TURKISH JOURNAL OF AQUATIC SCIENCES
RESEARCH ARTICLE/ARAŞTIRMA MAKALESİ ISSN: 2149-9659 E-ISSN: 2528-9462

VARIABILITY IN PHYTOPLANKTON PIGMENT COMPOSITION IN MERSIN BAY

Nebil YÜCEL[1,2], Zahit UYSAL[2], Süleyman TUĞRUL[2]

[1] Faculty of Marine Sciences & Technology, Iskenderun Technical University, Hatay-Türkiye

[2] Institute of Marine Sciences, Middle East Technical University, Mersin-Türkiye

ARTICLE INFO

Received: 28/11/2016

Accepted: 19/01/2017

Published online: 27/01/2017

Yücel et al., 32(1): 49-70 (2017)

doi: 10.18864/TJAS201705

Corresponding author:

Nebil YÜCEL, Faculty of Marine Sciences & Technology, Iskenderun Technical University Meydan Mah. 512, 31320, Iskenderun, Hatay-Türkiye

E-mail: nebilyucel@gmail.com

Keywords:

Pigments
HPLC
Phytoplankton
Nutrients
Northeastern Mediterranean

Abstract

Phytoplankton pigment composition and associated biochemical (chlorophyll *a*, nitrate+nitrite, ammonia, phosphate, silicate) and physical (temperature, salinity) parameters were studied seasonally in the river-fed eutrophic coastal and oligotrophic offshore waters of the Mersin Bay located on NE Mediterranean. Total chlorophyll *a* concentrations varied seasonally between 0.023-7.73 µg/L in the river fed coastal zone (salinity: 36.54-39.68), exhibiting less seasonality in the offshore station (0.01-0.91 µg/L). Seven different phytoplankton taxa (namely diatoms, cyanobacteria, prymnesiophytes, dinoflagellates, chlorophytes, chrysophytes, prochlorophytes), determined by High Performance Liquid Chromatography (HPLC), were consistently present in the coastal waters during 2008-2011. The predominant diatom and coccolithophorid taxa in the river-fed coastal waters were replaced by prokaryotic picoplankters (cyanobacteria & prochlorophytes) and prymnesiophytes in the offshore. Contribution of prokaryotic picoplankton and eukaryotic nanoflagellates to total chlorophyll (% 23 to 47 and % 28 to 43, respectively) increased by about 50% from the coastal to the offshore station. Contribution of large cells to total chlorophyll decreased from 49% to 10% towards offshore. Prochlorophytes were observed as the dominant group in relatively colder and darker deep waters.

INTRODUCTION

Results of algal biomass derived from chlorophyll *a* measurements by different methods have been widely used to assess the global distribution, composition and function of phytoplankton in the ocean system including seas and bays. High performance liquid chromatography (HPLC) provides precise and fast measurement of phytoplankton pigments (Jeffrey et al., 1997; Dos Santos et al., 2003). Since each main phytoplankton taxon has a specific marker pigment, these pigments can be used to assess taxonomic composition of marine phytoplankton (Jeffrey et al., 1997; Barlow et al., 1999). Peridinin (PER), fucoxanthin (FUC) and zeaxanthin (ZEA) have been commonly used to designate dinoflagellates, diatoms and cyanobacteria, respectively (Jeffrey et al., 1997). Microscopic identification methods are mainly used to quantify large size phytoplankton (size >15 micrometers), which make up the majority of algal standing stock in productive waters. Introduction of single cell analysis by flow cytometry and pigment analysis by HPLC enabled easy quantification of small-size phytoplankton (picoplankton, <2 μm), which are dominant in the less productive offshore waters (Li et al., 1993; Partensky et al., 1993), such as subtropical ocean gyres and the Mediterranean (Li et al.1993; Bell & Kalff 2001; Uysal, 2006).

Eastern Mediterranean is very oligotophic (Yılmaz & Tugrul, 1998; Ediger et al., 1998; Psarra et al., 2000; Krom et al., 2003; Tanaka et al., 2007) and annual phytoplankton blooms take place between late autumn and early spring as soon as the dissolved nutrients are mixed into photic zone (Krom et al., 2003). Perennial rivers located in the Cilician Basin constitute a large proportion of all available fresh water inputs into the entire oligotrophic eastern Mediterranean with a total fresh water flux of 27 km^3/yr (half of the present discharge of the Nile ; Gu et al., 2013). This freshwater input and associated nitrate and silicate discharge are primary drivers of the coastal algal production. Since the annual discharge of the River Nile was reduced by 90% in the 1960's, the freshwater input in Cilician Basin became the major fresh water and thus nutrient sources for NE Mediterranean (Kocak et al., 2010). The significant inputs of these rivers make the Cilician Basin a characteristic ROFI (regions of freshwater influence) region, where the changes in the coastal ecosystem, and the eutrophication processes are determined by coastal / open sea interactions and the interaction with the atmosphere. Phytoplankton is dominated by small sized individuals (cyanobacteria *Synechococcus* spp., prochlorophytes, pico and nano eukaryotes, small naked flagellates etc.,) in offshore waters (Li et al., 1993; Yacobi et al., 1995; Ignatiades, 1998; Psarra et al., 2005). In contrast, larger cells composed primarily of diatoms and dinoflagellates were reported as the most abundant group in the river-fed coastal waters of Cilician basin (Kideys et al., 1989; Eker et al., 2003; Koray, 1995; Eker & Kideys, 2000; Polat et al., 2000; Polat and Işık, 2002; Uysal et al., 2003). Since Cilician coastal waters fed by nitrate and silicate rich but SRP-depleted rivers (Figure 1), the coastal waters with less surface salinity have high nitrate and silicate concentrations especially during winter-spring period as the phosphate has consistently remained low (0.03-0.08 μM) during the year (Sağlamtimur & Tugrul, 2004). Atmospheric (wet+dry) deposition feeds the entire basin with high DIN/SRP (Dissolved Inorganic Nitrogen/Soluble Reactive Phosphate) ratios (Kocak et al., 2010). Nutrient enrichment experiments indicate either phosphorus or nitrogen & phosphorus co-limitation in the eastern Mediterranean (Krom et al., 2005, 2010; Zohary et al., 2005; Tanaka et al., 2007).

Dramatic increases in human population along the coastal regions and associated increase in nutrient inputs from industrial, domestic and agricultural sources have enhanced eutrophication in the coastal waters of Cilician basin. However, historical and systematic data in the region before the 1990's are very limited to assess long-term changes in the coastal ecosystem of Mersin Bay (Uysal et al., 2008). Limited studies on phytoplankton composition in Cilician basin have focused mainly larger phytoplankton (Yılmaz et al., 2003; Yılmaz, 2006; Yucel, 2008). The present study aims to provide a more comprehensive taxonomic approach by including almost all components of the flora.

MATERIAL AND METHODS

Study site and methodology

Two stations selected at the coastal (T27: N36.6833° – E34.8166°) and off-shore (T48: N36.38333° – E34.7000°) zones of Mersin bay (Fig. 1) were visited 12 times to collected water samples on board R/V Bilim-2 between September 2008 and October 2011. Despite the oligotrophic nature of the offshore station, the coastal station (depth: 30 m) receive substantial amount of river as well as anthropogenic input from the surrounding.

Niches of greatly varying properties occur along the coast as a result of this heterogeneous distribution of sources. Water samples from target depths were collected using 5 L Nansen closing bottles attached onto a Sea-Bird Model rosette + CTD probe system at each sampling and station during mid-day. CTD data obtained *in situ* were processed by the standard Sea-Bird data processing software to calculate salinity and temperature.

Water samples were analyzed for phytoplankton pigment composition and associated biological (Chl-*a*) chemical (nitrate (in fact; sum of nitrate+nitrite), phosphate, silicate and ammonia) and physical (temperature, salinity, and density) parameters. Water samples were filtered immediately onto Whatman 25 mm GF/F filters and kept frozen in liquid nitrogen tank to avoid pigment degradation (Mantoura, et al, 1997). Extraction was carried out with 90 % HPLC grade acetone under sonication. Samples were stored at 4 °C overnight for extraction and centrifuged to clarify the extract. The method applied in this study (Barlow et at., 1993) is a modification of the reverse-phase method described earlier by Mantoura & Llewelyn (1983). Pigment analysis was done with an Agilent 1100 HPLC system using a C8 column equipped. 500 µl of the extract was filtered through 0.2 µm pore size Millipore filters and mixed with 500 µl 1M ammonium acetate ion pairing solution for the measurement. Buffered extracts were injected (100µl) into a Thermo Hypersil MOS-2 C8 column (150x4.6mm, 3µm particle size, 120Å pore size and 6.5% carbon loading) using an Agilent HPLC system (Quaternary pump, manual injector) having 100 µl loop. Using a binary mobile phase system pigments were separated with linear gradient. Mobile phases used in the gradient elution consisted of primary eluent (A) consisting of methanol and 1M ammonium acetate (80:20 v/v), and a secondary eluent (B) consisting of 100% methanol. Pigments were separated at a flow rate of 1 mL min^{-1} by a linear gradient programmed as follows (minutes; % solvent A; % solvent B): (0;75;25), (1;50;50), (20;30;70), (25;0;100), (32;0;100). Then, the column was reconditioned to original conditions over a further 7 minutes. Ammonium acetate was used as an ion pairing reagent, and it is recommended that it should be present in both the sample and mobile phase to improve pigment separation and suppressed dissociation of isolated compounds. Fourteen different phytoplankton pigments of chemotaxonomic importance were detected by absorbance at 440nm using an Agilent variable wavelength detector (Mantoura & Llewellyn, 1983). Data collection and integration were performed using a PC-based Chemstation Chromatography Package. The HPLC system was calibrated for each pigment with commercial standards, namely, chlorophyll a, b provided by Sigma Co; chlorophyll c2, chlorophyll c3, peridinin, 19-butanoyloxyfucoxanthin, fucoxanthin, 19-hexanoyloxyfucoxanthin, diadinoxanthin (DIAD), alloxanthin, lutein, zeaxanthin, divinyl chlorophyll-a and β-carotene provided by VKI, Denmark. The detection limit for chl-a and marker pigments was about 0.005-0.007 µg/l. Concentration of each pigment was calculated according to the 'external standard' equation (Jeffrey et al., 1997).

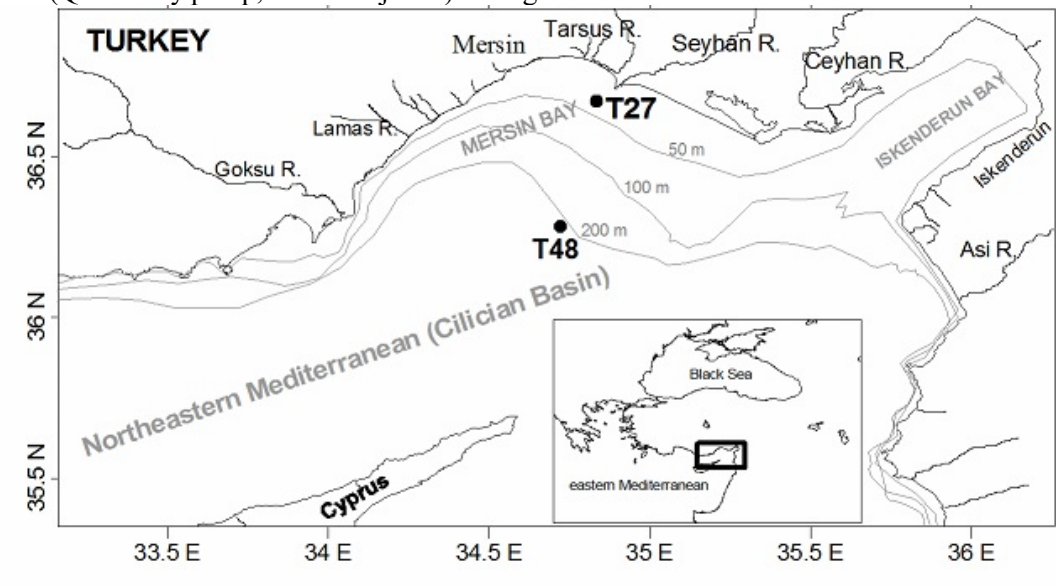

Figure 1. Location of sampling stations in Mersin bay (northeastern Mediterranean).

Pigments measured quantitatively by HPLC were used to assess relative abundance of seven major algal groups in filtered samples from the coastal and offshore waters of the Mersin Bay. In addition, the results of pigment clusters were used to classify the identified algae in three different size groups (Table 1) (Bidigare et al., 1990; Gibb et al., 2000).

Photoprotective (PPC = DIAD+ALLO+ZEA+β-CAR) and photosynthetic (PSC = PER+FUC+HEX+BUT) pigments together form the total carotenoids (TCAR).

Samples for dissolved nutrients were stored in 50-100 mL capacity high-density polyethylene bottles (pre-cleaned with 10% HCl). Samples for nitrate and phosphate analysis were kept frozen and samples for silicate were kept cool in dark. Nutrient measurements were carried out by using a Bran Luebbe model four-channel auto-analyzer; the methods followed were very similar to those described in Strickland & Parsons (1972) and Grasshoff et al., (1983). Detection limits for nitrate, phosphate, silicate and ammonia were 0.02, 0.015, 0.1 and 0.04 µM, respectively.

Statistical analyses

Pearson-product moment correlation analysis was applied to investigate possible relationships between pigment types and their concentrations and the ambient biological, physical and chemical variables using SPSS (**S**tatistical **P**ackage for the **S**ocial **S**ciences) version 15.0 for Windows.

RESULTS AND DISCUSSION

Changes in physical parameters

Temperature and salinity varied in the range of 15-30 ºC and 36.5-39.5 in the coastal and 16.4-29.7 ºC and 38-39.6, respectively in the offshore station throughout the sampling period (Figure 2). With the onset of spring, a gradual warming of the near-surface (1 m) waters was observed which further continued till late summer. Development of a well-defined seasonal thermocline during fall was observed at ≈ 40 m in the offshore station. The thermohaline gradient formed in the offshore water column was observed to disappear in winter due to cooling and vertical mixing of the upper layer. However, top 10 m of the coastal station was highly influenced by river discharges during winter-spring seasons and no well-mixed feature appeared in winter (Figure 2).

Spatio-temporal Variability in Nutrient Concentrations

Nutrient concentrations were higher in coastal waters (Figure 1) with maximum values of DIN and Si measured in February 2010. However, phosphate concentrations remained very low (0.02-0.05 µM) (Figure 3) as reported recently (Kocak et al. 2010). A strong decline was observed in spring due to increased autotrophic uptake. Higher ammonia concentrations varied between 0.1-2.43 µM in coastal waters with higher values at the hot points receiving domestic waste waters and contaminated river discharges, and then declined to its natural levels of 0.05-0.45 µM in the offshore waters having low values of phosphate (0.02-0.04 µM). Nitrate and silicate concentrations decreased with depth, when the surface layer was occupied by lower salinity waters (Figure 3).

Table 1. List of pigments used as biomarkers of different phytoplankton groups (Jeffrey et al., 1997).

Pigment	Abbreviation	Occurrence	Major groups
Zeaxanthin Divinyl Chlorophyll *a*	ZEA DIV*a*	Cyanobacteria Prochlorophytes	Prokaryotic picoplankton
Fucoxanthin Peridinin	FUC PER	Diatoms Dinoflagellates	Large eukaryotes
19'-Butanoyloxyfucoxan- 19'-Hexanoyloxyfucoxan- Chlorophyll *b*	BUT HEX CHL*b*	Chrysophytes, Prymnesio- Chlorophytes	Eukaryotic nanoflagellates

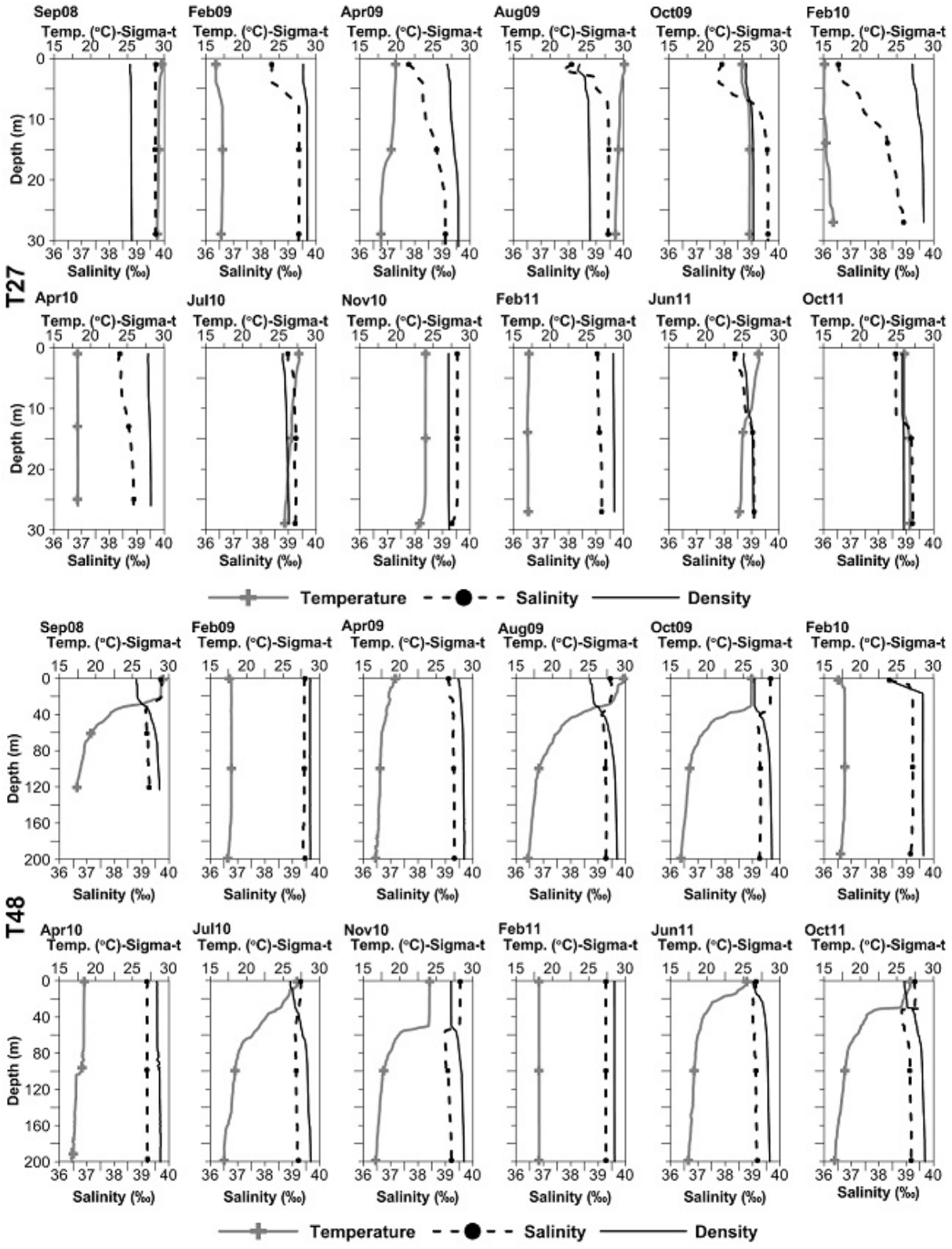

Figure 2. Temperature, salinity and density profiles of the coastal (T27) and offshore (T48) waters of Mersin bay (NE Mediterranean).

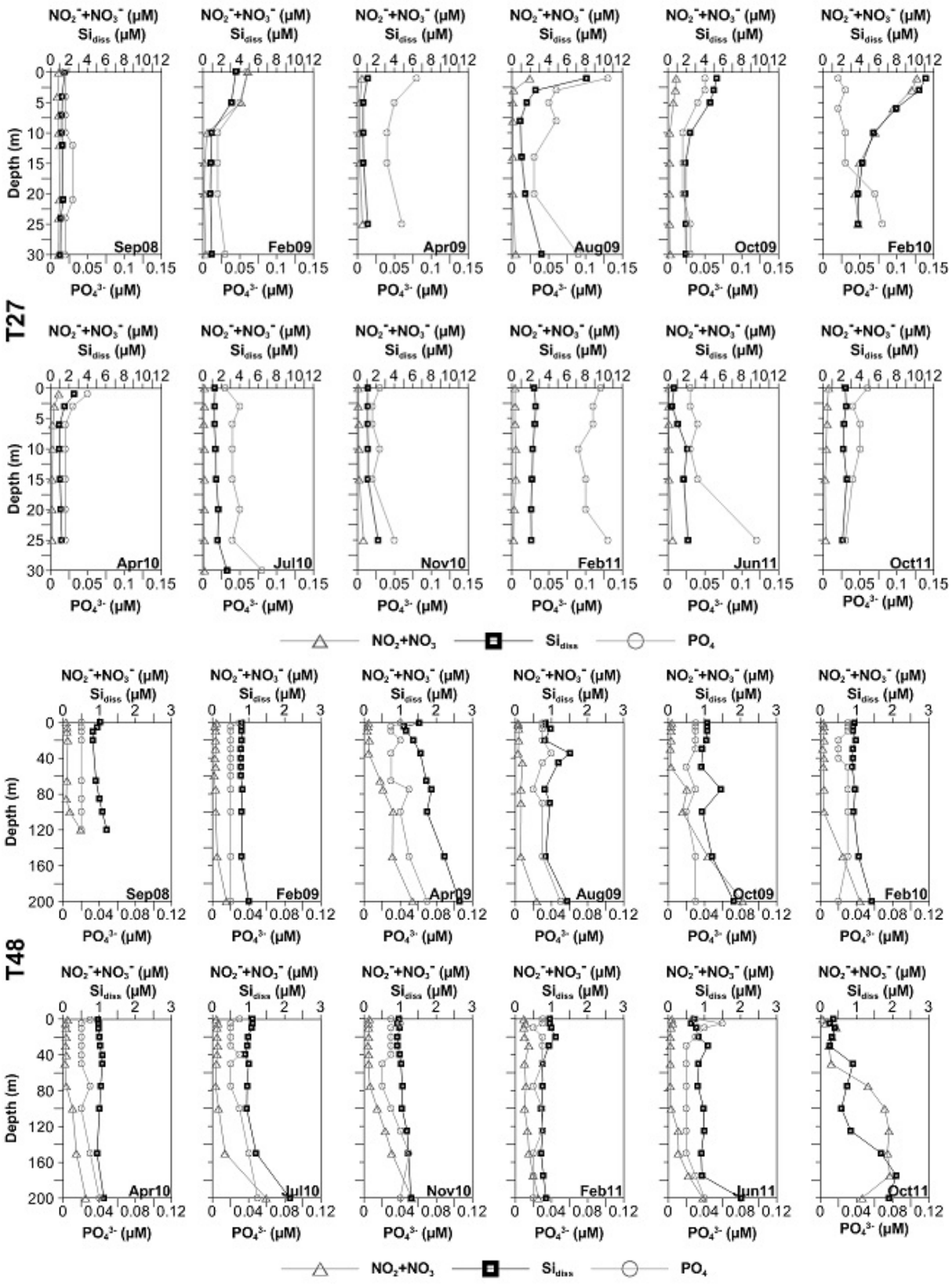

Figure 3. Nutrient profiles of the coastal (T27) and offshore (T48) waters of Mersin bay (NE Mediterranean).

No significant vertical variability appeared in the phosphate profiles due to limited SRP inputs by river discharges. However higher SRP concentrations in the near bottom water observed in February 2011 was due to sediment resuspension. The winter nitrate values decreased from 9.7 µM in the surface to 0.27 µM at 30 m of the coastal station. Greater Si concentrations were expectedly measured in the less saline near surface waters in August & October 2009 and February 2011, respectively (Figure 3). Similarly, the offshore surface nitrate changed seasonally from 0.05 in summer to 0.24 µM in winter when the upper layer was well mixed (Figure 3) whereas the coastal nitrate enhanced markedly (9.7 µM) in winter. Therefore, the coastal DIN/SRP (N/P) ratio exceeded 100 in late winter and then declined drastically to 6 during summer & autumn when the river inputs were at minimal levels and excess nitrate in surface water was consumed. However, the surface N/P ratio consistently remained low (<10) in the offshore station in all seasons (Figure 4). Similar spatio-temporal variations appeared in DIN/Si ratio; the coastal ratio decreased from 2 in winter to 0.1 in summer-autumn period, whereas the offshore surface ratio ranged merely between 0.2 in winter and 0.3 in summer due to excess silicate in seawater (Figure 3; Table 2). In the offshore station, the nitrate and silicate profiles displayed an increasing feature in the less saline deeper waters at 75-100 m, below the seasonal thermocline and euphotic zone (Figure 3).

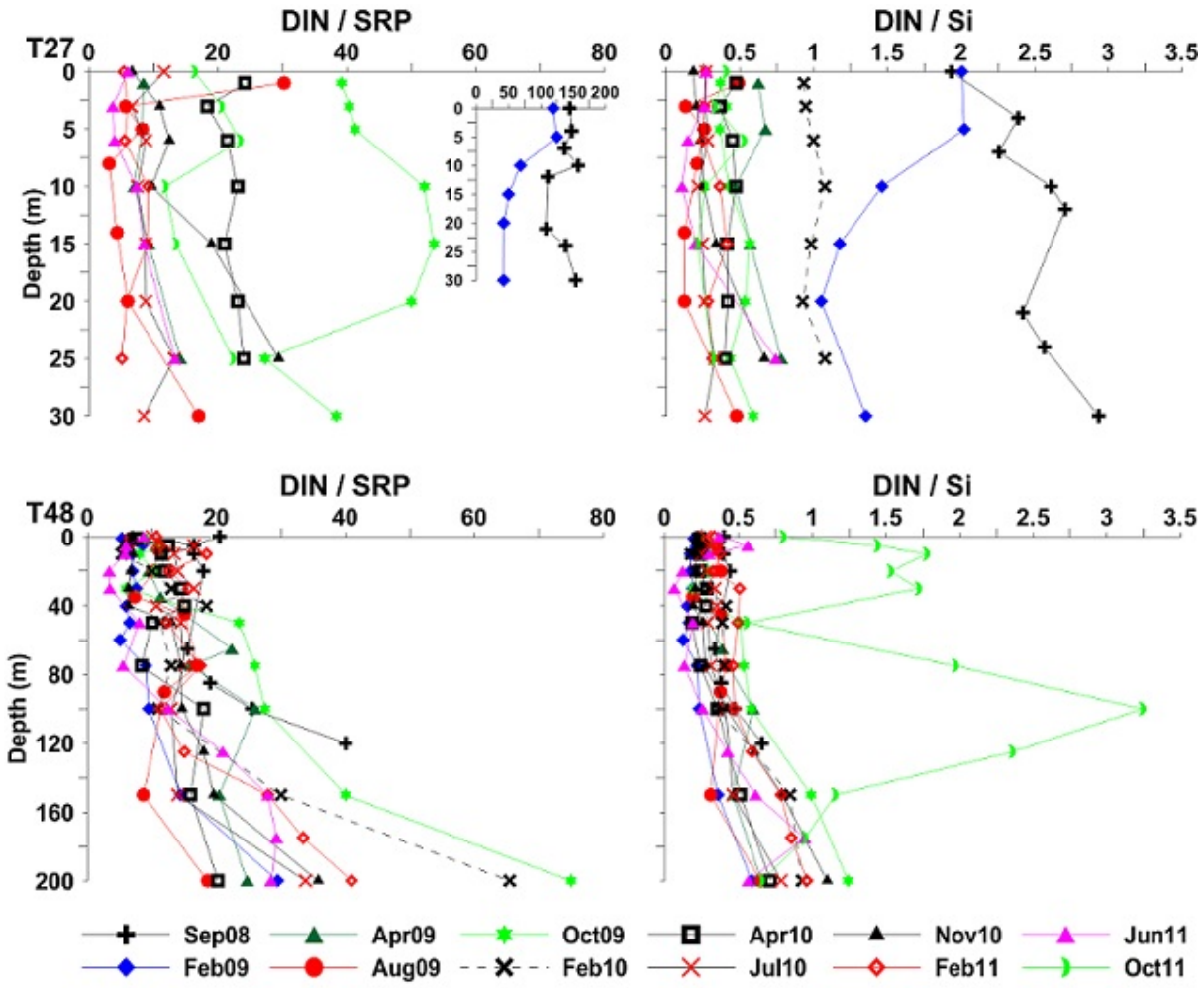

Figure 4. DIN/SRP and DIN/Si profiles of the coastal (T27) and offshore (T48) waters of Mersin bay (NE Mediterranean) (removed DIN/SRP values in Feb 2010 (higher than 500) from coastal graph).

Table 2. Seasonal variations in water column physical, chemical and biological parameters in Mersin Bay.

Parameters	Coastal (T27)								Offshore (T48)							
	Autumn		Winter		Spring		Summer		Autumn		Winter		Spring		Summer	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Temperature (ºC)	22.82	26.85	15.00	17.28	17.90	19.91	25.77	30.17	16.34	29.21	15.97	21.76	16.56	19.52	16.60	29.74
Salinty (‰)	37.80	39.68	36.54	39.40	37.77	39.10	37.77	39.46	38.83	39.65	38.39	39.99	39.12	39.80	39.00	39.53
NO_2+NO_3 (µM)	0.10	0.94	0.12	10.22	0.17	0.85	0.06	1.92	0.06	2.06	0.05	1.09	0.05	1.34	0.05	1.48
NH_4 (µM)	0.10	2.43	0.06	2.29	0.06	0.36	0.05	2.01	0.06	0.40	0.05	0.29	0.10	0.39	0.02	0.34
PO_4 (µM)	0.02	0.06	0.02	0.13	0.02	0.08	0.03	0.13	0.00	0.05	0.02	0.03	0.02	0.07	0.02	0.06
Si (µM)	1.05	5.33	0.81	11.14	0.60	2.57	0.43	8.08	0.25	2.10	0.71	1.41	0.94	2.64	0.64	2.14
DIN/SRP	7	158	5	519	7	24	3	30	3	75	5	66	8	26	3	34
DIN/Si	0.18	2.93	0.25	2.02	0.36	0.78	0.11	0.74	0.19	3.59	0.15	0.96	0.18	0.72	0.06	0.95
Chl a (µg/L)	0.023	3.336	0.068	1.815	0.063	0.528	0.741	7.727	0.027	0.908	0.037	0.284	0.012	0.101	*	0.818
FUC (µg/L)	*	0.484	0.009	0.513	0.037	0.184	*	0.359	*	0.072	*	0.026	*	0.011	*	0.014
ZEA (µg/L)	0.034	0.187	*	0.043	0.007	0.022	0.009	0.414	0.008	0.116	*	0.025	*	0.040	0.006	0.039
HEX (µg/L)	0.013	0.176	0.007	0.098	0.013	0.068	*	0.046	*	0.147	0.013	0.090	*	0.030	*	0.044
CHLL-b (µg/L)	*	0.105	*	0.166	*	*	*	*	*	0.070	*	0.057	*	0.000	*	0.018
DIV-a (µg/L)	*	0.084	*	0.039	*	0.009	*	0.028	0.008	0.095	*	0.031	*	0.045	*	0.038
PER (µg/L)	*	0.068	*	0.016	*	*	*	0.093	*	0.074	*	0.022	*	0.000	*	0.004
BUT (µg/L)	*	0.037	*	0.032	0.004	0.032	*	0.287	*	0.067	*	0.039	*	0.012	*	0.022
β-CAR (µg/L)	0.010	0.120	*	0.055	0.004	0.055	0.003	0.146	*	0.027	*	0.015	*	0.027	*	0.019
Large Eukaryotes(µg/L)	*	0.551	0.009	0.527	0.022	0.184	*	0.385	*	0.146	*	0.048	*	0.011	*	0.016
Eukaryotic Nanoflagellates(µg/L)	0.016	0.305	0.009	0.243	0.010	0.100	*	0.320	*	0.284	0.015	0.187	*	0.042	*	0.066
Prokaryotic Pikoplankton(µg/L)	0.042	0.260	*	0.075	0.003	0.023	0.016	0.414	0.018	0.192	*	0.056	0.002	0.085	0.006	0.075
PPC	0.043	0.430	0.009	0.290	0.032	0.248	0.012	0.713	0.010	0.198	*	0.064	*	0.074	0.007	0.049
PSC	0.024	0.761	0.051	0.604	0.046	0.299	*	0.473	*	0.360	0.015	0.149	*	0.080	*	0.079

* : Below Detection Limit

Phytoplankton Pigments

Distribution of chlorophyll *a*

Surface chlorophyll *a* (Chll-*a*) concentrations varied seasonally between 0.01-7.73 in coastal waters (depth <30 m) as the offshore concentrations varied in the range 0.01-0.91 µg L^{-1} during the period of February 2009-October 2011 (Figure 5), indicating about 10-fold decrease in the oligotrophic open waters. The surface Chll-*a* reached the maximum values in June 2011 in the coastal and offshore waters. Surface concentrations were generally below 2 and 0.2 µg L^{-1} in the coastal and offshore waters, respectively. There is no seasonality in surface chlorophyll concentrations in the study area. A characteristic Deep Chll-a Maximum (DCM) was formed at 75 m at the offshore station in June and October 2001. However, highest Chll-*values* were mostly observed in near surface waters of both stations (Figure 5). Higher concentrations were measured in the water column in June and October 2011.

Distribution of marker pigments and algal groups

Diatoms dominated the coastal zone with marked contributions extending from April 2009 to October 2011 (except July and November 2010 and July 2011; Figure 6). Diatom contribution to total chlorophyll exceeded 50% during February 2010. Diatoms also dominated the bulk algae composition in the near-bottom waters of coastal zone in April 2009, April and November 2010 (Figure 6). Diatoms were followed by cyanobacteria with higher concentrations in August and October 2009 and July 2010. Mean cyanobacteria contribution exceeded 33% during the dry months in coastal waters. Diatoms and Cyanobacteria are the dominant groups in coastal station with prymnesiophytes contributing at around 14%. Contribution of chlorophytes increased in October 2009 (11%) and February 2010 (19%) coincided with increased river discharge (Figure 2 and 6). Chrysophytes peaked in coastal in June 2011 while dominating coastal surface waters (69%). Eukaryotic nanoflagellates were generally dominated by prymnesiophytes (63%), followed by chrysophytes (24%) in coastal waters during the sampling periods. Chlorophytes increased rarely their contribution to flora (Octobers and February 2010). Coastal pigment composition was very diverse in August and October 2009, whereas only FUC and Chll-*b* were excessively dominant in February 2010. Contribution of prochlorophytes (DIV-*a*) to the prokaryotic picoplankton pool in coastal waters remained at negligible levels from April 2009 to October 2011 (except November 2010). Large eukaryotes dominated by diatoms were replaced by prokaryotic picoplankton and eukaryotic nanoflagellates occasionally as experienced in winter with increasing concentrations of nutrients and decreasing temperature (Figure 6). Eukaryotic nanoflagellates and large eukaryotes dominated (>90%) the total biomass of phytoplankton in February 2010; this feature was well pronounced in the upper 15 meters of coastal station waters (Figure 6). On the other hand, cyanobacteria-dominated prokaryotic picoplankton enhanced in the bulk biomass of algae in the coastal zone (<30m) in summer and autumn periods. Eukaryotic nanoflagellates increased their contribution to flora in winter and spring periods.

In the oligotrophic offshore waters of the bay, the pico (47%) and nanoplanktonic (43%) forms (cyanobacteria, prochlorophytes and prymnesiophytes especially the coccolithophorid *Emiliana huxleyi*) dominated the bulk of algae pool during the study period. Although, average values displayed similar contribution of each to overall composition, prokaryotic picoplankton (cyanobacteria and prochlorophytes) made significant contributions to the algae pool in summer whereas prymnesiophytes were observed more abundant in winter and fall (Figure 6). They peaked near surface and at the deep chlorophyll maximum (50-75 m). They shifted with each other in DCM in offshore waters followed by large cells. Large eukaryotes were almost missing from the oligotrophic offshore surface waters (Figure 6). Similarly, eukaryotic nanoflagellates and large eukaryotes were not observed at the upper 40 m of the offshore waters in July 2010. Nearly 70% of large eukaryotes were diatoms in offshore waters. Eukaryotic nanoflagellates dominated the flora in all Februaries (2009, 2010 and 2011) and November 2010 in the offshore. Contribution of dinoflagellates to total composition was negligible in offshore except November 2010 (12%). Prokaryotic picoplankton dominated the biomass in the hot and dry seasons. While the bulk biomass of upper 50 meters was dominated by cyanobacteria, contribution of *prochlorococcus* increased with decreasing light intensity in deeper part; however, their concentrations remained below 0.1 µg L^{-1} in the offshore waters.

Surface distribution of photosynthetic and photoprotectant pigments

PPC pigments protect organisms against high light exposures (Gibb et al., 2000). There are many studies about photoprotective carotenoids which were recorded in high amounts at least productive surface waters of tropics where small cells dominate (Gibb et al., 2000; Barlow et al., 2004; Sathyendranath et al., 2005). Photosynthetic carotenoids expectedly increase with increasing chlorophyll in eutrophic regions where large phytoplankters dominate the bulk (Barlow et al., 2002). As clearly shown in Figure 7, the concentrations of photoprotective (PPC) pigments decreased with increasing photosynthetic (PSC) pigments in winter and early spring. An apparent increase in PPC pigments was observed in July 2010, constituting over 95% of the total carotenoids in the offshore (where only ZEA and DIV-*a* were present). The contribution of large eukaryotes and eukaryotic nanoflagellates to the bulk remained very low. In October 2009, an apparent increase in PPS levels was recorded in the coastal zone. Contribution of PPC to the total carotenoid was much more variable in the offshore waters, ranging from 35-100% in summer to 26-55% in winter. Seasonality in the coastal PPC was less pronounced, constituting 30-66 of the total carotenoids, with increasing contribution in summer (July & August). Despite photosynthetic active radiation (PAR) intensity decreases in winter, the PPC pool was dominated by ZEA, exceeding PSC concentrations in the Mersin Bay coastal waters.

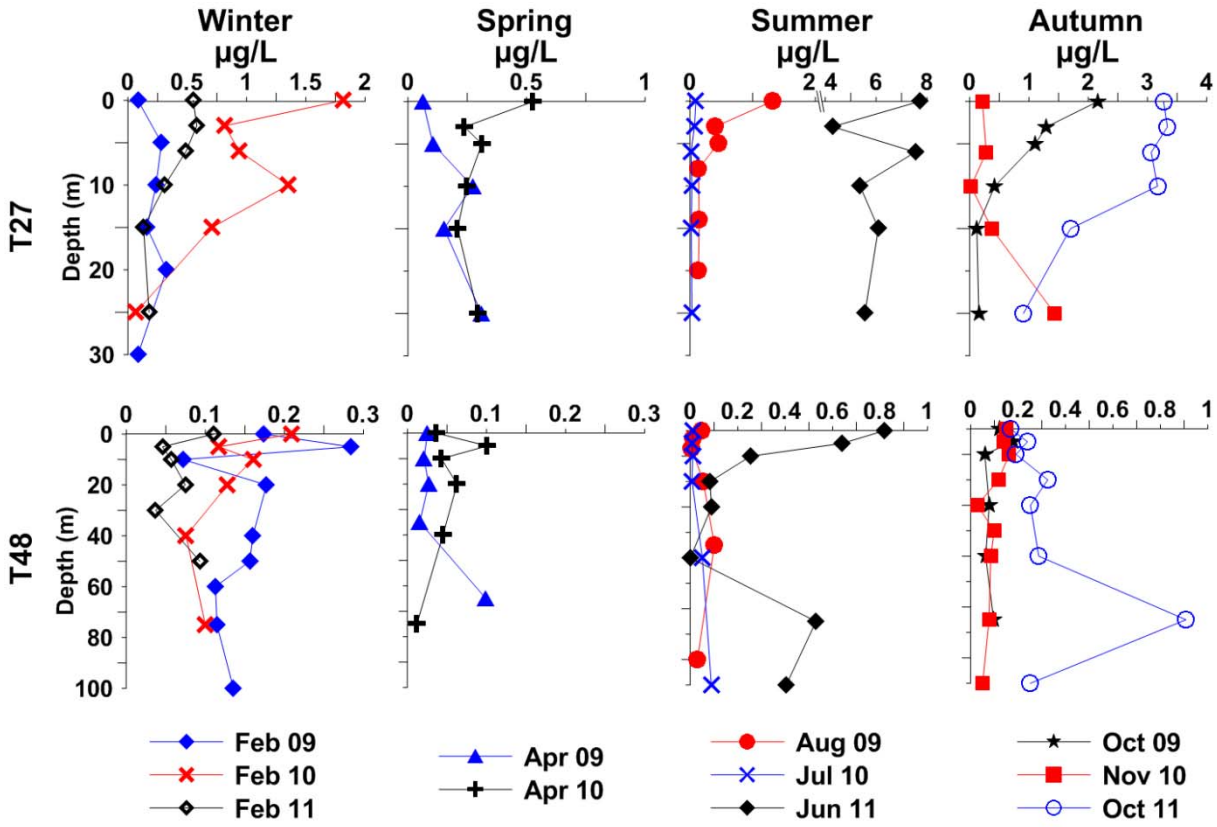

Figure 5. Chlorophyll *a* profiles of the coastal (T27) and offshore (T48) waters of Mersin bay (NE Mediterranean) (Scales are different).

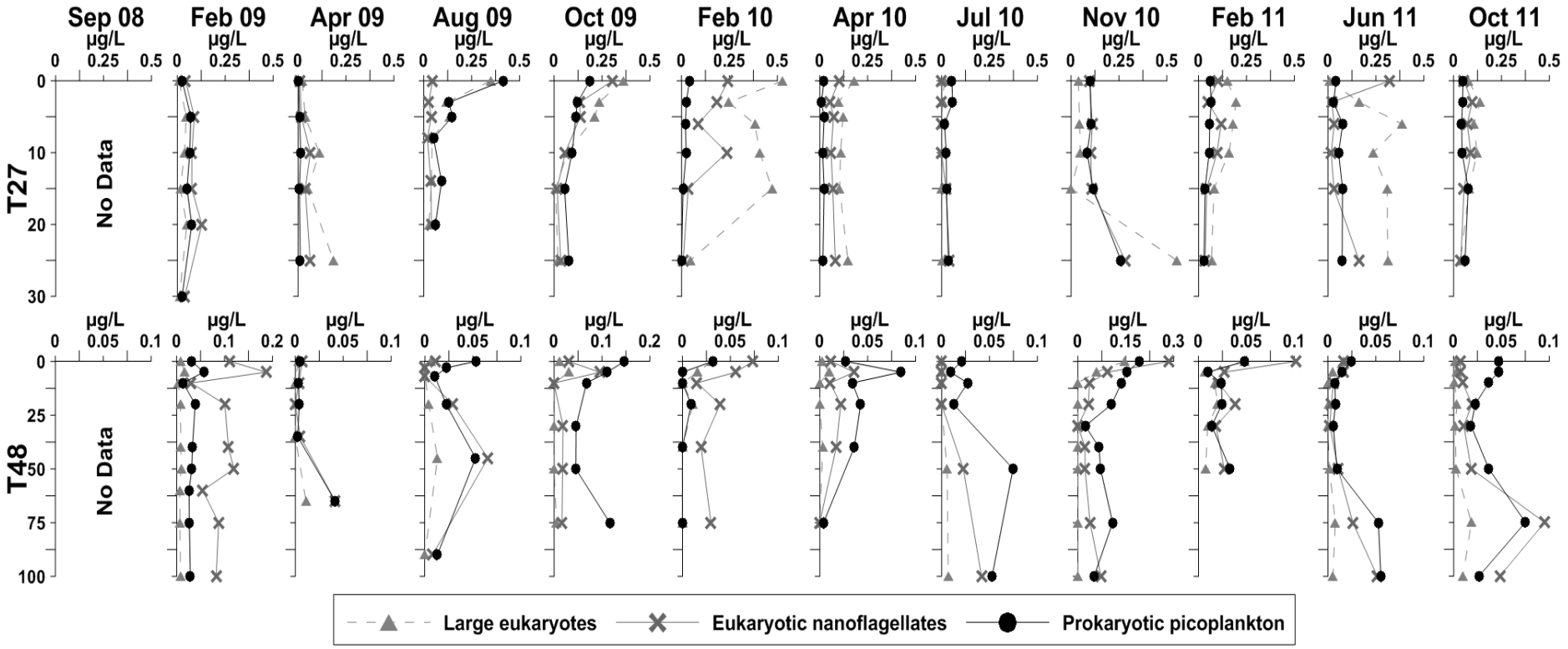

Figure 6. Vertical profiles of phytoplankton groups in Mersin bay (North eastern Mediterranean).

In the oligotrophic offshore waters of the bay, the pico (47%) and nanoplanktonic (43%) forms (cyanobacteria, prochlorophytes and prymnesiophytes especially the coccolithophorid *Emiliana huxleyi*) dominated the bulk of algae pool during the study period. Although, average values displayed similar contribution of each to overall composition, prokaryotic picoplankton (cyanobacteria and prochlorophytes) made significant contributions to the algae pool in summer whereas prymnesiophytes were observed more abundant in winter and fall (Figure 6). They peaked near surface and at the deep chlorophyll maximum (50-75 m). They shifted with each other in DCM in offshore waters followed by large cells. Large eukaryotes were almost missing from the oligotrophic offshore surface waters (Figure 6). Similarly, eukaryotic nanoflagellates and large eukaryotes were not observed at the upper 40 m of the offshore waters in July 2010. Nearly 70% of large eukaryotes were diatoms in offshore waters. Eukaryotic nanoflagellates dominated the flora in all Februaries (2009, 2010 and 2011) and November 2010 in the offshore. Contribution of dinoflagellates to total composition was negligible in offshore except November 2010 (12%). Prokaryotic picoplankton dominated the biomass in the hot and dry seasons. While the bulk biomass of upper 50 meters was dominated by cyanobacteria, contribution of *prochlorococcus* increased with decreasing light intensity in deeper part; however, their concentrations remained below 0.1 µg L^{-1} in the offshore waters.

Surface distribution of photosynthetic and photoprotectant pigments

PPC pigments protect organisms against high light exposures (Gibb et al., 2000). There are many studies about photoprotective carotenoids which were recorded in high amounts at least productive surface waters of tropics where small cells dominate (Gibb et al., 2000; Barlow et al., 2004; Sathyendranath et al., 2005). Photosynthetic carotenoids expectedly increase with increasing chlorophyll in eutrophic regions where large phytoplankters dominate the bulk (Barlow et al., 2002). As clearly shown in Fig. 7, the concentrations of photoprotective (PPC) pigments decreased with increasing photosynthetic (PSC) pigments in winter and early spring. An apparent increase in PPC pigments was observed in July 2010, constituting over 95% of the total carotenoids in the offshore (where only ZEA and DIV-*a* were present). The contribution of large eukaryotes and eukaryotic nanoflagellates to the bulk remained very low. In October 2009, an apparent increase in PPS levels was recorded in the coastal zone. Contribution of PPC to the total carotenoid was much more variable in the offshore waters, ranging from 35-100% in summer to 26-55% in winter. Seasonality in the coastal PPC was less pronounced, constituting 30-66 of the total carotenoids, with increasing contribution in summer (July & August). Despite photosynthetic active radiation (PAR) intensity decreases in winter, the PPC pool was dominated by ZEA, exceeding PSC concentrations in the Mersin Bay coastal waters.

Different groups of phytoplankton may require different environmental conditions for successful growth, such as excess nutrient concentrations, lower temperature and sufficient light intensity. Major rivers of NE Mediterranean, with their associated nutrient loads, flow into the wide shelf waters of Cilician Basin and control the shelf ecosystem of Iskenderun and Mersin bays. The eastern part of Mersin Bay receives fresh waters of Seyhan and Berdan rivers. Therefore, surface salinity decreased to 36-37 in near coastal waters (February 2010) as the surface temperature dropped to about 15 °C in winter. Expectedly, concentrations of dissolved nutrients peaked in February 2010 in the less saline coastal waters, leading to development of large-sized phytoplankton (diatoms). The present pigment results from the Mersin Bay, the bay coastal waters (<30 m) had diatom-dominated algae during winter and spring months, reflecting the role of nitrate and silicate-laden river discharges as experienced previously (Kideys et al., 1989; Eker et al., 2003; Koray, 1995; Eker & Kideys, 2000; Polat et al., 2000 ; Polat & Işık, 2002; Uysal et al., 2003).

Larger cells composed mainly of diatoms and dinoflagellates are more tolerant (opportunistic) and dominate the algal biomass in nutrient-enriched seas (Fogg, 1991). Similarly, highly negative/positive correlations were observed between temperatures, DIN, silicate versus diatom biomass in the bay waters. In summer the diatom flora was replaced by prokaryotic picoplankton (Cyanobacteria dominated) in the coastal waters with limited diatom biomass, due to high surface temperature (~30°C) and thus high light intensity in surface waters with low nutrient concentrations and also low N/Si ratio (0.5). Increased water temperature with enhanced light (PAR) intensity are known to favour production of smaller algal groups (as a cyanobacteria) in nutrient-depleted near surface waters since prokaryotic picoplankton retains photoprotective pigments (ZEA) to resist high light conditions

(Gibb et al., 2000). Picoplankton group with ability to regulate own pigment concentrations, can also grow faster under high light and temperature (Fig. 8) (Postius et al. 1998; Boumann et al., 2003). Prokaryotic picoplankton occasionally may become more abundant in nutrient-enriched near surface waters (Partensky et al. 1999; Polat & Uysal, 2009) and contribute significantly to total primary production during the warm period (Weisse 1993; Kormas et al. 2002), resulting in a significant positive correlation between prokaryotic picoplankton and temperature ($p<0.001$, Fig. 8). Moutin et al. (2002) suggest that prokaryotic picoplankton (*Synechococcus* sp.) display higher affinity for orthophosphate and significantly higher maximum uptake rates than heterotrophic bacteria and eukaryotic cells. Mesoscale Lagrangian phosphate-enrichment experiments conducted in Levantine Sea support this conclusion (Psarra et al., 2005).

The high positive correlation observed between cyanobacteria (ZEA) and dinoflagellate (PER) ($p<0.0001$) may be either linked to prey-predator relationship as previously suggested by Christaki et al. (1999; 2001) or their tolerance to increased temperature. Dinoflagellates in the study area can be autotrophic, heterotrophic, parasitic or endosymbionts of marine animals and protozoa (Tomas et. al.1997). They may act as producers or consumers or both in the same time in the food web (Gaines & Elbrächter, 1987). It is widely accepted that phosphorus is the limiting nutrient in primary production for both coastal and open waters of the northeastern Mediterranean (Tufekçi et al., 2013; Yılmaz & Tugrul, 1998; Krom et al., 2005). Despite the higher N/P ratios in deep water and external sources (Kocak et al. 2010), reactive phosphate has been detected at nanomalolar levels in the coastal and offshore waters of NE Mediterranean (Doğan-Sağlamtimur & Tugrul, 2004). Thus, low Nitrate/SRP ratios (<10) in the salty surface waters during spring-autumn period imply selective SRP inputs from faster regeneration of organic P in the photic zone of Eastern Mediterranean (Krom et al., 1992) and dry deposition of atmospheric dusts (Kocak et al., 2010).

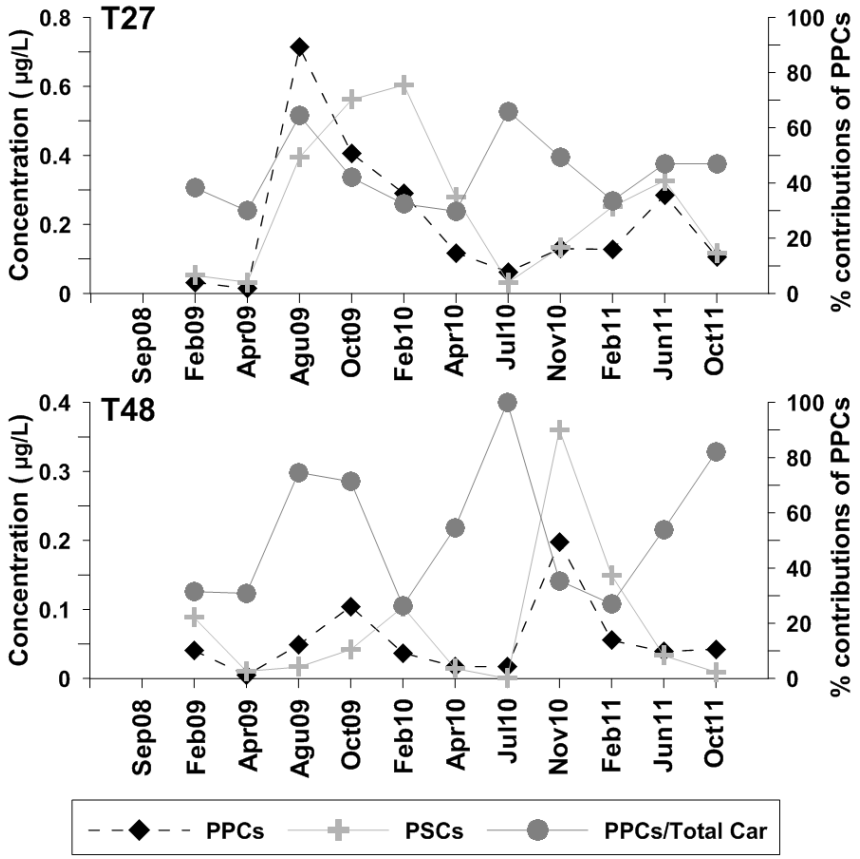

Figure 7. Seasonal variability in photosynthetic (PSC (PER + FUC + HEX + BUT)) and photoprotectant carotenoids (PPC (DIAD + ALLO + ZEA + β-CAR)) concentrations and relative contributions (%) of PPCs to total carotenoids in Mersin bay surface waters.

Production of large eukaryotes, especially diatoms, was limited by nitrogen in coastal waters of the bay (N/Sİ<1 and N/P <10) in April 2009 and then by temperature in summer. On the other hand, diatom production in the bay was limited by phosphorus in February 2010 when rivers fed the bay waters with high N/P ratios (>25). Coastal waters reached its minimum temperature (15 °C) in February 2010 and biomass of the diatoms displayed an increasing trend with decreasing temperature (Figure 8).

Chlorophytes increased their contribution to the biomass of the eukaryotic nanoflagellates in nutrient replete coastal waters (Oct 2009 and Feb 2010) as observed elsewhere (Mackey et al., 2002). But, in the rest of the study periods, their contribution to total phytoplankton biomass remained low (about) 4%) in spite of their high competitive ability (Hassen et al., 2009). However, predation might have limited chlorophytes in coastal waters (Hassen et al., 2009).

Prymnesiophytes including coccolithophorids (*Emiliana huxleyi*) were present consistently at both stations throughout the sampling period, with remarkably lower contribution to total chlorophyll in the coastal water (18%) than in the offshore (26%) where prokaryotic picoplankton dominated the total algae pool.

Eukaryotic nanoflagellates were mostly dominated by coccolithoporids except in February 2010 when the coastal waters contained surplus concentrations of DIN and silicate. Silicate was preferentially consumed by diatoms and coccolithophorids; when its concentration and DIN/Si ratio declined below threshold levels the composition of phytoplankton shifted to cyanobacteria (Ludwig et al., 2009).

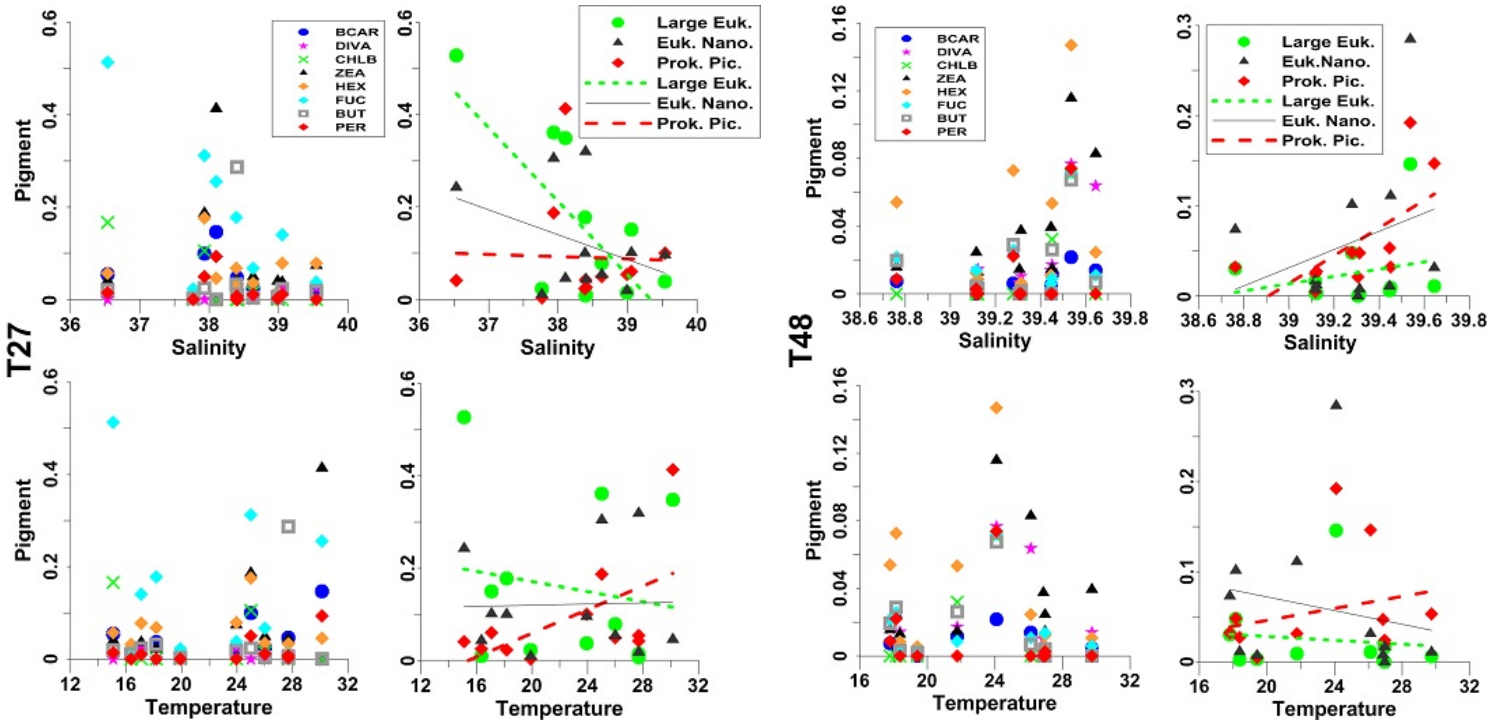

Figure 8. Phytoplankton pigment concentrations versus temperature & salinity in coastal (T27) and offshore (T48) surface waters.

Table 3. Observed correlations between physical, chemical and biological data for coastal and offshore stations.

Shelf	TEMP	SAL	DENS	PO_4	NO_2+NO_3	NH_4	Si	LARG	NANO	PROK	PER	BUT	FUC	HEX	ZEA	CHLL-*b*	DIV-*a*
Salinty	**.321***																
Density	**-.902****	-.018															
PO_4	-.048	-.154	.037														
NO_2+NO_3	**-.672****	**-.496****	**.470****	**.269***													
NH_4	-.190	.109	.232	.229	**.517****												
Si	**-.303***	**-.310***	.067	**.322****	**.687****	**.440****											
Large Eukaryotes	**-.300***	**-.462****	.081	.114	**.383****	.016	**.477****										
Eukaryotic Nanoflagellates	**-.362****	-.188	.192	-.138	**.432****	.248	**.306***	**.478****									
Prokaryotic Picoplankton	**.444****	**.423****	**-.430****	-.011	**-.315***	.164	.094	.126	**.278***								
PER	**.389****	-.097	**-.513****	**.267***	-.070	.049	**.288***	**.482****	**.251***	**.628****							
BUT	**-.488****	-.139	**.450****	-.004	**.407****	**.325****	.089	**.289***	**.760****	.033	.029						
FUC	**-.335****	**-.442****	.129	.094	**.396****	.045	**.467****	**.992****	**.468****	.099	**.442****	**.308***					
HEX	**-.384****	-.080	**.275***	-.226	**.396****	**.277***	**.273***	**.423****	**.856****	**.285***	.149	**.621****	**.443****				
ZEA	**.458****	.238	**-.520****	-.028	**-.271***	.073	.119	**.283***	**.365****	**.924****	**.744****	.068	**.260***	**.341****			
CHLL-*b*	-.117	**-.333****	-.132	.023	**.440****	.148	**.422****	**.393****	**.450****	.034	**.314***	.157	**.380****	**.363****	.211		
DIV-*a*	.048	**.719****	.179	-.104	-.227	.191	-.216	**-.508****	-.031	**.419****	-.180	.130	**-.488****	.127	.177	**-.275***	
CHLL-*a*	.031	**-.360****	-.218	-.007	.169	.060	**.305***	**.717****	**.512****	.311*	**.601****	.197	**.711****	**.413****	**.535****	**.446****	**-.405****

Offshore	TEMP	SAL	DENS	PO_4	NO_2+NO_3	NH_4	Si	LARG	NANO	PROK	PER	BUT	FUC	HEX	ZEA	CHLL-*b*	DIV-*a*
Salinty	**.423****																
Density	**-.907****	**-.328****															
PO_4	**.266***	-.018	**-.249***														
NO_2+NO_3	-.115	-.076	.111	**.312***													
NH_4	**.262***	-.166	-.201	**.457****	**.293***												
Si	-.233	.086	**.259***	**-.405****	-.227	**-.271***											
Large Eukaryotes	.039	.214	-.003	-.032	.060	-.214	.018										
Eukaryotic Nanoflagellates	-.144	**.279***	.094	-.090	.097	**-.297***	-.052	**.802****									
Prokaryotic Picoplankton	.226	**.441****	-.136	.008	.135	-.105	.028	**.563****	**.511****								
PER	.055	.182	-.007	-.019	.013	-.207	.025	**.956****	**.681****	**.510****							
BUT	-.108	**.315***	.076	-.092	.140	**-.254***	.001	**.799****	**.961****	**.561****	**.646****						
FUC	.023	.230	.001	-.045	.095	-.206	.013	**.961****	**.851****	**.565****	**.836****	**.880****					
HEX	-.177	.232	.136	-.100	.055	**-.323****	-.009	**.835****	**.978****	**.539****	**.718****	**.950****	**.880****				
ZEA	**.359****	**.444****	**-.259***	.093	.005	-.095	-.054	**.613****	**.454****	**.909****	**.608****	**.442****	**.566****	**.486****			
CHLL-*b*	-.087	**.271***	.010	-.053	.116	-.228	-.168	**.575****	**.866****	**.305***	**.506****	**.742****	**.594****	**.759****	**.312***		
DIV-*a*	.070	**.369****	-.002	-.071	.234	-.097	.098	**.427****	**.482****	**.923****	**.338****	**.584****	**.475****	**.502****	**.679****	**.251***	
CHLL-*a*	.044	-.193	-.074	.143	**.493****	.222	**.429****	.137	.220	.117	.031	.230	**.223**	.188	.112	.225	.105

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed). N = 63

As clearly seen from the surface distribution of DIN, SRP and Si, the nutrient-depleted saltier offshore waters (Figure 4, station T48) receive very limited inputs from external sources other than potential atmospheric deposition and small scale upwelling events (Krom et al., 2004; Kocak et al., 2010) and phytoplankton composition is dominated by small sized organisms (Li et al., 1993; Yacobi et al., 1995; Ignatiades, 1998; Psarra et al., 2005).

Prokaryotic picoplankton (cyanobacteria and prochlorophytes) and prymnesiophytes were dominant in offshore waters in dry summer-autumn seasons when the warmer surface mixed layer were depleted in nitrate and SRP, yielding lower N/P ratios. Eukaryotic nanoflagellates dominated by coccolithophorids became excessively important in the offshore flora in February (2009, 2010, and 2011); the cooler euphotic zone waters had variable nutrient concentration and N/P (5-27) ratios whilst DIN/Si ratio was low (0.02-0.96). Although there was no significant relation between temperature and eukaryotic nanoflagellates, direction of the relation was inverse (Figure 8). While concentration of cyanobacteria increased with increasing temperature, concentration of prymnesiophytes decreased in offshore waters. Also, cyanobacteria have photoprotectant pigment (zeaxanthin), it helps to resist in optically clear waters under the high light conditions. In addition, they are prokaryotes. They increase their enzymatic activity with increasing temperature. On the other hand, prymnesiophytes are eukaryotic and do not have photoprotectant pigments. Consequently, they replace each other according to prevailing physical conditions. Total pigment concentrations of bulk flora were dominated by prokaryotic picoplankton from August 2009 till October 2011 in the N, P depleted offshore waters. Prochlorophytes were observed as the dominant group in cold and dim light condition deep waters. Conversely, cyanobacteria dominated the warmer and highly illuminated upper layer waters of the offshore ($p<0.01$). Prochlorophytes seemed to be more adapted to oligotrophic conditions than other groups (Bastillos-Guzman et al., 1995; Dandonneau et al., 2006) in offshore waters. No significant relationship between prokaryotic picoplankton and nutrients was observed in offshore waters.

Algae biomass at the DCM formed between 50-75 m was generally dominated by prokaryotic picoplankton in the offshore. The contribution of *prochlorococcus* to composition averaged 33% in the DCM layer during the study period, reaching the maximum (64%) in November 2010. Contribution of *prochlorococcus* was higher than other prokaryotic picoplankton cyanobacteria (21%) in the DCM layer.

Surface biomass of the phytoplankton peaked in February and October 2009 and November 2010 in the offshore waters (>0.1 µg/l). Eukaryotic nanoflagellates mostly prymnesiophytes dominated composition when ammonia concentration decreased below 0.1 µM in surface waters in February 2009, November 2010 and February 2011. Collos and Harrison (2014) showed that prymnesiophytes are more sensitive to ammonium toxicity than cyanobacteria. Significant negative correlation between eukaryotic nanoflagellates and NH_4 was observed in this study (Table 3) corroborating previous findings or alternatively suggesting ammonium limitation on cyanobacteria (Muro-Pastor and Florencio, 2003).

CONCLUSIONS

Phytoplankton composition changes according to nutrient availability and physical conditions. Phytoplankton biomass in the coastal water, much exceeding the offshore concentrations, is enhanced by increasing nitrogen concentrations. Changes in chlorophyll content and composition of phytoplankton were primarily affected by the river inflow in the study area. Expectedly, diatoms were the most dominant group in the river-fed coastal waters of the bay, followed by coccolithophorids. However, in the oligotrophic offshore waters, prokaryotic picoplankton (cyanobacteria and prochlorophytes) dominated the bulk algae biomass, followed again by prymnesiophytes in oligotrophic offshore areas in northeastern Mediterranean. Prymnesiophytes was the cosmopolitan class of phytoplankton at all sampling events in the basin. Similarly, Uysal et al., (2008) reported the coccolithophorid *Emiliana huxleyi* (Prymnesiophyceae) as the dominant species in the Cilician basin. Phytoplankton composition changes according to nutrient availability and physical conditions. Phytoplankton biomass was found higher in coastal than offshore waters and enhanced by increasing nitrogen concentrations. Changes in chlorophyll content and composition of phytoplankton were primarily affected by the river inflow in the study area. From inner bay shore to the shelf break zone (offshore waters), prokaryotic picoplankton and eukaryotic nanoflagellates contribution to the total chlorophyll gradually increased from 23 to 47%

and from 28 to 43%, respectively, as the contribution of large eukaryotes decreased % 49 to 10%. Contribution of photoprotectant carotenoids (PPC) to total carotenoids ranged between 30 – 70% in the coastal and 25 – 100 % in the offshore. On the other hand, HEX (prymnesiophytes) was found to be the major contributor to photosynthetic carotenoids (PSC) in NE Mediterranean.

ACKNOWLEDGEMENTS

The authors would like to thank Sehmus Basduvar and Saim Cebe for their helps in pigment and nutrient measurements and crew of R/V Bilim-2 for their kind helps throughout the cruises. This study was supported by the Scientific and Technological Research Council of Turkey (TUBITAK) in framework of the SINHA-107G066 project and METU in framework of the BAP-07-01-2009-01 project.

REFERENCES

Barlow, R.G., Mantoura, R.F.C., Gough, M.A. & Fileman, T. W. (1993). Pigment signatures of the phytoplankton composition in the northeastern Atlantic during the 1990 spring bloom. *Deep Sea Research Part II: Topical Studies in Oceanography,* 40(1-2), 459-477. doi:10.1016/0967-0645(93)90027-k

Barlow, R. G., Mantoura, R. F. C., & Cummings, D. G. (1999). Monsoonal influence on the distribution of phytoplankton pigments in the Arabian Sea. *Deep Sea Research Part II: Topical Studies in Oceanography,* 46(3-4), 677-699. doi:10.1016/s0967-0645(98)00123-4

Barlow, R., Aiken, J., Moore, G., Holligan, P. & Lavender, S. (2004). Pigment adaptations in surface phytoplankton along the eastern boundary of the Atlantic Ocean. *Marine Ecology Progress Series,* 281, 13–26. doi:10.3354/meps281013

Barlow, R.G., Aiken, J., Holligan, P.M., Cummings, D.G., Maritorena, S. & Hooker, S. (2002). Phytoplankton pigment and absorption characteristics along meridional transects in the Atlantic Ocean. *Deep Sea Research Part I: Oceanographic Research Papers*, 49(4), 637–660. doi:10.1016/s0967-0637(01)00081-4

Bell, T. & Kalff, J. (2001). The contribution of picophytoplankton in marine and freshwater systems of different trophic status and depth. *Limnology and Oceanography*, 46(5), 1243-1248. doi:10.4319/lo.2001.46.5.1243

Bidigare, R.R., Ondrusek, M.E., Morrow, J.H. & Kiefer, D.A. (1990). In-vivo absorption properties of algal pigments. *Ocean Optics X.* Proc. SPIE Ocean Optics, X 1302, 290-302. doi:10.1117/12.21451

Booth, B.C. (1993). Estimating cell concentration and biomass of autotrophic plankton using microscopy. In: P.F. Kemp, B.F. Sherr, E.B. Sherr & J.J. Cole (eds) *Handbook of methods in aquatic microbial ecology*. Lewis Publishers, Boca Raton, FL, p 199–205. ISBN 0-87371-564-0.

Bouman, H.A., Platt, T., Sathyendranath, S., Li, W.K.W., Stuart, V., Fuentes-Yaco, C., Maass, H., Horne, E.P.W., Ulloa, O., Lutz, V. & Kyewalyanga, M. (2003). Temperature as indicator of optical properties and community structure of marine phytoplankton: implications for remote sensing. *Marine Ecology Progress Series*, 258, 19-30. doi:10.3354/meps258019

Bustillos-Guzmán, J., Claustre, H. & Marty, J. (1995). Specific phytoplankton signatures and their relationship to hydrographic conditions in the coastal northwestern Mediterranean Sea. *Marine Ecology Progress Series*, 124, 247-258. doi:10.3354/meps124247

Campbell, L. & Vaulot, D. (1993). Photosynthetic picoplankton community structure in the subtropical North Pacific Ocean near Hawaii (station ALOHA). *Deep Sea Research Part I: Oceanographic Research Papers*, 40(10), 2043-2060. doi:10.1016/0967-0637(93)90044-4

Christaki, U., Jacquet, S., Dolan, J.R., Vaulot, D. & Rassoulzadegan, F. (1999). Growth and grazing on Prochlorococcus and Synechococcus by two marine ciliates. *Limnology and Oceanography*, 44(1), 52-61. doi: 10.4319/lo.1999.44.1.0052

Christaki, U. (2001). Nanoflagellate predation on auto- and heterotrophic picoplankton in the oligotrophic Mediterranean Sea. *Journal of Plankton Research*, 23(11), 1297-1310. doi:10.1093/plankt/23.11.1297

Collos, Y. & Harrison, P.J. (2014). Acclimation and toxicity of high ammonium concentrations to unicellular algae. *Marine Pollution*

Bulletin, 80(1-2), 8-23. doi:10.1016/j.marpolbul.2014.01.006

Doğan-Sağlamtimur, N. & Tugrul, S. (2004). Effect of riverine nutrient on coastal water ecosystems: A case study from the northeastern Mediterranean shelf. *Fresenius Environmental Bulletin*, 13(11b), 1288-1294.

Dos Santos, A.C.A., Calijuri, M.C., Moraes, E.M.; Adorno, M.A.T., Falco, P.B. Carvalho,, D.P., Deberdt, G.L.B. & Benassi, S.F. (2003). Comparison of three methods for Chlorophyll determination: Spectrophotometry and Fluorimetry in samples containing pigment mixtures and spectrophotometry in samples with separate pigments through High Performance Liquid Chromatography. *ACTA Limnologica Brasiliensis*, 15(3), 7-18.

Dandonneau, Y., Montel, Y., Blanchot, J., Giraudeau, J. & Neveux, J. (2006). Temporal variability in phytoplankton pigments, picoplankton and coccolithophores along a transect through the North Atlantic and tropical southwestern Pacific. *Deep Sea Research Part I: Oceanographic Research Papers,* 53(4), 689-712. doi:10.1016/j.dsr.2006.01.002

Edıger, D., Tuğrul, S., Polat, Ç.S., Yılmaz, A. & Salihoğlu, İ. (1999). Abundance and Elemental Composition of Particulate Matter in the Upper Layer of Northeastern Mediterranean. In P. Malanotte-Rizzoli & V.N. Eremeev (Eds.), The Eastern Mediterranean as a Laboratory Basin for the Assessment of Contrasting Ecosystems (pp. 241-266). Volume 51 of the series NATO Science Series. Netherlands, Springer Netherland. ISBN 978-0-7923-5586-1, doi:10.1007/978-94-011-4796-5_17

Eker, E. & Kideyş A. E. (2000). Weekly variations in phytoplankton community structure of a harbour in Mersin Bay (north-eastern Mediterranean), *Turkish Journal of Botany*, 24, 13-24.

Eker-Develi, E., Kideyş, A.E., Tugrul, S., Yılmaz D. & Ediger D. (2003). Phytoplankton dynamics in the Northeastern Mediterranean with respect to relative dust deposition. Proceedings of "Second International Conference on Oceanography of the eastern Mediterranean and Black Sea:Similarities and differences of two interconnected basins, Oct 14-18, 2002, Ed. Yılmaz A., TÜBİTAK Publisher. Pp. 686-694.

Fogg, G.E. (1991). The phytoplanktonic ways of life. *New Phytologist*, 118(2), 191-232. doi:10.1111/j.1469-8137.1991.tb00974.x

Gaines, G. & Elbrächter, M. (1987). Heterotrophic nutrition. In F.J.R. Taylor (Eds), The biology of dinoflagellates. Botanical Monographs Volume 21, Blackwell Scientific Publications, Oxford. ISBN 0632009152Gibb, S., Barlow, R., Cummings, D., Rees, N., Trees, C., Holligan, P. & Suggett, D. (2000). Surface phytoplankton pigment distributions in the Atlantic Ocean: an assessment of basin scale variability between 50°N and 50°S. *Progress in Oceanography*, 45(3-4), 339-368. doi:10.1016/s0079-6611(00)00007-0

Goericke, R. & Repeta, D. (1993). Chlorophylls *a* and b and divinyl chlorophylls a and b in the open subtropical North Atlantic Ocean. *Marine Ecology Progress Series*, 101, 307-313. doi:10.3354/meps101307

Gu, J., Salem, A. & Chen, Z. (2013). Lagoons of the Nile delta, Egypt, heavy metal sink: With a special reference to the Yangtze estuary of China. Estuarine, *Coastal and Shelf Science*, 117, 282-292. doi:10.1016/j.ecss.2012.06.012

Hassen, M.B., Hamza, A., Drira, Z., Zouari, A., Akrout, F., Messaoudi, S., Aleya, L. & Ayadi, H. (2009). Phytoplankton-pigment signatures and their relationship to spring–summer stratification in the Gulf of Gabes. Estuarine, *Coastal and Shelf Science*, 83(3), 296-306. doi:10.1016/j.ecss.2009.04.002

Ignatiades, L. (1998). The productive and optical status of the oligotrophic waters of the Southern Aegean Sea (Cretan Sea), Eastern Mediterranean. *Journal of Plankton Research,* 20(5), 985-995. doi:10.1093/plankt/20.5.985

Jeffrey, S.W., Mantoura, R.F.C. & Wright, S.W. (1997). Phytoplankton pigments in oceanography: Guidelines to modern methods. UNESCO Publishing Paris, 37-84, ISBN 9231032755.

Kıdeyş, A.E, Ünsal M. & Bingel F. (1989). Seasonal changes in net phytoplankton off Erdemli, norteastern Mediterranean. *DOĞA, Turkish Journal of Botany*, 13(1), 45-54.

Krom, M.D., Brenner, S., Kress, N., Neori, A. & Gordon, L.I. (1992). Nutrient dynamics and new production in a warm-core eddy from the Eastern Mediterranean Sea. *Deep Sea Research Part A. Oceanographic Research Papers*, 39(3-4), 467-480. doi:10.1016/0198-0149(92)90083-6

Krom, M.D., Groom, S. & Zohary, T. (2003). The eastern Mediterranean. In K.D. Black & G.B. Shimmield (Eds.), Biogeochemistry of Marine Systems (pp. 91-126). Boca Raton, Blackwell Publishin. ISBN 1-84127-327-9

Krom, M.D., Herut, B. & Mantoura, R. F. C. (2004). Nutrient budget for the Eastern Mediterranean: Implications for phosphorus limitation. Limnology and Oceanography, 49(5), 1582–1592. doi:10.4319/lo.2004.49.5.1582

Krom, M.D., Woodward, E.M.S., Herut, B., Kress, N., Carbo, P., Mantoura, R.F.C., … Zodiatis, G. (2005). Nutrient cycling in the south east Levantine basin of the eastern Mediterranean: Results from a phosphorus starved system. *Deep Sea Research Part II: Topical Studies in Oceanography*, 52(22-23), 2879–2896. doi:10.1016/j.dsr2.2005.08.009

Krom, M.D., Emeis, K.-C. & Van Cappellen, P. (2010). Why is the Eastern Mediterranean phosphorus limited? *Progress in Oceanography*, 85(3-4), 236-244. doi:10.1016/j.pocean.2010.03.003

Koçak, M., Kubilay, N., Tuğrul, S., & Mihalopoulos, N. (2010). Atmospheric nutrient inputs to the northern levantine basin from a long-term observation: sources and comparison with riverine inputs. *Biogeosciences*, 7(12), 4037-4050. doi:10.5194/bg-7-4037-2010

Koray, T. (1995). Phytoplankton species succession diversity and nutrients in neritic waters of the Aegean Sea (Bay of Izmir). Turkish *Journal of Botany*. 19, 531-544.

Kormas, K., Garametsi, V. & Nicolaidou, A. (2002). Size-fractionated phytoplankton chlorophyll in an Eastern Mediterranean coastal system (Maliakos Gulf, Greece). *Helgoland Marine Research*, 56(2), 125-133. doi:10.1007/s10152-002-0106-2

Pazi, I. & Kucuksezgin, F. (2006). Circulation, hydrographic and nutrient characteristics of the Cilician Basin, Northeastern Mediterranean Sea. *Journal of Marine Systems*, 59(3-4), 189-200. doi:10.1016/j.jmarsys.2005.10.003

Li, W., Zohary, T., Yacobi, Y. & Wood, A. (1993). Ultraphytoplankton in the eastern Mediterranean Sea: towards deriving phytoplankton biomass from flow cytometric measurements of abundance, fluorescence and light scatter. *Marine Ecology Progress Series*, 95, 79-87. doi:10.3354/meps095079

Ludwig, W., Dumont, E., Meybeck, M. & Heussner, S. (2009). River discharges of water and nutrients to the Mediterranean and Black Sea: Major drivers for ecosystem changes during past and future decades? *Progress in Oceanography*, 80(3-4), 199-217. doi:10.1016/j.pocean.2009.02.001

Mackey, D., Blanchot, J., Higgins, H. & Neveux, J. (2002). Phytoplankton abundances and community structure in the equatorial Pacific. *Deep Sea Research Part II: Topical Studies in Oceanography*, 49(13-14), 2561-2582. doi:10.1016/s0967-0645(02)00048-6

Mantoura, R.F.C. & Llewellyn, C.A. (1983). The rapid determination of algal chlorophyll 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

Mantoura, R.F.C., Wright, S.W., Jeffrey, S.W., Barlow R.G. & Cummings, D.G. (1997). Filtration and storage of pigments from microalgae. In: S. W. Jeffrey, R. F. C. Mantoura and S. W. Wright, Phytoplankton Pigments in Oceanography: Guidelines to Modern Methods. UNESCO Monograph in Oceanographic Methods. SCOR WH 78. Paris:SCOR-UNESCO Monographs on Oceanographic Methodology.

Moore, L., Goericke, R. & Chisholm, S. (1995). Comparative physiology of Synechococcus and Prochlorococcus: influence of light and temperature on growth, pigments, fluorescence and absorptive properties. *Marine Ecology Progress Series*, 116, 259-275. doi:10.3354/meps116259

Moore, L.R. & Chisholm, S.W. (1999). Photophysiology of the marine cyanobacterium Prochlorococcus: Ecotypic differences among cultured isolates. *Limnology and Oceanography*, 44(3), 628–638. doi:10.4319/lo.1999.44.3.0628

Moutin, T., Thingstad, T.F., Van Wambeke, F., Marie, D., Slawyk, G., Raimbault, P. & Claustre, H. (2002). Does competition for nanomolar phosphate supply explain the predominance of the cyanobacteriumSynechococcus? *Limnology and Oceanography*, 47(5), 1562–1567. doi:10.4319/lo.2002.47.5.1562

Muro-Pastor, M.I. & Florencio, F. J. (2003). Regulation of ammonium assimilation in cyanobacteria. *Plant Physiology and Biochemistry*, 41(6-7), 595–603. doi:10.1016/s0981-9428(03)00066-4

Özsoy, E., Hecht, A., Ünlüata, Ü., Brenner, S., Sur, H.I., Bishop, J., … Oğuz, T. (1993). A synthesis of the Levantine Basin circulation and hydrography, 1985–1990. *Deep Sea Research Part II: Topical Studies in Oceanography*, 40(6), 1075-1119. doi:10.1016/0967-0645(93)90063-s

Partensky, F., Hoepffner, N., Li, W., Ulloa, O. & Vaulot, D. (1993). Photoacclimation of Prochlorococcus sp. (Prochlorophyta) Strains Isolated from the North Atlantic and the Mediterranean Sea. *Plant Physiology*, 101(1), 285-296. doi:10.1104/pp.101.1.285

Partensky, F., Blanchot, J., Lantoine, F., Neveux, J. & Marie, D. (1996). Vertical structure of picophytoplankton at different trophic sites of the tropical northeastern Atlantic Ocean. *Deep Sea Research Part I: Oceanographic Research Papers*, 43(8), 1191-1213. doi:10.1016/0967-0637(96)00056-8

Partensky, F., Hess, W.R. & Vaulot, D., (1999). Prochlorococcus, a Marine Photosynthetic Prokaryote of Global Significance. *American Society for Microbiology*, 63(1), 106-127.

Psarra, S., Tselepides, A. & Ignatiades, L. (2000). Primary productivity in the oligotrophic Cretan Sea (NE Mediterranean): seasonal and interannual variability. *Progress in Oceanography*, 46(2-4), 187-204. doi:10.1016/s0079-6611(00)00018-5

Psarra, S., Zohary, T., Krom, M.D., Mantoura, R. F.C., Polychronaki, T., Stambler, N., Tanaka, T., Tselepides, A. & Thingstad, F.T.. (2005). Phytoplankton response to a Lagrangian phosphate addition in the Levantine Sea (Eastern Mediterranean). *Deep Sea Research Part II: Topical Studies in Oceanography*, 52(22-23), 2944–2960. doi:10.1016/j.dsr2.2005.08.015

Polat, S. & Işık, O. (2002). Phytoplankton distribution, diversity and nutrients at the northeastern Mediterranean coast of Turkey (Karataş-Adana. *Turkish Journal of Botany*, 26, 77-86.

Polat, S., Avşar, D., Işık, O. & Çiçek, E. (2000). Interrelationships Between Phytoplankton Abundance and Physico-chemical Parameters in Yumurtalık Bight, Northeastern Mediterranean. *Ege University Journal of Fisheries and Aquatic Sciences*, 17(3-4) 129-142.

Polat, S. & Uysal, Z. (2009). Abundance and biomass of picoplanktonic Synechococcus (Cyanobacteria) in a coastal ecosystem of the northeastern Mediterranean, the Bay of İskenderun. *Marine Biology Research*, 5(4), 363-373. doi:10.1080/17451000802512275

Postius, C., Kenter, U., Wacker, A., Ernst, A. & Böger, P. (1998). Light causes selection among two phycoerythrin-rich Synechococcus isolates from Lake Constance. *FEMS Microbiology Ecology*, 25(2), 171-178. doi:10.1111/j.1574-6941.1998.tb00470.x

Sathyendranath, S., Stuart, V., Platt, T., Bouman, H., Ulloa, O. & Maass, H., 2005. Remote sensing of ocean colour: towards algorithms for retrieval of pigment composition. *Indian Journal of Marine Science*, 34, 333–340.

Strickland, J.D.H. and Parsons,T.R., 1972. A Practical Handbook of Seawater Analysis. Fisheries Research Board of Canada Bulletin 167, Ottawa.

Tanaka, T., Zohary, T., Krom, M.D., Law, C.S., Pitta, P., Psarra, S., Rassoulzadegan, F., Thingstad, F.T., Tselepides, A., Woodward, E.M.S., Flaten, G.A.F., Skjoldal, E.F. & Zodiatis, G.G. (2007). Microbial community structure and function in the Levantine Basin of the eastern Mediterranean. *Deep Sea Research Part I: Oceanographic Research Papers*, 54(10), 1721-1743. doi:10.1016/j.dsr.2007.06.008

Tandeau de Marsac, N. & Houmard, J. (1993). Adaptation of cyanobacteria to environmental stimuli: new steps towards molecular mechanisms. *FEMS Microbiology Letters*, 104(1-2), 119-189. doi:10.1111/j.1574-6968.1993.tb05866.x

Thingstad, T.F., Krom, M.D., Mantoura, R.F.C., Flaten, G. A. F., Groom, S., Herut, B., Kress, N., Law, C. S., Pasternak, A., Pitta, P., Psarra, S., Rassoulzadegan, F., Tanaka, T., Tselepides, A., Wassmann, P., Woodward, E.M.S., Wexels Riser, C., Zodiatis, G., & Zohary, T. (2005). Nature of phosphorus limitation in the ultraoligotrophic eastern Mediterranean. *Science*, 309, 1068-1071, doi:10.1126/science.1112632.

Tomas, C.R., Hasle, G.R. & Syvertsen, E.E., Steidinger, K.A., Tangen, K., Throndsen, J., & Heimdal, B.R. (1997). Identifying Marine Phytoplankton. Academic Press, San Diego. 858 pages, ISBN-10:012693018X.

Tugrul, S., Yemenicioğlu, S., Ediger, D., Uysal, Z., Mutlu, E., Doğan-Sağlamtimur, N., Yılmaz, D. & Devrimci, H.A. (2005). Ege ve Kuzeydoğu Akdeniz Kıyı Alanlarında Uzun Süreli Biyolojik İzleme, Değişim ve Uyum İzleme, Mersin Körfezi Ötrofikasyon İzleme Programı, MEDPOL FAZ III Final Report, ODTÜ-IMS, Erdemli-Mersin, unpublished..

Tüfekçi, V., Kuzyaka, E., Tüfekçi, H. & Avaz, G. (2013). Determination of limited nutrients in the Turkish coastal waters of the Mediterranean and Aegean Seas. Journal of Black Sea/Mediterranean Environment, 19(3), 299-311.

Uysal, Z. & Köksalan, İ. (2006). The annual cycle of Synechococcus (cyanobacteria) in the northern Levantine Basin shelf waters (Eastern Mediterranean). *Marine Ecology*, 27(3), 187-197. doi:10.1111/j.1439-0485.2006.00105.x

Uysal, Z., Senichkina, L., Kuzmenko, L., Georgieva, L. & Altukhov, D. (2003). Weekly changes in phytoplankton species composition, diversity, abundance, and biomass across the northern Levantine basin shelf waters. In Proceedings of the Second International Conference on "Oceanography of the Eastern Mediterranean and Black Sea: Similarities and Differences of Two Interconnected Basins". Yılmaz, A (ed.) 14–18 October 2002. METU Cultural and Convention Center, Tubitak Publishers, Ankara, Turkey. 680-686.

Uysal, Z., Latif, M.A., Özsoy, E., Tugrul, S., Kubilay, N., Beşiktepe, Ş.T., Yemenicioğlu, S., Mutlu, E., Ediger, D., Beşiktepe, Ş., Ediger, V., Ak Örek, Y., Örek, H., Demirel, M., Tunç, Ş.Ç. & Terbıyık, T. (2008). The Circulation, Removal, and Eutrophication of the Coastal Ecosystem in the Cilician Basin" YDABAG104Y277 coded TUBITAK project report, unpublished.

Weisse, T. (1993). Dynamics of autotrophic picoplankton in marine and freshwater ecosystems, In:Jones, J.G.(Eds.), Advances in microbial ecology. Springer US, New York, 13, pp.327-370. doi:10.1007/978-1-4615-2858-6_8, ISBN 978-1-4613-6238-8

Yacobi, Y.Z., Gitelson, A. & Mayo, M. (1995). Remote sensing of chlorophyll in Lake Kinneret using highspectral-resolution radiometer and Landsat TM: spectral features of reflectance and algorithm development. *Journal of Plankton Research*, 17(11), 2155-2173. doi:10.1093/plankt/17.11.2155

Yemenicioğlu, S., Tugrul, S. & Ediger, D. (2004). Ege ve Kuzeydoğu Akdeniz Kıyı Alanlarında Uzun Süreli Biyolojik İzleme, Değişim İzleme ve Uyum İzleme Programı, MEDPOL FAZ III Final Report, ODTÜ-IMS, Erdemli-Mersin, unpublished.

Yılmaz, A. & Tuğrul, S. (1998). The effect of cold- and warm-core eddies on the distribution and stoichiometry of dissolved nutrients in the northeastern Mediterranean. *Journal of Marine Systems,* 16(3-4), 253-268. doi:10.1016/s0924-7963(97)00022-5

Yılmaz, D., Ediger, D., Eker-Develi, E. & Kideys, A.E. (2003). Taxonomic composition and pigments of phytoplankton in the northern Levantine Basin. In: Yilmaz A. (Eds.), Oceanography of the Eastern Mediterranean and Black Sea. TUBITAK Publisher, Ankara, Turkey: 715-719.

Yılmaz, D. (2006). Temporal variation of phytoplankton in the northeastern shelf of Mediterranean Sea: Composition, pigment signature and production. Ph.D. Thesis, Institute of Marine Science, Middle East Technical University, Mersin, Turkey, 165 pp, unpublished.

Yılmaz D., Ediger D. & Tugrul, S., 2007. Phytoplankton assemblages in the northeastern shelf of Mediterranean Sea determined by CHEMTAX analysis of pigment data. 38. CIESM Congress, Istanbul,Turkey (online abstract copy-http://www.ciesm.org/online/archives/abstracts/).

Yucel, N. (2008). Phytoplankton pigment distribution in the Cilician basin (northeastern Mediterranean). MSc Thesis, Institute of Marine Science, Middle East Technical University, Mersin, Turkey, 175 pp, unpublished.

Zohary, T., Herut, B., Krom, M.D., Mantoura, R.F.C., Pitta, P., Psarra, S., Rassoulzadegan, F., Stambler, N., Tanaka, T., Thingstad, F.T., & Woodward, E.M.S.Malcolm S. Woodward, E. (2005). P-limited bacteria but N and P co-limited phytoplankton in the Eastern Mediterranean—a microcosm experiment. *Deep Sea Research Part II: Topical Studies in Oceanography*, 52(22-23), 3011-3023. doi:10.1016/j.dsr2.2005.08.011