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# Spatial and temporal variation in the distribution and abundance of *Synechococcus* spp., picoeukaryotes, nanoeukaryotes and Chlorophyll-*a* in the Eastern Black Sea

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The abundance and distribution of *Synechococcus* spp., picoeukaryotes, nanoeukaryotes, and chlorophyll-*a*, were studied through the water column (from 0 - 100 m depth) during four different seasons along the Eastern Black Sea coast. Based on annual average values, *Synechococcus* spp. were numerically dominant with an annual average of  $3.40 \times 10^4$  cells mL<sup>-1</sup>, ranging between 0.51 and  $9.93 \times 10^4$  cells mL<sup>-1</sup>, followed by picoeukaryotes with an annual average of  $0.79 \times 10^3$  cells mL<sup>-1</sup>, ranging from 0.05 to  $3.93 \times 10^3$  cells mL<sup>-1</sup>. The nanoeukaryotes were the least abundant group in the region, with an annual average of  $3.26 \times 10^2$  cells mL<sup>-1</sup> ranging between 0.12 and  $17.98 \times 10^2$  cells mL<sup>-1</sup>. The minimum and maximum values of *Synechococcus* spp. and picoeukaryotes were found at stations from Fatsaand Arhavi, while nanoeukaryotes had their minimum-maximum abundance at stations from Yomraand Ordu. Overall, the *Synechococcus* spp. seasonal cycle exhibited a bimodal distribution, with one peak in summer and the other in autumn. In contrast, picoeukaryote and nanoeukaryote seasonal cycles had unimodal distributions, with peaks during winter. The abundance of *Synechococcus* spp. was significantly negatively correlated with depth during winter, while picoeukaryotes was considerably negatively correlated with depth during winter, while picoeukaryotes and chlorophyll-*a*, implying their coexistence. However, the picoeukaryotes were significantly negatively-correlated with nanoeukaryotes.

[Keywords: Abundance, Chlorophyll-a, Distribution, Flow cytometry, Phytoplankton]

# Introduction

Photosynthetic picoplankton plays a vital role in the pelagic food web (*e.g.*, form food for small protozoan predators) and can significantly contribute to the total phytoplankton biomass and photosynthesis<sup>1.4</sup>. They can account for up to 80 % of the phytoplankton biomass and productivity in tropical and subtropical waters, with the greatest contribution in oligotrophic oceans<sup>3,5,6</sup>. Their dynamics and distribution are reported to be regulated by several environmental factors such as temperature<sup>7,8</sup>, salinity<sup>9,10</sup>, and the availability of nutrients<sup>11,12</sup>.

The seasonal changes in the distribution and abundance of phytoplankton have been scarcely documented in the Black Sea off the coast of Turkey. Although the data on the seasonal changes in abundance of *Synechococcus* spp. is available for spring, summer and autumn in episodes<sup>13-15</sup>. Also, Uysal<sup>16</sup> studied their pigment, size, growth and diurnal variability in detail, providing baseline data. However, no data are available for picoeukaryotes and nanoeukaryotes from this region.

This study quantified the abundance of *Synechococcus* spp., pico- and nanoeukaryotes during winter, spring, summer and autumn on the Eastern Black Sea coast to investigate the seasonal changes within these communities. Also, the concentration of Chlorophyll-*a* was determined for spring, summer and autumn. The results of this study could be incorporated into time series that will help to identify, understand and quantify the rate of climate change and its effects on phytoplankton communities<sup>12</sup>.

# **Materials and Methods**

# Study area and sample collection

Water sampling from the surface down to 100 m was conducted once every three months along 11 transects (totalling 40 stations), spanning over a 475 km stretch of the Eastern Black Sea coast between the towns of Arhavi in the east to Bafra in the west (Fig. 1). The water samples were collected during winter (February), spring (May) and summer (August) of 2013 from all stations. However, due to adverse weather conditions, during autumn



Fig. 1 — Map of the Black Sea with the study area

(December 2013), water samples were acquired only from Samsun and Ordu. The water temperature (°C), dissolved oxygen (mg L<sup>-1</sup>), salinity (ppt) and Sigma-t (kg m<sup>-3</sup>) were recorded with a Sea & Sun Tech M75 CTD profiler. The research vessel of the Trabzon Central Fishery Research Institute (SUMAE), SÜRAT Araştırma-1, was used for sample collection.

The sampling sites included a maximum of five offshore stations located (Fig. 1). Moreover, water samples were collected from the surface at 0, 10, 20, 30, 40, 50, 75 and 100 m depth with 24 - 10 L Ocean Test Equipment Niskin bottles attached to a Seabird SBE-32 carousel water sampler. From the sampled water, 50 mL was kept in opaque glass tubes fixed with glutaraldehyde (1 % final concentration) and stored at -20 °C for laboratory analysis by flow-cytometer.

# Flow-cytometry analysis

The procedures for flow-cytometry analysis were similar to those described by Feyzioglu *et al.*<sup>13</sup>. Analyses were performed using the BD Accuri C6 flow cytometer. *Synechococcus* spp. were discriminated and separated depending on their chlorophyll autofluorescence in bivariate scatter plots of red (FL3: 660-700 n) vs. orange fluorescence (FL2: 540-630 nm). The picoeukaryotes and nanoeukaryotes were determined through the relative cell size inside

scatter (SSC) vs. FL3 fluorescence bivariate scatter plots. The final cell counts (cells/litre) were determined from event counts in the phytoplankton regions and analysed volume.

# Statistical analysis

The data obtained with flow-cytometry as cells mL<sup>-1</sup> were analysed using several computer packages. Contour plots were generated using ODV 5.0.0 (Ocean Data View, http://odv.awi.de). The Principal Component Analysis (PCA) based on the Pearson correlation was used to highlight the relationships between different environmental parameters<sup>17,18</sup>. The data were standardised and then transformed  $(\log + 1)$  to remove the effect of outliers before PCA<sup>17</sup>. The statistical significance was tested using SigmaPlot version 13 (Systat Software, Inc., San Jose, California, USA, www.sigmaplot.com).

# Results

#### Abundance of phytoplankton groups

The phytoplankton community on the Eastern Black Sea coast is composed of three major groups: *Synechococcus* spp., picoeukaryotes and nanoeukaryotes. The data for each phytoplankton group are represented as contour plots. The mean ( $\pm 95$  % CI) abundance for each station during different seasons is also provided in Table 1 to facilitate the presentation of the results.

STATION	Synechococcus spp.(cells $mL^{-1}$ )			
	Winter (×10 <sup>4</sup> )	Spring (×10 <sup>4</sup> )	Summer (×10 <sup>4</sup> )	Autumn (×10 <sup>4</sup> )
		Samsun		
Bafra	$0.62\pm0.14$	$3.04 \pm 1.78$	$8.32\pm3.38$	$5.69 \pm 1.43$
Merkez	$3.09\pm2.34$	$2.05\pm0.60$	$9.07\pm4.02$	$7.10\pm3.27$
Çarşamba	$1.94 \pm 1.02$	$2.77\pm0.90$	$6.84\pm2.49$	$6.70\pm2.90$
		Ordu		
Merkez	$2.59\pm0.56$	$1.98\pm0.51$	$2.41\pm0.32$	
Fatsa	$0.51\pm0.17$	$1.93\pm0.78$	$2.57\pm0.79$	$2.79\pm0.70$
		Giresun		
Merkez	$1.73\pm0.48$	$1.27\pm0.42$	$1.71\pm0.38$	
		Trabzon		
Vakfikebir	$2.33\pm0.88$	$1.54\pm0.58$	$2.52\pm0.88$	
Değirmendere		$3.42 \pm 2.71$	$4.48\pm4.27$	
Yomra	$0.65\pm0.12$	$2.41\pm0.98$	$3.20 \pm 1.22$	
		Rize		
Çayeli	$1.96 \pm 1.18$		$5.94 \pm 1.26$	
		Artvin		
Arhavi	$1.18\pm0.23$	$9.50\pm 6.92$	$9.93\pm4.30$	
Overall	$2.06 \pm 0.36$	$237 \pm 0.45$	$523 \pm 0.84$	$5.46 \pm 0.94$



Fig. 2 — Contour plot of the vertical profile of the abundances of (a) *Synechococcus* spp.; (b) picoeukaryotes; and (c) nanoeukaryotes on the Eastern Black Sea coast, Turkey during 2013

#### Synechococcus spp.

The phytoplankton community was numerically dominated by *Synechococcus* spp., which contributed up to more than 92 % of the overall total abundance

(cells mL<sup>-1</sup>) and was found everywhere in the study area throughout the year (Fig. 2a). Their peak abundance appeared during summer and autumn, with the lowest abundance in winter (Table 1). Their highest concentration generally existed at 30 - 50 m depth in spring and summer and at 0 - 20 m depth, during autumn and winter.

#### Picoeukaryotes

Picoeukaryotes were the second most abundant phytoplankton group found throughout the year. From Samsun to Giresun, their minimum abundance appeared during winter, with peak abundance in spring. Between Trabzon and Rize, their maximum abundance was observed in winter (Table 2; Fig. 2b). The highest cell concentration was found at 30 - 40 m depth through the water, with the lowest cell counts (1.7 - 6.0 %) at 75 - 100 m depth.

#### Nanoeukaryotes

As compared to *Synechococcus* spp. and picoeukaryotes, in this study, nanoeukaryotes had the lowest cell count. From Samsun to Ordu, they had maximum abundance in winter, whereas after these stations, their highest cell concentration was found in spring (Table 3; Fig. 2c). Their maximum abundance was recorded at 0 - 30 m depth, with the lowest cell counts (4.0 - 14.7 %) at 50 - 100 m through the water column.

## Chlorophyll-a

\*T

The chlorophyll-*a* concentration was measured during spring, summer and autumn. The highest concentration of Chlorophyll-*a* was  $1.28\pm0.45$  during spring and  $1.61\pm1.08$  during summer (Table 4).

Maximum Chlorophyll-*a* concentrations were found above 50 m depth (Fig. 3).

## Hydrography

The water temperature (°C), dissolved oxygen (mg  $L^{-1}$ ), salinity (ppt) and Sigma-t (kg m<sup>-3</sup>) were measured at Samsun, Trabzon and Artvin stations that correspond to the west, middle and east extents of the study area (Fig. 4). The Sea Surface Temperature (SST) ranged from 8.6 – 26.5 °C for Samsun, 9.5 - 26.2 °C for Trabzon and 9.7 - 26.8 °C for Artvin; hence, the western side seems to be colder than the eastern part of the study area. The temperatures at 30 - 100 m depth were not notably different during all four seasons for each station (one-way ANOVA,  $F_{3,28} = 1.52$ , P = 0.232 for Samsun;  $F_{3,28} = 2.46$ , P = 0.083 for Trabzon and  $F_{3,28} = 2.04, P = 0.131$  for Artvin). However, they were remarkably distinct from each other ( $F_{11,84}$  = 2.77, P = 0.004). Generally, the highest dissolved oxygen concentration was measured at 50 - 60 m depth in summer and at 10 - 20 m depth during other seasons. The lowest concentration of dissolved oxygen was observed below 60 m depth. Salinity (range 17.5 - 20.8 ppt) showed a uniform distribution from 0 - 100 m depth during all four seasons, and the stations were not significantly different from each other  $(F_{11,120} = 1.182, P = 0.307)$ . Similarly, Sigma-t also showed a uniform distribution  $(F_{11,120} = 1.642, P = 0.095).$ 

Table 2 — Abundance (mean ±95 % CI) of picoeukaryotes, along the Eastern Black Sea coast during 2013

STATION		Picoeukary	otes (cells mL <sup><math>-1</math></sup> )	
	Winter (×10 <sup>3</sup> )	Spring (×10 <sup>3</sup> )	Summer (×10 <sup>3</sup> )	Autumn (×10 <sup>3</sup> )
		Samsun		
Bafra	$0.30\pm0.09$	$0.86\pm0.50$	$0.65\pm0.39$	$0.69\pm0.25$
Merkez		$0.49\pm0.22$	$0.40 \pm 0.21$	$0.85\pm0.28$
Çarşamba	$0.28\pm0.06$	$0.67\pm0.20$	$0.66\pm0.28$	$1.11 \pm 0.43$
		Ordu		
Merkez	$0.20\pm0.05$	$0.53\pm0.17$	$0.60 \pm 0.14$	
Fatsa	$0.05\pm0.03$	$1.02\pm0.39$	$0.90\pm0.33$	$0.80\pm0.24$
		Giresun		
Merkez	$1.92\pm0.54$	$0.39\pm0.13$	$0.68\pm0.28$	
		Trabzon		
Vakfıkebir	$2.58\pm0.88$	$0.42\pm0.14$	$0.63 \pm 0.25$	
Değirmendere		$0.64\pm0.37$	$0.57\pm0.31$	
Yomra	$0.83\pm0.16$	$0.69\pm0.36$	$0.47\pm0.15$	
		Rize		
Çayeli	$1.02\pm0.35$		$0.53\pm0.14$	
		Artvin		
Arhavi	$1.15\pm0.39$	$3.93 \pm 1.48$	$0.78\pm0.42$	
Overall	$0.92\pm0.16$	$0.77\pm0.15$	$0.69\pm0.12$	$0.80\pm0.15$
he mean ±95 % CI calcu	ulation included all cells (n	$hL^{-1}$ ) number from $0 - 100$	m depth at a station in a seas	son

STATION	Nanoeukaryotes (cells $mL^{-1}$ )			
	Winter ( $\times 10^2$ )	Spring ( $\times 10^2$ )	Summer ( $\times 10^2$ )	Autumn ( $\times 10^2$ )
		Samsun		
Bafra	$5.05\pm1.13$	$1.24\pm0.34$	$1.05\pm0.37$	$3.67 \pm 1.15$
Merkez		$1.60\pm1.33$	$6.60 \pm 5.34$	$2.98 \pm 1.68$
Çarşamba	$15.54\pm4.65$	$1.21\pm0.40$	$1.20 \pm 0.53$	$4.48\pm2.22$
		Ordu		
Merkez	$17.98\pm5.57$	$2.17\pm0.67$	$0.83\pm0.25$	
Fatsa	$2.03\pm0.66$	$4.58\pm0.281$	$1.08\pm0.62$	$3.20\pm1.31$
		Giresun		
Merkez	$1.88\pm0.49$	$2.03\pm1.00$	$1.17\pm0.37$	
		Trabzon		
Vakfıkebir	$1.34\pm0.39$	$2.23\pm0.76$	$1.23\pm0.67$	
Değirmendere		$2.90\pm2.04$	$2.63 \pm 1.97$	
Yomra	$0.12\pm0.09$	$2.47\pm0.99$	$1.93\pm0.75$	
		Rize		
Çayeli	$0.55\pm0.31$		$3.69 \pm 1.45$	
		Artvin		
Arhavi	$0.59\pm0.20$	$9.98 \pm 1.73$	$3.46 \pm 1.26$	
Overall	$5.55 \pm 1.31$	$2.40\pm0.37$	$1.94\pm0.33$	$3.61\pm0.70$

Table 4 — Abundance (mean ±95 % CI) of chlorophyll-a along the Eastern Black Sea coast during 2013

STATION	Chlorophyll- $a$ (µg L <sup>-1</sup> )			
	Spring	Summer	Autumn	
	Samsun			
Bafra	$0.66\pm0.17$	$0.47\pm0.16$	$0.85\pm0.18$	
Merkez	$1.21\pm0.66$	$1.61\pm1.08$	$0.69\pm0.23$	
Çarşamba	$1.09\pm0.29$	$0.49\pm0.12$	$0.50\pm0.15$	
	Ordu			
Merkez	$1.28\pm0.45$	$0.31\pm0.22$		
Fatsa	$1.19\pm0.39$	$0.23\pm0.07$	$0.53\pm0.20$	
	Giresun			
Merkez	$1.09\pm0.39$	$1.06\pm0.42$		
	Trabzon			
Vakfikebir	$1.15\pm0.39$	$0.66\pm0.20$		
Değirmendere				
Yomra	$0.85 \pm 0.24$	$0.75\pm0.25$		
	Rize			
Çayeli		$0.85\pm0.28$		
	Artvin			
Arhavi		$0.78\pm0.32$		
Overall	$1.05 \pm 0.12$	$0.68\pm0.09$	$0.71 \pm 0.11$	

\*The mean  $\pm 95$  % CI calculation included all cells (mL<sup>-1</sup>) number f

There was a considerable negative correlation between dissolved oxygen and depth and temperature and depth, whereas significant positive correlations were seen between salinity and depth, Sigma-t and depth (Fig. 5). Furthermore, dissolved oxygen and temperature tended to have a notable negative correlation with salinity and Sigma-t (p < 0.05).

# Principal components analysis

The first and second axes of the principal components explained 38.3 % and 33.6 % for winter, 43.8 % and 27.0 % for spring, 46.7 % and 21.8 % for summer, 58.8 % and 16.1 % for autumn, respectively, of the total variance of phytoplankton data (Fig. 6). There were no significant links seen between Synechococcus spp. and depth in all seasons except winter. The abundance of Synechococcus spp. was significantly negatively correlated with depth during winter with a Pearson correlation coefficient of -0.13 (p < 0.05). The abundance of Synechococcus spp. had significant correlations positive with picoeukaryotes,



Fig. 3 — Contour plot of chlorophyll-a concentration at the Eastern coast of the Black Sea, Turkey during 2013

nanoeukaryotes and Chlorophyll-*a* in all seasons, which implies their coexistence (Fig. 6).

There were no obvious connections found between picoeukaryote and depth in spring and summer. The abundance of picoeukaryotes was significantly negatively correlated with depth during autumn and winter with Pearson correlation coefficients of -0.35and -0.29, respectively (p < 0.05). Contrary, the abundance of nanoeukaryotes tended to be significantly negatively correlated with depth in all seasons, with Pearson correlation coefficients of -0.20 in winter, -0.29 in spring, -0.22 in summer, and -0.54 in autumn (p < 0.05). The nanoeukaryotes also showed a significant negative correlation with picoeukaryotes during winter, with a Pearson correlation coefficient of -0.32 (p < 0.05).

The chlorophyll-*a* showed no significant correlations with depth in spring, whereas chlorophyll-*a* tended to have significantly negative correlations with depth during spring and autumn, with Pearson correlation coefficients of -0.29 and -0.48, respectively (p < 0.05).



Fig. 4 — Depth profiles of temperature (°C), dissolved oxygen (mg  $L^{-1}$ ), salinity (ppt.) and Sigma-*t* (kg m<sup>-3</sup>) at the Eastern Black Sea coast, Turkey, in 2013



Fig. 5 — Bivariate scatter plots displaying *r* values of the Pearson correlation between different environmental variables. Asterisk (\*) indicates the significance level (p < 0.05)

# Discussion

This study observed uneven distribution and abundance of Synechococcus spp., Picoand nanoeukaryotes along the Eastern Black Sea coast. The maximum abundance of Synechococcus spp. was found during summer and autumn, exhibiting a bimodal annual cycle that agreed with the findings of previous studies from other regions<sup>3,8,19</sup>. This study is consistent with earlier studies regarding the maximum abundance of Synechococcus spp. to be at the surface during winter and below the surface (< 50 m) in summer. Previous studies recorded partial photoinhibition of phytoplankton growth in the warm period of a year<sup>20,21</sup>, which probably resulted in lower Synechococcus spp., and Picoeukaryotes in the upper mixed layer in summer, while nanoeukaryotes like coccolithophores resulted in high abundance due to photoadaptation mechanism. Another reason for this trend could be the immediate consumption of nutrients after winter that ultimately leads to the seasonal minimum of Synechococcus spp. and Picoeukaryotes concentrations in the upper mixed layer<sup>22</sup>.

The mean annual abundance  $(3.40 \times 10^4 \text{ cells mL}^{-1})$ , well as the mean summer abundance as  $(2.48 \times 10^4)$ cells  $mL^{-1}$ ) and autumn abundance  $(4.92 \times 10^4 \text{ cells mL}^{-1})$  at the surface, were consistent with that of Feyzioglu et al.<sup>13</sup> and Mukhanovi et al.<sup>23</sup>. However, they were lower than the values reported by Uysal<sup>15</sup>. He reported 1.09×10<sup>5</sup> cells mL<sup>-1</sup> for the Black Sea with a range of  $3.73 \times 10^4 - 2.11 \times 10^5$  cells mL<sup>-1</sup> of *Svnechococcus* spp. (at the surface). Although the abundance of Synechococcus spp. at the Eastern coast of the Black Sea was lower than Uysal<sup>15</sup>, it was still higher than in oligotrophic regions such as in the Sargasso Sea  $(1.7 - 8 \times 10^3)$ , north-west Mediterranean  $(1.7 - 13 \times 10^3)$  and the northern Levantine Basin (annual average  $1.7 - 13 \times 10^3$ )<sup>8,24,25</sup>. Hence, this study also supports the trend of decreasing abundance of Synechococcus spp. cell concentrations from eutrophic to oligotrophic regions<sup>26-28</sup>. The annual average of picoeukaryotes was lower than Mukhanovi et al.<sup>23</sup> annual average value of  $7.3\pm5.4\times10^3$  cells mL<sup>-1</sup> for Sevastopol Bay (the Black Sea). Furthermore, the picoeukaryote abundance at the Eastern Black Sea coast was also relatively lower than the averages observed in oligotrophic waters<sup>29-31</sup>.

this study, the peak abundance of In nanoeukaryotes appeared during winter with a maximum of  $1.80 \times 10^3$  cells mL<sup>-1</sup>. This contrasts the Western English Channel, where with maximum abundance was found during summer<sup>12</sup>. Usually, the peak abundance of nanoeukaryotes is observed in the colder autumn and winter, e.g., in the middle Black Sea in November 1993<sup>(ref. 32)</sup>, in the NE Black Sea in late December 2006<sup>(ref. 33)</sup>, in the NW Black Sea in February 2003, 2006, 2007, and October – November 2010<sup>(refs. 34,35)</sup>, and in the Dardanelles Strait in January 2004<sup>(ref. 36)</sup>. The winter nanoeukaryotes bloom is associated with phosphate availability in the Black Sea<sup>34,35</sup>. Contrary to these results, in the World Ocean, the most extensive blooms are observed in the surface layer in late spring-summer<sup>37-40</sup>.

The maximum chlorophyll-*a* concentrations observed in this study ranged from  $1.19 - 1.61 \ \mu g \ L^{-1}$  (in spring and summer, respectively), with annual means of  $0.83\pm0.07 \ (\pm 95 \ \% \ CI)$ . This range was consistent with the previously reported values  $(0.1 - 1.5 \ \mu g \ L^{-1})$  for the southern Black Sea<sup>41</sup>. Similar to Agirbas *et al.*<sup>42</sup> results, the maximum and minimum abundance of chlorophyll-*a* was recorded during spring and autumn, respectively.



Fig. 6 — Principal component analysis (PCA) plots to depict the relationship depth, *Synechococcus* spp., picoeukaryotes, nanoeukaryotes and Chlorophyll-*a* 

The vertical distribution of dissolved oxygen in the Black Sea is considered extremely important due to the existence of permanent H<sub>2</sub>S and the lack of dissolved oxygen below the pycnocline layer<sup>22,43,44</sup>. This study recorded the highest dissolved oxygen concentrations within 50 - 60 m depth in summer, which agrees with the findings of Alkan et al.<sup>44</sup>. On the other hand, in this study, the highest dissolved oxygen concentration within 50 m depth was 11.3 mg/L, which is inconsistent with that of Alkan et al.<sup>44</sup>, who recorded 13.48 mg/L dissolved oxygen at this depth. These results might show that the photosynthetic biological activity within the euphotic zone during summer resulted in high dissolved oxygen within the abovementioned depths. Furthermore, the subsurface chlorophyll-a maximum

layer (SCML) at 50 - 60 m coincided with this dissolved oxygen level (Fig. 3).

#### Conclusion

The previous studies from the Black Sea provided data only for spring, summer and winter in parts. At the same time, this study covered four different seasons (in the same year) for Samsun and Ordu and three seasons (winter, spring, and summer) for Giresun, Trabzon, Rize and Artvin provinces. The present study will provide baseline data for 2013 for constructing an oceanographic time series that will ultimately assess to understand and quantify the rate of climate change and its effects on phytoplankton communities in the Black Sea.

## **Conflict of Interest**

There is no conflict of interest.

# **Author Contributions**

Study conception and fieldwork by AMF & UD. Data analysis and manuscript preparation by UD. Both authors approved its final version.

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