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Journal of Marine Systems 24 (2000) 313–326

JOURNAL OF MARINE SYSTEMS

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Pigments, size and distribution of *Synechococcus* spp. in the Black Sea

Zahit Uysal *

Institute of Marine Sciences, Middle East Technical University, P.B. 28, 33731, Erdemli-Icel, Turkey

Received 5 February 1999; accepted 5 August 1999

Abstract

Pigments, size and distribution of Phycoerythrin-containing unicellular cyanobacteria *Synechococcus* spp. within the euphotic zone were studied for the first time in April–May 1994 in the western and southwestern Black Sea by epifluorescence microscopy and flow-cytometry. *Synechococcus* was present in varying quantities at every station and depth studied. Surface spatial distribution of *Synechococcus* revealed that cells were much more abundant in offshore waters than near coastal regions under the direct influence of the Danube river. Minimum and maximum cell concentrations ranged between 9×10^2 and 1.45×10^5 cells/ml at the surface, between 2×10^3 and 1.23×10^5 cells/ml at the chlorophyll sub-maximum layer, and between 1.3×10^2 and 3.5×10^2 at the nitrite maximum layer. Cells at the chlorophyll sub-maximum layer (based on in-situ fluorometer readings) fluoresce brighter and longer than the ones at the surface and lower depths. Spectral properties of chromophore pigment types of total 64 clonal isolates from different depths down to the lower layer of the euphotic zone (~ 60 m) in the southern Black Sea coast revealed that all have type 2 phycoerythrobilin in common, lacking in phycourobilin. In vivo fluorescence emission maxima for the phycoerythrobilin were about the same (~ 578 nm) for all isolates. All isolates examined showed in vivo absorption maxima at between 435 and 442 nm and at about 681 nm due to chlorophyll-*a*. Based on the flow cytometer mean forward light scatter data for size distribution, it could be concluded that cells at the surface mixed layer (0–10 m) were larger in cell size than the cells at lower depths (20–60 m).

Keywords: Black Sea; *Synechococcus* spp.; pigments; size; distribution; flow cytometry; epifluorescence microscopy

1. Introduction

The most striking discovery of the last two decades has been the great contribution of the picoplankton community to the total phytoplankton biomass and chlorophyll in the oceans, (Berman, 1975; Johnson and Sieburth, 1979; Waterbury et al., 1979; Li et al., 1983, 1992; Platt et al., 1983; Takahashi and Bienfang, 1983; Glover et al., 1986; Iturriaga and Mitchell, 1986; Booth, 1988), and to the pelagic food web of the ocean (Johnson et al., 1982; Iturriaga and Mitchell, 1986; Li and Platt, 1987). This group also possess high specific growth rates (Bienfang and Takahashi, 1983; Douglas, 1984; Landry et al., 1984). The first members of the picoplankton to be discovered were phycoerythrin (PE)-containing unicellular cyanobacteria *Synechococcus* (Johnson and Sieburth, 1979; Waterbury et al., 1979). These

* Tel.: +90-324-521-2406; fax: +90-324-521-2327.
E-mail address: uysal@ims.metu.edu.tr (Z. Uysal).

0924-7963/00/$ - see front matter
PII: S0924-7963(99)00092-5

are photosynthetic non-flagellated organisms that resemble eukaryotic algae in almost all aspects of their physiology and ecology, yet are undoubtedly prokaryotes cytologically. In oligotrophic oceans this group contributes up to an estimated 25% of photosynthetic carbon fixation (Waterbury et al., 1986) and accounted for 64% of the total photosynthesis in the North Pacific Ocean (Iturriaga and Mitchell, 1986).

The in vivo spectral properties of clonal isolates of marine *Synechococcus* have revealed three major pigment groups; clones lacking PE, clones containing a PE composed of phycoerythrobilin and phycourobilin chromophores and clones containing a PE composed of only phycoerythrobilin chromophores (Wood et al., 1985). These differences in pigmentation can also be detected analytically by both epifluorescence microscopy and flow cytometry in natural waters (Yentsch et al., 1983; Olson et al., 1985, 1990; Wood et al., 1985). Epifluorescence microscopy was used to complement flow cytometry in enumeration of cyanobacteria (Li and Wood, 1988).

The Black Sea, forming the World's largest meromictic basin, is known to be a region of moderate to high productivity, since it is fed by a rich supply of nutrients compared with other parts of the world's oceans (Koblentz-Mishke et al., 1970). Nearly 87% of the Black Sea water volume are anoxic and contain high levels of hydrogen sulphide at depths of 100 to 200 m below the surface. Increased nutrient loads from major rivers have created intense eutrophication, leading to hypoxia and occasional anoxia in the northwest shelf area. Long-term modifications and alteration of the biological structure of the ecosystem have been well documented for this particular zone. The maximum spring–autumn primary productivity (60% of the western Black Sea production) is found in the northwest shelf where 87% of the total fresh water input reaches the Black Sea from major rivers, contributing large amounts of nutrients and detritus onto the shallow shelf region, reducing the surface salinity and transparency. Next to this high productivity area in the northwest shelf region, the highest primary productivity is reported to occur along the Romanian (western) and the Anatolian (southwestern) coasts, and extends into the central region separating the eastern and western gyres.

Eutrophication is considered to play a key role in the substantial alterations in the structure and function of phytoplankton as the first target of the increased nutrient load in the northwest shelf (Petrova-Karadjova, 1984, 1990, 1992; Bodeanu, 1991, 1993, 1995; Moncheva, 1991, 1992; Moncheva et al., 1991). The northwest shelf receives the bulk discharge of nutrient rich freshwater from the Danube, Dniestr and Dniepr rivers. Since the 1960s, riverine nitrogen and phosphorus loads dramatically increased respectively by a factor of 3 and 10, as a consequence of urban and industrial development and intensive use of fertilisers in agriculture (Tolmazin, 1985; Mee, 1992). The Danube itself, introduces about 60,000 tons of total phosphorus/year and some 340,000 tons of total inorganic nitrogen/year (GESAMP, 1990). Over the same period, silicon carried out by rivers significantly decreased as a result of hydraulic management programmes. Changes that occurred in the nutrient environment of the northwestern Black Sea have had direct influence on phytoplankton both qualitatively and quantitatively. Inorganic forms as expressed by the N:P:Si ratios show an excess of phosphorus over nitrogen with respect to phytoplankton requirements and over silicon with respect to diatoms requirements (Eeckhout and Lancelot, 1997). Results of recent investigations suggest that the interplay of the Danube anthropogenic load with the natural hydrological fronts and gradients (river plume dynamics, coastal upwelling and mixing and downwelling over the shelf break and slope) provides opportunities for enhanced phytoplankton productivity, thus contributing to the global environmental changes in the Black Sea northwest shelf (Aubrey et al., 1995).

As an immediate response to nutrient enrichment, both the frequency and the amplitude of algal blooms were increased in the 1970s (Bodeanu, 1984; Bologa et al., 1995). Before this period diatoms were the dominating group and early spring was the usual period for maximum phytoplankton growth and biomass sustained. After 1970, numerous phytoplankton blooms occurred during the summer period and the phytoplankton community was dominated by multiple blooms of non-siliceous phytoplankton. Modification of the ionic ratios of the major nutrients, especially the decrease in the Si:P and Si:N ratios led to increased blooms of dinoflagellates,

euglenophytes and coccolithophorids, most with mixotrophic affinities. As reported by Bodeanu (1992), 46 algal blooms occurred in the Roumanian coastal waters from 1981 to 1990. Formation of massive spring and summer red tides along the Rumanian and Bulgarian coasts by dinoflagellates (Sorokin, 1983, Sukhanova et al., 1988) has been well documented.

Similar changes in biodiversity of phytoplankton species as well as structural and succession aspects of phytoplankton development in the Bulgarian Black Sea coastal zone especially in spring and summer periods were observed (Moncheva, 1991, 1992; Petrova-Karadjova and Moncheva, in press). The eutrophication has dramatically modified the base of the marine food chain, favouring the development of almost monospecific dense blooms of nanoplankton, some of which have been cited as toxic. The shift in the biodiversity of the blooming species towards phytoplankters producing high DMSP that might affect the climate at least on a local basis, is also noteworthy (Moncheva and Krastev, 1997). Additional summer blooms with predominance of dinoflagellates and coccoliths (*Emiliana huxleyi*) have been increasingly observed in the southwestern Black Sea (Bologa, 1986; Hay and Honjo, 1989; Hay et al., 1990, 1991) as well as winter blooms of diatoms (Uysal and Sur, 1995).

Dramatic changes in the ecological status of the Black Sea due to the anthropogenic impact has drawn the attention of most scientists to the eutrophication phenomenon and its negative consequences to the ecosystem and to plankton dynamics in recent decades. Phytoplankton investigations conducted in the Black Sea mainly dealt with species composition (biodiversity and taxonomic structure), spatio-temporal and seasonal dynamics and vertical distribution, long-term alterations in relation to variability of environmental factors, eutrophication induced changes (especially on the northwestern continental shelf), phytoplankton blooms, primary production and trophic relationships. However, almost nothing is known about the trophic interactions within the microbial food web that supports metazoan food webs via biomass production of both heterotrophic and autotrophic tiny cells. Further studies are needed to understand the role of heterotrophic bacteria and autotrophic picoplankton as the major food source for small protists especially to ciliates and heterotrophic/-mixotrophic nanoflagellates. It is the aim of this study to provide background information on the least studied picoplankton component of the phytoplankton in the most eutrophic region of the Black Sea.

2. Material and methods

2.1. Sampling

Sampling was done between April 24th and May 15th, 1994 aboard the Turkish R/V *Bilim* of the Institute of Marine Sciences — Middle East Technical University, in the area extending from approximately 28°E to 35°E and 41°N to 45°N in the western and southwestern Black Sea (Fig. 1). Water samples from the surface mixed layer, the chlorophyll sub-maximum (based on in-situ fluorometer readings) and the nitrite maximum layer (sigma theta 15.8) were collected through a rosette sampler equipped with a CTD probe. Water samples were drawn from the closing bottles into 100 ml dark coloured polyethylene bottles and preserved with 4% buffered formalin.

2.2. Epifluorescence microscopy

A total of 10 ml aliquots from each sample were filtered onto 25 mm diameter, black, polycarbonate, nuclepore membrane filters with a 0.2-μm pore-diameter. The filters were then placed onto glass slides for counting on a Leitz Laborlux S epifluorescent microscope at $1000\times$ with a Leitz M2 filter set at 546 nm excitation, >580 nm emission. Cells were counted in at least 40 randomly chosen microscope fields. In addition we had the chance to compare epifluorescent microscope cell counts with those of flow cytometer on 11 samples (four preserved, seven unpreserved). Highly significant correlation (r_s significant at $P_{0.002}$) was found between the epifluorescence microscope and the flow cytometer cell counts.

2.3. Cultures

Another set of depthwise water samples at 10-m intervals down to the lower layer of the euphotic

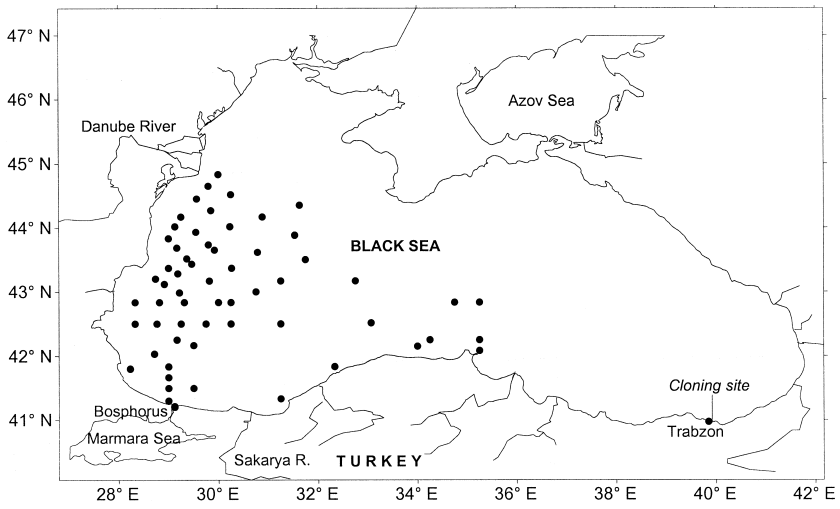


Fig. 1. Location of sampling stations in the Black Sea.

zone (60 m) from a single station (see Fig. 1) in the southern Black Sea near Trabzon (40°58′N; 39°51′E) on November 10, 1994 was taken with closing bottles for isolations. Samples were brought to USA via DHL in an ice box. Immediately after, subsamples were sent to Canada for flow cytometric analysis. Live samples were further diluted into corning cell-wells for isolations. From there, one drop from each well was streaked onto presterilized Petri plates with sloppy agar (1%) prepared with BG11 and F/4-silica supplemented with ammonium chloride media for further purification. Plates were kept in subsaturating light in the range of 30–50 μE m^{-2} s^{-1} at a constant room temperature (18°C). They were illuminated with cool white fluorescent lamps on light:dark cycles (10:14 h) over a couple of weeks until the colonies were big enough to be picked up under a dissecting microscope. Isolates were grown in f/4-silica medium supplemented with 100 μM ammonium chloride at 18°C in subsaturating light (50 μE m^{-2} s^{-1}) and kept in exponential growth by repeated transfer to new liquid media.

2.4. Flow cytometry

In addition to four preselected samples (preserved with 4% buffered formalin) from the April 1994 cruise, depthwise, fresh, unpreserved water samples at 10-m intervals down to the lower layer of the euphotic zone (60 m) from a single station in the Southern Black Sea were sent to Canada as soon as possible for flow cytometric analysis of *Synechococcus* spp. Analysis were performed with a FACSort Becton Dickinson flow-cytometer. Only phytoplankton cells (i.e., no bacteria, etc.) were counted because the instrument was instructed to reject all particles with low levels of red fluorescence (i.e., from chlorophyll-*a*). Cell size was estimated from a 488-nm laser light scattered in the forward narrow angle direction which is related to the cross-sectional area of each particle. The calibration of the light scatter signals was done utilising commercially available plastic spherical beads of known diameters. The results are therefore given in terms of ESD 'Equivalent Spherical Diameter' in micrometers.

2.5. Spectrofluorimetry

In vivo fluorescence emission and excitation spectra of pigments were measured on a SLM Aminco Bowman Series 2 Luminescence Spectrometer. The instrument settings for chlorophyll excitation, and PE excitation and emission were as follows:

Chlorophyll excitation: Scan rate adjustment was 3 nm/s in the range of 380–640 nm, emission at 680 nm with a band pass of 4 nm for excitation and emission.

PE excitation: Scan rate adjustment was 3 nm/s in the range of 450–570 nm, emission at 578 nm with a band pass of 2 nm for excitation and emission.

PE emission: Scan rate adjustment was 1 nm/s in the range of 555–700 nm and excitation at 545 nm with a band pass of 2 nm for excitation and emission.

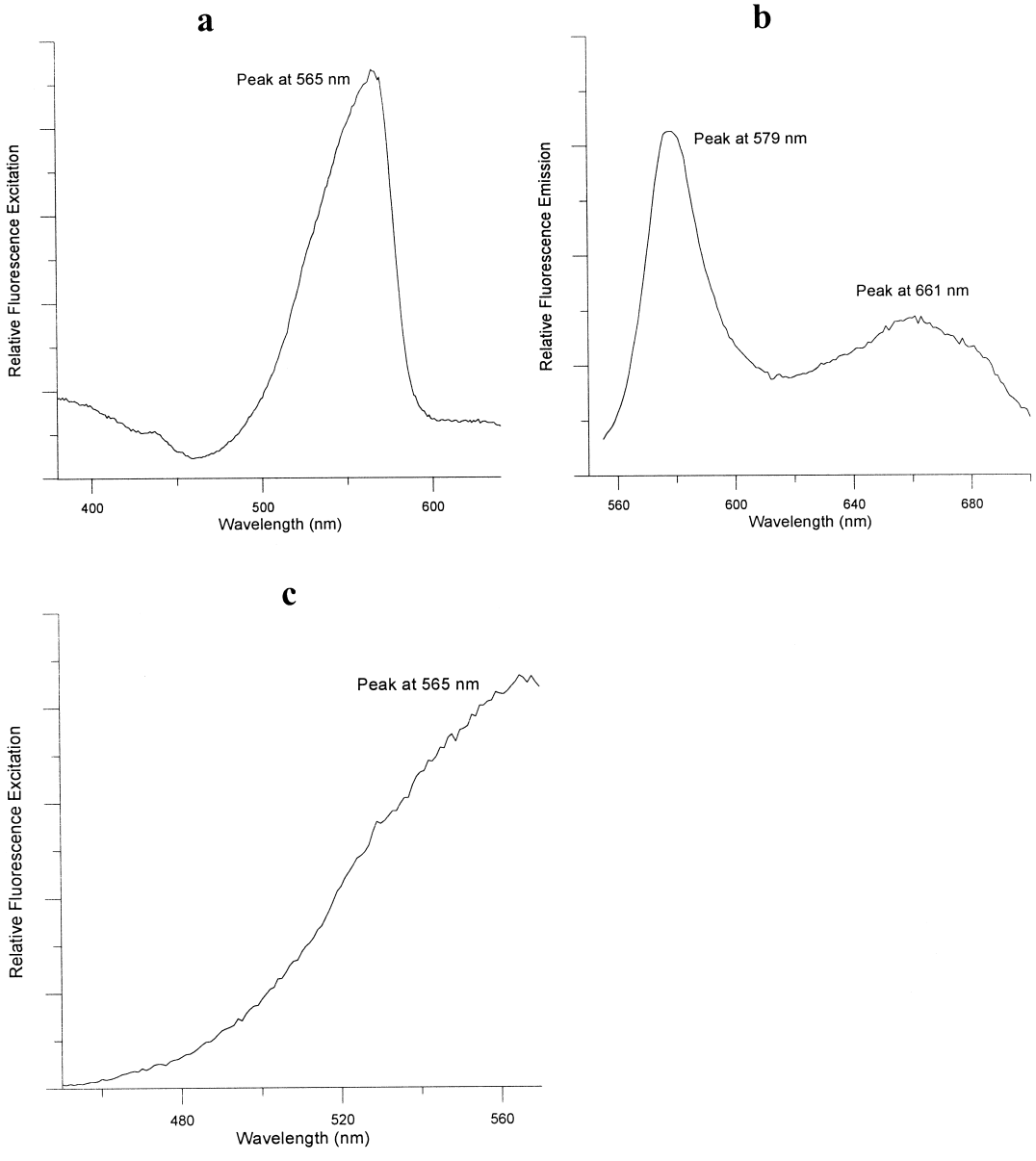


Fig. 2. Energy-corrected fluorescence excitation spectrum for chlorophyll-*a* emission at 680 nm (a) fluorescence emission spectrum with excitation at 545 nm (b) and excitation spectrum for PE emission at 578 nm (c) for exponentially growing Black Sea surface *Synechococcus* clone BS0101.

2.6. Absorption spectrometry

In vivo absorption spectra for the samples were measured with a Beckman DU 640 Spectrometer. Scatter correction was made by filling half the volume of the sample with glycerol. All samples were scanned between 350 and 770 nm at a scan speed of 1200 nm/mn.

3. Results and discussion

A total of 64 clonal isolates of PE-containing Black Sea *Synechococcus* spp. have been examined for their characteristic in vivo absorption and fluorescence emission and excitation properties. All measurements were performed on exponentially growing cultures whose the growth was monitored by daily fluorometric measurements of chlorophyll.

Representative fluorescence emission and excitation spectra for the exponentially growing Black Sea surface *Synechococcus* clone BS0101 are shown in Fig. 2a–c and the spectral features for all Black Sea clonal isolates are summarised in Table 1. Excitation at 545 nm, absorbed essentially by the phycoerythrobilin chromophores results in in vivo fluorescence emission maximum at $\sim 578 \pm 1$ nm in almost all clonal isolates. A close in vivo fluorescence emission maximum (576 ± 1 nm) was found for two types of PE containing clonal cultures (WH7805 and WH8018; Wood et al., 1985). Such a slight difference was also observed in excitation maximum for in vivo fluorescence emission at 578 nm. The excitation peak for PE emission at 578 nm was found at 566 ± 1 nm for the Black Sea cultures, a value very close to that of 567 ± 3 nm found for clones WH7805 and WH8018.

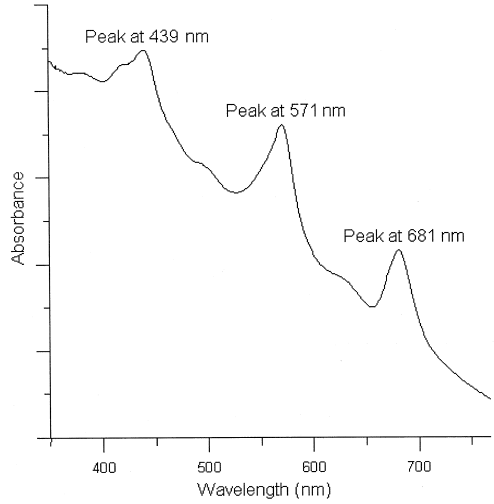


Fig. 3. In vivo absorption spectrum from exponentially growing Black Sea *Synechococcus* clone BS0101.

One representative in vivo absorption spectrum from exponentially growing Black Sea surface *Synechococcus* clone BS0101 is shown in Fig. 3. All examined strains showed absorption maxima between 435 and 442 nm and at about 681 nm due to chlorophyll-*a*. Similar results within the same ranges have also been observed for the clones WH7805 and WH8018. One in vivo absorption peak due to PE was found at 570 ± 1 nm.

Based on the flow cytometer mean forward light scatter data for size distribution, it could be con-

Table 1
Summary of spectral characteristics for the Black Sea isolates of *Synechococcus* spp. Spectral maxima (± 1 SD) are given in nm; *n* is the number of cultures used to measure mean and SD

Principal phycobiliprotein	Phycoerythrobilin
In vivo fluorescence emission maxima for different depths	
Surface	578 ± 1 ($n = 10$)
10 m	579 ± 1 ($n = 10$)
20 m	578 ± 1 ($n = 10$)
30 m	578 ± 1 ($n = 14$)
40 m	576 ± 1 ($n = 4$)
50 m	578 ± 2 ($n = 14$)
60 m	578 ± 1 ($n = 2$)
Excitation maxima for in vivo fluorescence emission at 578 nm	
Surface	565 ± 1 ($n = 10$)
10 m	566 ± 1 ($n = 10$)
20 m	568 ± 2 ($n = 10$)
30 m	565 ± 1 ($n = 14$)
40 m	566 ± 1 ($n = 4$)
50 m	564 ± 2 ($n = 14$)
60 m	566 ± 1 ($n = 2$)
In vivo absorption maxima	
Surface (corrected)	570 ± 1 ($n = 10$)
10 m (corrected)	571 ± 1 ($n = 9$)
20 m	569 ± 1 ($n = 6$)
30 m	571 ± 1 ($n = 6$)

cluded that cells at the surface mixed layer (0–10 m) were larger in size than cells at lower depths (20–60 m). Results supported an apparent decrease in cell size with depth. As shown in Fig. 4, most of the cells at the surface mixed layer fall into 0.7–0.8 μm ESD size group whereas the dominant cell size between 20 and 60 m depth is about 0.5 μm ESD. It is noteworthy that the surface samples taken from Bosphorus exit of the Black Sea (Fig. 1) on April 1994 comprised two different size groups, a major one of about 0.7 μm ESD and the remaining of about 2 μm ESD (Fig. 5a). Similar composition was also observed at the station 28°14′E and 41°48′N where two different sizes were present at the surface (Fig. 5b). This, however, was not true for the cells at 25 m depth in the same station dominated by long fluorescing spherical cells (Fig. 5c) as well as those found at 30 m depth at the offshore station 30°45′E and 43°N, located in the western gyre (Fig. 5d).

In the near Bosphorus region, analysis by epifluorescence microscopy revealed the presence of a significant amount of large elongate–elliptical cells in the sample. Only in this station such extraordinary large cells have been observed. This is also well reflected in flow cytometer forward scatter data for this particular region (see Fig. 5a). Further studies are needed to distinguish genetically distinct subpopulations of *Synechococcus* spp. in this junction area.

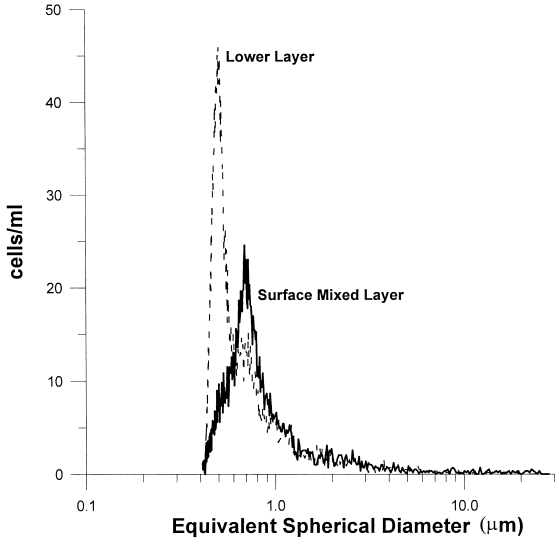


Fig. 4. Comparison of cell sizes of Black Sea *Synechococcus* spp. between the surface mixed layer and the lower layer.

3.1. Synechococcus counts

A total number of 101 samples from the surface mixed layer, the chlorophyll sub-maximum (based on in-situ fluorometer readings) and the nitrite maximum layer (sigma theta 15.8) were enumerated by epifluorescence microscopy for their *Synechococcus* contents. *Synechococcus* was present in varying quantities at every station and depth studied. Cell concentrations ranged from 9×10^2 to 1.45×10^5 cells/ml at the surface level, from between 2×10^3 and 1.23×10^5 cells/ml at chlorophyll sub-maximum layer and from between 1.3×10^2 and 3.5×10^2 at depth of the nitrite maximum.

Spatial heterogeneity was more pronounced at the surface than below. Surface cell counts of *Synechococcus* revealed that cells were much more abundant in offshore waters than near coastal regions under the direct influence of the Danube river (Fig. 6a). Changes in surface temperature are shown in Fig. 6b. Surface salinity seemed to (Fig. 6c) have a greater impact than temperature on the abundance distribution. Based on Spearman's rank correlation analysis, highly significant positive correlation ($P = 0.00$, $n = 61$, $r = 0.75$) was observed between the surface cell counts and the salinity values. Such a strong response to salinity gradient by *Synechococcus* was also observed in Florida Bay (Phlips and Badylak, 1996). Despite this highly significant correlation with salinity, no correlation was observed with temperature. Minimum cell density (9×10^2 cells/ml) was observed at colder (10.5°C) and less saline (15) waters near the Danube inflow area. In contrast, the maximum (1.45×10^5 cells/ml) was characteristic of the much warmer (15°C) and saline (18.5) offshore waters where the surface average for the whole area was 4.5×10^4 cells/ml.

Cell counts were as low as 1000 cells/ml at the Danube inflow area and progressively increased towards much saline and colder offshore waters to a maximum 1.45×10^5 cells/ml. From here we can also conclude that cell concentrations were lower along the cyclonic boundary current and higher in the western gyre (for details on the formation of different water masses see, Oguz et al., 1993). The

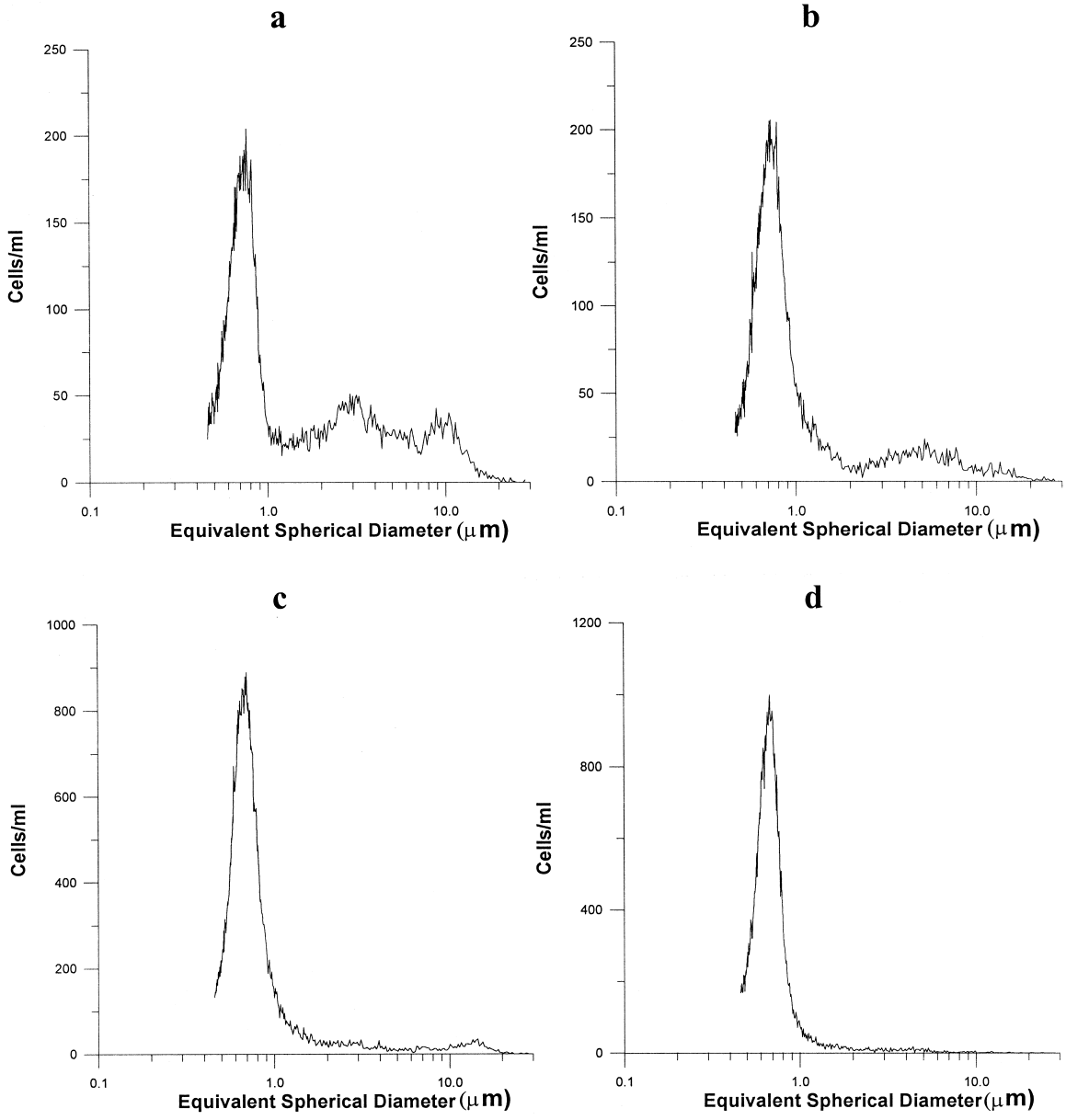
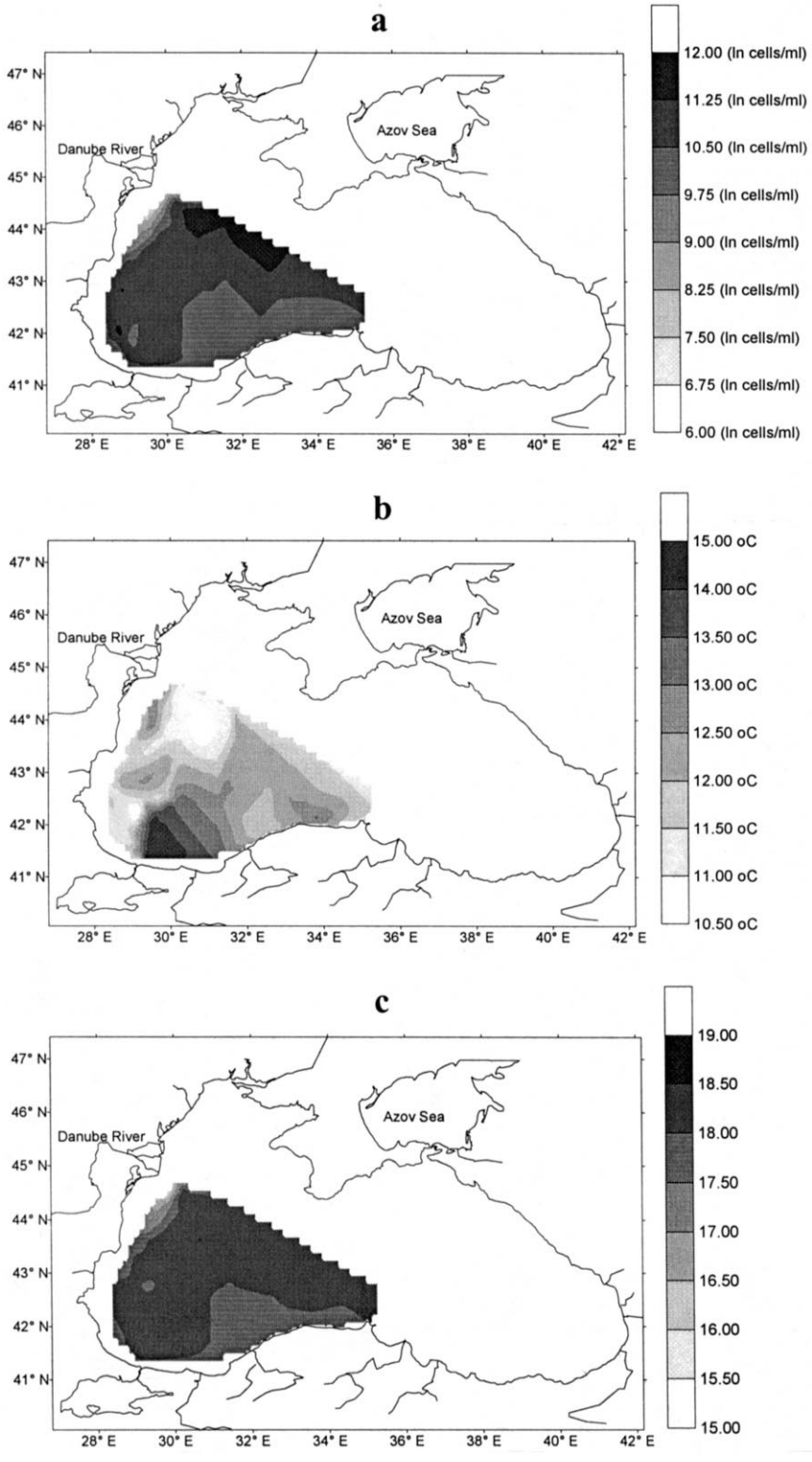


Fig. 5. Comparison of *Synechococcus* spp. cell sizes at different sites; (a) surface sample at the Bosphorus exit to Black Sea, (b) surface sample at station 28°14′E and 41°48′N, (c) at 25 m depth at station 28°14′E and 41°48′N, and (d) at 30 m depth at the offshore station 30°45′E and 43°00′N, located in the western gyre.

highest counts were recorded in the northern part of the western gyre. This, in fact, contrasts with other findings in the North Atlantic and Pacific Oceans (Waterbury et al., 1979; Glover, 1985; Olson et al.,

Fig. 6. Surface spatial distribution of *Synechococcus* spp. cell abundance in ln of cells/ml (a) temperature in °C (b) and salinity (c) in the study region.


a
Danube River
Azov Sea
47° N
46° N
45° N
44° N
43° N
42° N
41° N
28° E
30° E
32° E
34° E
36° E
38° E
40° E
42° E
12.00 (ln cells/ml)
11.25 (ln cells/ml)
10.50 (ln cells/ml)
9.75 (ln cells/ml)
9.00 (ln cells/ml)
8.25 (ln cells/ml)
7.50 (ln cells/ml)
6.75 (ln cells/ml)
6.00 (ln cells/ml)
b
Danube River
Azov Sea
15.00 oC
14.00 oC
13.50 oC
13.00 oC
12.50 oC
12.00 oC
11.50 oC
11.00 oC
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c
Danube River
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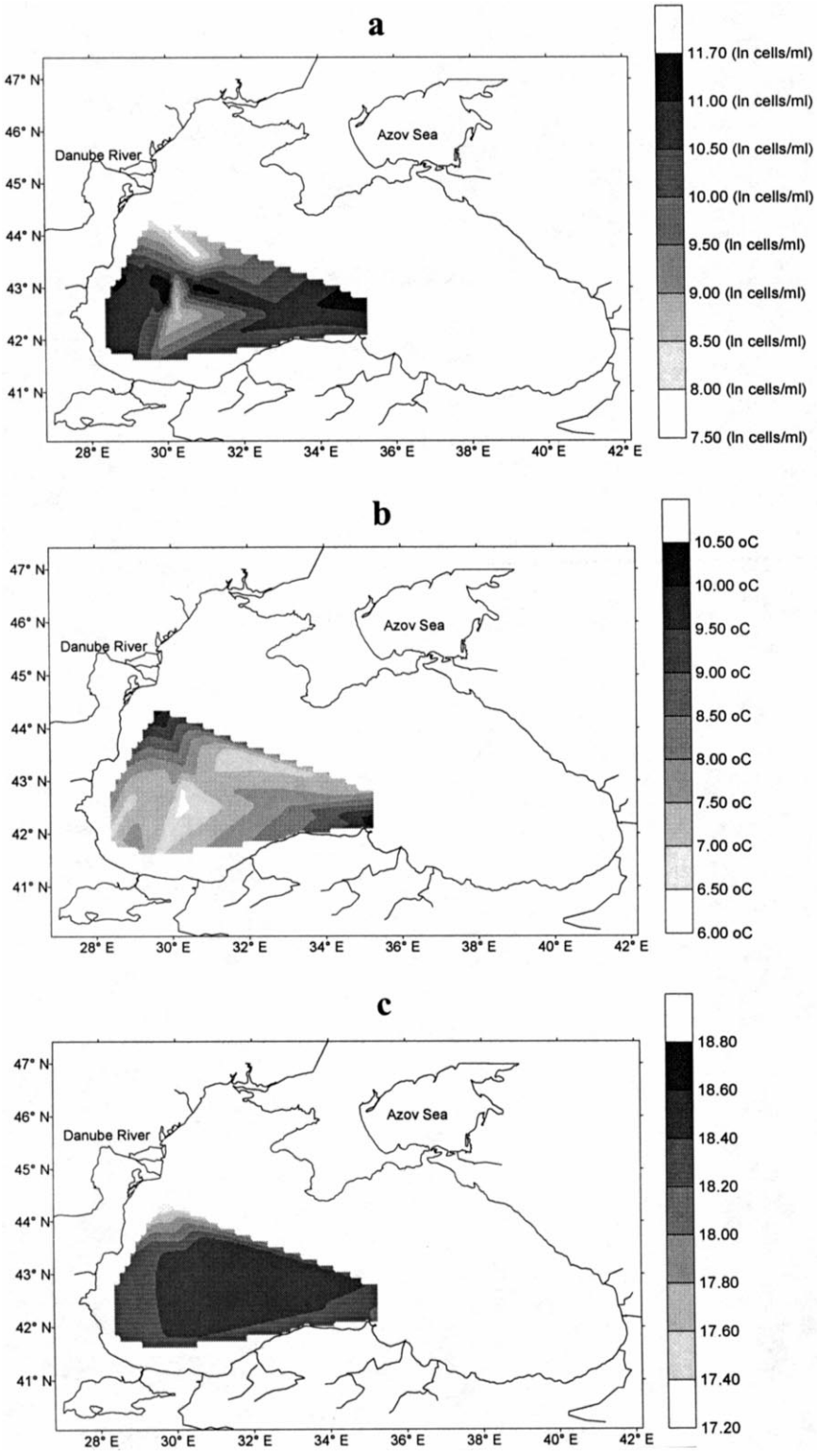
a
47° N
46° N
45° N
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42° N
41° N
Danube River
Azov Sea
28° E
30° E
32° E
34° E
36° E
38° E
40° E
42° E
11.70 (ln cells/ml)
11.00 (ln cells/ml)
10.50 (ln cells/ml)
10.00 (ln cells/ml)
9.50 (ln cells/ml)
9.00 (ln cells/ml)
8.50 (ln cells/ml)
8.00 (ln cells/ml)
7.50 (ln cells/ml)
b
Danube River
Azov Sea
10.50 oC
10.00 oC
9.50 oC
9.00 oC
8.50 oC
8.00 oC
7.50 oC
7.00 oC
6.50 oC
6.00 oC
c
Danube River
Azov Sea
18.80
18.60
18.40
18.20
18.00
17.80
17.60
17.40
17.20

1990), where the lowest concentrations were characteristic of the central oligotrophic oceans as well as with our recent unpublished data based on cell counts along the Turkish coast only. This further depicts that the *Synechococcus* cell abundance is not directly related to the nutrient availability especially in this region of the Black Sea. As stated in the Introduction, the Danube inflow area is fed by a rich supply of nutrients as well as detritus and terrigenous pollutants. The role of pollutants in this respect is uncertain and needs to be studied in detail. In the region, inorganic forms as expressed by the N:P:Si ratios show an excess of phosphorus over nitrogen with respect to phytoplankton requirements and over silicium with respect to diatom requirements. In recent years, nutrient measurements done in the Sulina branch of the Danube between 1988 and 1992 revealed that there was a five- and two-fold increase, respectively, of the total inorganic nitrogen and phosphate loads of the Danube River, whereas the riverine input of silicate decreased to about one-third (Cociasu et al., 1996). In response to nutrient enrichment, both the frequency and the amplitude of algal blooms were increased especially along the cyclonic boundary of the current. However, this does not apply to the *Synechococcus* population during the sampling period.

There may be other factors affecting cell concentrations other than physico-chemical ones. This may be due to differences in grazing pressure among sites or timing of the sampling schedule during the day. Recent data gathered from field and microcosm experiments conducted on board R/V *Bilim* in the Black Sea have shown that synchronous division occurs between noon and midnight with an apparent grazing pressure from midnight to noon along the southern Black Sea coast. (Uysal et al., 1998). Similar results have also been obtained from the Arabian Sea (Sherry and Uysal, 1995). Studies in the Arabian Sea have shown that the diurnal change was found greater than the differences between physically and spatially discrete water masses. In addition, *Synechococcus* spp. have been shown to exhibit varying

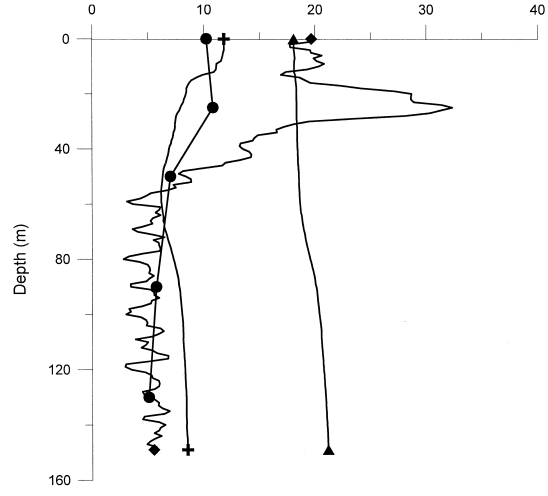

Fig. 8. Abundance (ln of cells/ml) of *Synechococcus* spp (●) as a function of depth compared to vertical profiles of temperature (°C, +), salinity (▲) and fluorescence (a.u., ♦) at the station located offshore Bosphorus (41°50′N; 29°00′E). a.u.: Arbitrary unit.

degrees of diurnal periodicity in cell division rates both in culture (Campbell and Carpenter, 1986) and in incubation experiments (Carpenter and Campbell, 1988; Kudoh et al., 1990).

Cell concentration within the chlorophyll submaximum layer (Fig. 7a) ranged from 2×10^3 (at 10.4°C and at 17.26 salinity) to a maximum of 1.23×10^5 cells/ml (at 7.46°C and at 18.52 salinity) in the region with an average of 4.3×10^4 cells/ml. The northern sector covering mainly offshore waters and its southern extension located offshore between the Bosphorus and Sakarya River were characterized by the lowest abundance. Despite the high range of temperature variations at that depth (Fig. 7b), there was little change in salinity (Fig. 7c) and the magnitude was much smaller than the one observed at the surface. Even such small change in salinity seemed to cause spatial heterogeneity in cell concentrations. A strong response of *Synechococcus* to weak changes in salinity (15–20) has been well demonstrated by Phlips and Badylak (1996). Based on Spearman's

Fig. 7. Spatial distribution of *Synechococcus* spp. cell abundance in ln of cells/ml (a) temperature in °C (b) and salinity (c) within the florescence maximum layer in the study region.

rank correlation analysis, negative correlation ($P = 0.06$, $n = 23$, $r = 0.75$) was observed between the cell counts and the salinity values in the chlorophyll sub-maximum layer. It is difficult to suggest that any of these factors have more control on the cell concentrations than the other. Since we had only a few samples representing the nitrite maximum layer, we were unable to draw such figures for this layer. However, the lowest cell concentrations were characteristic of this layer for the Black Sea, like in the North Atlantic and Pacific Oceans (Olson et al., 1990).

The vertical profiles of the cell, temperature, salinity and fluorescence distributions at a station located offshore Bosphorus (41°50′N; 29°00′E) are given in Fig. 8. Cells are mainly present above the thermocline and within the chlorophyll sub-maximum layer. The abundance increased about two fold from surface (2.7×10^4 cells/ml) to the chlorophyll sub-maximum layer (5.0×10^4 cells/ml). It decreased to 10^3 cells/ml at 50 m and to 150 cells/ml at 130 m.

In conclusion, spectral characteristics of the pigment type, size, and abundance distribution (both horizontally and vertically) of cyanobacterium *Synechococcus* spp. have been reported for the first time for the Black Sea. Results have indicated phycoerythrobilin rich *Synechococcus* to be most abundant at the surface mixed layer in the western gyre. Cell densities increased towards offshore and decreased sharply below the chlorophyll sub-maximum layer. Larger cells were characteristic of the upper surface mixed layer.

Acknowledgements

I thank Dr. Michelle Wood (Univ. of Oregon, USA) for both the theoretical and technical guidance and Dr. W.K.W. Li (Bedford Inst. of Oceanography) for the flow cytometric analysis of the samples. This work was fully supported by the Turkish Scientific and Technical Research Council (TUBITAK) under the NATO postdoctoral fellowship programme. Sincere thanks go to Nelson Sherry, Mark Teiser, Eric Stenn and Errol Mustafa for their valuable suggestions and generous help.

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