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Picoplankton Dynamics during Late Spring 2010 in the South-Eastern Black Sea

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Abstract

Abundance and biomass of picoplankton which are major component of the marine microbial food web with physico-chemical environmental variables were monitored monthly in the south-eastern Black Sea during late spring 2010. According to our results *Synechococcus* spp. abundance range from 3.67 x10^5 - 6.58 x10^8 cells L^{-1} whereas biomass range from 0.15-23.9 µg C L^{-1}. Maximum abundance and biomass of *Synechococcus* spp. were found at 1% light level at June 2010. The minimum and the maximum heterotrophic bacterial abundance and biomass ranged between 1.14x10^9- 3.63x10^9 cells L^{-1} and 6.24-76 µg C L^{-1}, respectively. Heterotrophic bacteria made a higher contribution to picoplankton biomass on the region, while picophytoplankton became more important within the microbial food web at 30 m in June. The ratio of bacterial carbon (integrated 30 m) to phytoplankton carbon was 0.69, 0.24, 0.49 at April, May and June, respectively. In the top of 20 m water column phytoplankton carbon biomass was 2-3 times higher than picoplankton. Below this depth, however, picoplankton represented the dominant fraction in terms of carbon biomass (50-75%).

Keywords: Cyanobacteria, heterotrophic bacteria, carbon biomass, microbial loop, Black Sea.

Güney Doğu Karadeniz'de 2010 Yılı Baharının Son Periyodu Boyunca Pikoplankton Dinamikleri

Özet

Mikrobiyal besin zincirinin önemli katılımcısı olan pikoplankton bolluğu ve biyokütlesi çevresel parametreler ile birlikte 2010 yılı geç ilkbahar periyodunda aylık olarak izlenmiştir. Çalışmanın sonuçlarına göre; *Synechococcus* spp. bolluğu 3,67 x10^5 - 6,58 x10^8 hücre L^{-1}, biyokütlesi ise 0,15-23,9 µg C L^{-1}arasında değişmiştir. *Synechococcus* spp.'ye ait en yüksek bolluk ve biyokütle Haziran 2010'da %1'lik ışık derinliğinde gözlenmiştir. Minimum ve maksimum heterotrofik bakteriyel bolluk ve biyokütle, sırasıyla 1,14x10^9 - 3,63x10^9 hücre L^{-1} ve 6,24-76 µg C L^{-1}arasında değişmiştir. Heterotrofik bakteri bölgede pikoplankton biyokütlesine daha yüksek katkıda bulunurken, pikofitoplankton Haziran 2010'da 30 m'de mikrobiyal besin zincirinde daha önemli bir yer almıştır. Nisan, Mayıs ve Haziranda bakteriyel karbon (30 m'ye entegre edilmiş) fitoplankton karbon oranı sırasıyla, 0,69, 0,24, 0,49 olarak bulunmuştur. 20 m'lik üst su kolonunda fitoplankton karbon biyoması pikoplankton karbon biyokütlesinden 2-3 kat fazla bulunmuştur. Bununla birlikte, bu derinliğin altında ise pikoplankton karbon biyokütlesinde (%50-75) baskın duruma geçmiştir.

Anahtar Kelimeler: Siyanobakteri, heterotrofik bakteri, karbon biyokütle, mikrobiyal döngü, Karadeniz.

Introduction

In plankton communities, an important indicator of ecological dynamics is the relative balance between the biomass of heterotrophic bacteria and autotrophic phytoplankton (Odum, 1971). Picoplankton which consist heterotrophic and autotrophic bacteria are base of the food web and also are a significant percentage of the total biomass of the ocean. Microbial community acts as a sink for organic C respired as CO_2 in the eutrophic systems where as a direct link between trophic levels in the oligotrophic systems (Pomeroy *et al*., 2007).

The Black Sea is characterized by a permanently stratified water column and represents the largest anoxic basin in the world. So it provides an ideal site for exploration of microbial processes (Murray *et al*., 1995; Oguz *et al*., 1999). The Black Sea fed by many large rivers runoff which carry considerable loads of anthropogenic nutrients and contaminants, and the shelf waters are generally more turbid and polluted than the gyre waters (Murray, 1991; Sur *et al*., 1996).

Last three decade, many of study indicated some changes at trophic status of the Black Sea (Oguz *et al*., 2006). These shifts may affect the distribution and production of microbial communities which is major component of microbial loop (Bouvier *et al.*, 1998; Jackson, 2001).

Recently microbial food web has been a significant part of biological oceanography research (Ducklow, 2001). Especially, contribution of heterotrophic and autotrophic bacterial carbon to total carbon biomass is important. However, the picoplankton in the region has been less well-studied than elsewhere. The previous studies generally focused on photosynthetic picoplankton or only related to bacteria. Uysal (2001) reported the abundance and diel fluctuations of *Synechococcus* spp.in the western and southern Black Sea. Bird and Karl (1991) studied microbial biomass and population diversity; Sorokin *et al.* (1995) studied the biomass, production and activity of bacteria; Morgan *et al*. (2006) studied microbial abundances and production in western part of the Black Sea. On the other hand, studies based on both heterotrophic and autotrophic picoplankton variation in the Black Sea during the same sampling period were remained poorly. In the present study, spatial distributions and temporal variations of cell abundance and carbon biomass were determined with environmental parameters. Moreover, a particular attention was also paid to reveal preliminary investigations on the major microbial loop in the study area. Contribution of picoplanktonic carbon to coastal food web was also discussed at late spring in the south-eastern Black Sea.

Materials and Methods

Investigation Area and Sampling

Sampling were performed at two stations which are located coastal and offshore waters in the south-eastern part of Black Sea during late spring 2010 on board R/V DENAR (Figure 1).Water depths of the stations were 250 m (YK1) and 750 m (AD1). Water samples collected with Niskin bottles mounted on a Seabird SBE 50 rosette sampler from surface to 60 m depths with 10 m intervals. Temperature, salinity, pH and dissolved oxygen profiles were obtained with a conductivity-temperature-depth-oxygen profiler (CTD, General Oceanic Idranaut 316). Light penetration was measured by using a Li-Cor LI-193SA Spherical Quantum Sensor and LI-190SA Quantum Sensor as $\mu Em^{-2}s^{-1}$ and the 1% light penetration depth (compensation depth) was calculated from the profile for each month around noon.

Nutrient Analysis

Water samples for dissolved inorganic nutrients (NO_3-N, NO_2-N, PO_4-P and SiO_4) were collected 10intervals from surface to 60 m depths and then filtered through 0.45 µmcellulose acetate membranes. The filtrate was collected in 100 ml acid-washed high-densitypolyethylene bottles and kept frozen until analysis. The analyses were conducted by standard Spectrophotometric methods (Parsons *et al*., 1984).

Bacterial Cell Count and Carbon Biomass Estimation

10 ml seawater were immediately fixed with glutaraldehyde (final conc. 1%) and stored at 4°C in the dark for further analysis. All samples were processed within 2 weeks. For the estimation of picoplankton number the acridine-orange direct count method according to Hobbie *et al.* (1977) was applied. Cell counts were performed under a Nikon E 600 epifluorescence microscope with a filter combination of B-2A (blue excitation, dichroic mirror DM 505, excitation filter EX 450-490, barrier filter BA 520) and G-1A (green excitation, dichroic mirror DM575, excitation filter EX 546/10, barrier filter BA 580). Bacterial cells in at least 30 microscopic fields

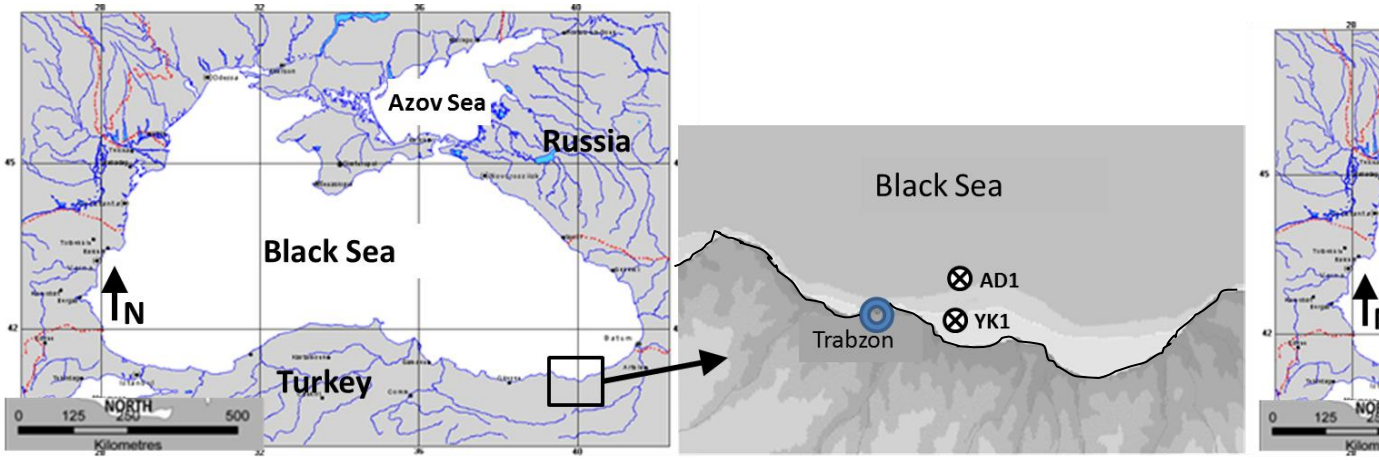

Figure 1. Sampling stations.

were counted. Mean cell volumes were estimated using image analysis system composed of a digital camera, computer and the image analysis software. To calculate carbon content of bacteria and *Synechococcus* spp., 77 and 123 fg carbon per cubic micron were used, respectively (Carlson *et al*., 1999; Waterbury *et al*., 1986)

Phytoplankton Carbon Biomass Estimation and Chlorophyll a

For microscopic analysis, depending on the phytoplankton density, 1 L seawater samples were fixed in a borax-buffered 4% formalin seawater-solution. Samples were concentrated to 10 ml by sedimentation methods. The major taxonomic groups were determined (Rampi and Bernhard, 1978; Spector, 1984; Tomas, 1996) and enumerated by using a Sedgewick- Rafter cell under a phase contrast binocular microscope (Nikon E600). Phytoplankton biomass as a carbon was estimated for diatoms, dinoflagellates and coccolithophores using the relationship described by Menden-Deuer and Lessard (2000):

$$Diatoms = 0.288 \times V_{0.811}$$
$$Dinoflagellates = 0.760 \times V_{0.819}$$
$$Other\ groups = 0.216 \times V_{0.939}$$

where phyto-carbon is the mass of carbon (pg C cell^{-1}), then converted to µg C cell^{-1} and V the volume (µm^3). The volume of each cell was calculated by measuring appropriate morphometric characteristics (Menden-Deuer and Lessard, 2000).The Chl-*a* concentration was determined spectrophotometrically according to Parson *et al*. (1984)

Results

Hydrography

Vertical profile of water temperature, salinity, dissolved oxygen and pH profiles obtained during sampling period are shown in Figure 2. Over the sampling period, sea surface temperatures fluctuated from 9.52 to 22.42°C and seasonal thermocline was observed at 30 m depth in May. As a seasonal pattern, temperature was lowest in April, increased rapidly as the seasonal progressed, reaching >20°C in June. The salinity ranged from 16.83‰ to 17.71‰. In the layer shallower than 20 m depth, less saline water were observed in May and June indicating that runoff and/or rain fall influenced salinity conditions. Dissolved oxygen value ranged between 275 and 331 µM at surface water. No significance differences were found at CTD profiles between coastal and offshore station. The thickness of the compensation depth (define as the depth of 1% of the surface light) ranged between 24 and 30 m during late spring.

Nitrate + Nitrite concentrations in sampling stations ranged from 0.25 µM to 14.2 µM and showed two peaks at 30 m and 50 m depth in offshore stations. Phosphate concentration was less than 0.017 µM at coastal station, and higher than 0.01 µM at off-shore station from April to June 2010. Silicate concentration was shown similar trend throughout the water column at both of stations and was change between 0.21 µM-11.04 µM.

Autotrophic Bacterial Abundance and Cell Volume

Vertical distribution of *Synechococcus spp*. is shown in Figure 3. The *Synechococcus* spp. abundance during this period in the top 60 m range from 3.67 x10^5 cells L^{-1} to 6.58 x10^8 cells L^{-1}. *Synechococcus* spp. abundance distributed uniform in water column at April 2010. The maximum cell density was observed in subsurface (20-30 m) in May and June, just after the beginning stratification. Highest cell counts of *Synechococcus* spp. (6.58 x10^{8}cells L^{-1}) were attained at 1% light level (30 m) in offshore waters at June 2010 where the surface cell density was 3.67x10^{5}cells L^{-1}. A sharp decline in cell numbers below 30 m was evident. The cell density of autotrophic picoplankton was lower at coastal station than at offshore.

Mean cell volumes (MCV) were variable in the surface layers ranging from 0.16-1.18 µm^3 (Figure 4). A pronounced temporal variation in MCV was noticed with respect to largest cells occurring in April (1.33 µm^3) and with smallest cells from May to June.

Autotrophic Bacterial Biomass

Bacterial biomass values were based on abundances and mean cell volumes, using allometric volume-based carbon conversion factors ranging from 0.15 µg C L^{-1} to 23.9 µg C L^{-1}. Biomass was distributed uniform throughout water column from April to May. The biomass of *Synechococcus* spp. showed peak that coincided with compensation depth (% 1 light level) in the June. The depth integrated (0-60 m) carbon biomass ranging from 0.57 µg C L^{-1} to 4.99 µg C L^{-1}and was approximately threefold higher in June than in April and May. Biomass generally tended to increase with depth till 1 % light level, and decreased gradually.

Heterotrophic Bacterial Abundance and Cell Volume

Vertical distribution of heterotrophic bacteria is shown in Figure 5. The heterotrophic fraction of picoplankton, composed of bacteria proved to be very important in south-eastern part of the Black Sea both as numbers and as carbon biomass. Cell density of heterotrophic bacteria during late spring period at top 60 m ranged between 1.14x10^9 cells L^{-1} and 3.63x10^9 cells L^{-1} with on average 1.95 x10^9 cells L^{-1}.

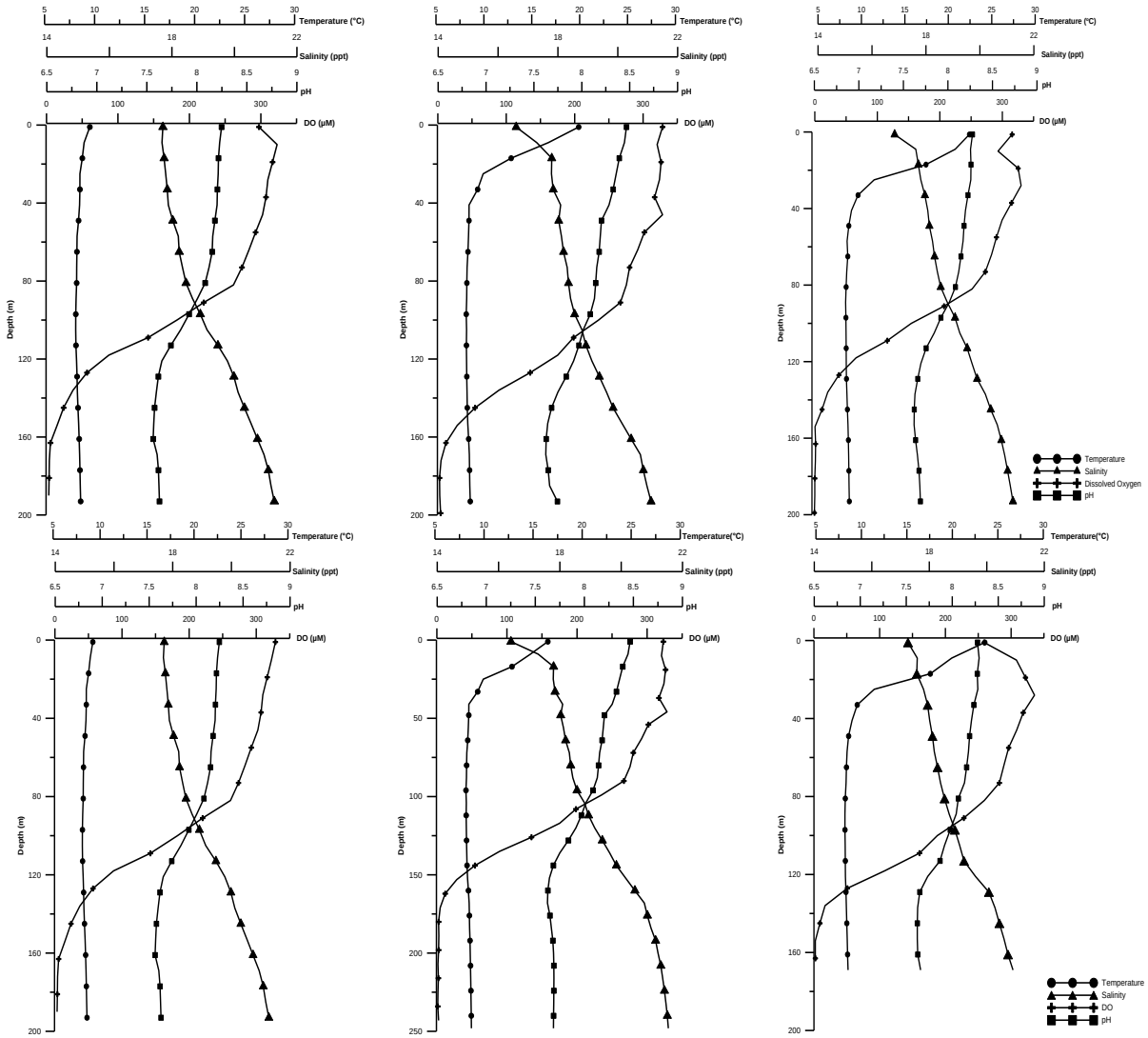

Figure 2. Vertical distribution of temperature (°C), salinity (ppt), Dissolved Oxygen (µM) and pH at sampling stations (A, B, C, coastal stations, D, E, F, offshore stations at April, May, June 2010, respectively).

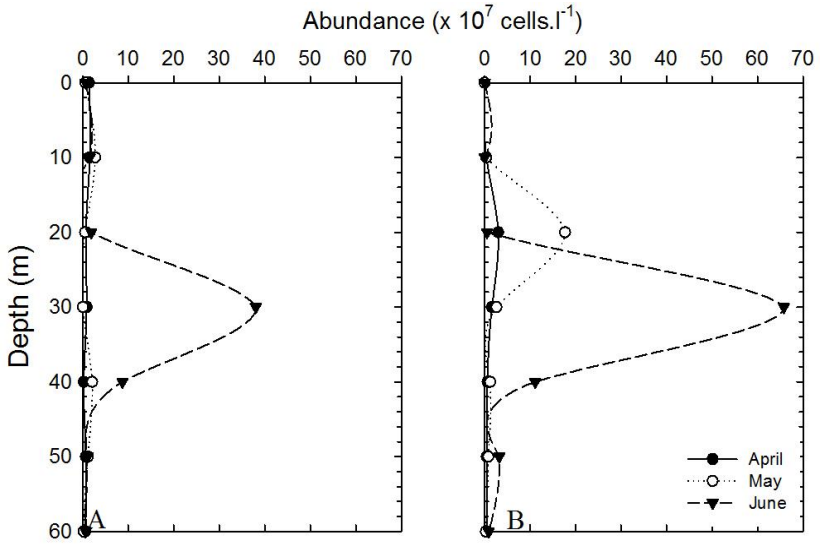

Figure 3. Vertical distribution of *Synechococcus* spp. (A, Coastal, B, Offshore stations).

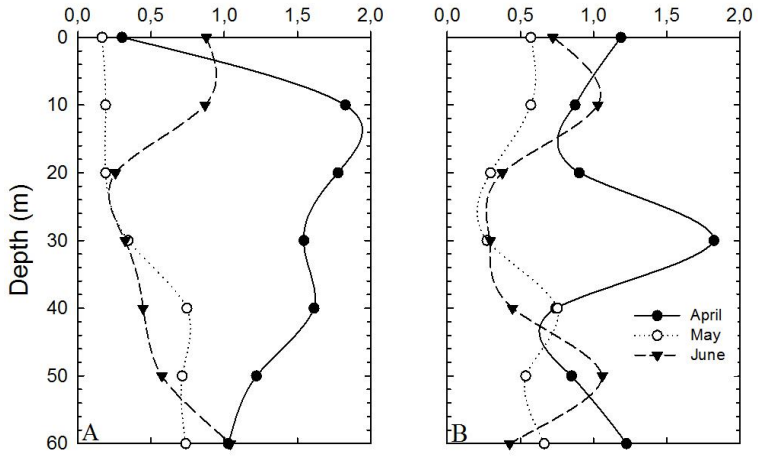

Figure 4. Temporal variation of mean cell volume (μm^3) of *Synechococcus* spp. at sampling stations (A: Coastal station, B: Offshore station).

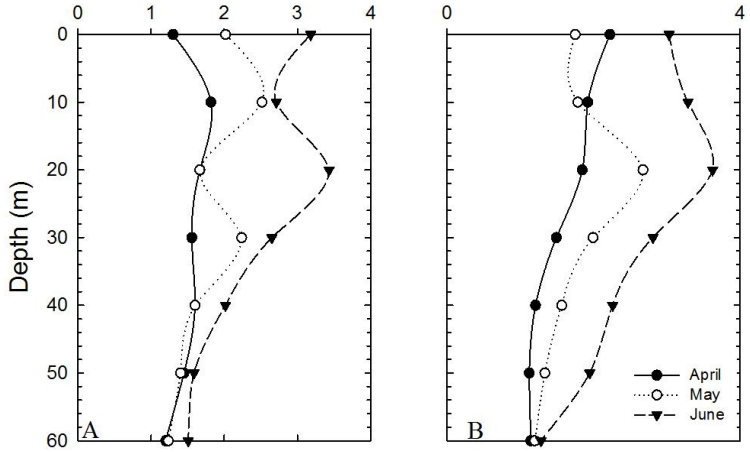

Figure 5. Vertical distribution of heterotrophic bacteria abundance (x10^9 cells.L^{-1}) at sampling stations (A: Coastal station, B: Offshore station).

Cell numbers of bacteria increased from April to June and decreased gradually with depth below 30 m. No significant statistical difference was observed between sampling stations. The maximum abundance of bacteria (3.63x10^9 cells L^{-1}) was found at above thermocline stratified water in June. The depth integrated bacterial number showed similar trend both of coastal and offshore area, and ranging from 1.51x10^9 cells L^{-1} to 2.6x10^9 cells L^{-1}.

Most of the bacteria were cocci or very small short rods at top 60 m. MCV were variable in the surface layers ranging from 0.05 μm^3 to 0.25 μm^3 (Figure 6). A pronounced temporal variation in depth integrated MCV was noticed with respect to largest cells occurring in April (0.38 μm^3). In June the smallest bacteria (mean cell volume< 0.09 μm^3) was about 75% of the entire bacterial population.

Heterotrophic Bacterial Biomass

The heterotrophic bacterial carbon contents range from 6.24 μg C L^{-1}-76 μg C L^{-1}. The maximum carbon biomass was measured at 20 m in April at coastal waters. However, bacterial carbon biomass generally distributed uniform at water column. The seasonal distribution of biomass resulted quite difference. The 0-60 m column integrated heterotrophic carbon biomass ranging from 15.64 μg C L^{-1} to 45.47 μg C L^{-1}and was approximately threefold higher in April than May and June.

Picoplankton –Phytoplankton Carbon Ratio and Chlorophyll

During sampling period phytoplankton biomass (integrated to 30 m) ranged from 41.11 μg C L^{-1} to 74.45 μg C L^{-1}, whereas bacterial biomass varied from 16.46 μg C L^{-1} to 46.88 μg C L^{-1}. Over the sampling period Chl-*a* values varied between 0.43 and 3.92 μg L^{-1}. Phytoplankton biomass was generally much higher than bacterial biomass within euphotic zone. However, relative importance of

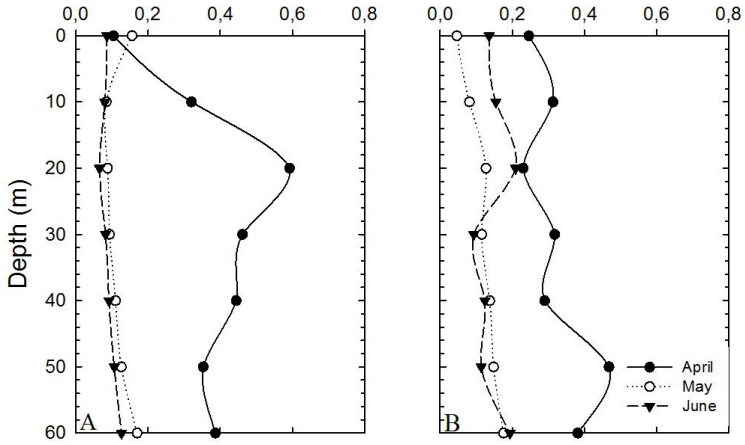

Figure 6. Temporal variation of mean cell volume (μm^3) of heterotrophic bacteria at sampling stations (A: Coastal station, B: Offshore station)

bacterial biomass increased with increasing depth and below 30 m depth became much more important (Figure7). The ratio of bacterial carbon (integrated 30 m) to phytoplankton carbon was 0.69, 0.24, and 0.49 at April, May and June, respectively.

Discussion

One of the most important changes in the Black Sea recorded within last decade is the changing major phytoplanktonic group's ratio. The increase in the ratio of dinoflagellates might be related to the change in nutrient balance and the temperature regime of the seawater (Feyzioglu and Seyhan, 2007; BSC, 2008; Bat *et al*., 2011). Microbial communities can affect directly or indirectly depending on any changes at upper trophic levels. As much as 90% of the sinking POM in the upper water column is mineralized in the sub-mixed euphotic zone and oxycline (Oguz *et al*., 1999); suggesting that bacteria in the subsurface layers can efficiently demineralize sinking POM in the upper water column.

Heterotrophic bacteria numerically dominated the picoplankton community with abundance and biomass during late spring varying from 1.12x10^9 to 3.63x10^9 cells L^{-1} and gradually decreased with depth, while cell volume increased. Sorokin *et al*. (1995) reported very high surface bacterial standing stocks ranging from 80 to 90 μg C L^{-1} on the northwest shelf. Bouvier *et al*. (1998) found lower summer bacterial biomass ranging from 20 to 54 μg C L^{-1}. in the Danube mixing zone and from 14 to 19 mg C m^{-3} in the open portion of the shelf. Similarly, Becquevort *et al*. (2002) reported relatively low biomass values on the open shelf during spring and summer, ranging from 5.6 to 26.7 μg C L^{-1}. Morgan *et al*. (2006) reported much higher bacterial abundance (2.4-2.9x10^9 cells L^{-1}) and biomass (30-38 μg C L^{-1}) in the northwest shelf than shelf break and central basin. In the south-western Gyre, Bird and Karl (1991) found surface-layer bacterial abundances of about 0.08x10^9 cells L^{-1}, while Sorokin *et al*. (1995) reported much higher abundances ranging from 0.9 to 1.63x 10^9 cells L^{-1}. In our study, surface bacterial abundance on the south-eastern shelf during the late spring, ranged from 1.3x10^9 to 3.18x10^9 cells L^{-1}, and biomass ranged from6.24 to 42.08 μg C L^{-1}. These values are generally much higher than values for the northwest shelf and central basins. In the south-eastern Black Sea, salinity declined during early summer was accompanied by an increase in phytoplankton abundance, as shown by the highly significant negative correlation between salinity and Chl-*a* ($r = -0.5512$, $P<0.05$) and between salinity and autotrophic picoplankton ($r = 0.5409$, $P<0.05$). However, with respect to heterotrophic bacterial abundance, a direct influence of salinity or an indirect one via phytoplankton abundance was not observed. During late spring, no direct correlations were also found between picoplankton abundance and nitrate, nitrite, phosphate, and silicate.

Since heterotrophic bacteria depend in large measure on the organic matter produced by autotrophic organisms, one would expect a positive correlation between the variables of phyto and bacterioplankton. Such as similar correlation, however, was not observed in the south-eastern Black Sea during this investigation. Instead, a large scattering of bacterial abundance regardless of Chl-*a* concentration was found. For example, a significant correlation between both groups of organisms was reported by Bird and Kalff, (1984) and Cole *et al*., (1988). On the other hand no significant correlation was found by Malone and Ducklow (1990) Cho *et al*. (1994). The lack of a significant correlation between the abundances of phyto and bacterioplankton is probably due to several other reasons.

Sorokin *et al*. (1995) reported mean bacterial volume as 0.28 μm^3 in the oxycline zone. In our study, mean bacterial volume was 0.2 μm^3 and tended to decrease from April to June. During April, bacterivore population in this region may be too low

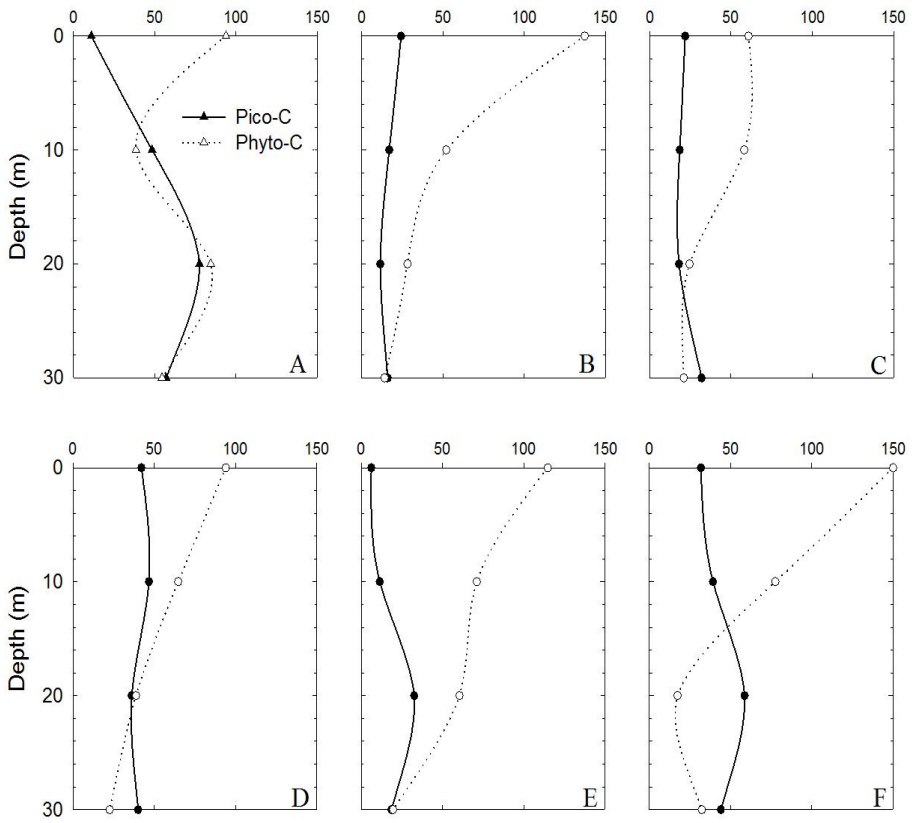

Figure 7. Contribution to carbon biomass of picoplankton and phytoplankton above 1% light level in sampling stations (A: April, B: May, C: June at coastal station, D: April, E: May, F: June at offshore station).

to exert a strong grazing pressure which may increase with increasing temperature and bacterial number end of the late spring. Nevertheless, the effects of predation in the natural environment are difficult to demonstrate as many other factors can affect the picoplankton size and distribution.

Uysal (2001) reported maximum cell abundance of *Synechococcus* spp. $1.25x10^8$ cells L^{-1} at surface water in the Batumi anticyclone. In the same study, maximum abundance was observed within euphotic zone and decreased sharply. Feyzioglu *et al*. (2004) found lower bacterial cell number ($6x10^6$ cells L^{-1}) in the south-eastern coast. In our study surface *Synechococcus* spp. abundance ranged from $3.67x10^2$ to $1.3x10^7$ cells L^{-1}, and biomass ranged from 0.02 to 0.73 µg C L^{-1}. The similar surface variation ($9x10^5$ cells L^{-1}- $1.45x10^8$ cells L^{-1}) also were reported by Uysal (2006). *Synechococcus* spp. tended to increase after spring bloom of nano and microphytoplankton its due to nutrients was exhausted and 1% light depth became deeper. The water stratification and thickness of euphotic zone are dominant factors determining the vertical distribution of cyanobacteria (Glover *et al*.; 1986, Miyazono *et al*., 1992). Similar situation was also observed in the south-eastern Black Sea during this investigation. Expansion of euphotic zone might help to increase C biomass of *Synechococcus* spp. under well mixed water column from April to June 2010. Increase in biomass, manifested by change in cell numbers but without increase in mean cell volume in June.

The cell densities of autotrophic and heterotrophic bacteria were integrated above 60 m layer respectively and mean percentage composition of each groups were calculated. The heterotrophic fraction of picoplankton, composed of bacteria proved to be very important in the south-eastern part of Black Sea both as numbers and as carbon. Heterotrophic bacterial carbon biomass (~80%) surpassed the autotrophic bacterial biomass throughout the water column during the whole period. Integrated biomass ratios of autotrophic to heterotrophic picoplankton (i.e. *Synechococcus* spp. to heterotrophic bacteria) were <0.3. This reveals that heterotrophic bacteria made a higher contribution to picoplankton biomass on the region, while picophytoplankton became more important within the microbial food web at 30 m in June.

A comparison of the concentrations of bacterial and phytoplankton carbon in the south-eastern Black Sea revealed that the ratio of bacterial carbon to phytoplankton carbon was 0.46 and 0.48 (seasonal mean of the carbon ratios) at the coastal station and offshore stations, respectively. This value was somewhat higher than previous studies. Artigas (1998) reported a ratio close to 0.25 in the Gironde Estuary and Gocke *et al*. (2004) reported a ratio 0.44 in the hypertrophic tropical lagoon Ciénaga,

Columbia. The ratio of bacterial carbon to phytoplankton carbon was 0.69, 0.24 and 0.49 at April, May and June, respectively. In the top of 20 m waters phytoplankton carbon biomass was 2-3 times higher than picoplankton. Below this depth, however, picoplankton represented the dominant fraction in terms of carbon biomass (50-75%).

Consequently, picoplankton especially below compensation depth in the south-eastern Black Sea represents an important source of carbon for consumers, through the functioning of the microbial food web. Future studies should examine the physiological and trophic bases for the dominance of bacterial biomass and incorporate them into models of the Black Sea ecological and biogeochemical dynamics.

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