, G8: Giresun 8 miles, T2: Trabzon 2 miles, T8: Trabzon 8 miles, T15: Trabzon 15 miles, C2: Camburnu 2 miles, C8: Camburnu 8 miles, P2: Pazar 2 miles, P8: Pazar 8 miles, P15: Pazar 15 miles, K2: Kemalpaşa 2 miles, K8: Kemalpaşa 8 miles).

40 m with 10 m intervals by using SBE32 Carousel rosette sampler. *In-situ* Chl-*a* and other parameters (e.g. temperature, salinity etc) were obtained by using SBE 25 CTD probe.

Nutrient Analysis

A 250 ml seawater samples for dissolved inorganic nutrients (NO₃-N, NO₂-N, PO₄-P and SiO₂-Si) were filtered through 0.45 µm cellulose acetate filters. The filtrate was collected in 100 mL acid-washed high-density polyethylene bottles and then was kept frozen (-20°C) until the analysis. The analyses were conducted by a SEAL auto-analyser in Central Fisheries Research Institute (CFRI) in Trabzon.

Pigment Analysis

Water samples (1 L) for pigment analysis filtered through GF/F filters (nominal pore size 0.7 µm and 47 mm diameter), and stored in liquid nitrogen (-196°C) with cryo vials until HPLC analysis. Phytoplankton pigments were determined by HPLC analysis, using methods reported by Barlow, Cummings, & Gibb (1997) and Llewellyn, Fishwick, & Blackford (2005). In the laboratory, the frozen filters were extracted in 5 ml 90% HPLC grade acetone, ultrasonicated (Bandelin Sonopuls HD 2070) for 60 s and centrifuged for 10 min at 3500 rpm to remove cellular debris. Pigment separations were achieved using a C8 column (Thermo Hypersil MOS-2, 150 x 4.6 mm, 3 µm particle size, 120 Å pore size and % 6.5 carbon load) connected to a Shimadzu LC-20 AT/Prominence HPLC system equipped with solvent pump (flow rate 1 ml min⁻²), auto sampler, a UV absorbance, fluorescence and a diode array detector (DAD) at two different wavelengths (450 and 665 nm) and LC solution software. Eluant A consisted of 100% methanol: 1M ammonium acetate (80:20

v/v) and eluant B was composed of 100% methanol. Pigments were identified using retention time and spectral match using PDA (Jeffrey *et al.*, 1997), and pigment concentrations were calculated using response factors generated from calibration using a suite of pigment standards (DHI Water and Environment, Denmark). Seven major pigments are thus selected as being representative of distinct phytoplankton groups (Figure 2). These seven pigments are fucoxanthin, peridinin, 19-hexanoyloxyfucoxanthin, 19-butanoyloxyfucoxanthin, alloxanthin, chlorophyll b and divinyl chlorophyll b, and zeaxanthin (Uitz, Claustre, Morel, & Hooker, 2006).

Deriving Phytoplankton Size Classes from Diagnostic Pigments

The relative biomass proportions of phytoplankton size classes [picoplankton (< 2 µm); nanoplankton (2-20 µm) and microplankton (20-200 µm)] were determined depending on the approaches of Uitz *et al.* (2006) and Aiken *et al.* (2009).

$$DP_w = 1.41[Fuco] + 1.41[Perid] + 1.27[Hex - fuco] + 0.35[But - fuco] + 0.60[Allo] + 1.01[TChlb] + 0.86[Zea]$$

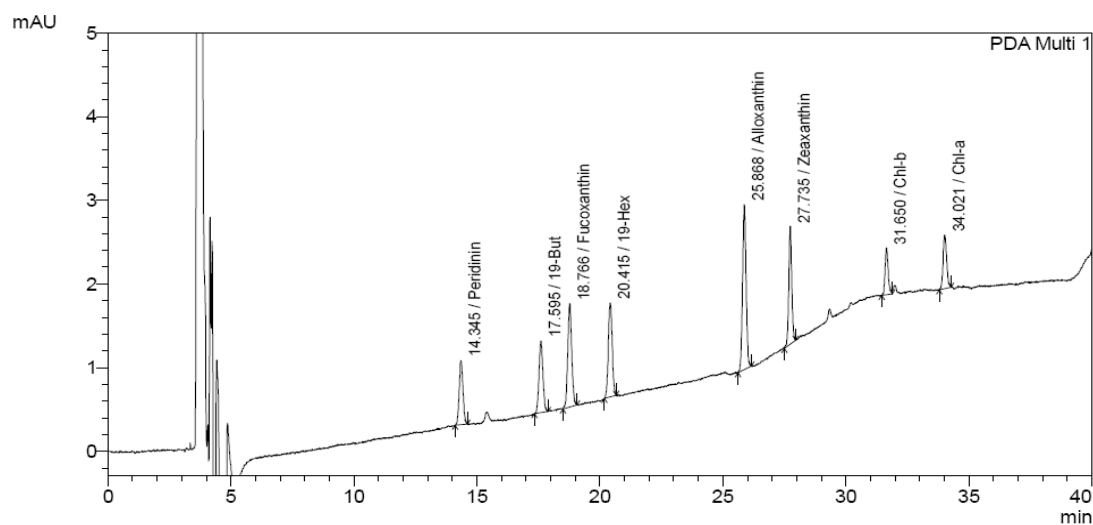
where DP_w represents the chlorophyll a concentration, which can be reconstructed from the knowledge of the concentration of the seven diagnostic pigments. The fractions of the three pigment-based phytoplankton size classes are computed following equations:

$$f_{micro} = (1.41[Fuco] + 1.41[Perid])/DP_w$$

$$f_{nano} = (1.27[Hex - fuco] + 0.35[But - fuco] + 0.60[Allo])/DP_w$$

$$f_{pico} = (1.01[TChlb] + 0.86[Zea])/DP_w$$

The actual chlorophyll a concentration associated with each class is derived from following



1 PDA Multi 1/440nm 4nm
Figure 2. Mix chromatogram for the pigment standards.

equations:

$$\text{Micro} - [\text{Chla}] = f_{\text{micro}} \times [\text{Chla}]$$

$$\text{Nano} - [\text{Chla}] = f_{\text{nano}} \times [\text{Chla}]$$

$$\text{Pico} - [\text{Chla}] = f_{\text{pico}} \times [\text{Chla}]$$

Statistics

One way analysis of variance (ANOVA) was used on normally distributed data to test for significant differences in phytoplankton, pigment compositions and nutrient concentrations for each station. The ANOVA critical significance value P was given in the text to indicate the level of difference. To

reveal correlations between phytoplankton communities and environmental factors, Canonical Correspondence Analysis (CCA) was also performed by using CANOCO 4.5 software (Ter Braak and Smilauer *et al.*, 2002).

Results

Hydrography

Temperature and salinity profiles along the stations revealed a typical hydrographic pattern of the Black Sea (Figure 3 and Figure 4). Sea surface temperature (SST) ranged from 8.58°C (February) to

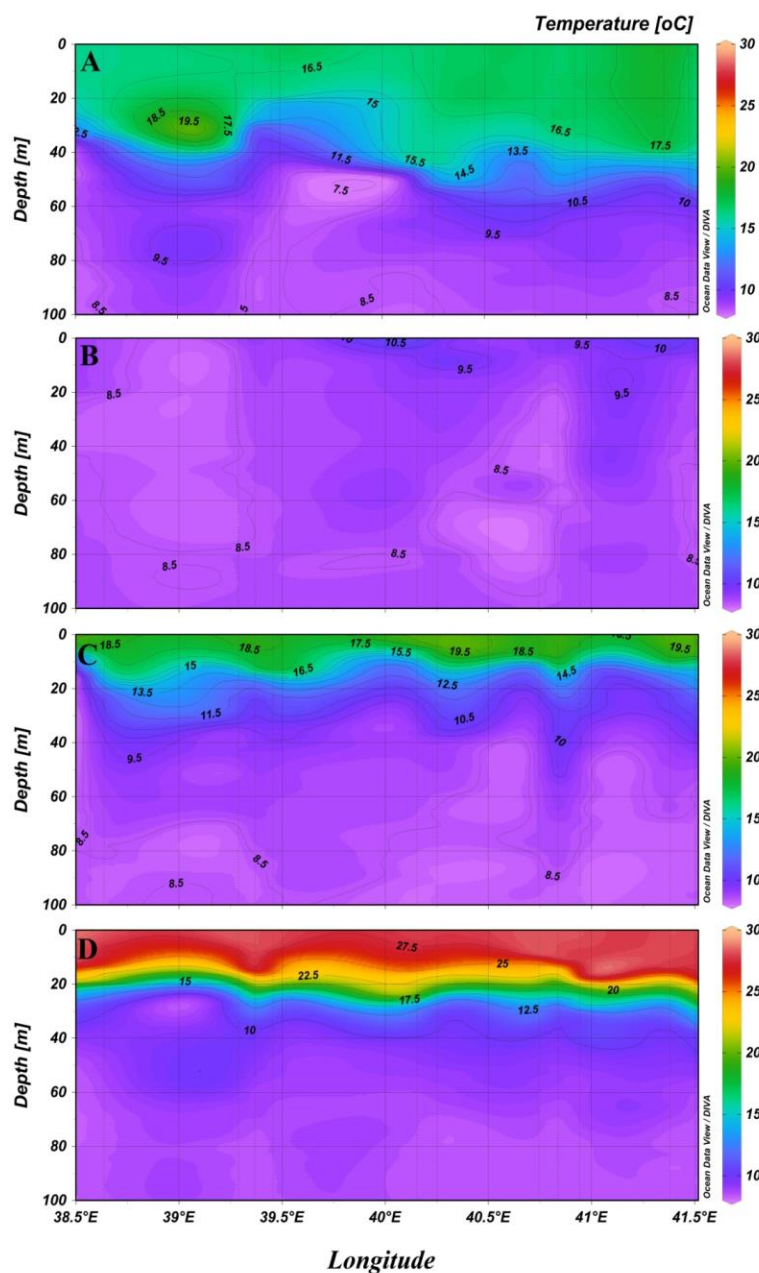


Figure 3. Spatio-temporal variation of temperature along the study area (A: Autumn 2014, B: Winter 2015, C: Spring 2015 and D: Summer 2015).

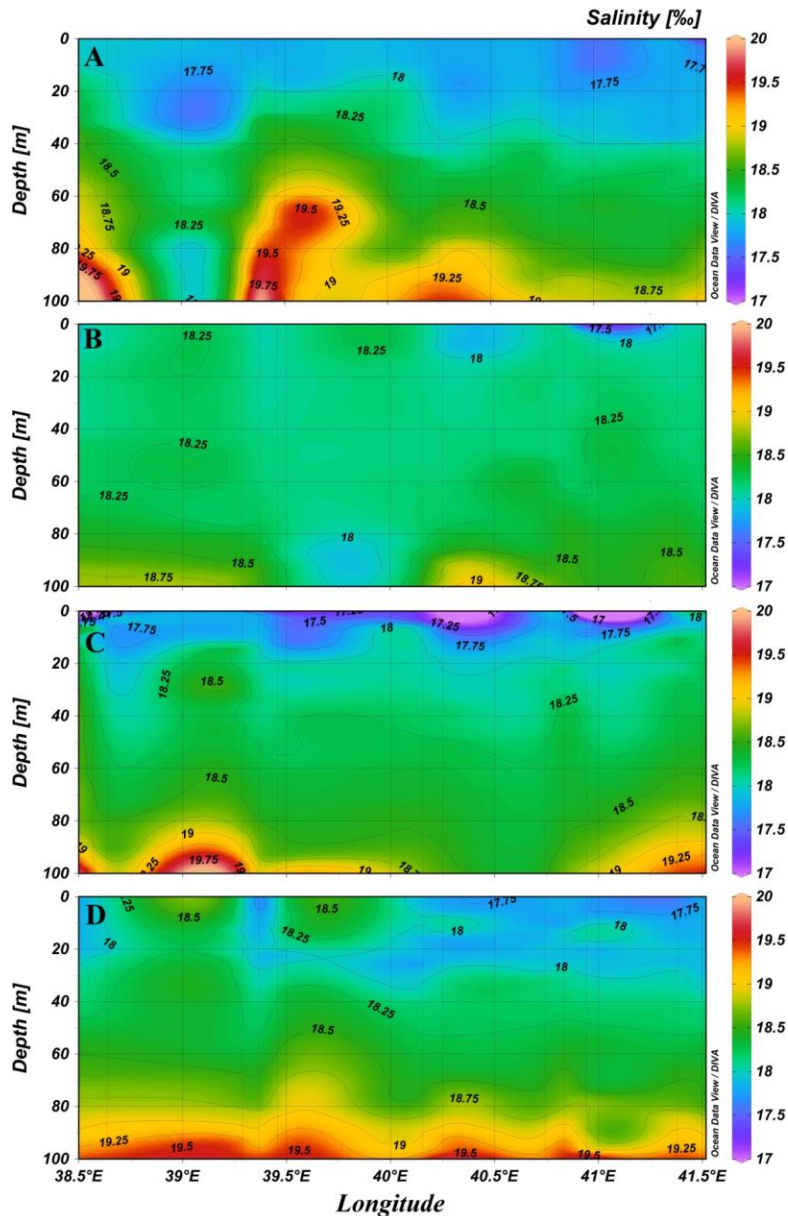


Figure 4. Spatio-temporal variation of salinity along the study area (A: Autumn 2014, B: Winter 2015, C: Spring 2015 and D: Summer 2015).

28.41°C (August) along the study area (Figure 3) with statistically significant difference (ANOVA, $p < 0.001$). In general, a well-mixed water column was formed in February, whereas stratification was observed in November and August. The permanent thermocline was detected between 20 and 50 m depths during the study period. Surface salinity varied from 16.13‰ (May) to 18.18‰ (August; Figure 4) and revealed seasonal difference (ANOVA, $p < 0.001$).

Spatio-Temporal Distribution of Nutrients

Nutrient distribution (i.e. nitrite+nitrate, phosphate and silicate) fluctuated seasonally during the study period with statistically significant difference (ANOVA, $P < 0.001$; Figure 5, Figure 6 and

Figure 7). In May and August, nutrient concentrations were found to be relatively uniform and low when compared to November and February. Extensive vertical mixing process during autumn and winter seasons resulted in high nutrient concentrations within the water column. In general, nutrient concentrations were patchy in November and February along the stations.

The highest nitrite+nitrate concentration (2.12 μM) was recorded in November, whereas the lowest one (0.001 μM) was measured in May and August at all stations (Figure 5). Phosphate concentrations along the stations were generally low and never exceeded 0.3 μM . The highest phosphate concentrations were measured as 0.28 μM in November and, lowest ones were obtained in May and August (Figure 6). Silicate

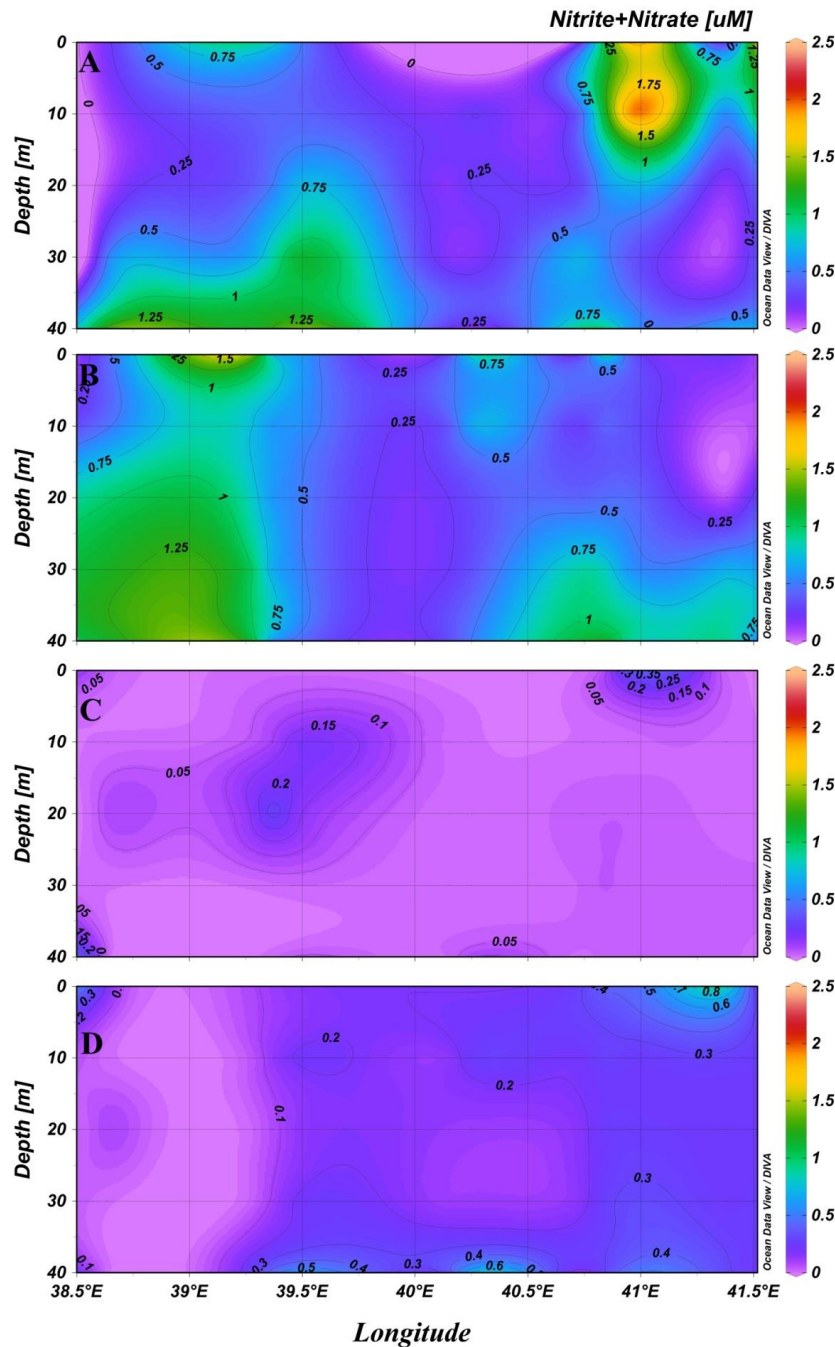


Figure 5. Spatio-temporal variation of Nitrite+Nitrate concentrations along the study area (A: Autumn 2014, B: Winter 2015, C: Spring 2015 and D: Summer 2015)

concentrations typically increased with depth and were represented in higher concentrations (Figure 7). The highest silicate concentration was recorded as $13.63 \mu\text{M}$ in November at the deepest part of the stations, whereas the lowest ones ($0.01 \mu\text{M}$) stations) were measured during February and May at surface waters.

The ratio of nutrients (e.g. N/P and Si/N) revealed statistically significant difference (ANOVA, $p < 0.001$) along the stations (Table 1). While the highest N to P ratio recorded in May whereas the highest ratio of Si to N detected in August. The ratio

of N to P increased from 122 to 606 in May, and decreased in November, which revealed a shift in P limitation to N limitation from summer to autumn. Similarly, an increase in the ratio of Si to N was detected in May and August, which suggested N limitation in spring and summer. Moreover, key nutrients were also statistically correlated with phytoplankton size classes (Table 2). Overall, microplankton were negatively correlated with phosphate and silicate in November and February (Pearson rank correlation, $p < 0.05$; $r = -0.27$ and -0.45 , respectively), positively correlated with silicate in

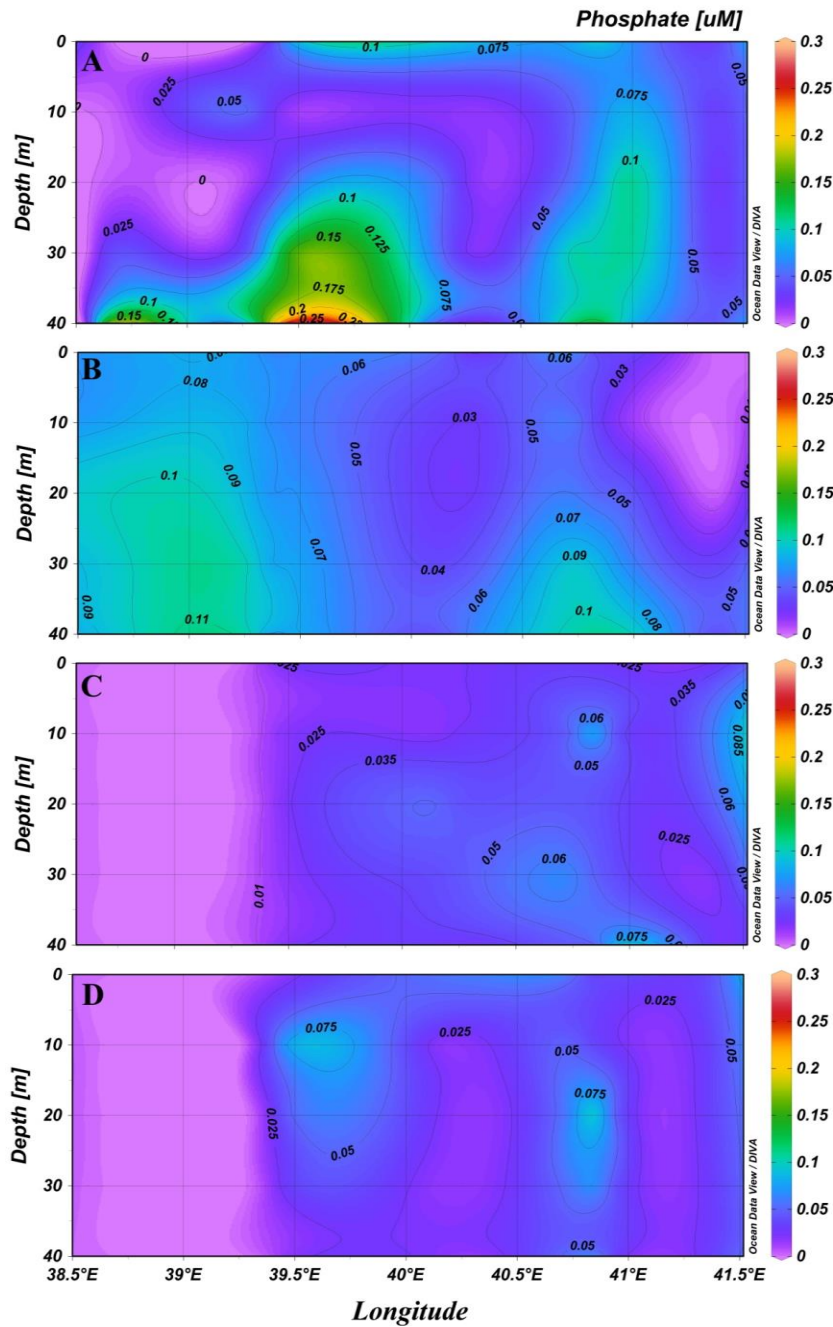


Figure 6. Spatio-temporal variation of Phosphate concentrations along the study area (A: Autumn 2014, B: Winter 2015, C: Spring 2015 and D: Summer 2015)

August (Pearson rank correlation, $p < 0.05$; $r = 0.45$). On the other hand, nanoplankton positively correlated with phosphate in February (Pearson rank correlation, $p < 0.05$, $r = 0.37$). Picoplankton positively correlated with phosphate in November (Pearson rank correlation, $p < 0.05$; $r = 0.29$), and negatively correlated with silicate in August (Pearson rank correlation, $p < 0.05$; $r = -0.44$).

Spatio-Temporal Distribution of *In-situ* Chl-*a*

A large range in *in-situ* Chl-*a* concentrations was observed along the study area (Figure 8), varied

from 0.35 to 4.57 $\mu\text{g}\cdot\text{l}^{-1}$ with statistically significant difference (ANOVA, $p < 0.001$). When the temporal variation evaluated in Chl-*a* concentrations, the levels showed fluctuations over the seasons, and the majority of the Chl-*a* was recorded in November and February (Figure 8A, Figure 8B). In general, Chl-*a* levels were high in coastal stations where was under the influence of river runoff. During the stratified periods (i.e. autumn and summer periods), Chl-*a* maxima coincided with seasonal thermocline, whereas during extensive vertical mixing process vertical profile of Chl-*a* concentrations revealed much patchy pattern along the stations (Figure 8B).

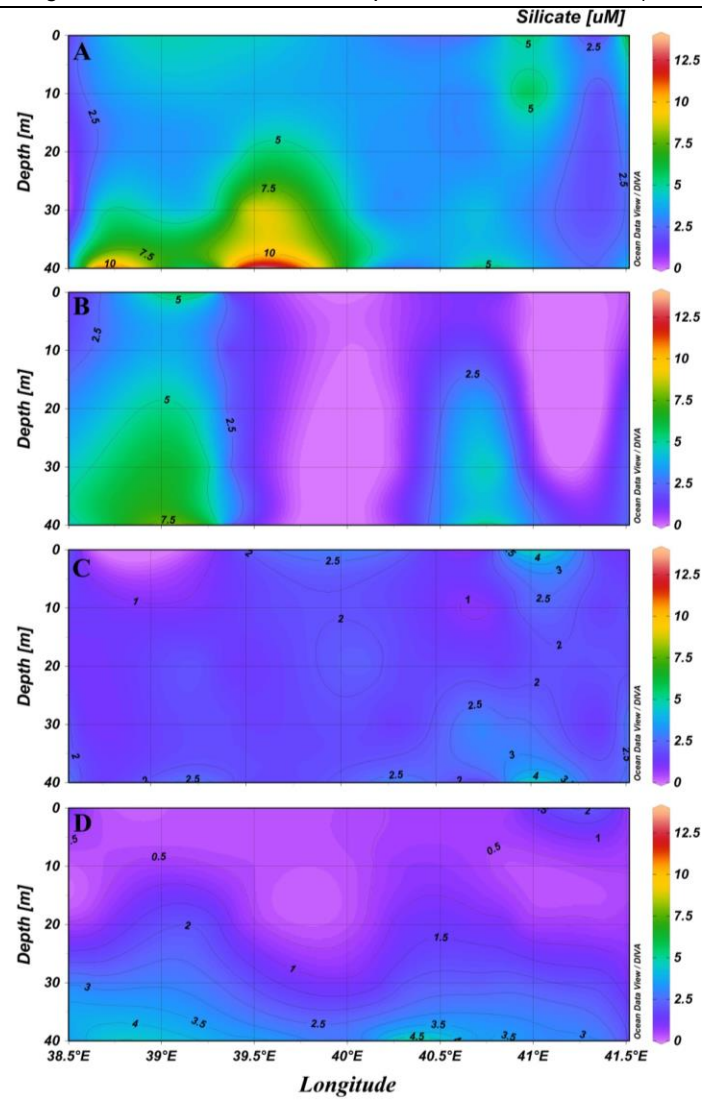


Figure 7. Spatio-temporal variation of Silicate concentrations along the study area (A: Autumn 2014, B: Winter 2015, C: Spring 2015 and D: Summer 2015)

Table 1. The ratio of key nutrients during the study period

Season	N/P	Si/N
Autumn	0,55-122	2,91-2297
Winter	1,91-246	0,03-1185
Spring	0,01-606	2,04-2920
Summer	1-387	0,65-4307

Table 2. Pearson rank correlation between nutrients and PSC

Season	PSC	NO ₂ +NO ₃	PO ₄ -P	SiO ₂ -Si
Autumn	Picoplankton	0,14	0,29	0,06
	Nanoplankton	0,06	0,13	0,02
	Microplankton	-0,12	-0,27	-0,05
Winter	Picoplankton	-0,02	0,20	0,18
	Nanoplankton	0,23	0,37	0,28
	Microplankton	-0,22	-0,45	-0,35
Spring	Picoplankton	-0,01	-0,04	0,04
	Nanoplankton	0,19	-0,03	0,03
	Microplankton	-0,13	0,05	-0,05
Summer	Picoplankton	0,18	0,22	-0,44
	Nanoplankton	-0,09	-0,15	-0,07
	Microplankton	-0,14	-0,14	0,45

PSC: Phytoplankton Size Classes, Bold ones indicate statistically significant correlation at the level of 95% confidence limit.

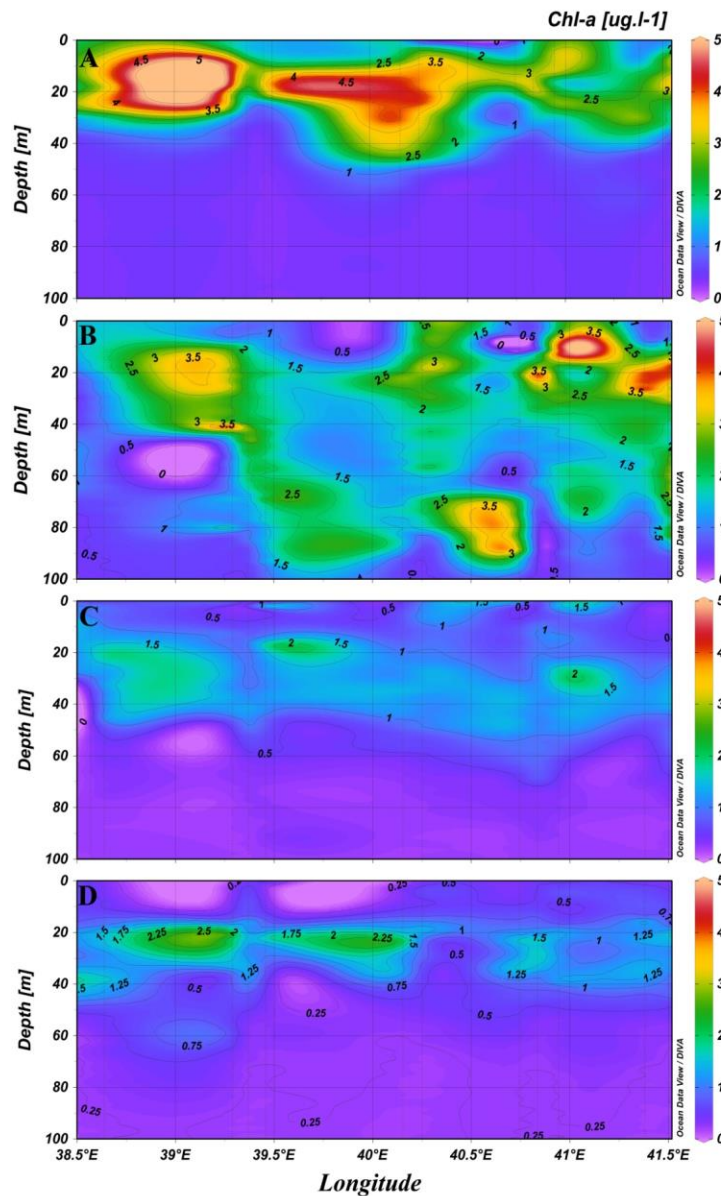


Figure 8. Spatio-temporal variation of *in-situ* chlorophyll-a along the study area (A: Autumn 2014, B: Winter 2015, C: Spring 2015 and D: Summer 2015)

Information about Chl-*a* concentration and its variation also reflect the trophic status for a given area. In order to reveal of seasonal differences, here, the *in-situ* Chl-*a* data were partitioned according to approximate trophic status: oligotrophic (picoplankton, Chl-*a* < 0.25 mg m⁻³), mesotrophic (nanoplankton, Chl-*a* > 0.25-1.2 mg m⁻³) and eutrophic (microplankton, Chl-*a* > 1.2 mg m⁻³) (Aiken *et al.*, 2009). In November and February, the study area was the eutrophic condition, especially first 50 m of the water column. In those periods, the system was dominated by microplankton and nanoplankton. During May and August, trophic conditions shifted towards to mesotrophic. Oligotrophic conditions were generally detected in stratified periods and below the Chl-*a* maximum with dominance of picoplankton.

Spatio-Temporal Distribution of Phytoplankton Size Classes (PSC)

There were notable differences in phytoplankton size classes (PSC) along the stations (One Way ANOVA, $p < 0.001$; Figures 9, 10, and 11). In general, the contribution of PSC to total phytoplankton ranged from 1% to 71%; from 1% to 92%; and from 8% to 93% for picoplankton, nanoplankton and microplankton, respectively. The phytoplankton were dominated by microplankton in autumn, winter and moderately in spring. On the other hand, their contribution decreased in summer (Figure 11). The second important group along the stations was nanoplankton. They were characterized with the high contribution in late winter and spring period at Chl-*a*

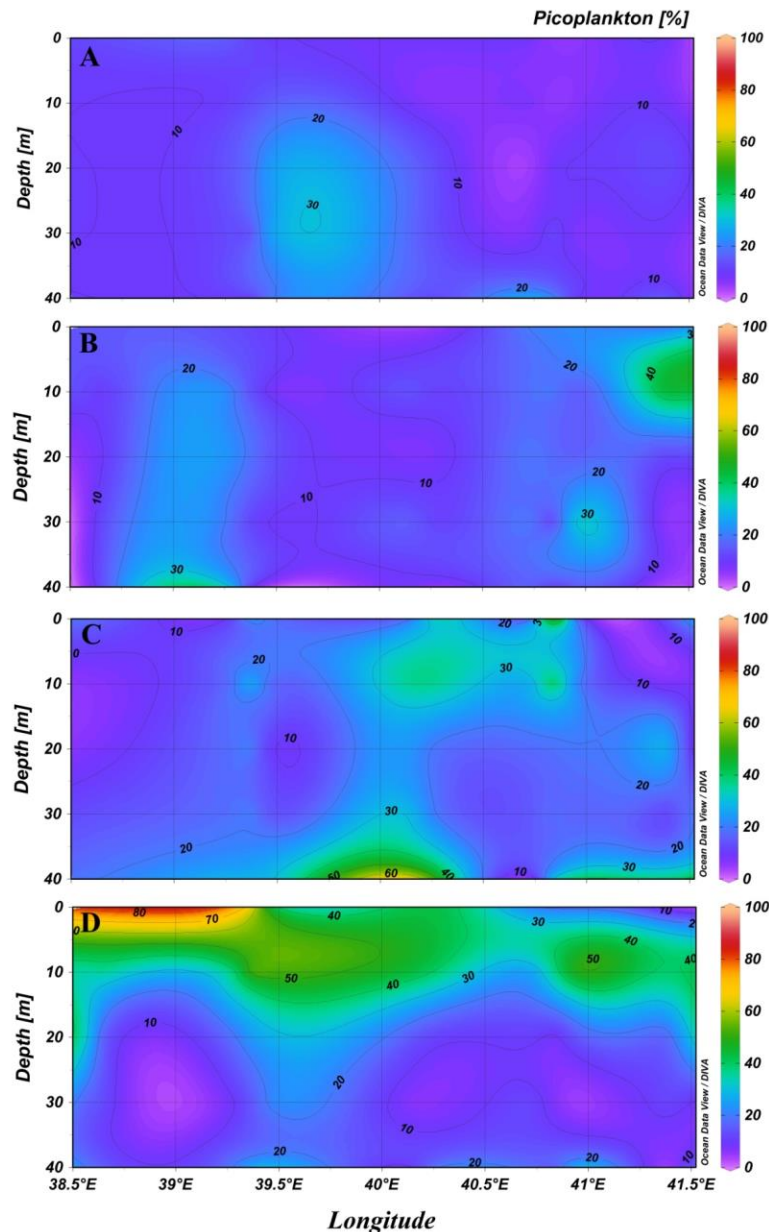


Figure 9. Spatio-temporal variation of picoplankton contribution (%) along the study area (A: Autumn 2014, B: Winter 2015, C: Spring 2015 and D: Summer 2015)

maxima (Figure 10). The contribution of picoplankton increased in surface waters of summer period (Figure 9).

Vertically, there were also significant differences in PSC along the stations. In November and February, microplankton was the prominent component of the PSC in the water column (Figure 11). Nanoplankton made substantial contribution to PSC in winter especially for Pazar and Hopa stations and in spring around 20 m (Figure 10). The contribution of picoplankton to PSC increased at surface layers in August, reached up to 50%. After 20 m, microplankton replaced with picoplankton along the study area.

Due to using Chl-*a* as a typically phytoplankton biomass, PSC correlated with size fractionated Chl-*a*

along the study area. The correlation between PSC and size fractionated Chl-*a* was also statistically significant (Figure 12). Interestingly, correlation between nanoplankton and nanoplankton fractionated Chl-*a* was much robust than micro- and picoplankton correlations ($p < 0.001$). Overall, statistically significant correlations indicate that pigment derived size fraction give an information about phytoplankton community composition in the south-eastern Black Sea.

Relationships between phytoplankton size classes and environmental factors

CCA revealed significant correlations between environmental variables (temperature, salinity, Chl-*a*,

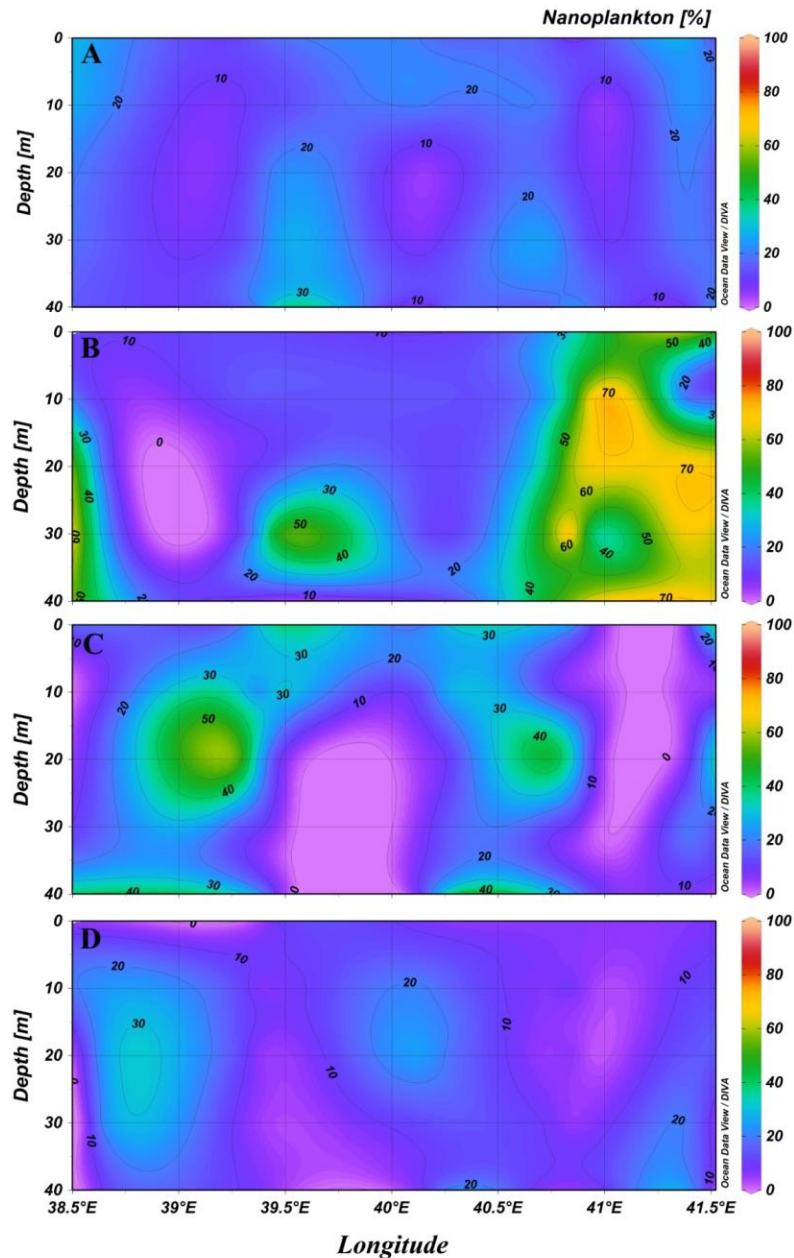


Figure 10. Spatio-temporal variation of nanoplankton contribution (%) along the study area (A: Autumn 2014, B: Winter 2015, C: Spring 2015 and D: Summer 2015)

nitrite+nitrate, phosphate, silicate, N/P and Si/N) and phytoplankton size classes ($p < 0.01$; Figure 13). Environmental variables explained 53.8% of the variance in phytoplankton size classes for autumn, 54.3% for winter, 70.7% for spring, and 64.9% for summer. In general, a notable separation between phytoplankton size classes was observed during the study period.

Microplankton were found to be positively associated with temperature, Chl-*a* and N/P, whereas nanoplankton and picoplankton were strongly associated with salinity, nitrite+nitrate and phosphate in autumn (Figure 12A). In winter, nanoplankton were positively associated with salinity, Chl-*a* and key nutrients. Picoplankton were strongly associated with

temperature, N/P and Si/P (Figure 12B). In spring, microplankton were positively associated with Chl-*a*. Nanoplankton were found to be positively associated with temperature, nitrite+nitrate and N/P and negatively associated with salinity. Picoplankton were positively associated with salinity and silicate, however negatively associated with temperature and other key nutrients (Figure 12C). In summer, microplankton positively correlated with Chl-*a*, silicate, N/P and Si/P, and negatively correlated with temperature. Nanoplankton were only correlated with salinity. Picoplankton were found to be positively correlated with temperature, and negatively correlated with Chl-*a* and key nutrients (Figure 12D).

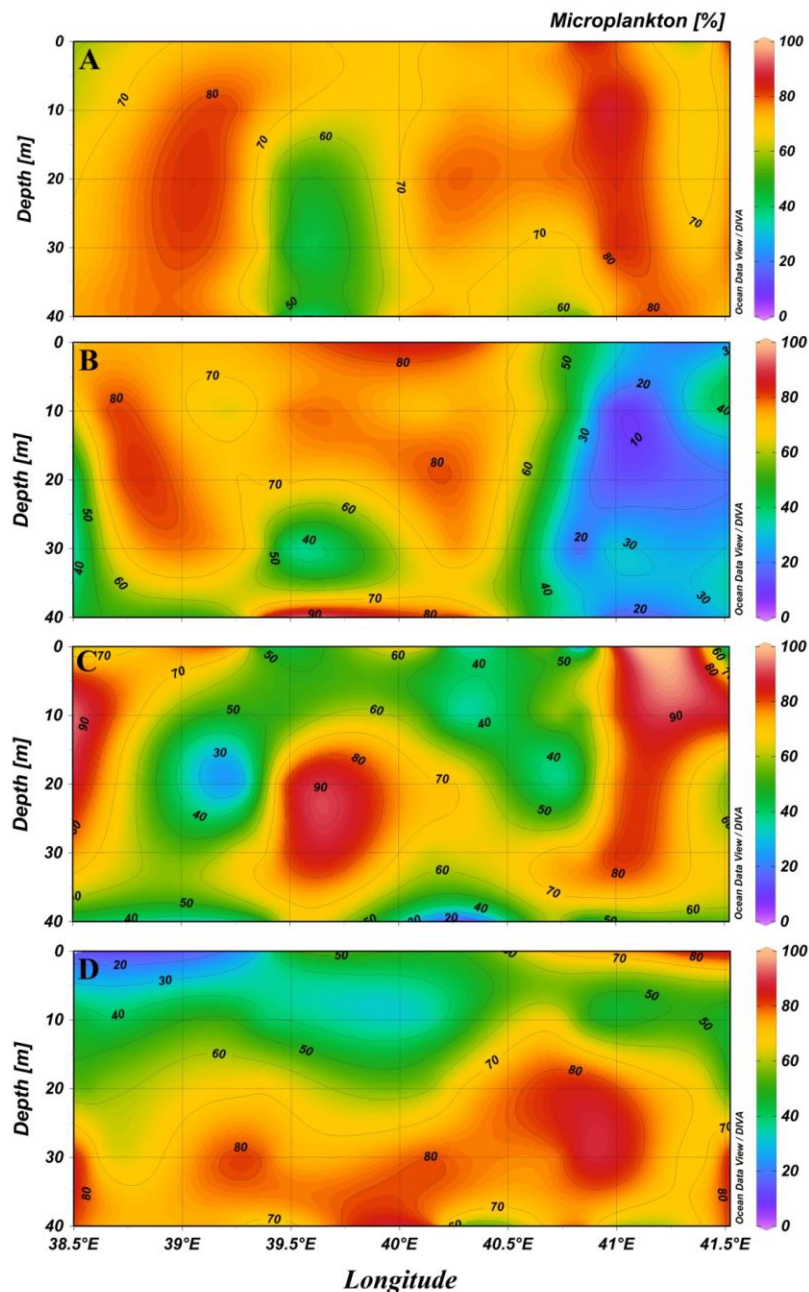


Figure 11. Spatio-temporal variation of microplankton contribution (%) along the study area (A: Autumn 2014, B: Winter 2015, C: Spring 2015 and D: Summer 2015)

Discussion

The data presented with this study makes a contribution to our understanding of the spatio-temporal distribution of phytoplankton size classes in the south eastern Black Sea. A large range of chlorophyll *a* concentrations was observed along the study area, ranged from 0.35 to 4.57 $\mu\text{g}\cdot\text{l}^{-1}$. The bulk of the Chl-*a* were recorded in November and February, although, the lowest concentrations recorded in August and below the thermocline. There was great variability in PSC over stations. Microplankton ratios were high in November and May. On the other hand, the concentrations were low

in August. Nanoplankton contribution was high in February and moderately in May. In contrast, picoplankton had high concentrations in August at surface waters. Overall, contribution of microplankton to PSC was higher than pico- and nanoplankton along the stations. These observations coincide with previous studies reported from the Black Sea (e.g. Agirbas *et al.*, 2015; Mikaelyan, Zatsepin, & Chasovnikov, 2013). Due to eutrophication, phytoplankton community composition, abundance, biomass, and bloom patterns of phytoplankton changed in the Black Sea. During that period, the biomass of dinoflagellates increased notably in the water column (Mikaelyan *et al.*, 2013). However,

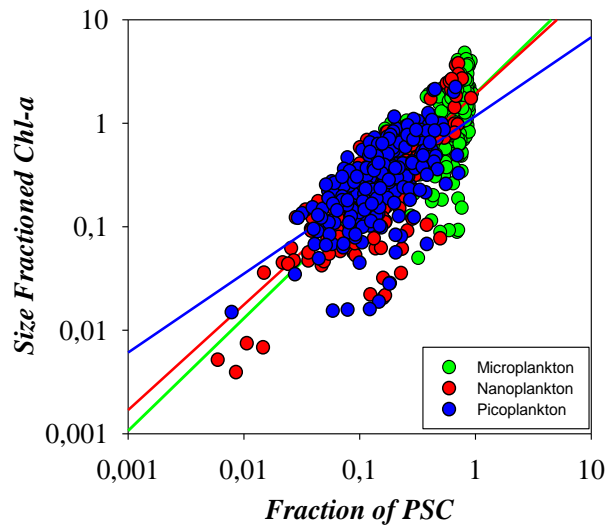


Figure 12. Linear regression between phytoplankton size classes (PSC) and size fractionated Chl-a (regression equations indicate picoplankton, nanoplankton and microplankton, respectively).

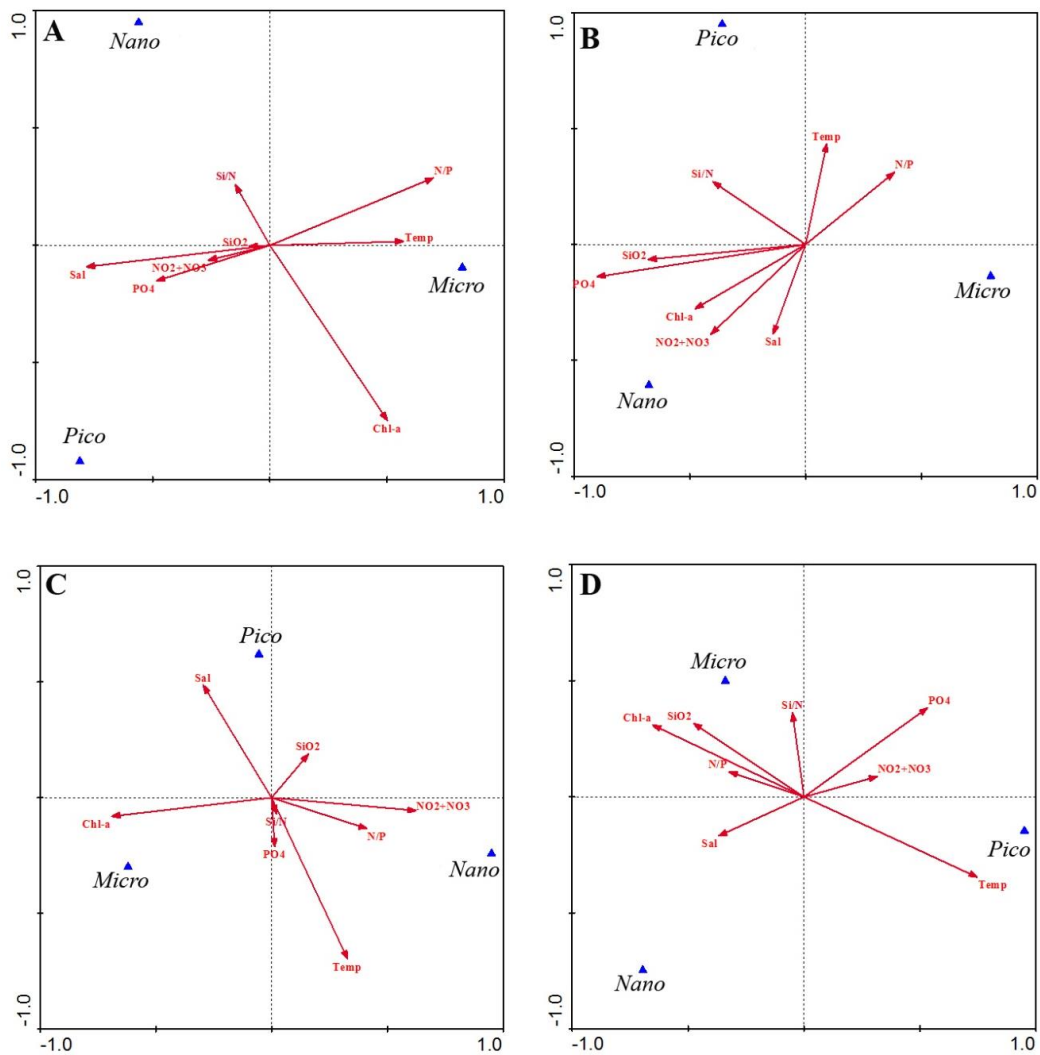


Figure 13. Canonical Correspondence Analysis ordination plots for environmental variables and phytoplankton size classes along the study area (A: Autumn 2014, B: Winter 2015, C: Spring 2015 and D: Summer 2015).

Black Sea has shown some signs of recovery (e.g. increases in diatoms abundance, decrease in the number of monospecific algal blooms etc.) in recent years (McQuatters-Gollop, Mee, Raitsos, & Shapiro, 2008). In the present study, dominance of microplankton supports the recovery reported from previous observations.

In the marine environment, the community composition of phytoplankton, species diversity and seasonal pattern are aspects of ecology that differ regionally; knowledge of this dynamics is core to understanding phytoplankton roles (Fishwick *et al.*, 2006). Phytoplankton community structure, and hence pigment ratios, adjust in response to changing environmental conditions (Trees, Clark, Bidigare, Ondrusek, & Mueller, 2000). On the other hand, phytoplankton size classes prosper different trophic status. Microplankton generally prosper high nutrient and have high Chl-*a*; nanoplankton are generally abundant in environments with some organic nutrients and have moderate Chl-*a*; picoplankton are generally abundant low nutrient environments and associated with lower Chl-*a* (Aiken *et al.* 2009; Maranon, 2009). In the present study, the majority of microplankton was detected in autumn and spring periods, when the highest nutrient concentrations were recorded (see also Figures 3-5). Analogously, statistically significant correlations obtained from CCA analysis between nutrients and PSC suggest that trophic status and environmental factors have a significant role on the phytoplankton community composition.

Aiken *et al.* (2009) reported that phytoplankton size classes are closely related to the trophic status of the environments. Picoplankton generally dominate the surface layers of warm, oligotrophic, low N waters; nanoplankton are more abundant in cooler, mesotrophic, moderate N waters; microplankton are dominant in eutrophic, high N waters. In the present study, similarly, high picoplankton contribution was observed in August at the surface waters, where the nutrients were depleted in the water column. On the other hand, nanoplankton contribution was generally substantial in winter and autumn below the Chl-*a* maxima, when the extensive vertical mixing occurred within the water column. Moreover, surface pigment as a proxy for PSC, adaptations are likely to be related to or controlled by the nutrient dynamics (Barlow *et al.*, 2004). Diatoms are opportunistic organisms that are able to respond quickly to nitrate enrichment (Fogg, 1991), and Chl-*a* molecules contain nitrogen atoms, while carotenoids do not (Porra *et al.*, 1997). Hence, the nitrogen characteristic of diatoms and chlorophylls probably explain high contribution of microplankton in nutrient rich waters during autumn and winter along the study area. It was also reported that the presence of diatom-nanoflagellate communities in upwelled waters in the Arabian Sea (Latasá & Bidigare, 1998; Barlow, Mantoura, & Cummings, 1999), and the dominance of the picoplankton in oligotrophic, low chlorophyll waters

towards the equator (Barlow *et al.*, 2004). Ondrusek, Bidigare, Sweet, Defreitas, & Brooks (1991) reported from across the north Pacific that the dominance of diatoms in nitrate-rich coastal waters, with cyanobacterial dominance in nitrate-poor mid-ocean regions. A strong relationship is reported between phytoplankton size and environmental factors (e.g. nutrients and light) that affect some metabolic activities (e.g. photosynthesis), dominance phytoplankton and succession (Chisholm, 1992; Bouman, Platt, Sathyendranath, & Stuart, 2005; Aiken *et al.*, 2008, Brewin *et al.*, 2010).

Field studies revealed that the vertical structure of phytoplankton size classes is governed by several mechanisms (Perez, Fernandez, Maranon, Moran, & Zubkov, 2006). Brewin (2010) reported that the percentage of picoplankton decreased with depth down to Chl-*a* maxima, and the nanoplankton percentage increased below the Chl-*a* maxima. Similar pattern was observed in oligotrophic gyres of south Pacific with great contribution of nanoplankton below the Chl-*a* maximum (Ras, Claustre, & Uitz, 2008). Claustre & Marty (1995) suggested that nanoplankton can develop close to the nutricline and their presence at very low light levels may be governed by nitrate availability as opposed to photoadaptation. They also suggested that reason for nanoplankton below the Chl-*a* maximum may include a decoupling between nitrate assimilation and CO₂ fixation, vertical migration and heterotrophic growth. Similarly in the present study, nanoplankton made main contribution below the Chl-*a* maximum especially in winter and spring.

Conclusion

Present study extends the previous knowledge of phytoplankton size classes over the spatial and temporal scale in the south-eastern Black Sea. Deriving phytoplankton size classes depending on HPLC-pigment analysis provides accurate and comprehensive information for a given area (Brewin *et al.*, 2010). However, the pigment based phytoplankton taxonomy does not strictly reflect the true size of phytoplankton communities. Some pigments are shared by various phytoplankton groups (e.g., fucoxanthin may also be found in some prymnesiophytes and pelagophytes), and also some phytoplankton groups may encompass a wide size range (e.g. diatoms are sometimes observed in the nanosize range, even if generally they belong to microplankton) (Uitz *et al.*, 2006). Hence, combining that method with other techniques (e.g. microscopy, flow-cytometry etc.) probably will give more comprehensive picture for the study area.

Statistically significant correlations among the variables (i.e. PSC, nutrients and *in-situ* Chl-*a*) clearly explain general pattern of PSC in relation to stations and environmental parameters. The differences observed in PSC may possibly be due to

phytoplankton adaptive strategy to environmental factors. These observations also suggested that the south eastern Black Sea was more oligotrophic in autumn and winter than in spring and summer. Additionally, the obtained results confirm that HPLC-derived PSC approach can be used for monitoring studies along the Black Sea.

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