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Chroococcoid cyanobacteria *Synechococcus* spp. in the Black Sea: pigments, size, distribution, growth and diurnal variability

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Phycoerythrin-containing unicellular cyanobacteria Synechococcus spp. were studied for the first time during April–May, 1994 and September–October, 1996, in the western and southern Black Sea for pigments, size and abundance distribution via spectrometry, epifluorescence microscopy and flow cytometry. Abundance distribution in the surface mixed layer in April–May, 1994, revealed that cells were more concentrated in offshore waters than in coastal regions under the direct influence of the river Danube. However, in the south, higher surface cell concentrations were characteristic of the nearshore areas during September–October, 1996. A highly significant correlation was observed between cell abundance and ambient physico-chemical parameters with depth. Visual inspection of the individual cells under the epifluorescence microscope revealed that cells at the subsurface, chlorophyll a maximum layer (SCML, based on in situ fluorometer readings) fluoresce more brightly and for longer than those at the surface and at lower depths. Spectral properties of a total of 64 Synechococcus spp. clonal isolates from different depths within the euphotic layer (about the top 60 m) in the southern Black Sea coast showed that all have type 2 phycoerythrobilin in common, lacking phycourobilin. In vivo fluorescence emission maxima for phycoerythrobilin were about the same (~578 nm) for all isolates. All isolates had in vivo absorption maxima at between 435 and 442 nm, and at about 681 nm due to chlorophyll a. It was shown from the flow cytometer mean forward light scatter data for size distribution that cells at the surface mixed layer (0–10 m) were larger than cells at lower depths (20–60 m). Based on in vivo fluorescence measurements, significant differences in the acclimated growth rates of clones from different depths were observed. Time versus cell count plots showed that cells of the cyanobacteria Synechococcus spp. are under grazing pressure, from midnight until noon, and slowly begin to rebuild their population in the afternoon by dividing throughout the evening.

INTRODUCTION

The picoplanktonic, chroococcoid cyanobacteria, *Synechococcus* spp., are known to be major contributors to the total photosynthetic biomass in the oceans (Berman, 1975; Waterbury *et al.*, 1979; Johnson and Sieburth, 1979; Li *et al.*, 1983; Platt *et al.*, 1983; Takahashi and Bienfang, 1983; Iturriaga and Mitchell, 1986; Glover *et al.*, 1986; Booth, 1988; Li *et al.*, 1992), especially in the more oligotrophic regions such as the Mediterranean Sea (Li *et al.*, 1993; Magazzu and Decembrini, 1995; Agawin and Agusti, 1997). The group also possesses high specific growth rates (Bienfang and Takahashi, 1983; Douglas, 1984; Landry *et al.*, 1984). The first members of the picoplankton to be discovered were the phycoerythrin-containing, unicellular cyanobacteria, *Synechococcus* (Waterbury *et al.*, 1979; Johnson and Sieburth, 1979). In oligotrophic oceans, this group contributes up to an estimated 25% of photosynthetic carbon fixation (Waterbury *et al.*, 1986) and accounts 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 phycoerythrin (PE), clones containing PE composed of phycoerythrobilin and phycourobilin

chromophores, and clones containing 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; Wood *et al.*, 1985; Olson *et al.*, 1985, 1990). Epifluorescence microscopy was used to complement flow cytometry in enumeration of cyanobacteria (Li and Wood, 1988).

In recent decades, dramatic changes in the ecological status of the Black Sea due to the anthropogenic impact has drawn the attention of many scientists to the eutrophication phenomenon and its negative consequence on the ecosystem and on plankton dynamics. Phytoplankton investigations conducted in the Black Sea have 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 along the north-western continental shelf), phytoplankton blooms, primary production and trophic relationships. Almost nothing is known about the trophic interactions within the microbial food web which supports metazoan food webs via biomass production of both heterotrophic and autotrophic cells. Further studies are needed to understand the role of heterotrophic bacteria and autotrophic picoplankton as the major food source for small protists, especially ciliates and heterotrophic/mixotrophic nanoflagellates. It is the aim of this study to provide baseline information on the least studied picoplankton component of the phytoplankton in the most eutrophic region of the Black Sea.

Study area

The Black Sea, forming the World's largest meromictic basin, comes within the category of semi-enclosed basins and has a surface area of 4.23×10^5 km^2. It is known to be a region of moderate to high productivity since it is fed by a rich supply of nutrients via major rivers, namely, the Danube, Dniestr and the Dniepr. Increased nutrient loads from these rivers have created intense eutrophication, leading to hypoxia and occasional anoxia in the north-west shelf area. The excess run-off maintains a highly stable brackish surface layer separated from deep waters by a permanent halocline at depths of 80–200 m, which seriously prevents vertical exchanges in general, and convective mixing in particular. Below the halocline, hydrogen sulphide is present.

Among the unique hydrodynamic features are the cyclonically meandering rim current, together with the interior western and eastern gyres, and several mesoscale anticyclonic eddies [Figure 1, redrawn from (Oguz *et al.*, 1993)]. The two most persistent eddies are the Batumi and the Sevastopol eddies. In the south, three other quasi-permanent anticyclonic eddies, namely, the Bosphorus, Sakarya and Kizilirmak eddies, are present. In addition to these, one or two more recurrent coastal anticyclonic eddies may occur in the region between Sakarya Canyon and Cape Sinop. The quasi-permanent Crimea and

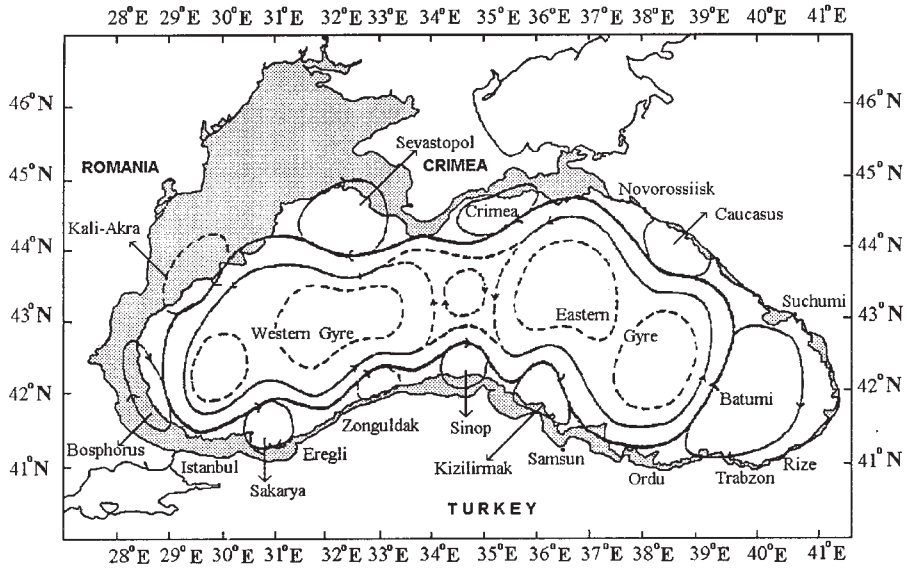

Fig. 1. Main features of the upper layer general circulation of the Black Sea [redrawn from (Oguz *et al.*, 1993)]. Solid (dashed) lines indicate quasi-permanent (recurrent) features of the general circulation.

Caucasus eddies are located on the north-eastern coast. Finally, the Kali-Akra eddy from the Bulgarian coast is another apparently recurrent anticyclonic feature from the shallow west coast.

The meandering nature of the boundary current is possibly responsible for these standing structures as well as transient features along the periphery. The strong boundary current limits water and material transfer across the flow, while jet-like instabilities, mesoscale eddies, filaments and mushroom-like structures play important roles in the cross-shelf exchanges (Uysal and Sur, 1995). 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) provide opportunities for enhanced phytoplankton productivity, thus contributing to the global environmental changes in the Black Sea north-west shelf (Aubrey *et al.*, 1995).

METHOD

Sampling was done between April and May, 1994 (Figure 2a) and September and October, 1996 (Figure 2b) 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 40°E and 41°N to 45°N in the western and southern Black Sea. Water samples from the surface mixed layer, the subsurface chlorophyll *a* maximum layer (SCML, based on *in situ* fluorometer readings) and the nitrite maximum layer (sigma theta 15.8) were collected through a rosette sampler with a CTD probe attached during the April–May, 1994 survey. The CTD profiler (Sea Bird Electronics, Model 9/11) was equipped with a CHELSEA Aquatrac II *in situ* fluorometer, a 25 cm Sea-Tech Transmissometer and a GO-FLO Rosette Sampler holding 12 bottles of 5 l each (General Oceanics). Exact sampling depths for each layer were determined with reference to the profiles obtained during the downcast, and samples were then collected during the upcast from these predetermined depths. For the SCML, samples were always collected from the peak points.

During the September–October 1996 cruise, 26 stations were visited and a total of 121 samples were collected from the surface, subsurface chlorophyll *a* maximum and chlorophyll *a* minimum layers, within the euphotic zone. Water samples were drawn from the Nansen bottles into 100 ml, dark-coloured, polyethylene bottles and preserved with 4% buffered formalin. Aliquots (10 ml) from each sample were filtered onto 25 mm diameter, black, polycarbonate, nuclepore membrane filters with a 0.2 mm pore diameter. The filters were then placed

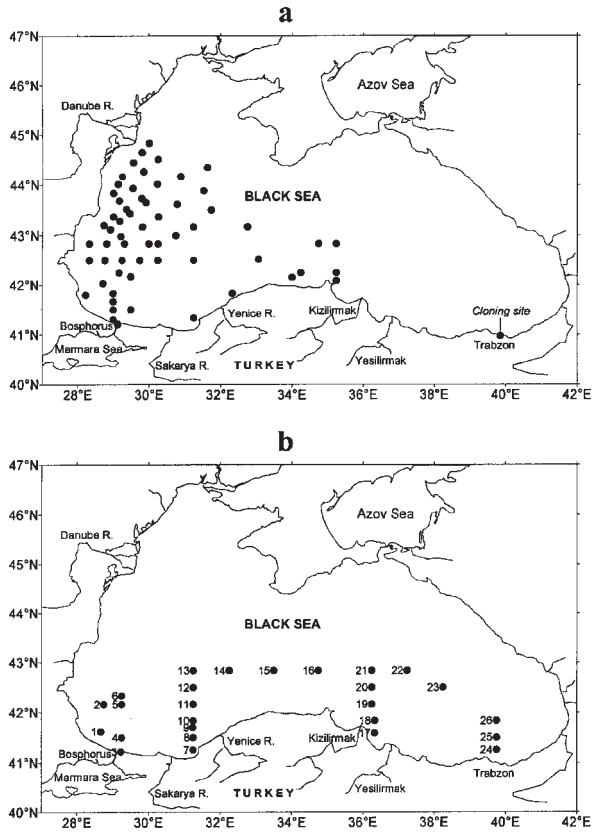

Fig. 2. Location of sampling stations in the Black Sea during (**a**) April–May, 1994 and (**b**) September–October, 1996 cruises.

onto glass slides for counting on a Leitz Laborlux S epifluorescent microscope at 1000× with a Leitz M2 filter set at 546 nm excitation, >580 nm emission. For the September–October 1996 cruise, live samples were filtered onto the same type of filters and kept frozen until counts were made under a Nikon epifluorescent microscope at 1000× with a filter combination of B-2A (DM 505, EX 450–490, BA 520) and G-1A (DM 575, EX 546/10, BA 580). Cells were counted in at least 40 randomly chosen microscope fields. Comparison of flow cytometer counts with epifluorescent microscope counts on a few samples (four preserved, seven unpreserved) resulted in a highly significant correlation (r_s significant at $P_{0.002}$).

Water samples for clonal culture of Black Sea *Synechococcus* spp. were taken from a single station (Figure 2a) near Trabzon (40°58′ N; 39°51′ E) at 10 m intervals down to the lower layer of the euphotic zone (60 m). Live samples were sent to the USA via DHL in an ice box. Immediately afterwards, subsamples were sent to Canada for flow cytometric analysis. Live samples were further diluted into corning-cell wells for isolation. Then, one drop from each well was streaked onto pre-sterilized Petri

plates containing sloppy agar (1%), prepared with BG11 and F/4-silica supplemented with ammonium chloride, for further purification. Plates were kept in subsaturating light ranging from 30–50 μEinst m^{-2} s^{-1} at a constant temperature room (18°C), illuminated with cool white fluorescent lamps, on a light:dark cycle (10:14 h) for 2 weeks until the colonies were big enough to be picked under a dissecting microscope. Isolates were grown in F/4-silica medium supplemented with 100 μmol l^{-1} ammonium chloride at 18°C in subsaturating light (50 μEinst m^{-2} s^{-1}) and kept in exponential growth by repeated transfer to new liquid media.

Acclimated growth rates for clones from different depths were determined by daily *in vivo* fluorescence measurements of chlorophylls. Measurements were made using a Turner Designs Model 10-AU-005 with a 10–040 optical kit to measure growth. Here, it is assumed that fluorescence from an *in vivo* culture is a linear function of the chlorophyll concentration, and that the concentration of chlorophyll per cell within the culture is constant. For the assessment of growth rates, clones from different depths were exposed to varying light ranging from 5 to 100 μEinst m^{-2} s^{-1} (5, 10, 20, 30, 40, 50, 75 and 100) at 18°C over a period of 25 days on a 10:14 h light:dark cycle. Daily growth rates were estimated only for the period of exponential growth. The stage of the culture in which the growth rate of a given day is less than the preceding day is assigned as the onset of the stationary phase, and the measurements made before this stage were assumed to remain within the exponential phase of the culture. For this, rates are calculated for each day and averaged over the entire exponential phase. The variance of the averaged growth rates for each clone was then compared using Student's *t*-test to determine whether the differences in growth rates for different clones were significant.

Flow cytometric analysis was 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 measured as 488 nm laser light scattered in the forward narrow angle direction, which is equivalent to the cross-sectional area of each particle. The calibration of the flow cytometric measures of light scatter was done using commercially-available, plastic spherical beads of known diameters. The results are therefore given in terms of 'equivalent spherical diameter' in micrometers.

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

Chlorophyll excitation. Scan rate adjustment was 3 nm s^{-1} in the range 380–640 nm, emission at 680 nm with a band pass of 4 nm for excitation and emission.
Phycoerythrin excitation. Scan rate adjustment was 3 nm s^{-1} in the range 450–570 nm, emission at 578 nm with a band pass of 2 nm for excitation and emission.
Phycoerythrin emission. Scan rate adjustment was 1 nm s^{-1} in the range 555–700 nm and excitation at 545 nm with a band pass of 2 nm for excitation and emission.

In vivo absorption spectra for the samples were measured with a Beckman DU 640 Spectrophotometer. Scatter correction was made by filling half the sample volume with glycerol. All samples were scanned in the range 350–770 nm at a scan speed of 1200 nm min^{-1}.

RESULTS AND DISCUSSION

Distribution

Direct counts performed by epifluorescence microscopy on 101 samples from different layers (the surface mixed layer, the SCML and the depth of nitrite maximum—at sigma theta 15.8) showed the presence of *Synechococcus* spp. in varying quantities at every station and depth studied in April–May 1994. Minimum and maximum cell concentrations ranged from 9×10^2 to 1.45×10^5 cells ml^{-1} at the surface, from 2×10^3 to 1.23×10^5 cells ml^{-1} at the SCML and from 1.3×10^2 to 3.5×10^2 cells ml^{-1} at the depth of nitrite maximum.

Surface cell counts suggested that cells are unevenly distributed in the study region, being low at the coastal sector under the direct influence of the river Danube and higher in the western central gyre (Figure 3a). Lowest cell density (9×10^2 cells ml^{-1}) was observed in colder (10.5°C, Figure 3b) and less saline (15 salinity, Figure 3c) waters near the Danube inflow area. Lower cell counts were also characteristic of the less saline Turkish coastal waters where temperature contrast was high. In general, the abundance distribution implies that the area occupied by the cyclonic boundary current (rim current) is relatively low in cell numbers in comparison with the western cyclone. Highest cell counts were attained in the northwestern central gyre. The cell density reached a maximum (1.45×10^5 cells ml^{-1}) in much warmer (15°C) and saline (18.5 salinity) offshore waters where the

surface average for the whole area was 4.5×10^4 cells ml^{-1}. This contrasts with the findings of other researchers in the North Atlantic and Pacific Oceans (Waterbury *et al.*, 1979; Glover, 1985; Olson *et al.*, 1990), where the lowest counts were characteristic of the central oligotrophic oceans. This further suggests that cell abundance is not directly related to nutrient availability, especially in the highly eutrophic north-western shelf area. It is well known that 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 requires further detailed studies. Recent nutrient measurements performed in the Sulina branch of the Danube between 1988 and 1992 have shown five- and twofold increases in total inorganic nitrogen and phosphate loads, respectively, of the river Danube, whereas the riverine input of silicate has 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 in the Black Sea north-west shelf (Sorokin 1983; Petrova-Karadjova, 1984, 1990, 1992; Sukhanova *et al.*, 1988; Bodeanu, 1991, 1993, 1995; Moncheva, 1991, 1992; Moncheva *et al.*, 1991; Bologa *et al.*, 1995) and further down in the south via the rim current along Turkish coastal waters (Uysal and Sur, 1995). However, this was not true for the *Synechococcus* population for this sampling period. Among the ambient water parameters, salinity rather than temperature seemed to have a greater impact on the surface spatial distribution of *Synechococcus* spp. A highly significant ($P = 0.00$, $n = 61$, $r = 0.75$) positive correlation (based on Spearman's Rank Correlation) is observed between cell abundance and salinity values. A similar strong response to the salinity gradient by *Synechococcus* was also reported in Florida Bay (Phlips and Badylak, 1996). Despite the highly significant correlation with salinity, no correlation was found with temperature.

Apart from physico-chemical factors, several other factors may effect *Synechococcus* abundance in a water body. These may be differences in grazing pressure among sites, or timing schedule of the sampling during the day. Recent field and on-board experimental data collected in the Black Sea have shown that a synchronous division occurs between noon and midnight, with an apparent grazing pressure from midnight to noon (Uysal *et al.*, 1998). A similar trend has also been obtained from the Arabian Sea (Sherry and Uysal, 1995). Moreover, the magnitude of the diurnal change was found to be greater than the differences between physically- and spatially-discrete water masses in the Arabian Sea. *Synechococcus* spp. have been shown to exhibit varying degrees of diurnal periodicity in cell division rates both in culture (Campbell and Carpenter, 1986) and in incubation

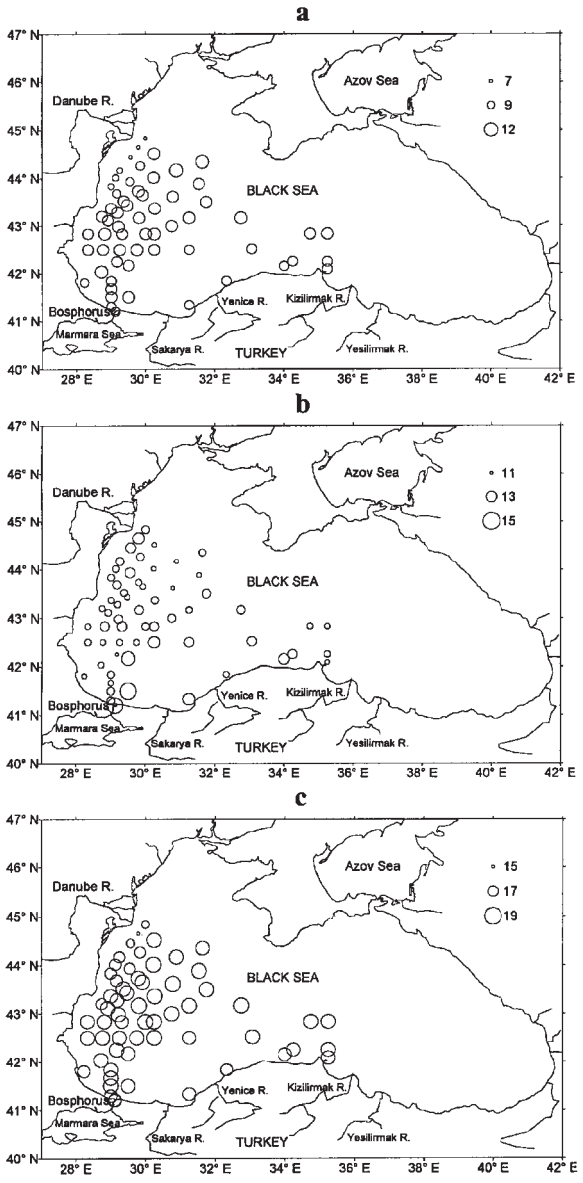

Fig. 3. Surface spatial distribution of *Synechococcus* spp. cell abundance (cells ml^{-1}) (**a**), temperature in °C (**b**) and salinity (**c**) during April–May 1994, in the western Black Sea.

experiments (Carpenter and Campbell, 1988; Kudoh *et al.*, 1990).

Synechococcus cell abundance in the SCML ranged from 2×10^3 (at 10.41°C and 17.26 salinity) to a maximum of 1.23×10^5 cells ml^{-1} (at 7.46°C and 18.52 salinity), with an average of 4.3×10^4 cells ml^{-1}. Abundance distribution of *Synechococcus* spp. cells in this layer is shown in Figure 4a. In contrast to the surface abundance distribution, the northern part of the western cyclone yielded the lowest counts in this layer.

To the west of the western cyclone, very low and high

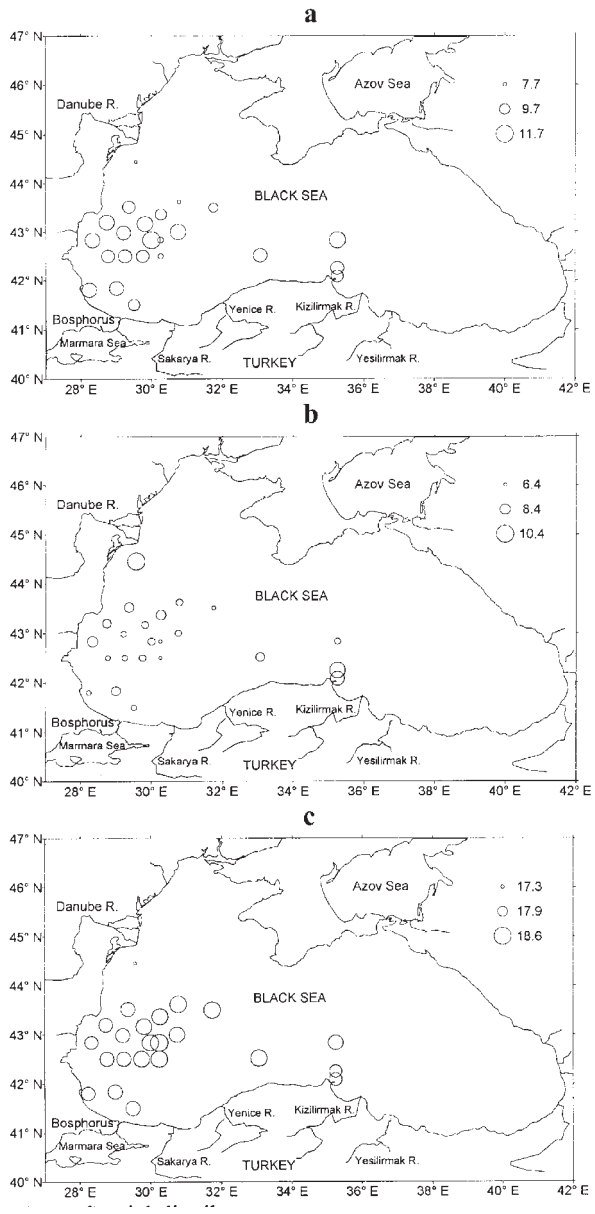

Fig. 4. Spatial distribution of *Synechococcus* spp. cell abundance (cells ml^{-1}) (**a**), temperature in °C (**b**) and salinity (**c**) in the SCML during April–May 1994 in the western Black Sea.

counts at neighbouring stations were observed. It is apparent from Figures 4a and 4b that these three stations with the lowest counts have colder temperatures in common. The station located at the far north near the Danube had the lowest counts (~2000 cells ml^{-1}) in the SCML. Due to intense eutrophication, the depth of subsurface chlorophyll *a* maximum was 10 m at this station. It is for this reason that the temperature of this particular station was highest (10.41°C) and the salinity was lowest (17.26 salinity). Despite the high range of variation in temperature in this layer (Figure 4b) within the stations, there was little change in salinity (Figure 4c), and the magnitude was much smaller than that observed at the surface. However, small changes in salinity seemed to cause spatial heterogeneity in cell concentrations. A strong response to narrow range (15–20 salinity) changes in salinity of *Synechococcus* has been demonstrated by Phlips and Badylak (Phlips and Badylak, 1996). Based on Spearman's rank correlation analysis, a negative correlation ($P = 0.06$, $n = 23$, $r = 0.75$) is observed between cell count and salinity in the SCML. At this stage, it is not known whether any of these factors may have greater control over cell concentrations than the others. As we had few counts from the nitrite maximum layer, we were unable to draw such figures for this layer. Lowest cell concentrations were characteristic of this layer for the Black Sea, as was the case in the North Atlantic and Pacific Oceans (Olson *et al.*, 1990).

Changes in *Synechococcus* spp. abundance with depth in relation to ambient temperature and salinity profile are shown in Figure 5. The observed maximum at 25 m corresponded to the subsurface chlorophyll *a* maximum depth (based on *in situ* fluorometer readings). Cell numbers increased almost twofold from the surface (~2.7 $\times 10^4$ cells ml^{-1}) to 25 m (5.0×10^4 cells ml^{-1}) depth. A sharp decline in cell numbers towards 50 m to ~1000 cells ml^{-1} was evident. This then reduced to about 150 cells ml^{-1} at 130 m depth.

Synechococcus spp. cell counts during September–October 1996 also stressed the spatial heterogeneity in the region. The observed depthwise variations in counts led us to evaluate cell counts for three different layers, the surface mixed, the subsurface chlorophyll *a* maximum and the chlorophyll *a* minimum layer.

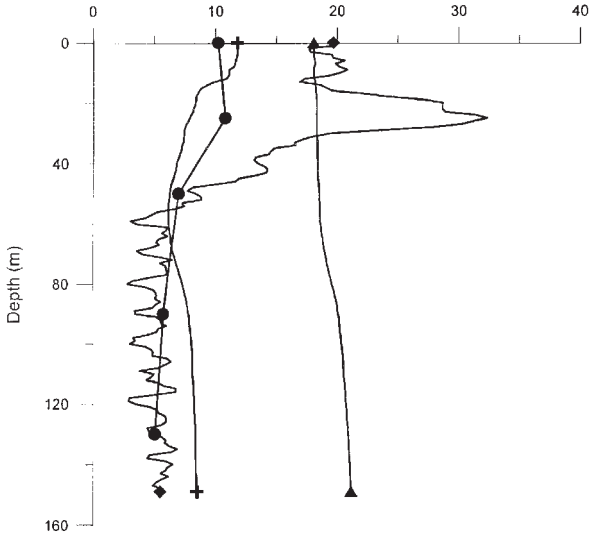

Fig. 5. Concentration (cells ml^{-1}) of *Synechococcus* (•) as a function of depth in relation to ambient temperature in °C (+), salinity (▲) and relative fluorescence (◆) at the station offshore Bosphorus (41°50′N; 29°00′E).

At all stations, the average cell number ml^{-1} at the surface was 1.09 × 10^5 and the minimum and the maximum counts ranged between 3.73 × 10^4 at station 2 in the west and 2.11 × 10^5 cells ml^{-1} at station 18 near Kizilirmak river (Figure 6a). The average for the surface in April 1994 was much less (4.45 × 10^4 cells ml^{-1}). About a sixfold difference was noted between the minimum and the maximum counts, which depicts the extent of variations in abundance between stations in the region. In contrast to the April 1994 cruise, nearshore stations have yielded higher counts than offshore stations. On four transects in Figure 6a, a decrease in abundance towards offshore is observed. In other words, cells were more abundant at relatively warmer (Figure 6b) and less saline (Figure 6c) coastal stations along the cyclonically-meandering rim current. Cell counts at stations located in the western and eastern central gyres remained low.

Synechococcus cell counts at the subsurface chlorophyll maximum layer varied little between stations compared with the surface layer. At this depth, the average cell concentration (1.26 × 10^5 cells ml^{-1}) was higher than the surface average. As seen in Figure 7a, the minimum and

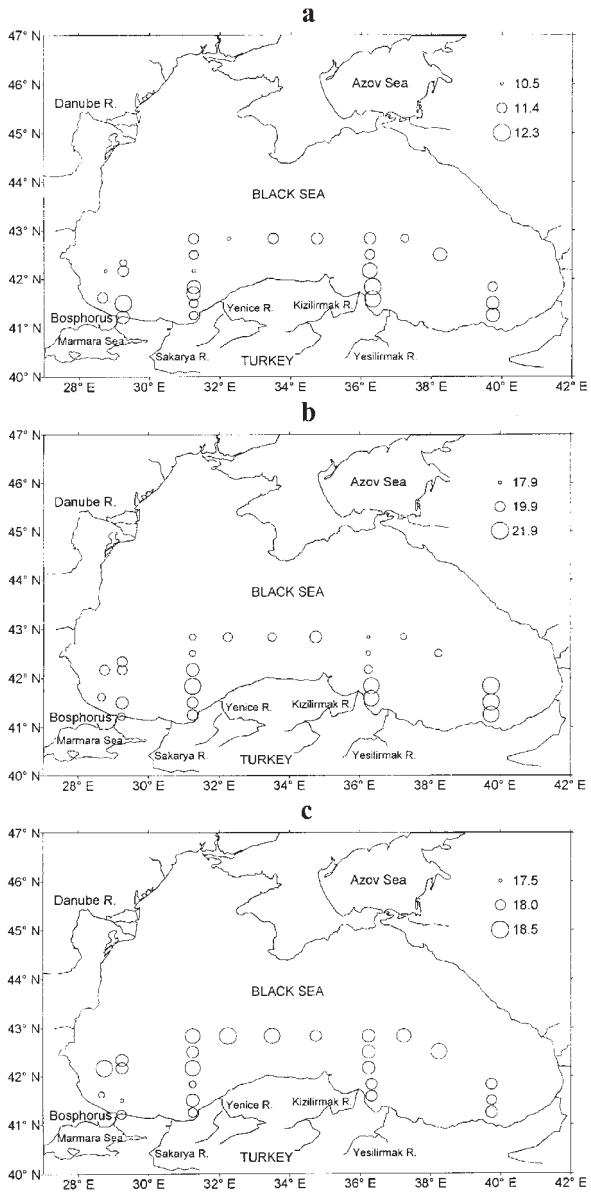

Fig. 6. Surface spatial distribution of *Synechococcus* spp. cell abundance (cells ml^{-1}) (**a**), temperature in °C (**b**) and salinity (**c**) during September–October 1996 in the study region.

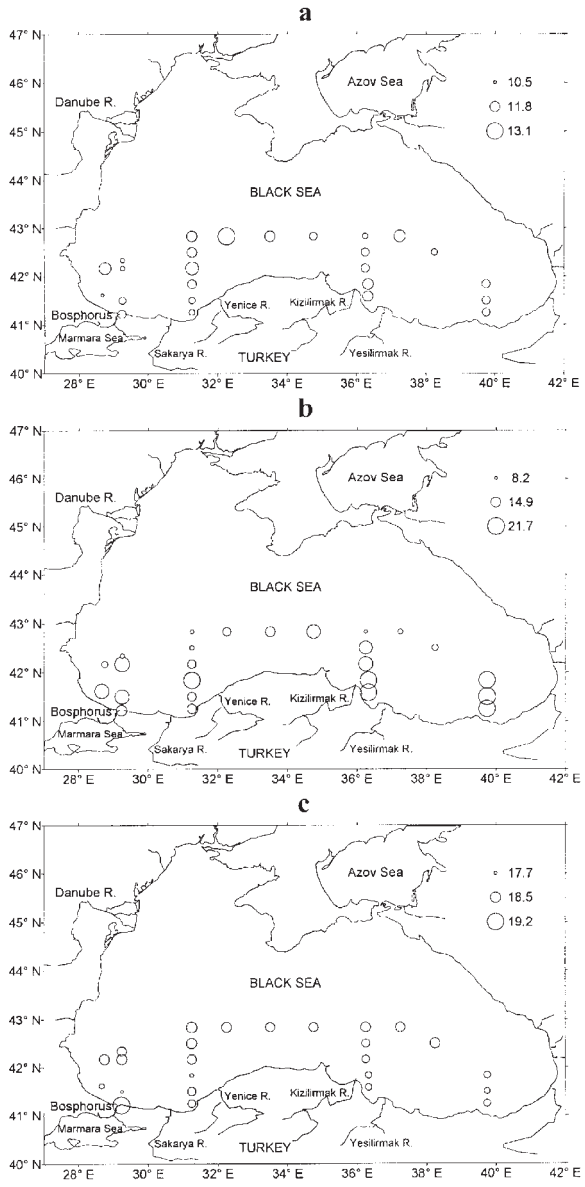

Fig. 7. Spatial distribution of *Synechococcus* spp. cell abundance (cells ml^{-1}) (**a**), temperature in °C (**b**) and salinity (**c**) in the SCML during September–October 1996, in the study region.

maximum cell concentrations ml^{-1} ranged between 3.63 $\times$ 10^4 (at station 1) and 5.19 $\times$ 10^5 (at station 14). In contrast to the observed higher surface cell counts obtained at coastal stations, relatively colder and more saline (Figure 7b, c) offshore waters, especially those included in the western central gyre, were more concentrated at SCML. Only the coastal stations of the transect between Kizilirmak and Yesilirmak rivers had higher abundances.

Maximal heterogeneity in cell abundance between stations is observed in the chlorophyll *a* minimum layer (Figure 8a). Average cell concentration was lowest (1.18 $\times$ 10^4 cells ml^{-1}) compared with the upper layers. Minimum and maximum counts ranged between 1.97 $\times$ 10^3 (at station 8) and 3.25 $\times$ 10^4 cells ml^{-1} (at station 24). Despite the apparent variation in cell abundances between stations, much homogeneity was observed in temperature and salinity levels (Figure 8b, c). Due to the inflow of Mediterranean waters through the Bosphorus to the Black Sea in this layer, the salinity rose to 35.18 at station 3.

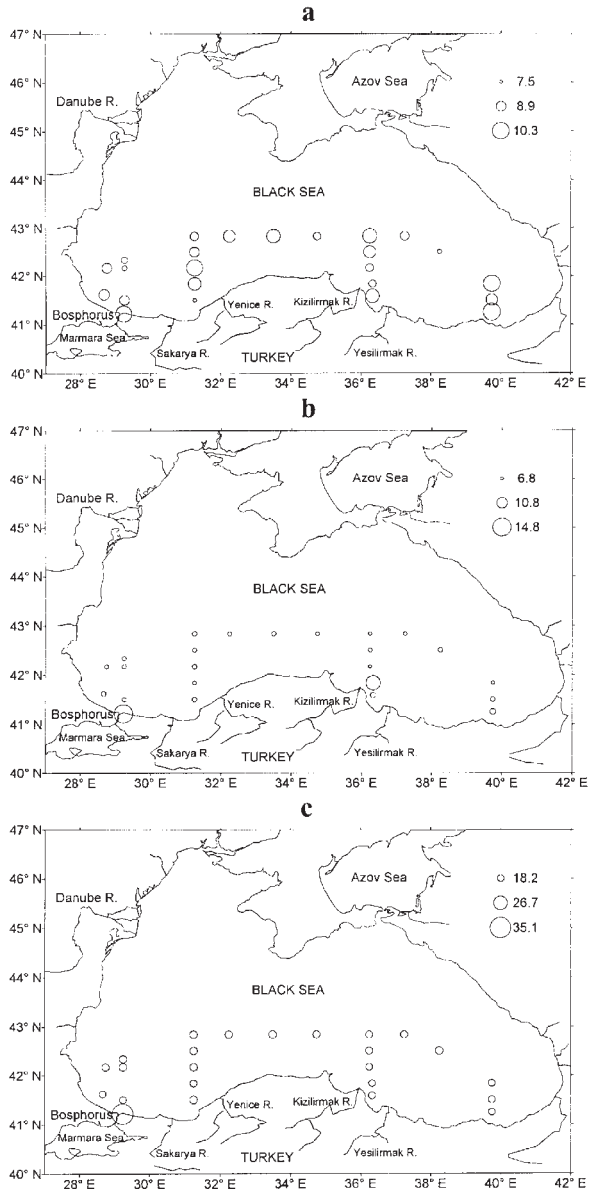

Fig. 8. Spatial distribution of *Synechococcus* spp. cell abundance (cells ml^{-1}) (**a**), temperature in °C (**b**) and salinity (**c**) in the chlorophyll minimum layer during September–October 1996, in the study region.

The vertical profiles of cell distribution for the rim current, western and eastern gyres, and for the Batumi anticyclone (Figure 9), in relation to temperature, salinity, dissolved oxygen, fluorescence and light transmission, show that cells are much more abundant in the surface mixed layer above the thermocline. At coastal station 1, included in the rim current, the majority of the cells were suspended in the surface mixed layer, reaching a maximum of 9.76 $\times$ 10^4 cells ml^{-1} at the surface level. This then gradually decreased to 7.95 $\times$ 10^4 cells ml^{-1} at 10 m, and at 20 m, to a level of 3.63 $\times$ 10^4 cells ml^{-1}.

In contrast to other profiles, *in situ* fluorescence readings were high and showed no distinct sub-maximum within the surface mixed layer; the light transmission was slightly weaker due to higher biogenic production along the rim current. It was also apparent from the dissolved oxygen concentrations that higher levels of photosynthetic activity took place in the surface mixed layer and within the thermocline. Highest concentrations attained within the thermocline can be partly attributed to increased solubility of dissolved oxygen at lower temperatures. Concentrations of 4–8 ml l^{-1} indicate higher photosynthetic activity (Parsons *et al.*, 1984). Cell numbers dropped to 1.42 $\times$ 10^4 cells ml^{-1} just below the thermocline at 30 m depth, and at 40 m there were only 5.26 $\times$ 10^3 cells ml^{-1}. There were no significant changes in abundance at lower depths.

A similar vertical profile was observed for station 6 located in the western gyre, except that there was an apparent subsurface chlorophyll maximum at around 28 m depth where the dissolved oxygen concentration was also highest (11.77 ml l^{-1}). Cells were most abundant at the surface level and decreased gradually towards the base of the thermocline; they showed an increase below the thermocline at 28 and 42 m depths. The abundance decreased from the surface (5.87 $\times$ 10^4 cells ml^{-1}) to 20 m (2.28 $\times$ 10^4 cells ml^{-1}) and again, increased twofold at 28 m (4.58 $\times$ 10^4 cells ml^{-1}). It further decreased to 1.88 $\times$ 10^3 cells ml^{-1} at 75 m and to 145 cells ml^{-1} at 108 m.

At station 21 located in the eastern gyre, cells were most abundant in the top 23 m, reaching a maximum of 1.1 $\times$ 10^5 cells ml^{-1} at 17 m. Below the thermocline at 27 m, the abundance decreased halfway to 5.8 $\times$ 10^4 cells ml^{-1} and to 1.94 $\times$ 10^4 cells ml^{-1} at 46 m. This was the depth corresponding to the bottom of the euphotic layer. At this

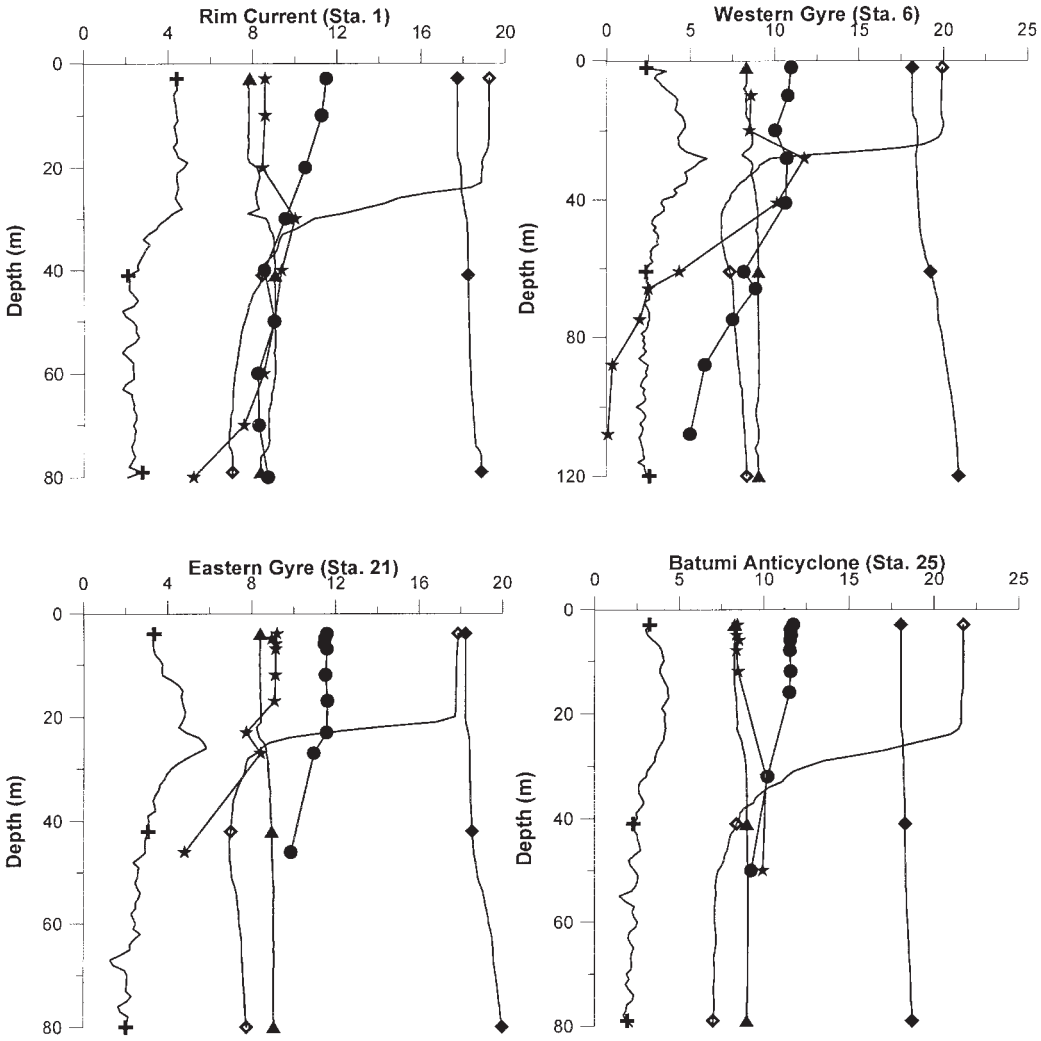

Fig. 9. Abundance (cells ml^{-1}) of *Synechococcus* spp (●) as a function of depth relative to those of temperature in °C (◇), salinity (◆), dissolved oxygen in ml l^{-1} (★), fluorescence in a.u. (+) and light transmission as % value (▲) at stations 1, 6, 21 and 25 in September–October 1996. a.u.: arbitrary unit. One tenth of the actual light transmission values obtained from the 25 cm Sea-Tech Transmissometer installed on the CTD probe are plotted to fit in the scale.

station only, the dissolved oxygen concentration showed no submaximum within the thermocline.

Maximum cell abundance at the surface level (1.25×10^5 cells ml^{-1}) was found at station 25 in the Batumi anticyclone, reaching a mean value of 1.06×10^5 cells ml^{-1} for the surface mixed layer. This then decreased sharply to 2.75×10^4 cells ml^{-1} at 32 m and to 1.03×10^4 cells ml^{-1} below the euphotic layer at 50 m. The bottom line for the euphotic layer was just below the thermocline at 32 m in the Batumi anticyclone. This indicates that on average, almost 90% of the existing *Synechococcus* spp. biomass is either being grazed or microbially-degraded within the euphotic layer during formation of the seasonal thermocline [late spring and summer (Oguz *et al.*, 1992)] in the Black Sea. The mean abundance for the western gyre was lowest for the surface mixed layer (4.34×10^4 cells ml^{-1}). The mean value for the eastern gyre was close (1.04×10^5 cells ml^{-1}) to that of the Batumi anticyclone and remained at a much lower level (7.11×10^4 cells ml^{-1}) in the rim current.

Significant correlations ($r > P_{0.01}$) have been observed between cell counts and physical and chemical parameters (Table I). Highly significant negative correlations were found between cell counts and salinity, depth and nutrient salts (phosphate, nitrate, silicate). This can be summarized as the decrease in cell counts and increase in the parameters listed above with depth. The positive correlation between cell counts and temperature, dissolved oxygen, *in situ* fluorescence and chlorophyll *a* denotes a decrease in all with depth in parallel. Briefly, we

Table I: Relationships between (a) physical and (b) chemical parameters and Synechococcus spp. abundance based on Spearman's rank correlation coefficient

(a)

	Temperature	Salinity	Dissolved oxygen	Depth	Time
Cell	0.6695	0.6292	0.3307	–7164	0.0548
abundance	117	117	117	117	117
	0.0000	0.0000	0.0004	0.0000	0.5552

(b)

	Phosphorus	Nitrogen	Silicium	Fluorescence	Chl *a*
Cell	–0.2980*	–0.6761	–0.5947	0.7132	0.6880
abundance	117**	117	117	117	46
	0.0013***	0.0000	0.0000	0.0000	0.0000

*Correlation coefficient; **Sample number; ***Significance level.

can conclude that, besides grazing pressure and the timing schedule of sampling during the day, the abundance distribution of the cyanobacteria *Synechococcus* spp. in the water column is much dependent on the ambient physicochemical factors.

Pigments

Representative fluorescence emission and excitation spectra for the exponentially-growing Black Sea *Synechococcus* clone BS021 are shown in Figure 10. A summary of spectral characters for all the Black Sea isolates is given in Table II. Excitation at 545 nm, absorbed maximally by the phycoerythrobilin chromophores, yielded an *in vivo* fluorescence emission maximum at 578 ± 1 nm in all isolates. In a similar study, Wood *et al.* (Wood *et al.*, 1985) found an *in vivo* fluorescence emission maximum at a similar range (at 576 ± 1) for type 2 PE-containing WH7805 and WH8018 clonal cultures obtained from the Center for Culture of Marine Phytoplankton (Bigelow Laboratory for Ocean Sciences, Maine). A similarly negligible difference was also observed in the excitation maximum for *in vivo* fluorescence emission at 578 nm (Figure 10c). The excitation peak for PE emission at 578 nm was around 566 ± 1 nm for the Black Sea cultures, which falls in the same range (567 ± 3) as those found for clones WH7805 and WH8018 (Wood *et al.*, 1985).

The *in vivo* absorption spectrum from the exponentially-growing Black Sea *Synechococcus* clone BS021 obtained from 20 m depth is shown in Figure 11. All clones examined 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 obtained for the clones WH7805 and WH8018. The *in vivo* absorption maximum for phycoerythrobilin (PE) was about 570 ± 1 nm for Black Sea clones and about 569 ± 2 nm for clones WH7805 and WH8018.

Size

Information gathered from the flow cytometer mean forward light scatter data revealed that *Synechococcus* spp. cell size differs with depth. Results indicated an apparent decrease in cell size with depth. Cells at the surface mixed layer (0–10 m) were larger than the cells at lower depths (20–60 m). As shown in Figure 12, the majority of the cells in the surface mixed layer fell within the 0.7–0.8 μm size group, whereas the dominant cell size at lower depths of 20–60 m was about 0.5 μm.

Growth

Significant differences (based on Student's *t*-test) in the acclimated growth rates of clones from different depths were observed. It is interesting to note that the clones isolated from near the surface were the slowest growers at the higher irradiance levels tested. Growth of the 10 m clone at all light intensities was the slowest compared with the others (Figure 13). At 5 and 10 μEinst m^{-2} s^{-1}, light level maximal growth rates were obtained for the 20 m clone. In addition, this clone behaved totally differently from the others in that the increase in chlorophyll content was much faster at all light intensities in clone BS20m. With increasing light intensity, clone BS50m started to exhibit higher growth rates. In general, clonal isolates

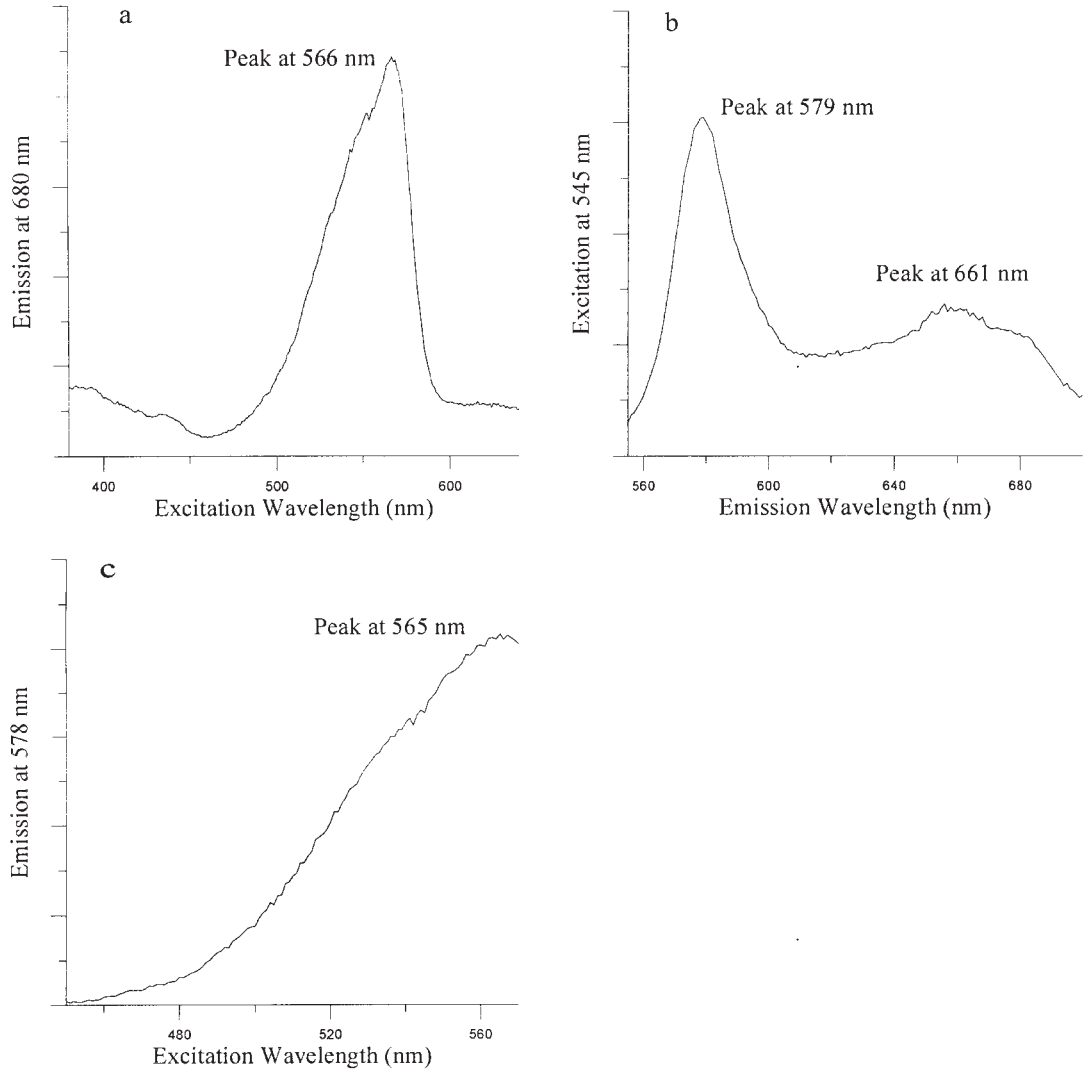

Fig. 10. Energy-corrected fluorescence excitation spectrum for Chl *a* emission at 680 nm (**a**), fluorescence emission spectrum with excitation at 545 nm (**b**) and excitation spectrum for PE emission at 578 nm (**c**) for exponentially-growing Black Sea *Synechococcus* spp. clone BS021 isolated from 20 m depth.

from deeper parts of the euphotic zone had higher growth rates at all light intensities than those obtained from the surface mixed layer, except clone BS20m. Although the spectral properties of all clones were the same, growth rate data show how diverse the clones are. Further studies are necessary to determine whether there is genetic diversity among these clones. Studies of isolates and field samples suggest that physiologically and genetically different *Synechococcus* groups may exist at the same site (Palenik, 1994).

Diurnal variability

From our field and on-board microcosm experiments conducted on board R/V Bilim in the Black Sea (Uysal *et al.*, 1998) and field data collected during the Arabian Sea expedition (Sherry and Uysal, 1995), we found that cells of *Synechococcus* spp. are, in general, under grazing pressure between midnight and noon; the population slowly begins to rebuild in the afternoon by dividing throughout the evening. In other words, cell division dominates during the latter half of the day, even if grazing continues throughout the day. This feature is apparent at the surface layer and is also true for the SCML (Figure 14) and the chlorophyll minimum layer. Similar diel variations in *Synechococcus* abundance were also observed in the equatorial Pacific (DuRand and Olson, 1996) and Sargasso Sea (Olson *et al.*, 1990), where the maximum was found near dusk. Similar to this study, the amplitude of the diel variation tended to decrease

Table II: Spectral characteristics of Black Sea 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 maximum (average for all depths)	
0–60 m	578 ± 1 (*n* = 64)
Excitation maximum for *in vivo* fluorescence emission at 578 nm	
0–60 m	566 ± 1 (*n* = 64)
In vivo absorption maximum	
0– 0 m	570 ± 1 (*n* = 31)

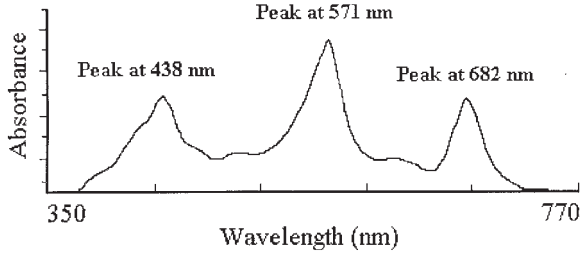

Fig. 11. *In vivo* absorption spectrum from exponentially-growing Black Sea *Synechococcus* spp. clone BS021.

with depth in the equatorial Pacific (DuRand and Olson, 1996).

In conclusion, it has been clearly shown that *Synechococcus* spp. are most abundant in the surface mixed layer of the Black Sea. Visual inspection of the individual cells under the epifluorescent microscope revealed that cells in the subsurface chlorophyll *a* maximum layer (SCML—based on *in situ* fluorometer readings) fluoresce brighter and for longer than those at the surface and at lower depths. Spectral properties of *Synechococcus* spp. clonal isolates from different depths within the euphotic layer (about top 60 m) on the southern Black Sea coast showed that all have type 2 phycoerythrobilin in common and lack phycourobilin. It was shown from the flow cytometer mean forward light scatter data for size distribution that cells in the surface mixed layer (0–10 m) were larger in size than the cells at lower depths (20–60 m). Lastly, clones of *Synechococcus* isolated from different depths were of the same pigment type, but showed significant differences in acclimated growth rates as a function of irradiance.

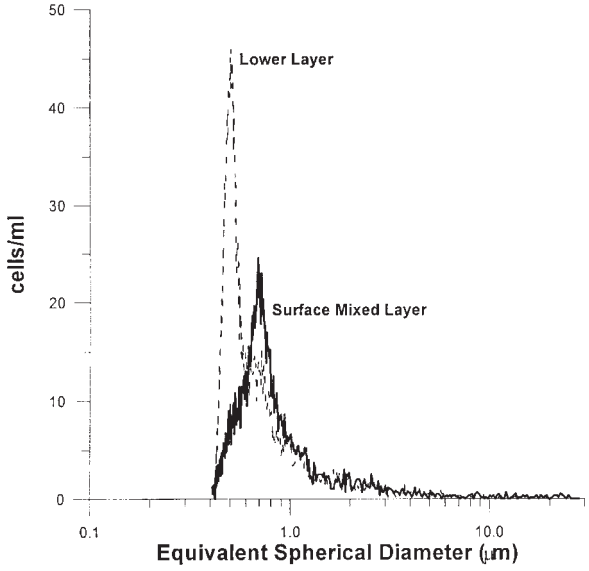

Fig. 12. Comparison of cell sizes of Black Sea *Synechococcus* spp. between the surface mixed layer and the lower layer.

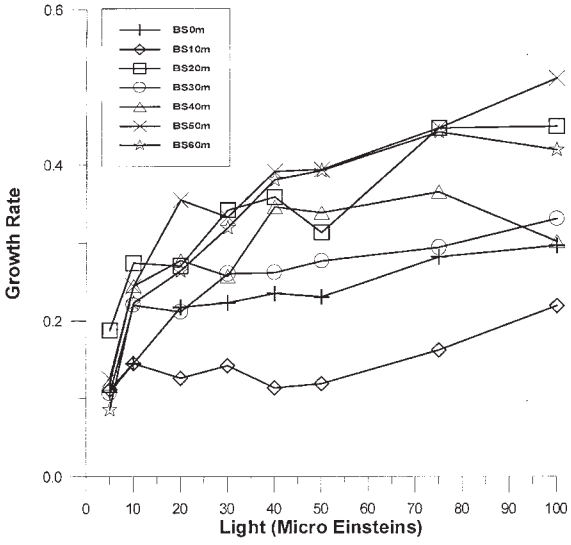

Fig. 13. Growth rate versus irradiance (μEinst m^{-2} s^{-1}) curves for clonal isolates of Black Sea *Synechococcus* spp. from different depths. Clones were grown at 18°C.

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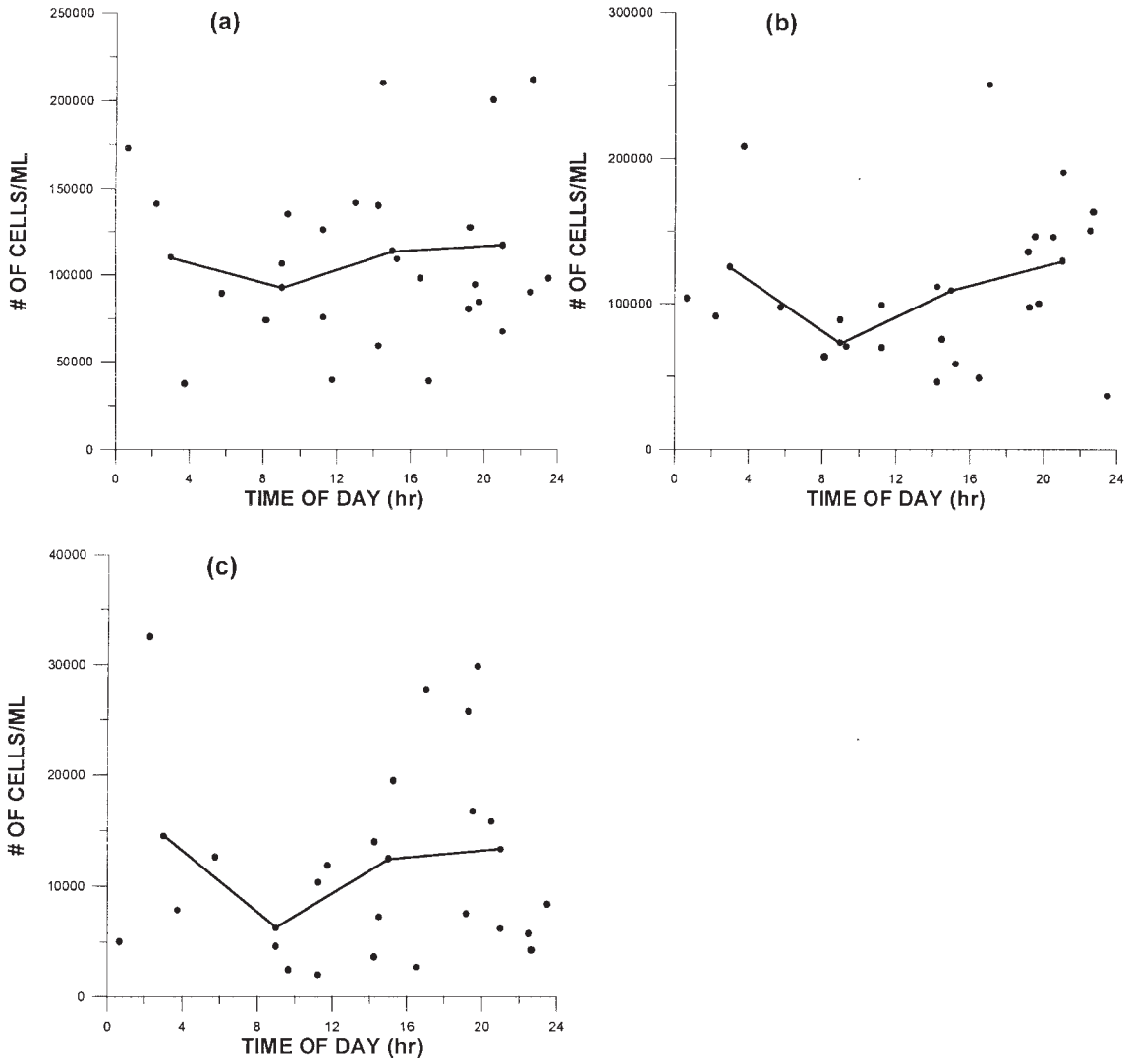

Fig. 14. Diurnal variability of *Synechococcus* spp. cell abundances at the surface (**a**), at the SCML (**b**) and at the chlorophyll minimum layer (**c**) in September–October 1996 in Turkish coastal waters of the Black Sea. Points on the line denote averages of counts for every consecutive 6 h period.

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