Succession of Picoplankton (Coccolid Cyanobacteria) in the
Southern Black Sea (Sinop Bay, Turkey)

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Abstract: Annual variations in abundance, biomass, shape and size spectrum of picoplankton (coccolid cyanobacteria) were investigated to explain the blooming of diatom and dinoflagellates in the surface mixed layer in the Sinop Bay of the Southern Black Sea during the period between January 1998 and December 1998. At the end of this study, it was observed that the coccolid cyanobacteria were spherical in shape and 1.5-2.0 μm in diameter. It was determined that picoplankton were predominant in the phytoplankton, averaging 99.2% of the total phytoplankton abundance. The abundance and biomass of coccolid cyanobacteria dominated in the picoplankton, averaging 99.1 and 99.2% of the total picoplankton, respectively. The picoplankton biomass varied between 535 and 1347 ng m⁻³. Cell numbers of coccolid cyanobacteria were generally high and varied between 1.3×10⁶ in April and 3.3×10⁷ cells L⁻¹ in June. High cell numbers of 2.1×10⁶ and 2.3×10⁷ cells L⁻¹ were also observed in October and December, respectively. A monthly average of cell number and biomass was estimated as 9.5×10⁵ cells L⁻¹ and 3961 ng m⁻³, respectively. Picoplankton abundances and biomass were not significantly related to physicochemical parameters. During the study period, the abundance (9.0×10⁵ cells L⁻¹) and biomass (3961 mg m⁻³) of coccolid cyanobacteria in the southern Black Sea was generally more than the data for different seasons and areas of the other marine systems. Abundance and biomass in the surface mixed layer in research period, also revealed that picoplankton cells were more concentrated in the shallow waters than offshore waters.

Key words: Southern Black Sea, Sinop Bay, picoplankton, coccolid cyanobacteria, succession

INTRODUCTION

The first investigation that discusses the role of the small fraction in phytoplankton communities in the Black Sea was published by Zaika and Yashin[1]. Investigations of the last few years have shown that the picoplankton in the oligotrophic waters of the ocean, a fraction of phytoplankton 2-3 μm in size, often accounts for up to 50-80% of primary production and chlorophyll[2][3]. Picoplankton predominates in the phytoplankton and averages 85% of total amount of algae in the open area of the Black Sea in winter[4]. Coccolid cyanobacteria predominates in the picoplankton both in abundance and biomass. The abundance and biomass of coccolid cyanobacteria are on average, 98.8 and 95.0% of total picoplankton, respectively. The number of picoplankton cells reaches 1.5×10⁷ cell L⁻¹ in the eastern Black Sea in February[5].

Autotrophic picoplankton can constitute the majority of the biomass and productivity of photosynthetic organisms in marine and freshwater systems. Indirect evidence has indicated that mortality of autotrophic picoplankton occurs principally at night in the open ocean, but continuously in coastal water. The predominant view of the fate of autotrophic picoplankton production in the ocean is that they are consumed by heterotrophic nanoflagellates[6][7]. Estimated heterotrophic nanoflagellate bacterivory accounted for 45-87% of bacterial production in oligotrophic Mediterranean Sea. Small protists (<3 μm) dominated the bacterivore assemblage and accounted for more than 90% of the heterotrophic bacterial consumption[8].

According to Skolka and Bodeani[9], from the taxonomic point of view, Black Sea phytoplankton comprise 1200 species, containing 63% diatoms, 16% dinoflagellates and 21% species belonging to other phyla. These are largely eurytem and euryhaline and 15% of the species are of fresh-water origin. They show a characteristic regional distribution and ecological grouping.

It is known that Sinop Bay in the Southern Black Sea (Fig. 1) is characterized by unstable hydrological conditions such as frequent change of onshore-offshore currents and is characterized as an upwelling area. Moreover, it is known that the Bay is a area of rapid succession in the blooms of individual species of algae
Fig. 1: The sampling stations in the Sinop Bay of the Southern Black Sea

replacing one another in plankton. Diatoms and dinoflagellates in the Sinop Bay were more abundant both qualitatively (96%) and quantitatively (96%) than the other taxonomic groups. Diatoms and dinoflagellates were conspicuous as the two most diverse groups with 49 and 46% in total species number, respectively. Although diatoms and dinoflagellates were almost equally diverse groups in terms of species number, the quantitative proportion of diatoms (75%) in the total community was higher than the quantitative proportion of dinoflagellates (21.9%)

According to investigation in the period of 1995-1996 in the Sinop Bay, the most of the blooming species are generally boreal-tropical and arcto-boreal forms among the diatoms and cosmopolitan forms among the diatoms. It is known that some of the arcto-boreal dinoflagellates blooming in the Black Sea are *Proasterion ballotii* (Lohmann) A.R.Loebl. (9.0×10⁶ cells L⁻¹ in July), *Heterocapsa triquetra* (Ehrenb.) J.R.Stein (6.2×10⁵ cells L⁻¹ in March) and *Grevillea trochoidea* (J.R.Stein) A.R.Loebl. (7.8×10⁵ cells L⁻¹ in June) observed in several periods of the year. However, some important cosmopolitan diatom forms in number are *Pseudonitzschia delicatissima* (Cleve) Heiden (9.0×10⁵ cells L⁻¹ in April), *Cylindrotheca closterium* (Ehrenb.) Ehlmann and Lévin (7.4×10⁵ cells L⁻¹ in July), *Cleveceras danicus* Cleve (2.7×10⁵ cells L⁻¹ in January) and *Pseudonitzschia pungens* (Grunow ex Cleve) Hasle (1.2×10⁶ cells L⁻¹ in August)

To evaluate the spatial and temporal changes in the abundance and biomass of picoplankton (coccolid cyanobacteria) and their role, an investigation was carried out concomitantly with blooming of diatom and dinoflagellates in the Sinop Bay of the Southern Black Sea between January 1998 and December 1998. This investigation is one of the first detailed studies on picophytoplankton on the Turkish Black Sea coast.

The Black Sea with a maximum depth of 2258 m, an average depth of about 1240 m and water volume of 537,000 km³, constitutes one of the world’s largest inter-continental seas exhibiting anoxic water volume (4.6×10⁷ km³, about 87% of the sea volume) below a thin layer (about 100 m) of oxygenated surface waters. The cyclonically meandering rim current which is driven by wind stress and further modified by thermohaline fluxes and bathymetry is a single basin-scale feature.

Biophysical properties of Black Sea: Vinogradov et al. separated the pelagic ecosystem of the Black Sea into two parts: the aerobic and chemobiotic zone. The aerobic zone is reached around ø (sigma teta) equal to 15.4 unit (about 100-150 m). The chemobiotic zone includes the suboxic zone just above the H₂S-layer in depths between 80-150 m and the anoxic or H₂S zone below the suboxic layer. The zone which is permanently formed in the pycnocline is defined as oxygen minimum zone. The suboxic zone is generally located between density surface of 15.4 and 16.2 and relatively thin interface (~10-15 m)

The whole deep basin after the depth of density 16.2 is anoxic because a sharp halocline established below the Cold Intermediate Layer (CIL) prevents ventilation of deep waters.

The distribution of pelagic flora and fauna is related to the boundaries of the oxygen minimum zone. The
surface waters of the Black Sea are biologically productive because of high run-off from rivers located on the north-western part of the basin. About 55% of the terrigenous matter is supplied by the Danube alone and about 25% is transported by the rivers in the south-eastern Black Sea. The terrigenous matter deposition is higher close to shore and reduces transparency and is associated with phytoplankton standing crop particularly in the north-western part of the Black Sea[15-21].

Over the rest of the basin hydrological properties and phytoplankton crop are more stable and much smaller, respectively[21, 22]. The depth of euphotic zone (1% light) in May in the anticyclonic regions and cyclonic region are around 35-40 m and 30-35 m deep in the Black Sea, respectively[23]. Phytoplankton and fishes are absent below the oxicline, where daytime mesozooplankton aggregation occurs. The principal components of the suboxic layer are some zooplankton species such as Sagitta setosa, Pleurobrachia pileus, Pseudocalanus elongatus and Calanus euxinus[24], which have maximum biomass among zooplankton species of the Black Sea[26].

According to the investigation between February and April 1991, in the Central and the Eastern Black Sea[9], the abundance of picophytoplankton varied from 10⁹ to 10¹⁰ cells L⁻¹ in the water column. The peak of abundance (6.0x10⁹ cells L⁻¹) and the minimum (2.5x10⁶ cells L⁻¹) were observed in the eastern Black Sea in February and the center of eastern cyclonic gyre in late-March, respectively. The major portion of the biomass of cyanobacteria was present in a layer over the main pioenclione. Picophytoplankton biomass uniformly decreased from the surface to the pionecline layer and maximum peak generally occurred in upper mixed layer in the Black Sea[26].

MATERIALS AND METHODS

Studies were carried out between January 1998 and December 1998 at 6 stations located mainly in the Sinop Bay (Fig. 1). The studies were combined with hydrographic and chemical observations in all period. To count diatom and dinoflagellates, the 5.0 L water samples were taken with a Hydro-bios universal series water sampler from mixed surface layer (0.5 m) at sampling stations (A1, A2, B1, B2, B3 and B4) in the Sinop Bay of the Southern Black Sea (35°07'05" and 35°17'05" E, 41°57'05" and 42°0'100" N) at monthly intervals. Samples fixed in Lugol (for 5 L, 12.5 mL) were preserved at 2-4°C pending microscopic examination. For enumeration of the phytoplankton species, Utermühl Sedimentation Chambers and Neubauer and Sedgwick-Rafter Counting Slides were used in combination according to the dimensions of the organisms[21-27].

For enumeration of the coccolid cyanobacteria, a surface water sample of 20-25 mL in volume was fixed by glutaraldehyde to the final concentration of 1%. The cells were sedimented in nuclear filters with 0.2 μm pores and stained with prillulin[28]. They were previously stained by Sudan black for recording the filter luminescence. Counts were made under an epifluorescence microscope at 1000x magnification (oil immersion). Cells less than 2 μm was counted on a part of the filter area corresponding to variable of filtered water for coccolid cyanobacteria.

The water column was also sampled with Nansen bottles to determine physico-chemical parameters. Temperature and salinity were measured with water quality checker of a U-10 model Horiba. The major nutrient analyses, inorganic nitrate (NO₃⁻-N), nitrite (NO₂⁻-N) and ammonia nitrogen (NH₄⁺-N); inorganic orthophosphate phosphorus (PO₄³⁻-P) were made according to the standard procedures of Strickland and Parsons[29].

Spectrophotometric measurements of chlorophyll-a used the acetone extracts of membrane-filtered seawater. Fluorescence intensities were obtained at 660 nm emission and 425 nm excitation wavelengths with a bandwidth of 60 nm by a Turner Model 430 Spectrophorometer. Calibration was performed with concentrated acetone extracts of seawater samples which were measured spectrophotometrically.

The biomass was estimated from counting cells and their diameters. From the cell count data for each species transformed in this way, the cell volume (biomass) for the total population is calculated using the formula: ΣV = (V₁ _ N₁) + (V₂ _ N₂) +………… + V₈ _ N₈ where, V is the total cell volume, m equals the number of species found, i represents species i (i = 1, 2, ..., m) in terms of its mean cell volume (Vi) and Ni represents the number of individuals of species i[30]. The mean volume estimate is then transformed into to a "live weight" estimate after assuming of a density of L⁻³[31].

RESULTS

Physical structure: During the sampling period, the first detectable increase in the temperature in the surface water was in April and it continued to increase until August. The temperatures in the surface water varied between 19.0 and 24.7°C in summer. The maximum temperature was observed in July and August. After the maximum of 24.7°C in July and August, temperature decreased to the minimum value (6.5°C) in March (Table 1).

The salinity varied between 16.2-18.1 ppt in the surface waters in the Sinop Bay during the sampling
period. The salinity decreased from January (16.8 ppt) to May (16.2 ppt). Due to heavy spring rains and effect of fresh water inputs, salinity remained lowest in late spring (especially in May) (16.2 ppt) (Table 1). The fresh water originates from major rivers, such as the Danube, the Dniester and the Dnieper, which carry industrial and domestic waste waters, reduce surface salinity and contribute large amounts of nutrients, detritus and pollutants. These factors create a large but variable area with a lower surface salinity, with a reduced transparency and an increased phytoplankton crop in the north-western part of the Black Sea. This section of the Black Sea receives 87% of the total freshwater input entering the whole system. Over the rest of the basin, especially in the southern and eastern part (Turkish Coasts), the hydrological properties are more stable than in other areas and the phytoplankton crop is much smaller.

### Distribution of nutrients and chlorophyll-a

During the year, nitrite and nitrate concentrations showed some fluctuations in the Sinop Bay (Table 1). While the maximum concentration of nitrite in the annual cycle were observed in February (mean: 0.168 μM), the maximum concentration in nitrate was observed in October (mean: 1.045 μM). After the maximum peak in October, nitrate dramatically fell down and subsided the lowest concentration in December (mean: 0.184 μM) (Table 1).

Like the nitrite and nitrate, phosphate levels were the highest in autumn and winter (Table 1). Phosphate levels decreased between January (mean: 0.605 μM) and April (mean: 0.224 μM) and increased between August (mean: 0.289 μM) and October (mean: 0.722 μM). After the maximum concentration in October, phosphate concentrations particularly remained constant in the period of October and January (0.605-0.722 μM).

Contrary to the other nutrient concentration, ammonia values were lowest in winter and variations were more evident during the year. The first detectable increase of ammonia in the research area was in April (means: 9.444 μM) and ammonia continued to increase until June and occurred maximum peak in this period (means: 19.722 μM). After the peak, it started to decrease until March (means: 2.677 μM) (Table 1).

Soluble inorganic silicate concentrations were lowest during the summer (3.804-4.217 μM) and peaked in early winter (mean: 12.06 μM) (Table 1). The silicate concentration was also low in April (mean: 3.424 μM) due to probably their uptake by diatom *P. delicatissima* which occurred very excessive bloom in this period. The increases in winter (especially in December) were probably related to the silica that reached the euphotic zone with remineralization.

Chlorophyll-a concentrations during the sampling period exhibited a major peak in April (3.75±1.05 μg L⁻¹) and a minor peak in June (3.25±0.45 μg L⁻¹). Although variations were not evident, after the minor peak, chlorophyll-a decreased until September (0.95±0.12 μg L⁻¹) and then fluctuated between September and March. The high chlorophyll-a concentrations were showed in spring (April) and summer period in June (Table 1). We observed a good correlation between chlorophyll-a and phytoplankton cell density (r=0.585, n=72) and biomass (r=0.885, n=72).

### Succession and size spectrum of picoplankton (coccolid cyanobacteria)

Cyanobacteria of regular spheroidal shape (1.0-1.5 μm) among prokaryote cells were dominant. Moreover, ellipsoid cells (1.0-1.8 μm) occurred often combined of various length. Shapeless colonies were also observed containing up to hundreds of spherical cells each with a diameter of about 2.0 μm. Coccolid cyanobacteria were characterized by a spherical shape with a diameter of 1.5-2.0 μm in Sinop Bay of southern Black Sea in the investigation period.

The abundance and biomass of coccolid cyanobacteria predominated in the picoplankton,
averaging 99.1 and 99.2% of the total picoplankton, respectively. The average picoplankton biomass varied between 745 in April and 10833 mg m$^{-3}$ in June (average 3961 mg m$^{-3}$) during the research period. The average biomass of coccoид cyanobacteria varied from 96.4 (in February) to 99.9% (in June and December) of the picoplankton biomass (Fig. 2).

The highest biomass was observed in June at shallow station B3 (13477 mg m$^{-3}$) which is one of the intensive aquaculture areas of the Southern Black Sea. However, the lowest biomass was observed in April at other shallow station B1 (535 mg m$^{-3}$) which is one of the beach area of the Bay. We also revealed that picoplankton cells were more concentrated in the shallow water (except January and October) than offshore waters in the Sinop Bay (Fig. 3).

During the research period, picoplankton predominant in the phytoplankton, averaging 99.2% of the total phytoplankton abundance. The abundance of coccoид cyanobacteria were generally high in the mixed surface layer in the Bay. The abundance of coccoид cyanobacteria varied between $10^7$ and $10^8$ cells L$^{-1}$ in the mixed surface layer. In the Bay, the peak of abundance ($3.3 \times 10^7$ cells L$^{-1}$) was observed at shallow station B3 in late June and the minimum was observed at station B1 in the middle of April ($1.3 \times 10^6$ cells L$^{-1}$). High cell numbers of $2.1 \times 10^7$ and $2.3 \times 10^5$ cells L$^{-1}$ were also observed in October (st.A1) and December (st.B3), respectively. A monthly average of cell number has been estimated as $9.5 \times 10^6$ cells L$^{-1}$ in surface waters (Fig. 4 and 5).

The declines of small diatoms were followed by increases of coccoид cyanobacteria and dinoflagellates in
the Bay. A relationship seems possible between the development of *P. delicatissima* and that of coccolid cyanobacteria. When the *P. delicatissima* bloom occurred in April (all the phytoplankton biomass in April was almost contributed by this species), cyanobacteria abundance remained minimal both density and biomass (Fig. 4). It was simply due to an association of cyanobacteria with autumn-winter (plus June) and an association of diatoms and dinoflagellates with late summer, winter and spring (for diatoms). A result of the condition, there was a negative correlation (*r* = -0.5245, n = 72) between a diatom *P. delicatissima* and picophytoplankton (coccolid cyanobacteria).

The results showed that the mean cell numbers of diatoms and dinoflagellates in surface mixed layer were high in April (3.9×10^6^ cells L⁻¹) and July (1.1×10^7^ cells L⁻¹) and low in February (9.3×10^6^ cells L⁻¹). The monthly average cell number was estimated to be 7.3×10^6^ cells L⁻¹ in surface waters. Maximum phytoplankton abundance in April was only represented by the diatom *P. delicatissima* (Fig. 4).

**DISCUSSION**

To evaluate the spatial and temporal changes in the succession of coccolid cyanobacteria and their role in the phytoplankton community, an investigation was carried out to their connect with the blooming of diatom and dinoflagellates in the Sinop Bay of the Southern Black Sea in the period of January 1998 and December 1998. Also, it was determined size spectrum of the coccolid cyanobacteria. At the end of the investigation, the average diameter of coccolid cyanobacteria (1.5-2.0 μm) in the Sinop Bay was bigger than open waters of the Black Sea, according to the data on composition and distribution of picophytoplankton in the open area of the Black Sea in winter⁴⁵. But, big size spectrum of coccolid cyanobacteria can be affect of growth rates in Sinop Bay. However, picophytoplankton with a diameter of 2-3 μm are dominant in eutrophic waters of Sevastopol Bay of the Black Sea⁴⁰ and in the eutrophic waters of the Baltic Sea.⁷⁷ Picophytoplankton in oligotrophic waters generally have diameter of 0.5-1.0 μm⁹. As a result, I can say that the structural-functional characteristics of picophytoplankton of eutrophic and oligotrophic waters are not identical, confirming above international data information.

During the study period, the abundance (9.0×10^6^ cells L⁻¹) and biomass (3961 mg m⁻²) of coccolid cyanobacteria in the Bay was relatively more higher than the data for different seasons and areas of the Black Sea and other marine systems. Abundance and biomass distribution in the surface mixed layer in research period (except January and October), also revealed that picophytoplankton cells were more concentrated in the shallow water than offshore waters in the Sinop Bay (Fig. 3 and 5). In late spring in the open waters of the Black Sea, the amount of picophytoplankton in the surface layer varied from 10⁴ to 10^¹⁰^ cells L⁻¹. The highest abundance of picophytoplankton in the center of the western cyclonic gyre reached 5.0×10^⁶^ cells L⁻¹ in the maximum surface layer in summer⁶⁹. A similar abundance (5.0×10⁶^ cells L⁻¹) was recorded in autumn in the center of eastern cyclonic gyre in the Black Sea⁶⁹. However, abundance of coccolid cyanobacteria in surface mixed layer of the Sinop Bay was higher (1.3×10⁷^3.3×10⁷^ cells L⁻¹) than in the open waters of the Black Sea (1.5×10²-1.5×10³ cells L⁻¹)⁹. On the contrary, 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 Danube⁴⁶.

According to Kokurkina and Mikaelryan⁴⁶, the abundance and biomass of picophytoplankton in the eastern central Black Sea reached 1.5×10⁹^ cell L⁻¹ and 4922.0 mg m⁻² in February, respectively. The high abundance and biomass of coccolid cyanobacteria in the Bay is in full conformity with a previous suggestion about the winter minimum of picophytoplankton abundance in the western part of the Black Sea⁴¹. It is also in full conformity with the concept of the seasonal dynamics of picophytoplankton in the temperate latitudes, where the peak of abundance was observed in summer and autumn and minimal abundance was observed in winter⁴²-⁴⁴. As stated by Waterbury et al.⁴⁶, the abundance of coccolid cyanobacteria is also limited by temperatures. In like manner, there is a similar tendency in our data, because the high and lower abundance of cyanobacteria (Fig. 3 and 4) was observed at high temperatures in June (19.0°C) and low temperatures in March (6.5°C), respectively (Table 1).

It could be said that the decline of small diatoms such as *P. delicatissima* was associated with an increases in coccolid cyanobacteria. A negative correlation (*r* = -0.5245, n = 80) was observed between a diatom *P. delicatissima* bloom and coccolid cyanobacteria in April. It was simply due to an association of cyanobacteria with autumn-winter (plus June) and an association of diatoms and dinoflagellates with late summer, winter and spring (for diatoms). In some studies on picophytoplankton abundance with excessive bloom of diatom *N. delicatula* (*P. delicatissima*) by Mikaelryan⁴⁶ demonstrated that the abundance in picophytoplankton was low during the bloom of *P. delicatissima*. Considering the available data on possible heterotrophic feeding of cyanobacteria⁴⁶, suggests a relationship between
development of cyanobacteria and that of small diatoms in diameter such as *P. delicatissima*. Moreover, the highest abundance of synechococcus (10^5 cells L^-1) was recorded during the spring bloom of large phytoplankton off the California coast[47]. Furthermore, cyanobacteria can come under intense grazing pressure by phagotrophic protists in eutrophic systems such as the Black Sea. The low growth of coccolid cyanobacteria in spring in the Bay was due to the bloom of a small diatom *P. delicatissima* (maximum density 9.0x10^6 cells L^-1 at station A1) (Fig. 3). It can be concluded that the relationship between cyanobacteria and small diatoms in diameter such as *P. delicatissima* is related to inverse association rather than competition for nutrient. Nonetheless, nutrient limitation is probably important in the eventual decline of the coccolid cyanobacteria bloom. It is known that there is a tendency for more cyanobacteria and diatoms with increasing nutrient[48]. The results provided in Lake Kinneret show evidence that (i) non-grazeability of cyanobacterium *Aphanizomenon ovalisporum* Forti enabled it to gain a competitive advantage over grazeable phytoplankton and (ii) that nutrient limitation, but not grazing, was probably important in the eventual decline of the *A. ovalisporum* bloom[49].

During the year, nutrient concentrations showed some fluctuations in the Sinop Bay (Table 1). Although variations were not particularly marked, all measured nutrient concentrations (except ammonia) in the surface water of the Sinop Bay were highest in autumn and winter than in spring and summer (Table 1). However, the lowest two value of ammonia were showed in early spring period (2.677 μM) and winter (3.33 μM), the highest was in June (19.722 μM). The high nutrient content (except ammonia) in winter and autumn is related to nutrient regeneration, but the minimums in spring (April) and summer (June) is due to nutrient consumption by phytoplankton blooms (*P. delicatissima*, 9.0x10^6 cells L^-1). In the Sinop Bay, picoplankton abundances and biomass were no important correlated with physicochemical parameters. In contrast, in central (17°S) and southern (20°S) shelf waters of the Great Barrier Reef, Synechococcus and Prochlorococcus abundances were better correlated with salinity, shelf depth and chlorophyll a concentration, than with concentrations of NH_4^+ , NO_3^- (i.e., NO_3^- + NO_2^-), or PO_4^-[39].

The high chlorophyll-a concentration (3.75 μg L^-1) in April was perhaps mainly due to excessive bloom of a diatom *P. delicatissima*. The other noteworthy feature of chlorophyll-a is that the low concentrations in autumn and winter were probably due to the complex of hydrodynamic structure and low temperature and irradiance, respectively.

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