



Seasonal Changes of Phytoplankton Chlorophyll a, Primary Production and their Relation in the Continental Shelf Area of the South Eastern Black Sea

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Received 31 October 2013
Accepted 11 August 2014

Abstract

We describe spatio-temporal variability of primary production (PP) and chlorophyll-a (Chl-a) concentration with environmental parameters during March-December 2010 period in the south-eastern coast of the Black Sea. PP was measured by a combination of ¹⁴C *in-situ* incubation experiments on natural phytoplankton assemblages. Primary production rates, varied notably within water column with 0.1-40 mg cm⁻³ d⁻¹, were always determined in the upper part of the euphotic zone down to the 10% of light intensity depth. The depth-integrated production rates ranged from 285 to 565 mg cm⁻² d⁻¹ for the coastal station and from 126 to 530 mg cm⁻² d⁻¹ for the offshore station (ANOVA, P>0.05). The average Chl-a concentrations within the euphotic zone ranged from 0.30 to 3.57 µg L⁻¹ for the coastal station and from 0.25 to 3.45 µg L⁻¹ for the offshore station (ANOVA, P<0.01), whereas the levels of depth-integrated Chl-a concentrations in the offshore station (9-80 mg m⁻²) were greater than in the coastal station (10 to 68 mg m⁻²) (ANOVA, P>0.05). The correlation between integrated PP and Chl-a values for the coastal station (r²= 0.98; P<0.01) was better than for the offshore station (r²= 0.12; P>0.05).

Keywords: Black Sea, primary production, chlorophyll-a, phytoplankton, euphotic zone.

Güney Doğu Karadeniz Kıta Sahanelğinde Fitoplanktonik Klorofil-a ve Birincil Üretimin Mevsimsel Değişimi ve İlişkileri

Özet

Bu çalışmada Mart-Aralık 2010 döneminde Güney Doğu Karadeniz kıyılarında birincil üretim ve klorofil-a'nın zamansal- alansal değişimi ve çevresel parametreler ortaya konulmuştur. Birincil üretim doğal fitoplankton topluluklarında yerinde C-14 inkübasyon deneyleriyle gerçekleştirilmiştir. Birincil üretim hızları su kolonu içerisinde 0,1-40 mg cm⁻³ d⁻¹ aralığında önemli bir şekilde değişim gösterirken, daima fotik bölgenin üst tabaklarından ışık yoğunluğunun %10'a kadar düştüğü bölgede ölçülmüştür. Derinliğe bağlı integre edilmiş birincil üretim değerleri kıyı istasyonunda 285-565 mg cm⁻² d⁻¹, açık istasyonda ise 126-530 mg cm⁻² d⁻¹ aralığında değişim göstermiştir (ANOVA, P>0,05). Öfotik bölgedeki ortalama klorofil-a konsantrasyonları kıyı ve açık istasyonlar için sırasıyla 0,30-3,57 µg L⁻¹ ve 0,25-3,45 µg L⁻¹ arasında değişim göstermiştir (ANOVA, P<0,01). Ancak derinliğe bağlı integre edilmiş klorofil-a konsantrasyonları (9-80 mg m⁻²) açık istasyonda kıyı istasyonundan (10 to 68 mg m⁻²) daha yüksek bulunmuştur (ANOVA, P>0,05). İntegre edilmiş birincil üretim ve klorofil-a değerleri arasındaki korelasyonlar değerlendirildiğinde kıyı istasyonu (r²= 0,98; P<0,01) açık istasyondan daha güçlü ilişkiler sergilemiştir.

Anahtar Kelimeler: Karadeniz, birincil üretim, klorofil-a, fitoplankton, öfotik bölge.

Introduction

Plankton are closely coupled to environmental changes (e.g. eutrophication), making them sensitive indicators of ecological disturbance (McQuatter *et al.*, 2007). In general, total phytoplankton standing stock is estimated as Chl-a concentration, which has long been recognized as a phytoplankton biomass (Boyce *et al.*, 2010). Therefore, tracing temporal variations in

Chl-a, an index of phytoplankton biomass, as well as PP, is necessary to identify changes induced by human activity or by natural fluctuations (Yuney *et al.*, 2002). Moreover, the determination of seasonal variations in Chl-a and PP has also great importance for developing ecosystem models (Finenko, 1979; Raymont, 1980; Chebotarev *et al.*, 1983; Vedernikov and Demidov, 1997).

Black Sea is a biologically productive and

unique marine environment representing the largest landlocked/semi-enclosed and the deepest anoxic basin in the world oceans (Yuney *et al.*, 2002; Yilmaz *et al.*, 2006), and receives considerable amount of chemicals, organic matter, and nutrients from the rivers around it (Eker-Develi and Kideys, 2003; Yilmaz *et al.*, 2006). However, the presence of a strong cyclonic rim current system prevents water and material transport from coastal waters to the interior waters of the basin. On the other hand, a number of anti-cyclonic eddies (e.g. Sevastopol, Crimea, Batumi) also influence coastal zone productivity (Oguz *et al.*, 1993); therefore, it affects overall productivity of the basin. Salinity nowhere exceeds 17‰, and excess precipitation together with runoff from the rivers (e.g. Danube, Dniester, and Don) creates a surface low salinity layer overlying a halocline at about 100 m (Longhurst, 2007). The presence of the permanent and strong halocline interface inhibits vertical mixing. Moreover, density gradient provides the major reasons for the anoxic and sulphidic condition of the sub halocline waters Ozsoy and Unluata, 1997).

The Black Sea ecosystem also provides a vital habitat for many commercial fish species and supports a large-scale fishery for around countries (Kideys, 2002; Agirbas *et al.*, 2010). Until recently, the Black Sea has supported fisheries almost 5 times richer than those of the neighbouring Mediterranean (Anonymous, 2000). Overall, PP in the Black Sea displays two phytoplankton peaks throughout the year; the major bloom of mainly diatoms occur in early spring while a secondary bloom of mainly coccolithophores appear during autumn in both the coastal and open waters (Sorokin, 1983; Vedernikov and Demidov, 1997). Recently, additional summer blooms of dinoflagellates and coccolithophorids (mainly *Emiliana huxleyi*) have frequently been reported (Hay *et al.*, 1990; Sur *et al.*, 1996; Yilmaz *et al.*, 1998; Yayla *et al.*, 2001). The most important changes in the Black Sea ecosystem recorded over the last decade are changing of the ratio of major phytoplankton groups (Bat *et al.*, 2011; BSC 2008; Feyzioglu and Seyhan, 2007) resulted from the deterioration of the Black Sea ecosystem (Kideys, 2002; Kopelevich *et al.*, 2002).

The primary production rates are relatively high in NW shelf of the Black Sea, ranges from 570 to 1200 mg cm⁻²d⁻¹, whereas values vary from 320 to 500 mg cm⁻²d⁻¹ in the regions of continental slope, 100 to 370 mg cm⁻²d⁻¹ in the central deep-sea regions during 1960-1991 (Vedernikov and Demidov, 1997; Bologna *et al.*, 1986; Demidov, 2008). On the other hand, production rates for the southern coasts of the Black Sea were estimated as 247-1925 mg cm⁻²d⁻¹ for spring and 405-687 mg cm⁻²d⁻¹ for summer-autumn period during 1995-1996 (Yilmaz *et al.*, 2006). Despite the significant roles of phytoplankton, earlier studies conducted in the Black Sea mainly focused on several distinct areas (e.g. NW shelf); however,

information about PP and Chl-a for the southern Black Sea has been remained poorly studied (Yilmaz *et al.*, 1998, 2006; Yayla *et al.*, 2001).

Therefore, the present study describes the spatial and temporal variability of PP and Chl-a associated with phytoplankton biomass, light penetration and dissolved inorganic nutrients in the continental shelf area of the south eastern Black Sea during 2010. Present study also constitutes the first measurement of *in-situ* primary productivity for the study area and mainly focuses on determination of seasonal dynamics of primary production in south eastern coasts of the Black Sea.

Materials and Methods

Study Area and Sampling Procedures

Samplings were carried out bi-monthly period from March 2010 to December 2010 at two stations (coastal and offshore) in the south eastern coasts of the Black Sea. The sampling locations were selected by examining bottom topography and mainly by considering circulation properties of the Basin (Oguz *et al.*, 2003). Coastal station (40°57'19" N; 40°11'35" E), has 400 m of water depth, was selected as away 2 nautical miles from the shore. On the other hand, offshore station (41°03'12" N; 40°09'07" E), has 730 m water depth, was located 8 nautical miles away from the shore (Figure 1). Temperature and salinity profiles were obtained at each station by using an Idronaut Ocean Seven 316 Plus CTD (Conductivity, Temperature and Depth) probe. A Li-Cor LI-193SA Spherical Quantum Sensor and LI-190SA Quantum Sensor was used for Photosynthetically Active Radiation (PAR) within the range of 400-700 nm, in μE m⁻²s⁻¹ unit in the euphotic zone. The mixed layer depth (MLD) was calculated from the density profiles obtained from CTD measurements. MLD was defined as the depth at which the difference with the surface density was greater than 0.125 (Levitus, 1982). The stations were assumed to be in well-mixed waters when $Z_{eu}/Z_{MLD} < 1$, and in a stratified water column if $Z_{eu}/Z_{MLD} > 1$ (Uitz *et al.*, 2006).

Sea water samples were obtained by using 5-L Niskin bottles mounted on a SBE 32 Carousel Water sampler. Water samples for dissolved inorganic nutrients (NO₃-N, SiO₄ and reactive PO₄-P) were collected in 5 m interval from surface to 60 m depths and then filtered through 0.45 μm cellulose acetate membranes. The filtrate was collected in 100 ml acid-washed high-density polyethylene bottles until analyses. The analyses were conducted by standard spectrophotometric methods (Parsons *et al.*, 1984).

Chl-a and phytoplankton samples were taken from surface to base of the euphotic depth. A 1-L subsample from each depth was preserved in a borax-buffered 4% formalin seawater-solution for microscopic analysis after the cruise. Samples were concentrated to 10 ml by sedimentation methods after



Figure 1. Study area and sampling points.

keeping the samples immobile for 2 weeks in dark and cool place till the analysis (Eker-Develi *et al.*, 2008). The excess seawater after settling was gently removed with pipette. The phytoplankton present in a subsample of 1 ml taken from the 10 ml sample and counted using a Sedgewick-Rafter cell under a phase contrast binocular microscope with 40, 100 and 400 magnifications (Nikon E600). The major taxonomic groups were identified based on the study of Rampi and Bernard (1978), Balech (1988), Fukuyo *et al.* (1990), Tomas (1996).

Phytoplankton biomass as a carbon was estimated for diatoms, dinoflagellates and coccolithophores using the relationship described by Menden-Deuer and Lessard (2000):

$$\begin{aligned} \text{Diatoms} &= 0.288 \times V^{0.811} \\ \text{Dinoflagellates} &= 0.760 \times V^{0.819} \\ \text{Other groups} &= 0.216 \times V^{0.939} \end{aligned}$$

where phyto-C is the mass of carbon (pgC cell⁻¹), then converted to µgC cell⁻¹ and V the volume (µm⁻³). The volume of each cell was calculated by measuring appropriate morphometric characteristics.

Chl-a analyses were conducted by HPLC technique. A 1-L seawater were filtered through GF/F glass fibre filters (25 mm diameter, nominal pore size 0.7 µm). After the filtering, the filters were immediately frozen by storage in liquid nitrogen until the HPLC analyses. The frozen filters were extracted in 90% HPLC grade acetone for one night at refrigerator before the analysis. The method chosen in this study (Barlow *et al.*, 1993) was modification of the reverse-phase method described in Mantoura and Llewellyn (1983). The analyses were carried out with a Shimadzu LC-20 AT/ Prominence HPLC system using C8 column equipped with degasser pump, a water pump (flow rate 1 ml/min), a 717 plus auto sampler, a UV absorbance, fluorescence and a photodiode array detector. The HPLC system was calibrated for each pigment with commercial standards (DHI Water and Environment, Denmark).

The rate of carbon fixation by phytoplankton in the samples was estimated from the incorporation of ¹⁴C radio-tracing technique (Steemann-Nielsen, 1952), with slight modifications (Richardson, 1991). The water samples for the incubation were taken from 75%, 36%, 10% and 1% surface light depths, immediately after collection, each bottle (2 light and 2 dark) was inoculated with 50 µl (1 µCi; 2220000 dpm) NaH¹⁴CO₃⁻ and placed in the light depths at noon (between 10.⁰⁰ and 14.⁰⁰ pm), from which water was sampled, using a free-floating buoyed. After *in-situ* incubation period (2-4 hours), each sample was filtered through 25-mm diameter 0.2 µm pore-size polycarbonate filters at very low vacuum (<50 mm Hg). Removal of inorganic ¹⁴C that had not been incorporated by phytoplankton as organic carbon from the filters was achieved by exposing them to concentrated hydrochloric acid (HCl) fumes for 12 h and dried for 24 hours. Then the filters were transferred to scintillation vials to which 4ml of scintillation cocktail were added. The carbon rates of each sample were measured in liquid scintillation counter (LSC, Perkin-Elmer TriCarb 1550). Dark-bottle values were subtracted from the counts obtained in the light samples. The integrated values of primary production and Chl-a were obtained by trapezoidal integration of the volumetric data down to the depth of bottom sampling.

$$Pt = \frac{dpm(a) \cdot total\ CO_2(c) \cdot 12(d) \cdot 1.05(e) \cdot 1.06(f) \cdot k_1 \cdot k_2 \cdot k_3}{dpm(b)}$$

where Pt = carbon uptake, mg cm⁻³h⁻¹

- (a) = sample dpm – background dpm = net dpm/sample
- (b) = the activity of the added ¹⁴C solution dpm
- (c) = concentration of total CO₂ in experimental water, mM dm⁻³
- (d) = 12: the atomic weight of carbon, converts mMdm⁻³ to mg dm⁻³
- (e) = a correction for the effect of ¹⁴C discrimination
- (f) = a correction for the respiration of organic matter produced during the experiment

(k1) = a correction factor for sub-sampling

(k2) = a time correction factor

(k3) = a unit conversion factor

Data Analyses

One-way analysis of variance (ANOVA) tests were used to test for significant differences in Chl-a and PP values among the stations over the study period. Kolmogorov–Smirnov tests were used to check whether the distribution of Chl-a and PP data were normal which was log-transformed until no significant difference was found among the distributions. Test for correlation between the Chl-a and PP was conducted by linear regression analysis using Sigma-Plot 11.

Results

Hydrography

Temperature, salinity and density profiles in the stations revealed a general pattern of the Black Sea

(Figure 2). The profiles demonstrated that a nearly isohaline and relatively cool, isothermal water mass exists below the seasonal pycnocline. The thickness of the Cold Intermediate Layer (CIL) was observed to be larger (65-130 m) in the coastal station than the offshore station (50 m), and the salinity varied greatly within the range of 18 to 21‰ in the CIL during the study period.

The surface temperature ranged from 9.52°C (March) to 27.52°C (July) during the sampling period in the coastal station. When the surface waters cooled down to 7°C, the upper layer was thoroughly homogenised by convective mixing down to 135 m. The seasonal thermocline formed above 50 m in the late spring and deepened (down to 50-60 m) in autumn. The surface salinity ranged from 15.90‰ (March) to 17.67‰ (December). Although, the permanent halocline was observed between 80 and 120 m depths, the permanent pycnocline formed at surface waters (e.g. 20-40 m), which are controlled by salinity gradient due to continuous intrusion of more saline Mediterranean waters. In the offshore station, the surface temperature ranged from 9.50°C (March)

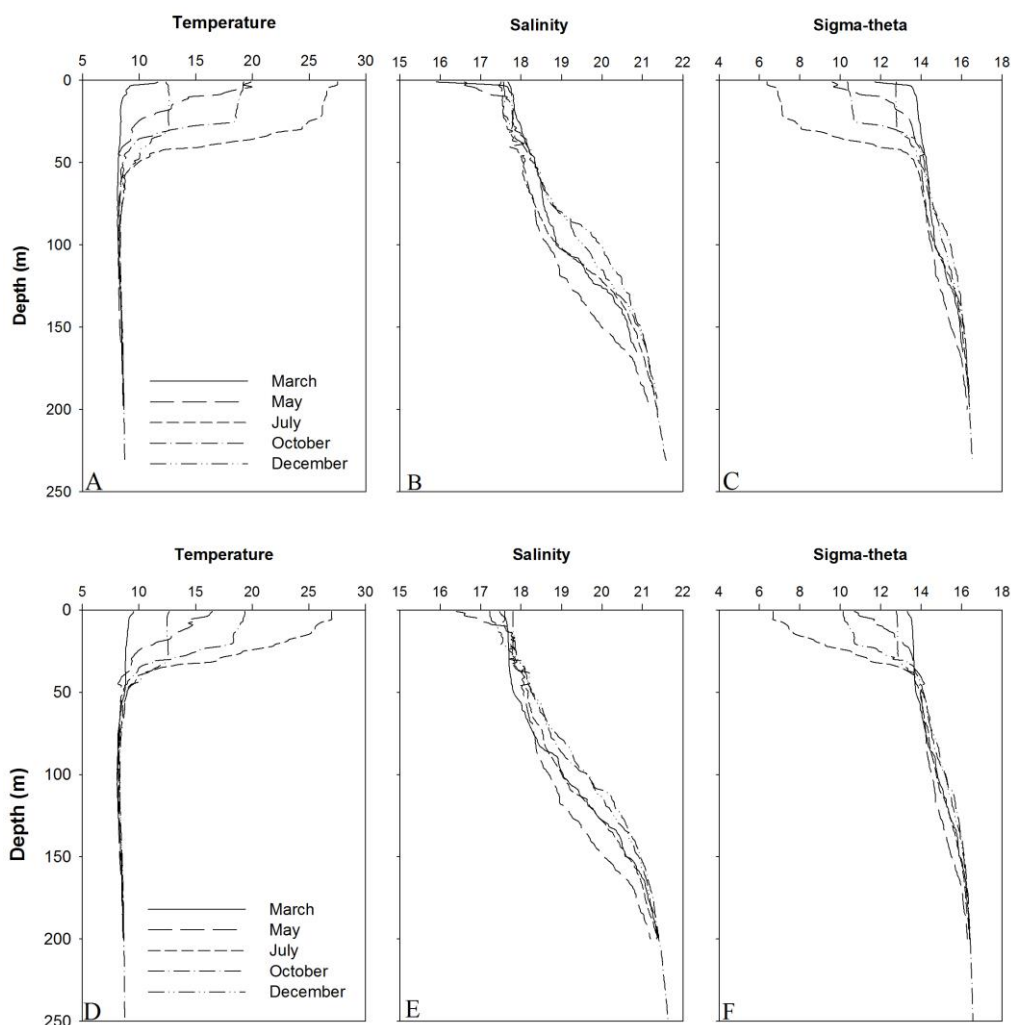


Figure 2. Temperature (°C), salinity and sigma-theta profiles in the upper layer of the south-eastern Black Sea for the study period (A, B, C for coastal station; D, E, F for offshore station).

to 27.20°C (July) during the sampling period. The seasonal thermocline was observed above 40 m during May-October in the both stations. The surface salinity ranged from 16.40‰ (May) to 17.80‰ (December). The permanent halocline was observed around 80-130 m depths.

The mixed layer depth in the stations revealed nearly a similar pattern, ranged from a maximum of 38-31 m during December (i.e. mixing most of the water column) to a minimum of 14-22 m during July and May in the coastal and offshore station, respectively (Figure 3). The water masses showed stronger stratification throughout the year except for March and December. Convictional cooling during the late autumn resulted in the mixed layer deepening with complete vertical mixing during study period. The measured light penetration (PAR) in the upper part of water column during the study period indicated the thickness of the euphotic zone (defined as the depth of 1% of the surface light) ranged from 24 to 35 m in the stations. The euphotic layer depth was always deeper than the mixed layer between May and December in the stations.

The average attenuation coefficient ranged from 0.14 to 0.23 m^{-1} . The highest average attenuation coefficient ($K_d = 0.23 m^{-1}$) was recorded in March and December, in the coastal and offshore stations, respectively (Table 1). The less energetic, high wavelength component of the incoming light was observed in the upper surface layer (the top 10 m), where the highest (downward) attenuation coefficient

($K_d = 0.20-0.49 m^{-1}$) was obtained. Below this layer the solar light penetrated with a constant K_d , which varied monthly and regionally between 0.15 and 0.19 m^{-1} . The highest estimated K_d value (0.49 m^{-1}) was observed in the coastal station, corresponding to euphotic layer of 31 m. Similar light penetration characteristics were also observed in the offshore station ($K_d = 0.41 m^{-1}$), corresponding to a euphotic zone thickness of 25 m (data not shown).

Nutrients

The vertical distribution of nitrate, silicate and phosphate concentrations measured in the study area were presented in Figure 4. Extensive vertical mixing processes during the winter months resulted in almost a uniform vertical distribution of nutrients. Averaged nitrate concentrations ranged from 0.42 to 0.71 μM in the coastal and from 0.48 to 1.07 μM in the offshore station over the study period. Averaged nitrate concentrations ranged from 0.34 to 0.91 μM in the coastal and 0.26 to 1.18 μM in the offshore station. The highest nitrate concentrations (0.91 and 1.18 μM) were obtained at 55 and 50 m depth in the coastal and offshore stations, respectively. There was no much variation in vertical nitrate profiles in the coastal station. Depending on the sampling periods, the maximum nitrate concentrations (0.71-1.07 μM) were obtained in March and October in the coastal and offshore stations, respectively. The minimum concentrations (0.42-0.48 μM) were obtained in May

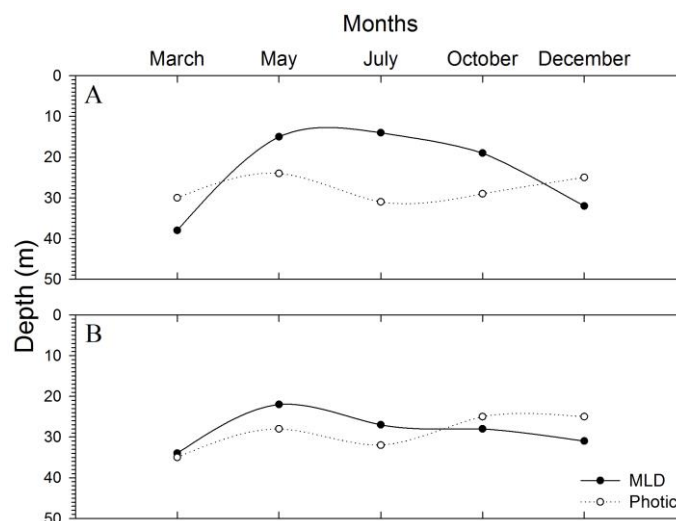


Figure 3. The distribution of MLD, photic depth and biochemical layers at the stations (A: coastal, B: offshore).

Table 1. The average light indices in the study area

Station	March		May		July		October		December	
	D	K_d	D	K_d	D	K_d	D	K_d	D	K_d
Coastal	30	0.23±0.08	24	0.20±0.02	31	0.14±0.02	29	0.16±0.02	25	0.20±0.06
Offshore	35	0.19±0.03	28	0.19±0.05	32	0.15±0.01	25	0.22±0.05	25	0.23±0.05

(D: Depth of the 1% surface light, m; K_d : Light attenuation coefficient, m^{-1})

and July in the coastal and offshore stations, respectively. In general, the average nitrate concentrations in the coastal station were lower than in the offshore station.

Silicate concentrations increased with depth in the stations. The concentrations were highest in winter months to $> 0.1 \mu\text{M}$ and declined in spring and summer months to $< 0.1 \mu\text{M}$ (Figure 4). Average

silicate concentration of the coastal station was lower than offshore station, except for surface and 60 m depths. Average silicate concentrations during the sampling periods varied from 0.06 to 0.27 μM for the coastal and from 0.07 to 0.37 μM for the offshore station. Averaged silicate concentrations varied from 0.09 to 0.28 μM for the coastal and 0.10 to 0.26 μM for the offshore station. During the sampling period,

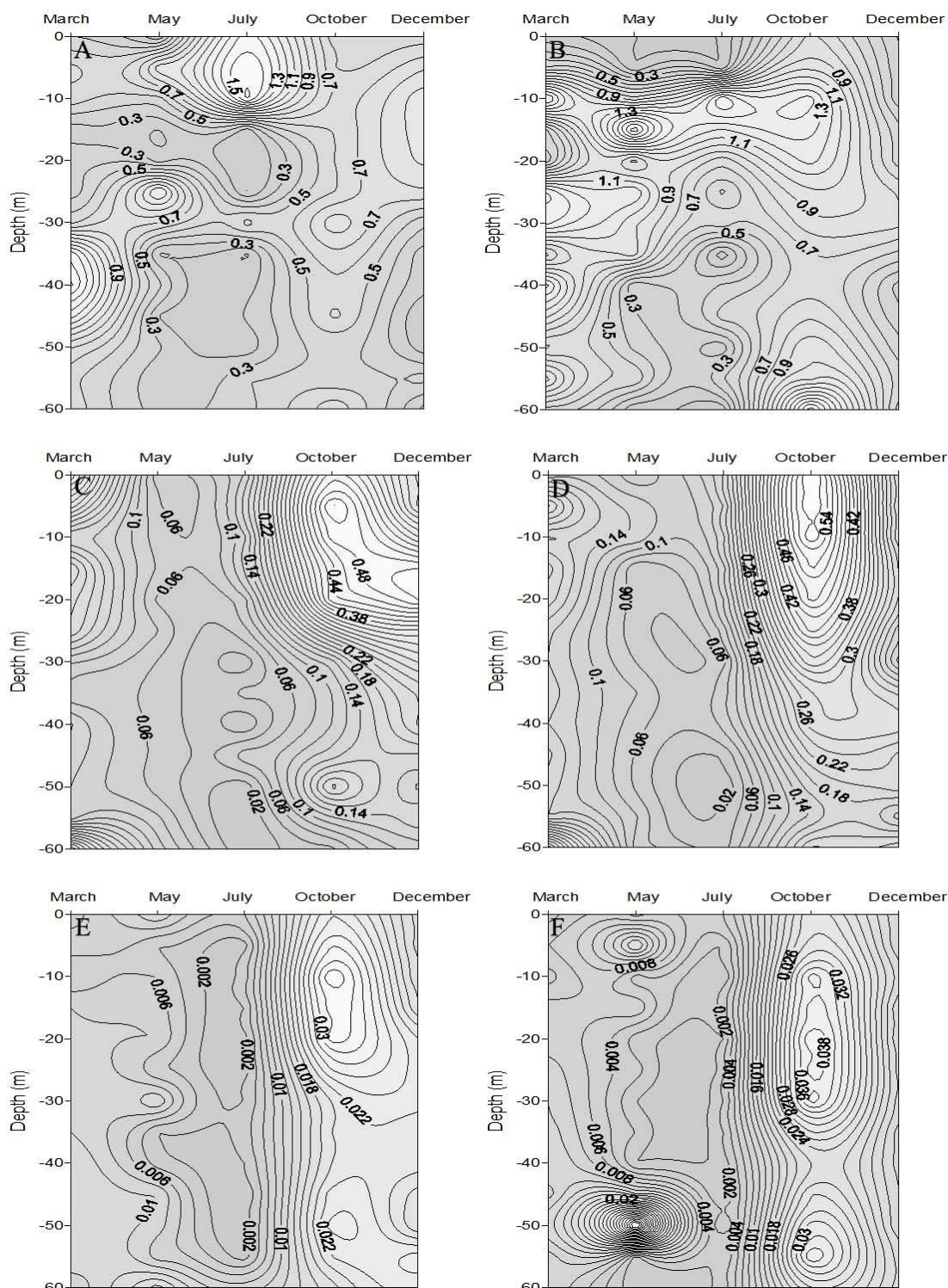


Figure 4. Spatiotemporal distribution of nutrients for the coastal and offshore stations at the left and right panel respectively (A and B donate nitrate; C and D donate silicate; E and F donate phosphate).

the highest concentrations (0.27-0.37 μM) were obtained in October and December in the coastal and offshore stations, respectively. The lowest concentrations (0.06-0.07 μM) were recorded in July and May in the coastal and offshore stations, respectively.

Throughout the sampling period, phosphate concentrations were generally low in the study area. Averaged phosphate concentrations over the months ranged from 0.002 to 0.025 μM for the coastal and 0.002 to 0.032 μM for the offshore station. Averaged phosphate concentrations ranged from 0.011 to 0.015 μM for the coastal and 0.01 to 0.023 μM for the offshore station. The highest phosphate concentrations were obtained at 40-45 m depths in the coastal and offshore stations, respectively. Averaged phosphate concentrations in the coastal station were generally lower than in the offshore station. The highest concentrations were recorded in October in the both stations. The lowest concentrations were recorded in July in both stations. The introduction of deep nutrient replete waters into surface mixed layer (30-60 m) during the winter months may be lead to higher concentrations of phosphate in the surface water of the offshore than coastal water.

Phytoplankton, Chlorophyll-a and Primary Productivity

A total of 89 species were identified in the stations throughout the March-December 2010 period. During study period, almost 71% of these were dinoflagellate species, 23% were diatom species and 6% was consisted of others species, mainly coccolithophores. The overall contribution of Prmysnesiophyta to total phytoplankton cell number was substantial made up by coccolithophores (mainly *Emiliana huxleyi*), ranged from 66% for the coastal station to 62% for the offshore station. Dinoflagellates were the second important group in the area. In general, contribution of dinoflagellates (as a group) to total phytoplankton abundance varied between 20 and 28% in the coastal and offshore stations, respectively. On the other hand, contribution of diatoms to total phytoplankton abundance was generally low and varied between 14 % and 10% in the coastal and offshore stations, respectively (data not shown). Moreover, there was a great variability in phytoplankton biomass (phyto-C) during the study period. In general, contribution of the coastal station to biomass (ranged from 56 to 209 $\mu\text{g L}^{-1}$) was greater than the offshore station (ranged from 54 to 198 $\mu\text{g L}^{-1}$). Coastal station was characterized with higher phyto-C during March, May and July. The phyto-C revealed concomitant increase with PP, and then declined ($r^2=0.98$, $P<0.01$). The Highest phyto-C was recorded in July that coincided with higher PP. Relatively, offshore station was characterised by lower phyto-C. On the contrary, phyto-C in the offshore station was greater than in the coastal station

during October and December, whereas correlation between PP and phytoplankton abundance was not straightforward ($r^2=0.38$; $P>0.05$) (Figure 5).

Chl-a concentrations exhibited statistically significant variance (ANOVA, $P<0.01$) throughout the study period (Figure 6). Chl-a concentrations varied from 0.30 to 3.57 $\mu\text{g L}^{-1}$ for the coastal and from 0.25 to 3.45 $\mu\text{g L}^{-1}$ for the offshore station. Chl-a concentrations for the coastal station were higher than for the offshore station within the water column. The highest concentrations ($>1 \mu\text{g L}^{-1}$) in the euphotic zone were obtained during March, May and July. On the other hand, concentrations were relatively low ($<1 \mu\text{g L}^{-1}$) during October and December. In general, higher Chl-a concentrations were recorded in the surface stratified layer, when the mixed layer depth was shallower than that of the euphotic layer (Figure 6).

PP rates remarkably varied with regard to time and depth (Figure 6). The coastal station had higher production rates associated with high concentrations of Chl-a in the upper parts of water column. Overall, PP well correlated with Chl-a over the study period, except for December in the coastal station and July in the offshore station. However, statistically significant regressions were observed only in the offshore station during March, which the water column stratification did not occurred (Table 2). The rates of PP fluctuated throughout study period in the stations; the highest rates, varied seasonally and regionally from 0.1 to 40 $\text{mg cm}^{-3}\text{d}^{-1}$, were always determined in the upper part of euphotic zone down to the 10% light intensity depth or the top 10–20 m of the water column. Below this layer, PP decreased markedly with depth and dropped to negligible rates at the 1% light intensity depth. During the study period, the highest PP was observed in March, May and July in the coastal station. Besides, an additional autumn bloom was recorded in the offshore station with the highest PP rate. In the coastal station, surface production rates interestingly showed marked decrease in December. This situation was probably due to light-limitation of the assemblages below surface layers. In these months, MLD depth was greater than photic depth.

Chl-a and PP were integrated to estimate the contribution of each sampling periods in the stations to the total biomass and productivity. The depth integrated Chl-a concentrations ranged from 10 to 68 mg m^{-2} for the coastal and from 9 to 80 mg m^{-2} for the offshore station. Overall, the highest depth-integrated Chl-a values were recorded in March and July in the both stations, but the magnitude was different. Interestingly, offshore station had higher integrated Chl-a concentrations during all periods, except for July and October (Table 3). On the other hand, no significant difference was found between coastal and offshore station-depth integrated Chl-a values (ANOVA, $P>0.05$).

The depth-integrated PP measured in the stations exhibited great fluctuation during the study period.

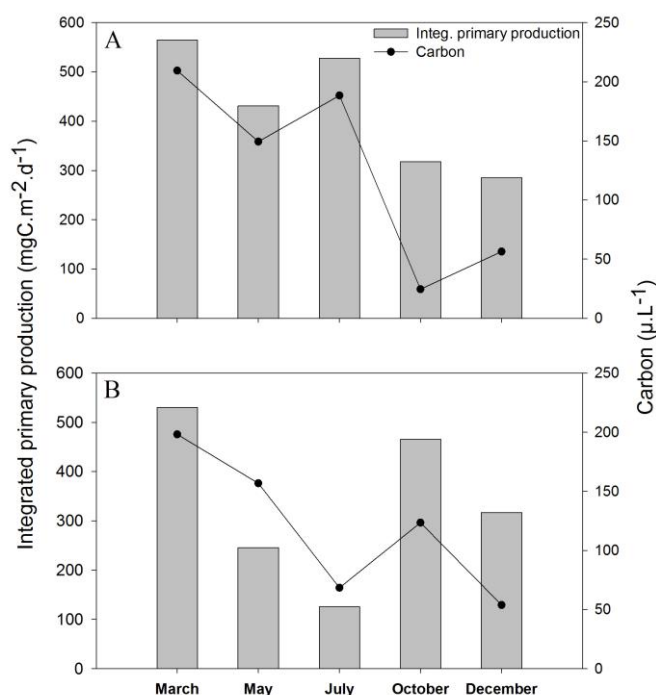


Figure 5. Depth-integrated primary production and abundance at the stations (A: Coastal station, B: Offshore station).

The integrated production rates for the coastal station were always higher than offshore station, except for October and December (Table 3) without significant differences (ANOVA, $P > 0.05$). During these months, water column was homogenised by convective mixing process resulted in MLD was greater than photic depth.

The depth integrated PP rates ranged from 285 to 565 $\text{mg cm}^{-2}\text{d}^{-1}$ for the coastal and from 126 to 530 $\text{mg cm}^{-2}\text{d}^{-1}$ for the offshore station (Table 3). In general, the highest production rates were recorded in the coastal station in March, May and July during the strong stratified periods. On the other hand, during the mixed periods (e.g. October and December), production rates in the coastal station were lower than in the offshore station. The values for the coastal station exhibited statistically significant correlation between integrated PP and Chl-a values ($r^2 = 0.98$; $P < 0.01$). On the other hand, no significant correlation were found for offshore station ($r^2 = 0.12$, $P > 0.05$). The resultant depth-integrated PP/Chl-a ratio was higher for the coastal station during the extensive stratified periods. Despite the highest production rates were recorded in the coastal station during March-July, interestingly, the highest integrated Chl-a values were observed in the offshore station for the same period. This was probably a consequence of the well-established stratification and low nutrient concentrations were observed in the coastal stations.

Discussion

The data presented here makes several noteworthy contributions to our understanding of

spatial and temporal variability in PP, Chl-a and phytoplankton biomass with related parameters (e.g. light penetration, dissolved inorganic nutrients) in the south eastern Black Sea during March-December 2010. The present study is also a first study, which was conducted using *in-situ* primary production measurements for the study area.

The hydrographic measurements in the study area revealed a nearly isohaline and relatively cool, isothermal water mass exists below the seasonal pycnocline. As reported from earlier studies that the surface waters of the southern Black Sea are always poor in terms of nutrients during the seasons when these waters are stratified (Basturk *et al.*, 1994; Bingel *et al.*, 1993; Codispoti *et al.*, 1991). The variations in nutrient concentration are mainly due to sub-surface nutricline and mixing process (Besiktepe *et al.*, 2000; Yayla *et al.*, 2001). In the spring-autumn period of 1995–1996, average concentrations in the euphotic zone ranged regionally and seasonally from 0.16 to 1.5 μM for TNOx (mainly NO_3) and from 0.03 to 0.35 μM for phosphate (Yilmaz *et al.*, 1998). In another study, concentrations in the euphotic zone were determined as $< 0.35 \mu\text{M}$ for PO_4 , $< 0.5 \mu\text{M}$ for $\text{NO}_3 + \text{NO}_2$ and 5 μM for reactive silicate during July 1997-September 1998 period in the southern Black Sea. The intense vertical mixing processes in winter months provides nutrient input from the nutricline, which may increase surface nitrate concentrations to 5 to 10-fold so that primary productivity becomes light limited (Yayla *et al.*, 2001).

The nutrient concentrations in the coastal waters were determined as 0.56 μM for $\text{NO}_3\text{-N}$; 0.17 μM for SiO_4 and 0.013 μM for $\text{PO}_4\text{-P}$. On the other hand, the

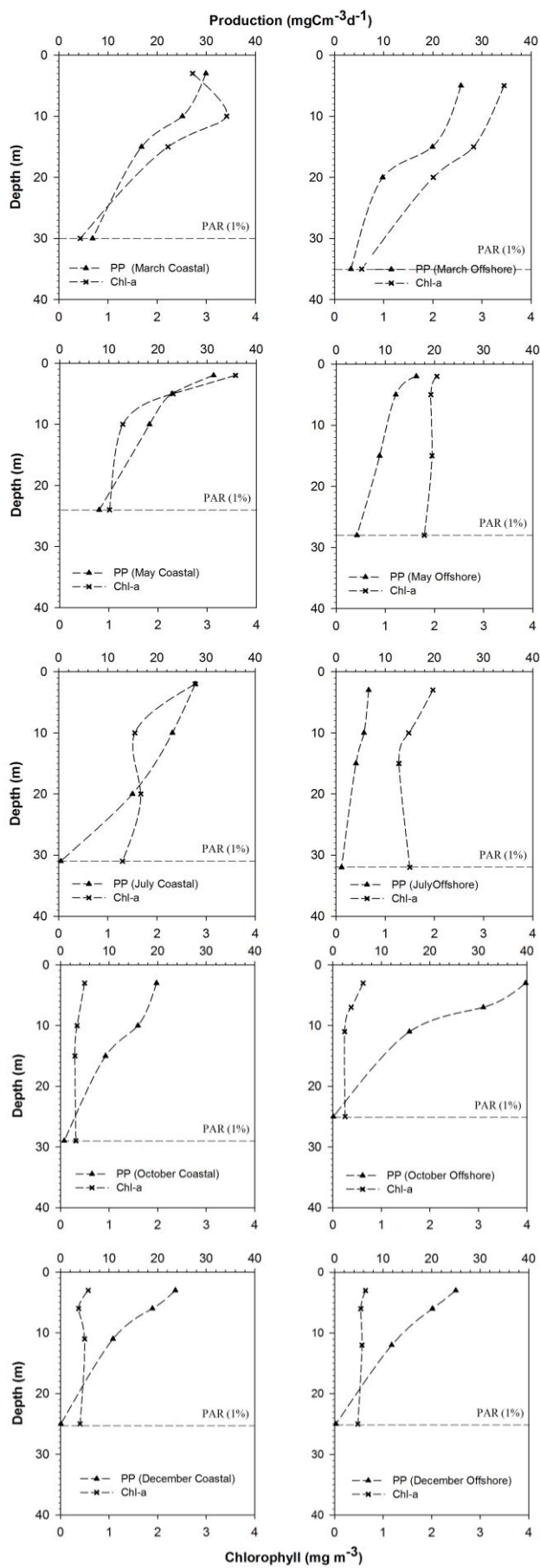


Figure 6. Vertical distribution of primary production (PP) and chlorophyll-a (Chl-a) at the stations (dashed lines indicate 1% PAR depth).

Table 2. Linear regression and ANOVA for Chl-a and Primary Production (PP) in the study area

Periods	Coastal	Offshore
March	PP = 4.078 + (7.099 * Chl-a), $r^2 = 0.80$, P>0.05	PP = -2.536 + (7.799 * Chl-a), $r^2 = 0.95$, P<0.05
May	PP = 4.150 + (7.814 * Chl-a), $r^2 = 0.88$, P>0.05	PP = -77.589 + (45.569 * Chl-a), $r^2 = 0.88$, P>0.05
July	PP = -8.374 + (13.687 * Chl-a), $r^2 = 0.56$, P>0.05	PP = -2.314 + (4.293 * Chl-a), $r^2 = 0.27$, P>0.05
October	PP = -12.578 + (66.344 * Chl-a), $r^2 = 0.53$, P>0.05	PP = -9.958 + (84.177 * Chl-a), $r^2 = 0.73$, P>0.05
December	PP = -11.261 + (53.585 * Chl-a), $r^2 = 0.22$, P>0.05	PP = -64.705 + (141.727 * Chl-a), $r^2 = 0.75$, P>0.05

(Significant regressions are in bold font, r^2 donates regression coefficients)

Table 3. Depth integrated primary production (PP_{int} , $mgCm^{-2}day^{-1}$) and chlorophyll a (Chl_{int} , mgm^{-2})

Period	Coastal		Offshore		PP_{int}/Chl_{int}	
	PP_{int}	Chl_{int}	PP_{int}	Chl_{int}	Coastal	Offshore
March	565	68	530	80	8.3	6.6
May	431	41	246	48	10.5	5.1
July	528	51	126	46	9.8	2.8
October	318	10	466	9	30.9	54
December	285	12	317	14	24.7	22.9

open waters concentrations were higher than coastal waters; the concentrations were determined as 0.71 μM for NO_3-N ; 0.18 μM for SiO_4 and 0.013 μM for PO_4-P . During the winter months, extensive vertical mixing processes resulted in the homogeneous nutrient profiles in the stations (see figure 5). The nitrate concentrations were generally low, and the highest concentrations were observed at 55 and 50 m depths in the coastal and offshore waters, respectively. The observed higher nitrate concentrations may possible due to the nitrification processes which reported from earlier studies (Yilmaz *et al.*, 1998, 2006, Yayla *et al.*, 2001). Phosphate concentrations were less variable in the coastal station. The phosphate maxima observed at surface and 10 m depths in the coastal and offshore station, respectively. Silicate concentration increased with depth in the stations. Our findings revealed nutrient concentrations in the study area were generally lower than other part of the Black Sea. During the study period, besides, low precipitation regime was also reported for the study area (Turkish state meteorological service data), lead to decreasing of nutrient input via the rivers along the south eastern Black Sea.

The importance of Chl-a for different regions of the southern Black Sea is well documented by different research groups (Yilmaz *et al.*, 1998; Coban-Yildiz *et al.*, 2000; Yayla *et al.*, 2001). In the seasonally stratified surface waters of the Black Sea, Chl-a profiles display a sub-surface maximum at the base of the euphotic zone (Coban-Yildiz *et al.*, 2000). The concentrations in the euphotic zone were generally reported as low ($<0.5 \mu g L^{-1}$) varied from 0.1 to 1.5 $\mu g L^{-1}$ in the southern Black Sea during

spring-autumn period of 1995–1996 (Yilmaz *et al.*, 1998). In another study, Chl-a concentrations during the spring-autumn period of 1995–1996, ranged from 0.1 to 0.6 $\mu g L^{-1}$ for the Batumi anticyclone region whilst the concentrations in the western cyclone region, the Bosphorus region and off Sinop ranged from 0.3 to 1.5 $\mu g L^{-1}$. Relatively, lower concentrations, varied from 0.1 to 0.5 $\mu g L^{-1}$, were observed during March-April period of 1995-1996, revealed almost uniform vertical distributions (Yayla *et al.*, 2001). Chl-a concentrations measured in the present study exhibited different pattern between coastal and offshore stations in terms of seasonal stratification, chlorophyll maximum and depth integrated values. The highest concentrations ($>1 \mu g L^{-1}$) were measured during the strong stratified periods (e.g. May and July) when the mixed layer depth was shallower than that of the euphotic layer. On the other hand, the lowest values ($<1 \mu g L^{-1}$) generally observed during convectonal cooling period (i.e. late autumn). Chl-a concentration was used as an indicator of biomass and trophic difference between oligotrophic and meso-plus eutrophic regions, where oligotrophic regions were designated as water masses with Chl-a of $<0.25 \mu g L^{-1}$, and mesotrophic regions with Chl-a 0.25-1.2 $\mu g L^{-1}$ and eutrophic waters with Chl-a $>1.2 \mu g L^{-1}$ (Aiken *et al.*, 2009). The results of this work clearly illustrate, south eastern coasts of the Black Sea can be considered as between mesotrophic and eutrophic trophic level based on measured Chl-a concentrations. Obtained Chl-a concentrations for the study area were higher than other parts of southern Black Sea. Instead, the observed high concentrations were mostly resulted from different study area (e.g. coastal waters),

different seasons and different analysis methods (e.g. fluorometric, spectrophotometric and HPLC).

The earlier studies on the primary production along the Black Sea showed two distinct periods (early spring and autumn) of enhanced PP and recently additional summer blooms have frequently been reported in the coastal and open waters (Hay *et al.*, 1990; Sur *et al.*, 1996; Yilmaz *et al.*, 1998). The spring bloom along the coastal waters is mainly dependent on riverine input and commences during early spring (i.e. April and May) when nutrient-rich shelf waters are sufficiently warm for phytoplankton growth (Yuney *et al.*, 2007; McQuatters-Gollop *et al.*, 2008). On the other hand, major nutrient source for the open waters comes from the nutricline (Yilmaz *et al.*, 2008) and experiences its bloom during autumn (Vinogradov *et al.*, 1999). Besides, summer and early autumn blooms are mainly characterized by coccolithophores with the strong thermocline and depletion of nutrients (Hay *et al.*, 1990; Vedernikov and Demidov, 1997; Yilmaz *et al.*, 1998). During the study period, we observed a large degree of variability in PP rates on the spatial and temporal scale. Based on river flow and increasing nutrient input during early spring, the highest production rates were observed in coastal waters with dominance of diatom species. Similarly, the highest PP rates in the offshore station were also observed during early spring (i.e. March). In addition, the highest rates in PP were observed in October and December in the offshore stations with dominance of coccolithophores. Overall, the observed bloom timing throughout the study period coincides with previously reported results.

The production rates reported from earlier studies for the southern Black Sea varied from 10 mg cm⁻³d⁻¹ to 180 mg cm⁻³d⁻¹. On the other hand, the depth-integrated rates were reported as 247-1925 for spring and 405-687 mg cm⁻²d⁻¹ for summer-autumn period (Yilmaz *et al.*, 1998, 2006). In another study, the depth-integrated rates were reported for the near shore as 785 mg cm⁻²d⁻¹ in July 1997 in the western Black Sea (Yayla *et al.*, 2001). The same author reported that the rates of PP for the deep regions of the Black Sea as 62-461 mg cm⁻²d⁻¹ during spring 1998 period, whereas in another study the rates of PP for the deep regions of the Black Sea were reported as up to 1200 mgC m⁻²d⁻¹ during February-March 1961-90 period (Vedernikov and Demidov, 1997). The production rates in the present study exhibited great spatial and temporal differences varied from 0.1 mg cm⁻³d⁻¹ to 40 mg cm⁻³d⁻¹. The depth-integrated rates were also calculated as 285-565 mg cm⁻²d⁻¹ and 126-530 mg cm⁻²d⁻¹ for the coastal and offshore station, respectively. The estimated production rates in the present study fall within the ranges those reported for the southern Black Sea (Yilmaz *et al.*, 1998). On the other hand, the differences in production rates between coastal and offshore stations clearly emphasize that physical conditions are main factor in the study area. Moreover, relatively higher production

rates were observed in the offshore station during the well-mixing periods (i.e. October, December). The coastal and open sea ecosystems of the Black Sea are notably distinct systems which are controlled by different factors (McQuatters-Gollop *et al.*, 2008). While coastal systems are mainly regulated by freshwater inflow and climatic processes (Bodeanu, 2002, 2004), open sea is controlled by stratification, upwelling and circulation systems (Sorokin, 2002).

The open Black Sea experiences its Chl-a maximum during autumn and winter with minimum levels reported during the summer months (McQuatters-Gollop *et al.*, 2008; Vinogradov *et al.*, 1999). It should be taken into consideration that the pattern of primary productivity in most of the Black Sea is determined by material transported via the cyclonic boundary Rim current and the frontal jet instabilities between the Rim current and the interior eddy fields (Yilmaz *et al.*, 1998). The results of this study indicate that the rate of PP is lower than other part of the southern Black Sea. Compared to previous studies performed in the other part of the Black Sea (e.g. NW shelf), production rates were quite low in the present study. The apparent lower values in PP rates are likely to be related to the different study area (e.g. coastal, cyclonic and anti-cyclonic areas etc.), study period and measurement techniques. It should be noted that the rate of PP in the present investigation was measured by *in-situ* incubation technique; however other research groups generally used deck incubator in their studies. Also, the obtained lower PP rates against high Chl-a values in the stations can also be due to smaller groups (e.g. picoplankton) which haven't been considered in the present study. If smaller groups are responsible for high Chl-a concentrations, it is not possible to mention at this stage, this means food chain is likely to be extended in the area. Hence, it leads to decrease in trophic efficiency of food web and PP rates. Moreover, the formation of the Batumi anti-cyclone convergence leads to low production, during summer periods (Oguz *et al.*, 1993) may also lead to lowering rate of primary production in study area. Besides, the lowering of PP also leads to decrease in fishery. This situation has great economic importance for Turkish fishery sector. Especially, anchovy fisheries were extremely important sources of income and protein, their collapse will have adverse effects on the economy and protein consumption of people, particularly those inhabiting the Black Sea coasts of Turkey (Kideys, 1994). It is well known that the seasonal dynamics of PP are governed by both the Chl-a concentration and relative photosynthetic activity. The increase in the depth-integrated PP/Chl-a ratio in October and December slows down PP rates relative to earlier periods, which coincides with decrease in the integrated Chl-a concentration in October and December; clearly indicate that PP is mostly governed by the Chl-a concentrations rather than by the specific photosynthetic activity in study

area. Moreover, the observed of lowest PP/Chl-a ratios during stratified periods (e.g. May, July) confirm the occurrence of a healthy phytoplankton or ecosystem, as was reported from earlier studies (Uysal *et al.*, 1997; Yilmaz *et al.*, 1998).

Finally, this work is intended to serve as a benchmark for current trends in PP and Chl-a in the area. Also, it is not intended to explain all of the coastal trends in the southern coasts of the Black Sea. Obtained higher Chl-a data with low PP rates for the study area allowed the discussion of how vary ecosystem parameters in terms of inter-regional (i.e. coastal and offshore) and inter-annual variability, additionally and more importantly indicate that south-eastern Black Sea can be classified as meso-eutrophic during the study period. Besides, the analysis suggests that smaller groups maybe prevailed during the study period. Therefore, the great variability in PP and Chl-a during the study period emphasises that need for more intensive and large-scale studies for the region. Moreover, the determination of correlations between the Chl-a and PP is also vitally important for developing ecosystem models and fisheries in the Black Sea. Improving the long-term understanding of changes in the pelagic ecosystem is only possible via continuous monitoring programs. In this regard, the present study undoubtedly documents that the study area needs such programs. This research may be helpful for future investigations to understand and evaluate the changes in structure of the Black Sea ecosystem.

Acknowledgments

Authors thank the officers and crew of RV DENAR I and all scientists for their assistance during the cruise. We are also thankful to Professor Aysen Yilmaz for valuable comments and to Dr. Huseyin Tüfekci for the radioactive counting. This work was funded by The Scientific and Technological Research Council of Turkey (TUBITAK, Project No: 108Y241).

References

- Agirbas, E., Seyhan, K., Kasapoglu, N., Feyzioglu, A.M., Ustundag, E. and Eruz, C. 2010. Recent changes of the Turkish anchovy fishery in the Black Sea with special reference to climate change. *Journal of Environmental Protection and Ecology*, 11(4): 1495–1503.
- Aiken, J., Pradhan, Y., Barlow, R., Lavender, S., Poulton, A., Holligan, P. and Hardman-Mountford, N. 2009. Phytoplankton pigments and functional types in the Atlantic Ocean: A decadal assessment, 1995-2005. *Deep-Sea Research Part II-Topical Studies in Oceanography* 56(15): 899-917. doi:10.1016/j.dsr2.2008.09.017
- Anonymous, 2000. Black Sea INCOM Program Science Plan. NATO Science Publications.
- Balech, E. 1988. Los dinoflaelados del Atlantico sudoccidental, Publ. Espec. Inst. Esp. Oceanogr., No 1 Madrid, 299 pp.
- Barlow, R.G., Mantoura, R.F.C., Gough, M.A. and Fileman, T.W. 1993. Pigment signatures of the phytoplankton composition in the northeastern Atlantic during the 1990 spring bloom. *Deep Sea Research II*, 40: 459-477. doi: 10.1016/0967-0645(93)90027-K
- Basturk, O., Saydam, C., Salihoglu, I., Eremeva, L.V., Kononov, S.K., Stoyanov, A., Dimitrov, A., Cociasu, A., Dorogan, L. and Altabet, M. 1994. Vertical Variation in the Principle Chemical Properties of Black Sea in the autumn of 1991. *Marine Chemistry*, 45: 149-165. doi: 10.1016/0304-4203(94)90099-X
- Bat, L., Sezgin, M., Satilmis, H.H., Sahin, F., Ustun, F., Birinci-Ozdemir, Z. and Baki, O.G. 2011. Biological Diversity of the Turkish Black Sea Coast. *Turkish Journal of Fisheries and Aquatic Sciences*, 11: 683-692. doi: 10.4194/1303-2712-v11_4_04
- Besiktepe, S. and Unsal, M. 2000. Population structure, vertical distribution and diel migration of *Sagitta setosa* (Chaetognatha) in the south-western part of the Black Sea. *Journal of Plankton Research*, 22: 669-683. doi: 10.1093/plankt/22.4.669
- Bingel, F., Kideys, A.E., Ozsoy, E., Tugrul, S., Basturk, O. and Oguz, T. 1993. Stock assessment studies for the Turkish Black Sea Coast. NATO-TU Fisheries, Final Report. METU, Institute of Marine Sciences.
- Bologa, A.S. 1986. Planktonic primary productivity of the Black Sea: A Review. *Thalassia Jugoslavica*, 21-22(1-2): 1-22.
- Bodeanu, N., Andrei, C., Boicenco, L., Popa, L. and Sburlea, A. 2004. A new trend of the phytoplankton structure and dynamics in the Romanian marine waters. *Cercetari Marine*, 35: 77–86.
- Bodeanu, N. 2002. Algal blooms in Romanian Black Sea waters in the last two decades of the 20th century. *Cercetari Marine* 34: 7–22.
- Boyce, D.G., Lewis, M.R. and Worm, B. 2010. Global phytoplankton decline over the past century. *Nature* 466 (7306): 591-596. doi:10.1038/nature09268
- BSC 2008. State of the Environment of the Black Sea (2001-2006/7). In: T. Oguz (Ed.), Publications of the Commission on the Protection of the Black Sea against Pollution (BSC) 2008-3, Istanbul, 448 pp.
- Chebotarev, Y., Moiseev, Y., Kopiliv, A., Sorokin, Y. and Mamaeva, T.I. 1983. Seasonal variation of nutrients and primary production in the littoral zone of the Black Sea near Gelendzhik Bay. In: Y. Sorokin, M.E. Vinogradov (Eds.), *Seasonal changes of plankton on the Black Sea*. Nauka, Moscow: 84–91 (in Russian).
- Coban-Yildiz, Y., Tugrul, S., Ediger, D., Yilmaz, A. and Polat, S.C. 2000. A Comparative Study on the Abundance and Elemental Composition of POM in three interconnected basins: the Black, the Marmara and the Mediterranean Seas. *Mediterranean Marine Science*, 1: 51-63. doi: 10.12681/mms.5
- Codispoti, L.A., Friederich, G.E., Murray, J.W. and Sakamoto, C.M. 1991. Chemical variability in the Black Sea: Implications of continuous vertical profiles that penetrated the oxic/anoxic interface. *Deep Sea Research* 38: 691–710. doi: 10.1016/S0198-0149(10) 80004-4
- Demidov, A.B. 2008. Seasonal Dynamics and Estimation of the Annual Primary Production of Phytoplankton in the Black Sea. *Oceanology* 5(48): 664–678. doi: 10.1134/S0001437008050068
- Eker-Develi, E., Kideys, A.E., 2003. Distribution of

- Phytoplankton in the Southern Black Sea in Summer 1996, Spring and Autumn 1998. *Journal of Marine Systems*, 39: 203-211. doi: 10.1016/S0924-7963(03)00031-9
- Eker-Develi, E., Berthon, J.F., Linde, D., 2008. Phytoplankton class determination by microscopic and HPLC-CHEMTAX analyses in the southern Baltic Sea. *Marine Ecology Progress Series* 359: 69-87. doi: 10.3354/meps07319
- Feyzioglu, A.M. and Seyhan, K. 2007. Phytoplankton Composition of South East Black Sea Coast. *J. Black Sea/Mediterranean Environment*, 13: 61-71.
- Finenko, Z.Z. 1979. Phytoplankton production. In: V.N. Greze (Ed.), *Principles of the biological productivity of the Black Sea*. Naukova Dumka, Kiev: 88-99 (in Russian).
- Fukuyo, Y., Takano, H., Chihara, M. and Matsuoka, K. 1990. Red tide organisms in Japon (An illustrated taxonomic guide). Uchida Rokakuho Publishing, Tokyo, 407 pp.
- Hay, B.J., Honjo, S., Kempe, S., Ittekkot, V.A., Degens, E.T., Konuk, T. and Izdar, E. 1990. Interannual variability in particle flux in the southwestern Black Sea. *Deep Sea Research*. 37: 911-928. doi: 10.1016/0198-0149(90)90103-3
- Kideys, A.E. 1994. Recent Dramatic Changes in the Black Sea Ecosystem: The Reason for the Sharp Decline in Turkish Anchovy Fisheries, *Journal of Marine Systems* 5: 171-181. doi: 10.1016/0924-7963(94)90030-2
- Kideys, A.E. 2002. Fall and Rise of the Black Sea Ecosystem. *Science*, 297. doi: 10.1126/science.1073002
- Kopelevich, O.V., Sheberstov, S.V., Yunev, O., Basturk, O., Finenko, Z.Z., Nikonov, S. and Vedernikov, V.I. 2002. Surface Chlorophyll-a in the Black Sea over 1978-1986 Derived from Satellite and in situ data. *Journal of Marine Systems*, 36: 145-160. doi: 10.1126/science.1073002
- Levitus, S. 1982. *Climatological Atlas of the World Ocean*, NOAA Professional Paper 13, U.S. Department of Commerce.
- Mantoura, R.F.C. and Llewellyn, C.A. 1983. The rapid determination of algal Chlorophyll-a and Carotenoid Pigments and Their Breakdown Products in Natural Waters by Reverse-Phase High Performance Liquid Chromatography. *Analytica Chimica Acta*, 151: 297-314. doi: 10.1016/S0003-2670(00)80092-6
- McQuatters-Gollop, A., Reid, P.C., Edwards, M., Burkill, P.H., Castellani, C., Batten, S., Gieskes, W., Beare, D., Bidigare, R.R., Head, E., Johnson, R., Kahru, M., Koslow, J.A. and Pena, A. 2011. Is there a decline in marine phytoplankton? *Nature*, 472(7342). doi: 10.1038/nature09950
- McQuatters-Gollop, A., Mee, L.D., Raitsos, D.E. and Shapiro, G.I. 2008. Non-linearities, regime shifts and recovery: The recent influence of climate on Black Sea chlorophyll. *Journal of Marine Systems*, 74: 649-658. doi: 10.1016/j.jmarsys.2008.06.002
- Menden-Deuer, S. and Lessard, E.J. 2000. Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. *Limnology and Oceanography* 45: 569-579. doi: 10.4319/lo.2000.45.3.0569
- Oguz, T., Latun, V.S., Latif, M.A., Vladimirov, V.V., Sur, H.I., Markov, A.A., Ozsoy, E., Kotovshchikov, V.V., Eremeev, V.V. and Unluata, U. 1993. Circulation in the surface and intermediate layers of the Black Sea. *Deep-Sea Research*, 40, 1597-1612. doi: 10.1016/0967-0637(93)90018-X
- Oguz, T., Cokacar, T., Malanotte-Rizzoli, P., Ducklow, H.W., 2003. Climatic warming and accompanying changes in the ecological regime of the Black Sea during 1990s. *G. Biogeochem. Cycles* 17 (3), 1088. doi: 10.1029/2003GB002031
- Parsons, T.R., Maita, Y., Lalli, C. 1984. *Manual of Chemical and Biological Methods for Sea Water Analysis*, Pergamon Press, Great Britain, pp. 173.
- Rampi, L. and Bernhard, M. 1978. Key for the determination of Mediterranean pelagic diatoms, Comit. Naz. Energia Nucleare, Roma, 72 pp.
- Raymont, J.E.G. 1980. *Plankton and productivity in the oceans*, 2nd ed., Phytoplankton. Pergamon Press, Oxford.
- Richardson, A. 1991. Comparison of 14C primary production determinations made by different laboratories. *Marine Ecology Progress Series*, 72: 189-201.
- Sorokin, Y.A. 2002. *The Black Sea: ecology and oceanography*. Biology of Inland Waters. Backhuys, Leiden, The Netherlands, 875 pp.
- Stemann-Nielsen, E. 1952. The use of radioactive carbon (14C) for measuring organic production in the sea. *J. Cons. perm. int. Explor. Mer.* 18: 117-140. doi: 10.1016/0146-6313(58)90015-7
- Sur, H.I., Ozsoy, E., Ilyin, Y.P. and Unluata, U. 1996. Coastal/deep ocean interactions in the Black Sea and their ecological environmental impacts. *Journal of Marine Systems*, 7: 293-320. doi: 10.1016/0924-7963(95)00030-5
- Tomas, C.R., 1996. *Identification Marine Diatoms and Dinoflagellates*, Academic Press, San Diego, 598 pp.
- Uysal, Z., Kideys, A.E., Senichkina, L., Georgieva, L., Altukhov, D., Kuzmenko, L., Manjos, L., Mutlu, E. and Eker, E. 1997. Phytoplankton Patches Formed along the Southern Black Sea Coast in the Spring and Summer of 1996. NATO TU- Black Sea Assessment Workshop, June, Crimea, Ukraine.
- Vedernikov, V.I. and Demidov, A.B. 1997. Vertical distribution of primary production and chlorophyll during different seasons in deep regions of the Black Sea. *Oceanology* 37: 376-384 (English Translation).
- Vinogradov, M., Shushkina, E., Mikaelyan, A. and Nezhlin, N.P. 1999. Temporal (seasonal and interannual) changes of ecosystem of the openwaters of the Black Sea. In: S.B. et al. (Ed.), *Environmental Degradation of the Black Sea: Challenges and Remedies*. Kluwer Academic Publishers, 109-129.
- Yayla, M., Yilmaz, A. and Morkoc, E. 2001. The Dynamics of Nutrient Enrichment and Primary Production Related to Recent Changes in the Ecosystem of the Black Sea. *Aquatic Ecosystem Health and Management*, 4: 33-49. doi: 10.1080/146349801753569261
- Yilmaz, A., Tugrul, S., Polat, C., Ediger, D., Coban, Y. and Morkoc, E. 1998. On the Production, Elemental Composition (C, N, P) and Distribution of Photosynthetic Organic Matter in the Southern Black Sea. *Hydrobiologia*, 363: 141-156. doi: 10.1023/A:1003150512182
- Yilmaz, A., Coban-Yildiz, Y. and Tugrul, S. 2006. Biogeochemical cycling and multilayer production in the Black Sea. *Geophysical Research Abstracts*, 8: 00541. doi: 1607-7962/gra/EGU06-A-00541
- Yunev, O., Vladimir, A., Basturk, O., Yilmaz, A., Kideys,

- A.E., Moncheva, S. and Konovalov, S.K. 2002. Long-term Variation of Surface Chlorophyll-a and Primary Production in the open Black Sea. *Marine Ecology Progress Series*, 230, 11-28. doi:10.3354/meps230011
- Yunev, O.A., Carstensen, J., Moncheva, S., Khaliulin, A., Aertebjerg, G., Nixon, S., 2007. Nutrient and phytoplankton trends on the western Black Sea shelf in response to cultural eutrophication and climate changes. *Estuarine, Coastal and Shelf Science* 74, 63–76. doi: 10.1016/j.ecss.2007.03.030