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ORIGINAL RESEARCH ARTICLE

Spatio-temporal pattern of phytoplankton and pigment composition in surface waters of south-eastern Black Sea

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KEYWORDS
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Summary Phytoplankton community, diatom to dinoflagellate ratio and pigment composition in surface waters with nutrient data from April 2013 to March 2014 were monitored in the south-eastern (SE) Black Sea using high performance liquid chromatography (HPLC) and microscopic analyses. Microscopic examination revealed a total of 71 species that consist of dinoflagellate (58%), diatoms (25%) and other groups (17%). Microscopy and HPLC-based pigment analyses revealed almost similar results which suggest that the phytoplankton community is mainly composed of diatoms, dinoflagellates and coccolithophores. Fucoxanthin (mean 0.35 $\pm$ 019 $\mu g\ L^{-1}$), peridinin (mean 0.18 $\pm$ 0.14 $\mu g\ L^{-1}$) and 19-hexanoyloxyfucoxanthin (mean 0.24 $\pm$ 0.15 $\mu g\ L^{-1}$) are prominent pigments which showed significant correlation with Diatom-C (r^2 = 0.63—0.71, $p < 0.05$), Dinoflagellate-C (r^2 = 0.49—0.80, $p < 0.05$) and Coccolithophore-C (r^2 = 0.72—0.82, $p < 0.05$), respectively. Mean carbon biomass of diatoms (36.50 $\pm$ 9.72 $\mu g\ L^{-1}$) was higher than that of dinoflagellates (33.32 $\pm$ 9.05 $\mu g\ L^{-1}$). Significant differences were also observed in nutrient ratio (N:P and Si:N) (One-way ANOVA, $p < 0.05$). Results illustrate that HPLC-based pigment approach can be used for taxonomic characterisation of phytoplankton groups in the SE Black Sea. Moreover, relatively high dinoflagellate species dominancy and significant correlations between Phyto-C and marker pigments indicate that phytoplankton community composition is shifting towards much smaller groups in SE coasts of the Black Sea.

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1. Introduction

Phytoplankton contribute at least one quarter of the biomass of the world's vegetation and constitute the base of food web in the aquatic ecosystems (Jeffrey and Vesk, 1997). They have crucial role in modulating the total CO_2 concentration, pH of the ocean and global carbon cycle (Brewin et al., 2010; Takahashi et al., 2002). Phytoplankton also impact the pelagic ecosystem throughout changing trophic transfer, food web and nutrient dynamics (McQuatters-Gollop et al., 2007; Nagata et al., 1996; Pedersen et al., 1999). Identifying changes in phytoplankton community composition and diversity is essential to improve our understanding of the responses to climate forcing (Rykaczewski and Dunne, 2011). Among the phytoplankton groups, diatoms and dinoflagellates have important roles in the ecosystem. Diatoms constitute the food web between the copepod and fish whereas dinoflagellates are generally considered trophic dead ends (McQuatters-Gollop et al., 2007; Verity and Smetacek, 1996). Additionally, shifts in phytoplankton community composition affect the abundance and diversity of marine organisms, eutrophication and food web structure in the ecosystem (Leterme et al., 2006; Micheli, 1999; Oguz, 2005; Richardson and Schoeman, 2004). Moreover, phytoplankton quickly responds to environmental disturbance, and can be used as indicator for trophic levels, eutrophication and different environmental circumstances (Brettum and Andersen, 2005; Margalef, 1978).

Studies on phytoplankton are classically conducted by using microscopy (Booth, 1993; Eker-Develi et al., 2008; Hasle, 1978; Utermöhl, 1958), which needs taxonomic expertise and time for counting samples. In addition, it is not possible to identify some small sized groups (e.g. picoplankton) with microscopy (Jeffrey and Vesk, 1997). Alternatively, some photosynthetic pigments (e.g. fucoxanthin, peridinin, 19-hexanoyoxifucoxanthin, Chl *b*, and zeaxanthin, etc.) for specific phytoplankton groups derived from high-performance liquid chromatography (HPLC) provide information about the composition of the phytoplankton community (Mantoura and Llewellyn, 1983; Wright and Jeffrey, 2006). In this method, a large number of samples can be processed, and it allows fast and easy separation of marker pigments and examination of the structure of phytoplankton assemblages based on pigments (Schlüter et al., 2000). Chl *a* is generally used as a convenient proxy for phytoplankton biomass. Additionally, other accessory pigments (e.g. carotenoids) can be evaluated for the chemotaxonomic association of phytoplankton assemblages (Gibb et al., 2000). Photosynthetic pigments are also considered as indicators for the physiological condition of a phytoplankton community, which indicates environmental condition and trophic status for a given area (Roy et al., 2006). The majority of phytoplankton carotenoids are photosynthetic. While photosynthetic carotenoids (PSC) absorb light energy and transfer it to chlorophyll, photoprotecting carotenoids (PPC) protect the organism against stressful high light conditions. PPC are capable of quenching excited radicals, and converting their excess energy to heat and dissipating it harmlessly. Moreover, the ratio of PSC to PPC may be used to provide some additional characterisation of transitions in phytoplankton community composition across biogeochemical province boundaries and hence the identification of these boundaries (Gibb et al., 2000). Furthermore, PPC and PSC absorb light in different remote sensing spectral bands (e.g. SeaWiFS). Thus, variability in their relative abundances has the potential to affect the performance of algorithms used to retrieve Chl *a* values from remotely sensed ocean colour data (Gibb et al., 2000).

The Black Sea is one of the largest anoxic marine ecosystems in the world (Tolmazin, 1985). It is a semi-enclosed and isolated environment, which has suffered from severe ecological deteriorations over the last three decades (Oguz, 2005). A considerable amount of chemicals, organic matter and nutrients via surrounding rivers (especially in the western Black Sea form the River Danube) affect the Black Sea ecosystem (Eker-Develi and Kideys, 2003; Yilmaz et al., 2006). Surface salinity never exceeds 17 psu, and excess precipitation together with run-off from the rivers Danube, Dniester, and Don creates a surface with low salinity layer overlying a halocline at about 100 m (Longhurst, 2007). Sea surface temperature (SST) exhibits typical seasonal characteristic with the highest in August and the lowest in February (Agirbas et al., 2015).

Studies on the phytoplankton in the Black Sea are mainly conducted along the north-western parts of the Black Sea, and substantially constituted by microscopic examinations (e.g. Bodeanu, 1989, 1993; Bologa, 1986; Cociasu et al., 1997; Ivanov, 1965; Moncheva and Krastev, 1997; Moncheva et al., 2001; Zaitsev and Alexandrov, 1997). Despite significant roles of phytoplankton communities in the Black Sea, information about their pigment composition, distribution and comparative studies using HPLC pigment analysis are limited (Agirbas et al., 2015; Ediger et al., 2006; Eker-Develi et al., 2012). Therefore, in this paper, particular attention has been paid to reveal the temporal pattern of phytoplankton community composition, diatom to dinoflagellate ratios, and pigment composition derived from HPLC analysis along SE coasts of the Black Sea. The objectives of this study are (i) to reveal whether or not diatom to dinoflagellate ratios and pigment composition have been similar in all stations throughout the year, (ii) to explore changes in the phytoplankton community composition, (iii) to investigate whether or not similar changes occur in pigment composition and (iv) to test applicability of HPLC technique in monitoring studies along the SE Black Sea.

2. Material and methods

Samplings were carried out monthly from April 2013 to March 2014 at four stations located in the SE coast of the Black Sea (Fig. 1). The sampling stations were determined by considering bottom topography and fisheries activities. For this purpose, we chose four coastal stations (Iyidere station, Derepazari station, Rize station and Gundogdu station), located one nautical mile from the shore with $<$50 m water depth. The study area is affected by intensive fisheries and anthropogenic activities. Seawater samples for pigment characterisation, phytoplankton and nutrient analyses were taken from surface (0.5 m) by using 5-L Niskin bottle.

2.1. Nutrient analysis

A 250 mL seawater samples for dissolved inorganic nutrients (NO_3-N, NO_2-N, PO_4-P and SiO_2-Si) were filtered through 0.45 μm cellulose acetate filters. The filtrate was collected

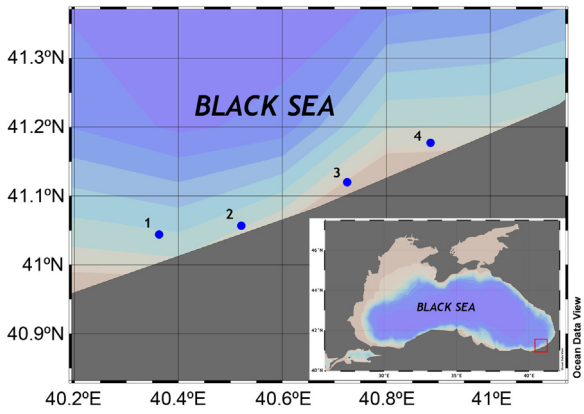

Figure 1 Geographic location of the sampling stations and bathymetry of the study area [1: Iyidere station; 2: Derepazari station; 3: Rize station; 4: Gundogdu station].

in 100 mL acid-washed high-density polyethylene bottles and then was kept frozen (−20°C) until the analysis. The analyses were conducted by a SEAL auto-analyser in Central Fisheries Research Institute (CFRI) in Trabzon.

2.2. Phytoplankton

Seawater samples (1 L) for microscopic enumeration were fixed with neutral Lugol's iodine solution and concentrated to 10 mL by sedimentation method (Utermöhl, 1958). The excess seawater after settling was gently removed by siphoning. The phytoplankton subsamples were counted using a Sedgewick-Rafter cell under an epiflourescence microscope with 10 × 20 magnifications (Leica DM4000 B). The phytoplankton groups (diatoms, dinoflagellates, coccolithophores) were identified according to Balech (1988), Fukuyo et al. (1990), Tomas (1996) and Rampi and Bernard (1978).

The phytoplankton biomass as carbon (Phyto-C) for diatoms (Diatom-C), dinoflagellates (Dinoflagellate-C) and others (haptophytes, flagellates, etc., other groups-C) was calculated by using following equations (Menden-Deuer and Lessard, 2000):

$$Diatom\text{-}C = 0.288 \times V^{0.811},$$

$$Dinoflagellate\text{-}C = 0.760 \times V^{0.819},$$

$$Other\ group\text{-}C = 0.216 \times V^{0.939},$$

where *Phyto-C* is the mass of carbon [pgC cell^{-1}], then converted to μgC cell^{-1} and *V* is the volume [μm^{-3}]. The volume of each cell was calculated by measuring appropriate morphometric characteristics (Menden-Deuer and Lessard, 2000).

2.3. Pigment analysis

Seawater samples (1 L) for pigment analysis were filtered through GF/F filters (nominal pore size 0.7 μm and 47 mm diameter), and stored in liquid nitrogen (−196°C) until the analysis. Pigments analysis was conducted according to Barlow et al. (1997, 2004) and Llewellyn et al. (2005). In the laboratory, the frozen filters were extracted in 5 mL 90% HPLC grade acetone, ultrasonicated (Bandelin Sonopuls HD 2070) for 60 s and centrifuged for 10 min at 3500 rpm to remove cellular debris. Pigment separations were achieved using a C8 column (ThermoHypersil MOS-2, 150 mm × 4.6 mm, 3 μm particle size, Å pore size and ‰ 6.5 carbon load) connected to a Shimadzu LC-20 AT/Prominence HPLC system equipped with solvent pump (flow rate 1 mL min^{-2}), auto sampler, a UV absorbance, fluorescence and a diode array detector (DAD) at two different wavelengths (450 and 665 nm) and LC solution software. Eluant A consisted of 100% methanol: 1 M ammonium acetate (80:20, v/v) and eluant B was composed of 100% methanol. Pigments were identified using retention time and spectral match using PDA (Jeffrey and Vesk, 1997), and pigment concentrations were calculated using response factors generated from calibration using a suite of pigment standards (DHI Water and Environment, Denmark).

2.4. Statistical analysis

One way analysis of variance (ANOVA) was used on normally distributed data to test for significant differences in phytoplankton, pigment compositions and nutrient concentrations for each station. The ANOVA critical significance value *p* was given in the text to indicate the level of difference. Pearson rank correlation was used on normally distributed data to test for significant differences between nutrients concentration and phytoplankton abundance. Linear regression was also fitted to assess trends among the variables.

3. Results

3.1. Nutrients

The study area exhibited typical hydrographic characteristic of the SE Black Sea (Agirbas et al., 2015). The highest Sea Surface Temperature (SST) was recorded in August (25.4°C) and the lowest one was obtained in February (10.3°C) during the study period (data not shown). Surface salinity was around 17‰ along the study area.

Nutrient concentration along the stations fluctuated depending on phytoplankton activity and seasonal vertical mixing process during the study period (Figs. 2 and 3). Especially extensive vertical mixing during early spring and late autumn resulted in high nutrient concentrations at surface layers. The highest nitrite concentration was 1.08 μM in September at Rize station, whereas the lowest one (0.01 μM) was measured in late spring and in early summer at all stations (Fig. 2A). No significant differences were found in nitrite concentrations among the stations (One-way ANOVA, $p > 0.05$).

Nitrate concentrations were higher than nitrite concentrations. The highest nitrate concentration was measured as 9.81 μM in October at Rize station (Fig. 2B). In general, the highest concentrations were recorded in October along the stations, except for Gundogdu station (in August). No significant differences were observed among the stations in terms of nitrate concentrations (One-way ANOVA, $p > 0.05$).

Phosphate concentrations were always low along the stations. The highest phosphate concentrations were measured

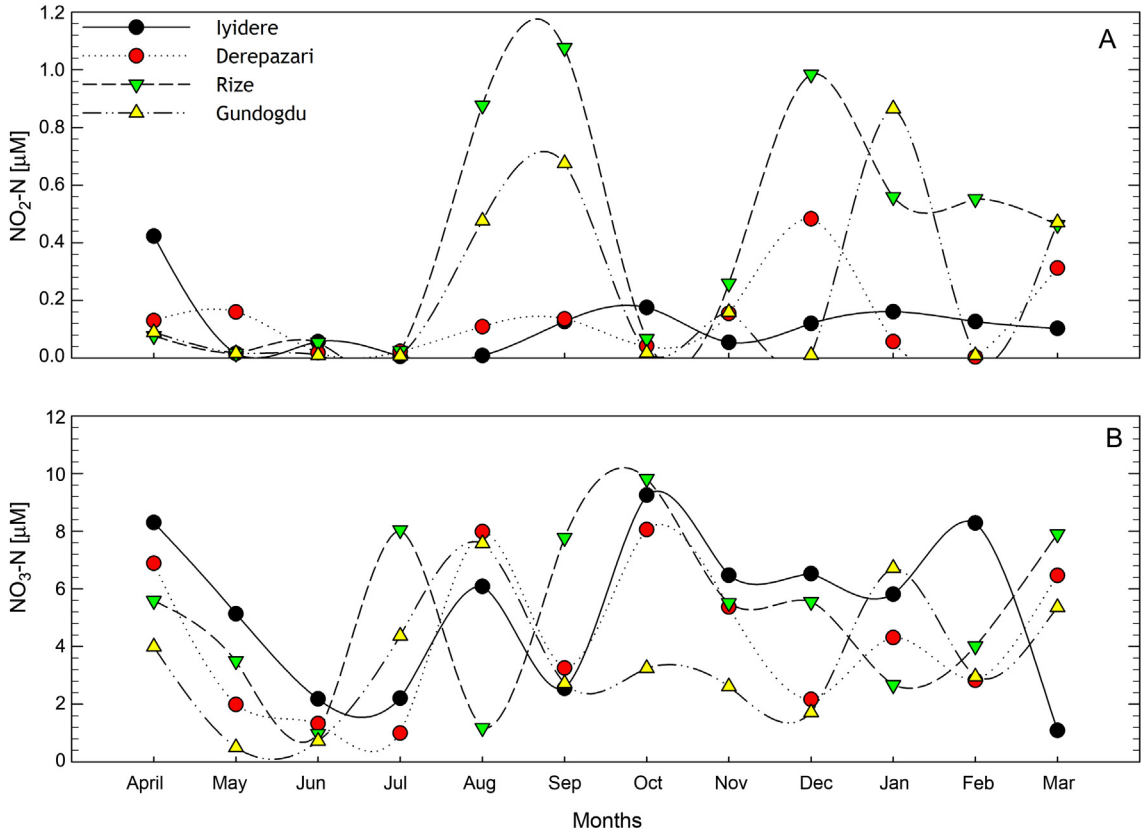

Figure 2 Monthly variation of NO_2-N [A] and NO_3-N [B] concentration along the stations.

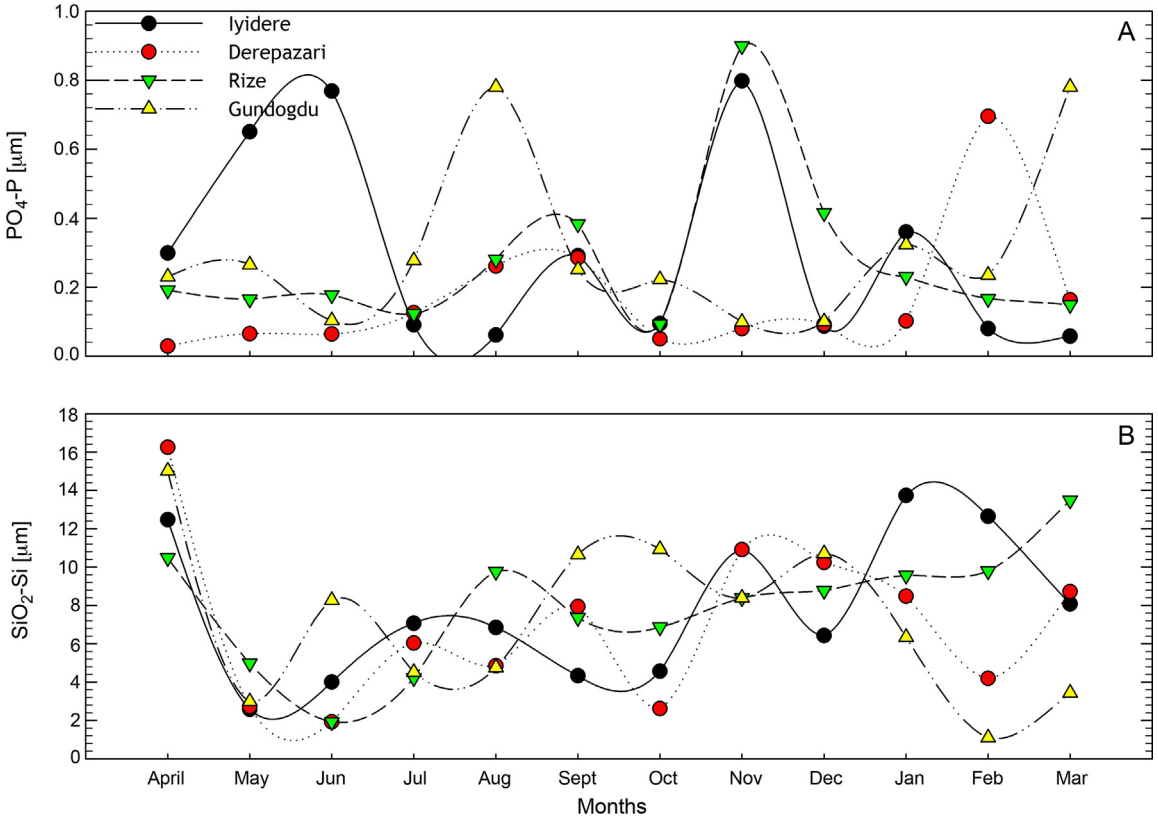

Figure 3 Monthly variation of PO_4-P [A] and SiO_2-Si [B] concentration along the stations.

as 0.90 μM and 0.80 μM in November at Rize station and Iyidere station, respectively (Fig. 3A). In general, phosphate concentrations were patchy throughout the study period, but not statistically different (One-way ANOVA, $p > 0.05$).

Silicate was always represented in higher concentrations and revealed nearly the same pattern in all stations (Fig. 3B). The highest silicate concentration was recorded in April at Derepazari station (16.25 μM), whereas the lowest one (1.92 μM at Rize and Gundogdu stations) was measured during summer.

The ratio of nutrients (N:P, Si:N, etc.) showed statistically significant different (One-way ANOVA, $p < 0.05$) patterns along the stations (data not shown). The ratio of N to P fluctuated over the time, and the highest ratios were generally recorded during mixing periods. The ratio of Si to N along the stations increased during mixing periods (i.e. early spring and late autumn). Moreover, key nutrients were also correlated with phytoplankton abundance. Overall, diatoms were negatively correlated with nitrate, silicate and phosphate, although dinoflagellates were significantly correlated with nitrite (r ranged from −0.55 to −0.64; Pearson rank correlation; $p < 0.05$). Moreover, statistically negative significant correlation between Phyto-C and nutrients was also observed in the present study (r ranged from −0.57 to −0.82; Pearson rank correlation, $p < 0.05$) except for phosphate which was positively correlated with other group Phyto-C (r ranged from 0.59 to 0.66; Pearson rank correlation, $p < 0.05$).

3.2. Phytoplankton

Microscopic enumeration revealed a total of 71 phytoplankton species belonging to dinoflagellates (58%), diatoms (25%) and other groups (17%). Phytoplankton abundance fluctuated over the study period. Despite having high qualitative contribution of dinoflagellates, they had a low abundance (in terms of quantitative contribution), ranged from 17% to 20%, among the taxonomic classes. Other groups, mainly coccolithophorid *Emiliania huxleyi*, dominated contributing to 50% (Iyidere station) and 42.7% (Gundogdu station) of the total phytoplankton abundance. Diatoms were the second important group in terms of abundance with a highest contribution of 18% (Gundogdu station) to 32% (Iyidere station).

Throughout the study period; diatoms, *Coscinodiscus granii*, *Proboscia alata*, *Pseudosolenia calcaravis*, *Thallassionema nitzschioides* constituted the highest contribution in May, July and August; whereas coccolithophores (*E. huxleyi*) were dominant in February, March and December. Dinoflagellates were mainly composed of *Ceratium fusus*, *Ceratium tripos*, *Ceratium furca*, *Dinophysis acuta*, *Dinophysis fortii*, *Dinophysis hastata*, *Gonyaulax spinifera*, *Prorocentrum compressum*, *Prorocentrum micans*, *Prorocentrum minimum*, and exhibited almost uniform distribution over the study period.

Stations revealed different pattern in terms of phytoplankton abundance and Iyidere station was represented with the highest abundance ($>1.8 \times 10^5$ cells L^{-1}) (Fig. 4). Diatoms were the only group significantly different among the stations (One-way ANOVA, $p < 0.05$). No significant differences were found in dinoflagellates and other taxonomic group abundances (One-way ANOVA, $p > 0.05$). Diatom bloom commenced in May along the stations, except for Gundogdu station. In June and July, diatom bloom gradually decreased and dinoflagellate reached the highest abundance in July and continued until late summer (Fig. 4), except for Rize station. Interestingly, dinoflagellate bloom in Rize station started in September. The second diatom bloom occurred in October; however, the magnitude of bloom was less than spring diatom bloom. More importantly, other taxonomic groups (haptophytes, flagellates, etc.) were the most abundant groups in summer and early autumn. These were also periods when the water column was stratified and key nutrients were depleted by mainly diatoms (opportunistic), and yielded dinoflagellates and other taxonomic groups (flagellates, etc.).

The ratio of diatoms to dinoflagellates (based on abundance) for the stations varied during the study period (Fig. 5). The highest ratio (0.09–6.74) was observed in late spring (i.e. May), with the highest abundance contribution of diatoms, and in late autumn (i.e. October and November). The lowest ratios were obtained in late summer (i.e. August) and early autumn (September), which coincided with Phyto-C fluctuations. In terms of stations, Iyidere and Derepazari stations were represented by the highest diatom to dinoflagellate ratio in late spring (i.e. May), whereas Gundogdu station was characterised by the lowest ratio throughout the study period.

3.3. Pigment

Chl *a* and accessory pigments (e.g. fucoxanthin, diadinoxanthin, peridinin, 19′-hexanoyloxyfucoxanthin, zeaxanthin, Chl *b*, etc.) concentration fluctuated over the study period (Figs. 6–9). Overall, Chl *a* was the dominant pigment ranged from 0.34 μg L^{-1} (September, Iyidere station) to 2.71 μg L^{-1} (May, Rize station), and fucoxanthin, peridinin and 19-Hex., were major accessory pigments along the stations. Fucoxanthin ranged from 0.07 μg L^{-1} (September, Iyidere station) to 0.90 μg L^{-1} (June, Rize station), and peridinin ranged from 0.04 μg L^{-1} (September, Rize station) to 0.77 μg L^{-1} (July, Rize station; Figs. 6 and 7). Concentrations of other accessory pigments were generally low (<0.5 μg L^{-1}) except for occasional high concentrations (Figs. 8 and 9). In general, low concentrations were recorded during spring (e.g. March and April). Especially, some photoprotecting carotenoids (e.g. β-Carotene), as an adaptive strategy against stressful high light conditions, were recorded in high concentrations during warm and stratified periods of the year.

3.4. Phyto-C

The Phyto-C (phytoplankton carbon biomass) for the stations exhibited a high degree of variability (31.79–193.62 μg L^{-1}) (Fig. 10). Overall, contribution of Diatom-C to Phyto-C was prominently high along the stations. Contribution of dinoflagellates to Phyto-C was also relatively high (Fig. 10). In general, the highest contribution of Phyto-C for diatoms were recorded in late spring (May), in early summer (June) and in late autumn (i.e. October and November), whereas the lowest ones were generally observed in early spring (April, March) and in early autumn (September). As to the dinoflagellates, the highest contribution to Phyto-C was observed

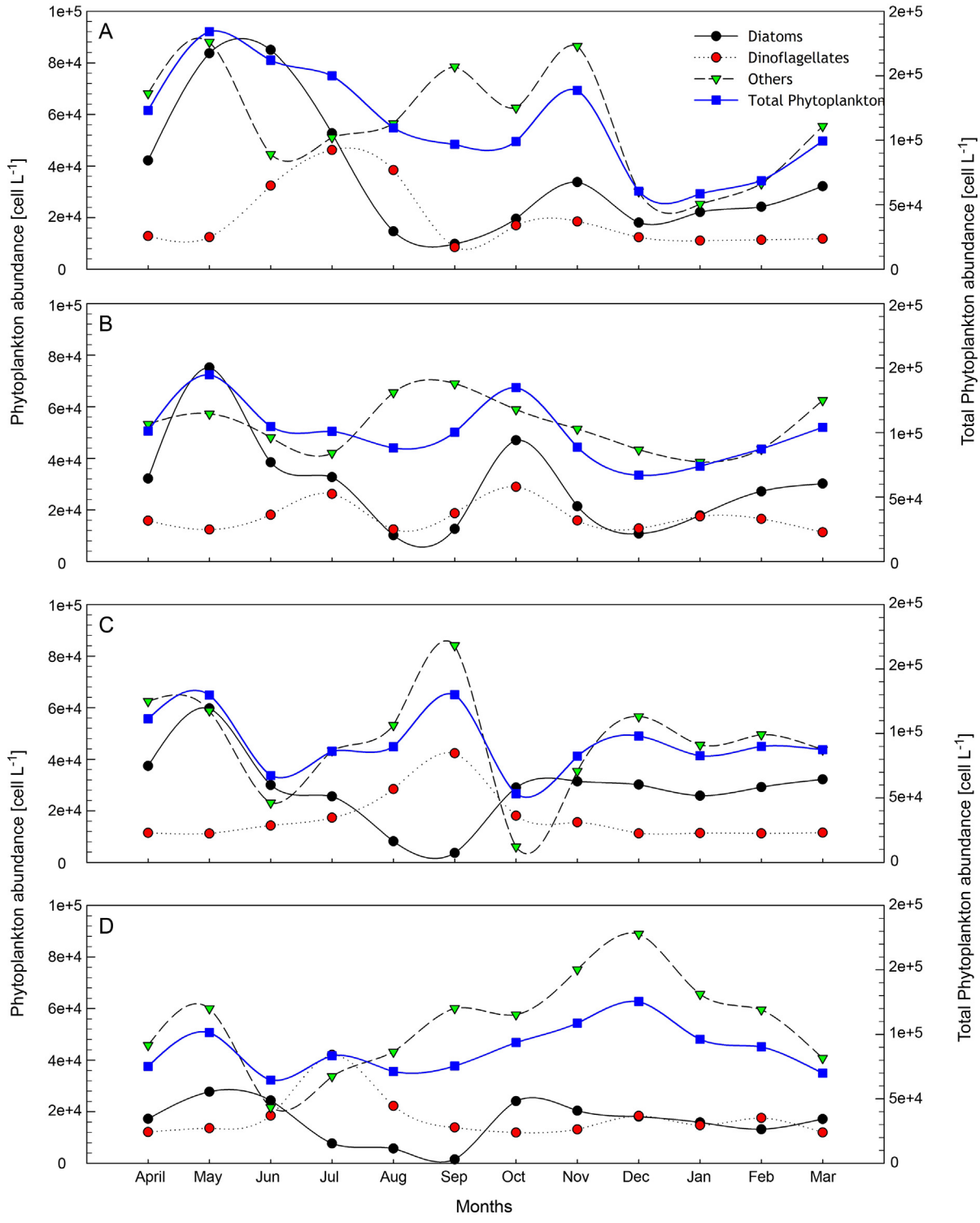

Figure 4 Monthly variation of phytoplankton abundance along the stations [A: Iyidere station; B: Derepazari station; C: Rize station; D: Gundogdu station].

in summer (July), and the lowest ones were recorded in September and February. The contribution of other phyto groups to Phyto-C was generally low during the study period. The highest contributions of other groups were recorded in autumn (November and October) and winter (January and February), whereas the lowest one was obtained in spring (March and April). In terms of mean Phyto-C, Iyidere (116.3 $\mu g\ L^{-1}$) and Rize (96.42 $\mu g\ L^{-1}$) stations were higher than Derepazari (87.8 $\mu g\ L^{-1}$) and Gundogdu (65.86 $\mu g\ L^{-1}$) stations, but difference was not statistically significant (One-way ANOVA, $p > 0.05$). Total Phyto-C ranged from 37 $\mu g\ L^{-1}$ to 194 $\mu g\ L^{-1}$ for Iyidere, from 48 $\mu g\ L^{-1}$ to 152 $\mu g\ L^{-1}$ for

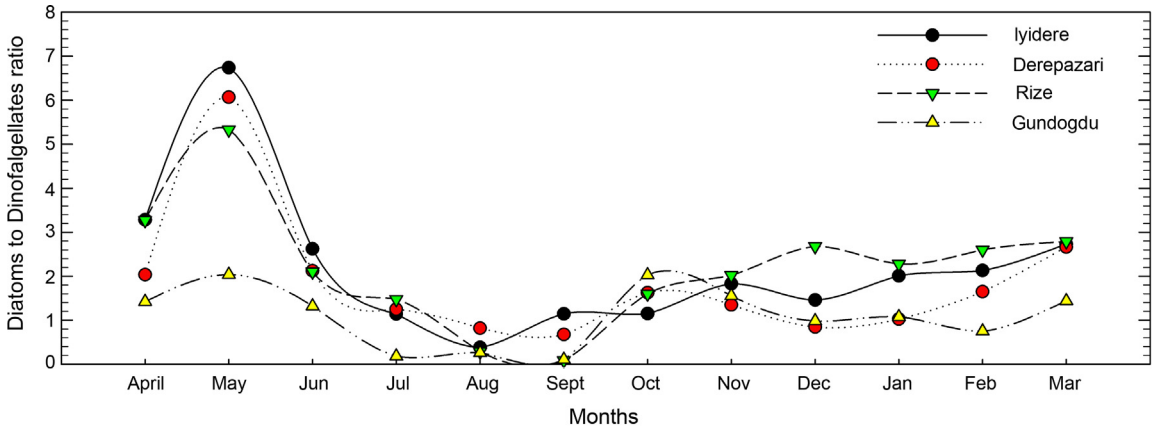

Figure 5 Monthly variation of diatom to dinoflagellate ratio (based on abundance) along the stations.

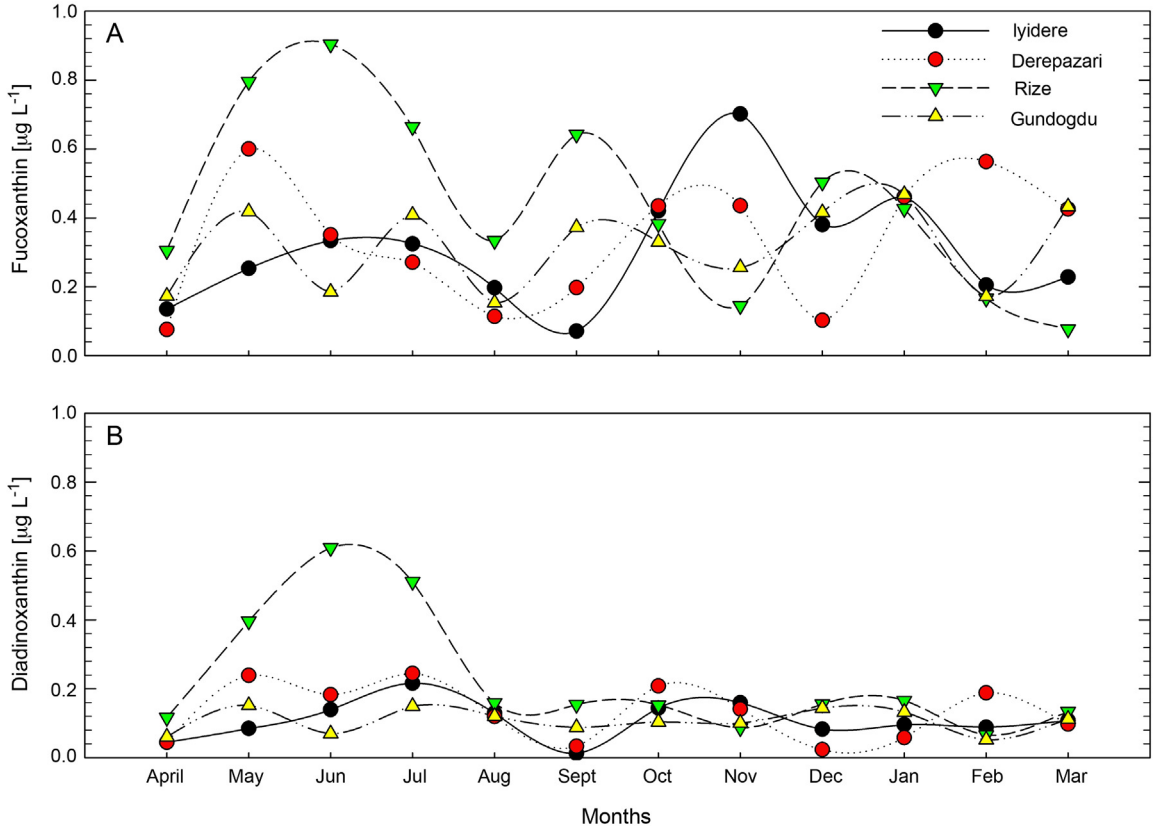

Figure 6 Monthly variation of fucoxanthin and diadinoxanthin along the stations.

Derepazari, from 55 μg L^{-1} to 154 μg L^{-1} for Rize and from 32 μg L^{-1} to 101 μg L^{-1} for Gundogdu station with statistically significant difference (One-way ANOVA, $p < 0.05$; Fig. 10). In general, Iyidere station was characterised with high Phyto-C contribution and followed by Rize station.

3.5. Phyto-C and pigment relationship

A good correlation was found between Phyto-C and group specific accessory pigments (Figs. 11–13). Diatom-C strongly correlated with fucoxanthin along the stations, the correlation for Iyidere ($r^2 = 0.70$, $p < 0.001$) and Gundogdu ($r^2 = 0.71$, $p < 0.001$) stations was higher than others (Fig. 11). There was also significant correlation between Dinoflagellate-C and peridinin, particularly in Iyidere ($r^2 = 0.75$, $p < 0.001$) and Rize ($r^2 = 0.80$, $p < 0.001$) stations (Fig. 12). The correlation between others Phyto-C (mainly *E. huxleyi*) and 19′-Hex was also prominent along the stations (Fig. 13). This is likely due to *E. huxleyi*, because this species accounted for majority of other phytoplankton groups along the stations. Moreover, total phytoplankton carbon significantly correlated with total Chl *a* for the stations except for

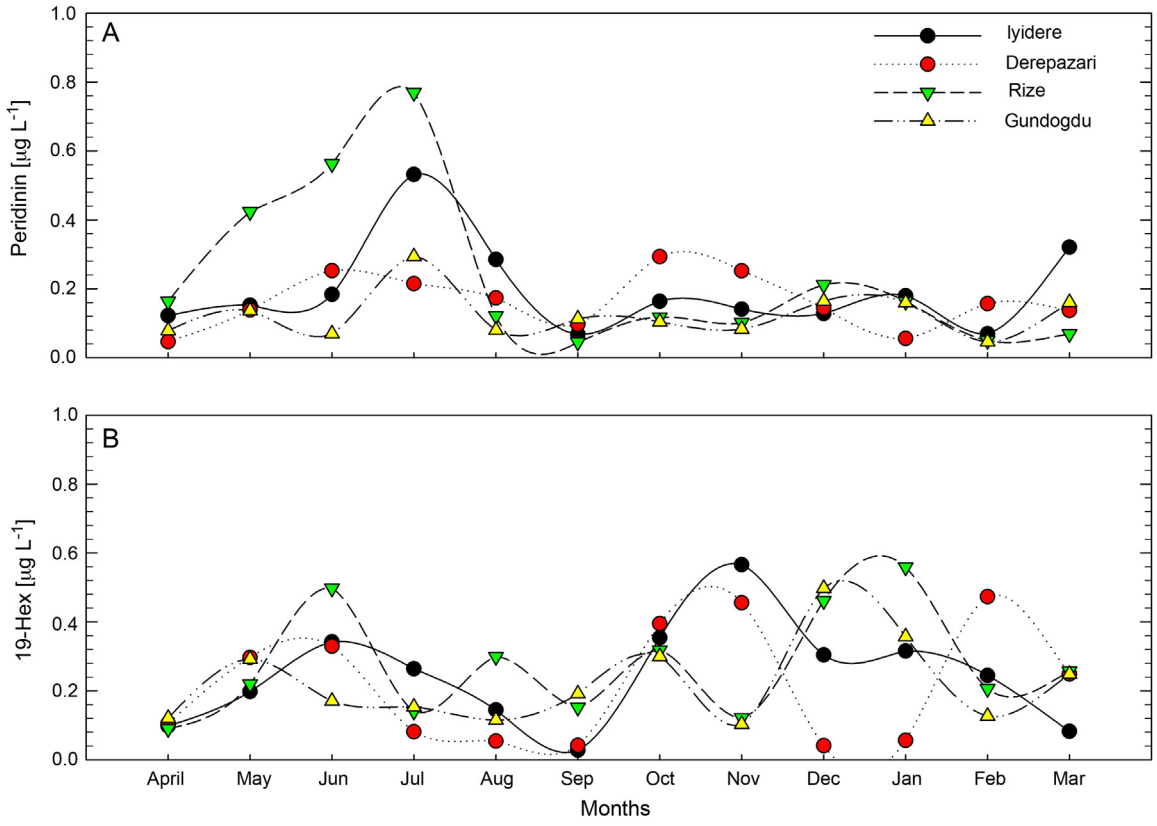

Figure 7 Monthly variation of peridinin and 19-hexanoyloxyfucoxanthin along the stations.

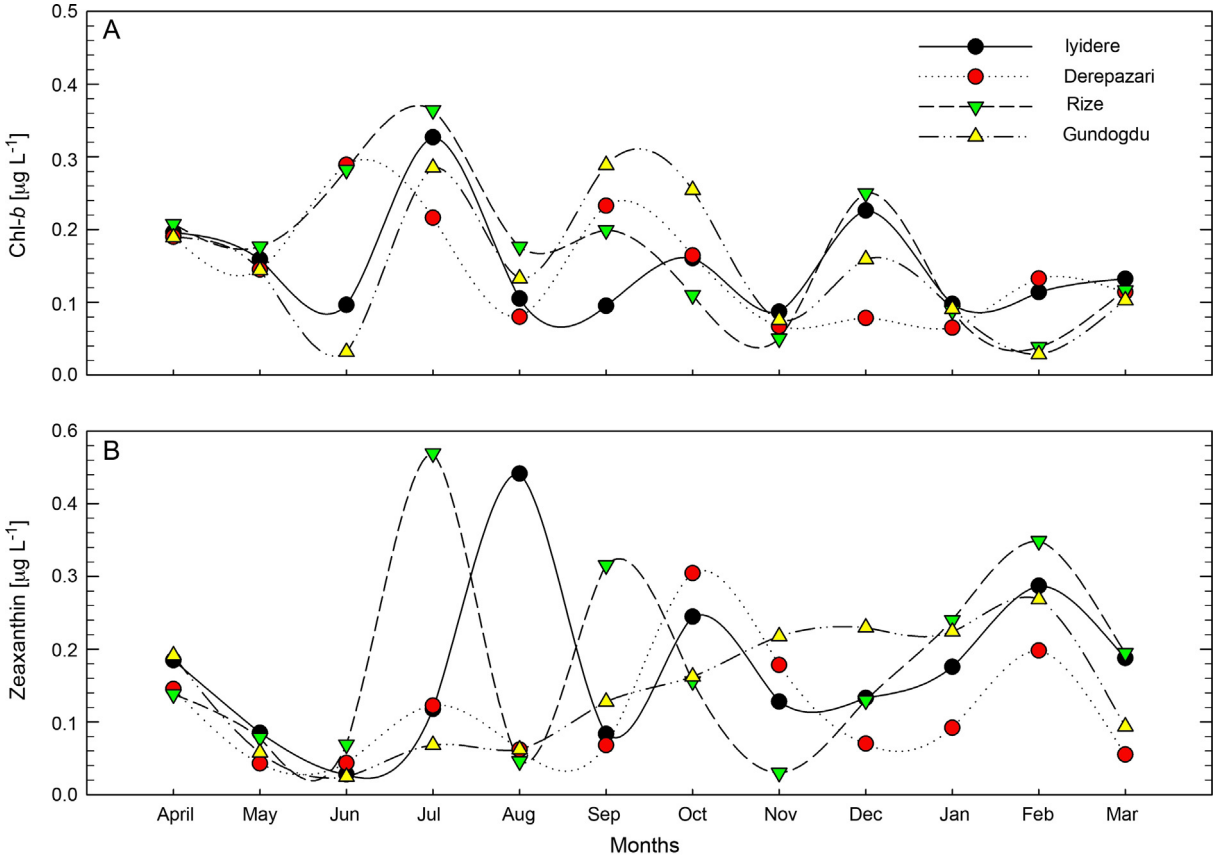

Figure 8 Monthly variation of Chl-*b* and zeaxanthin along the stations.

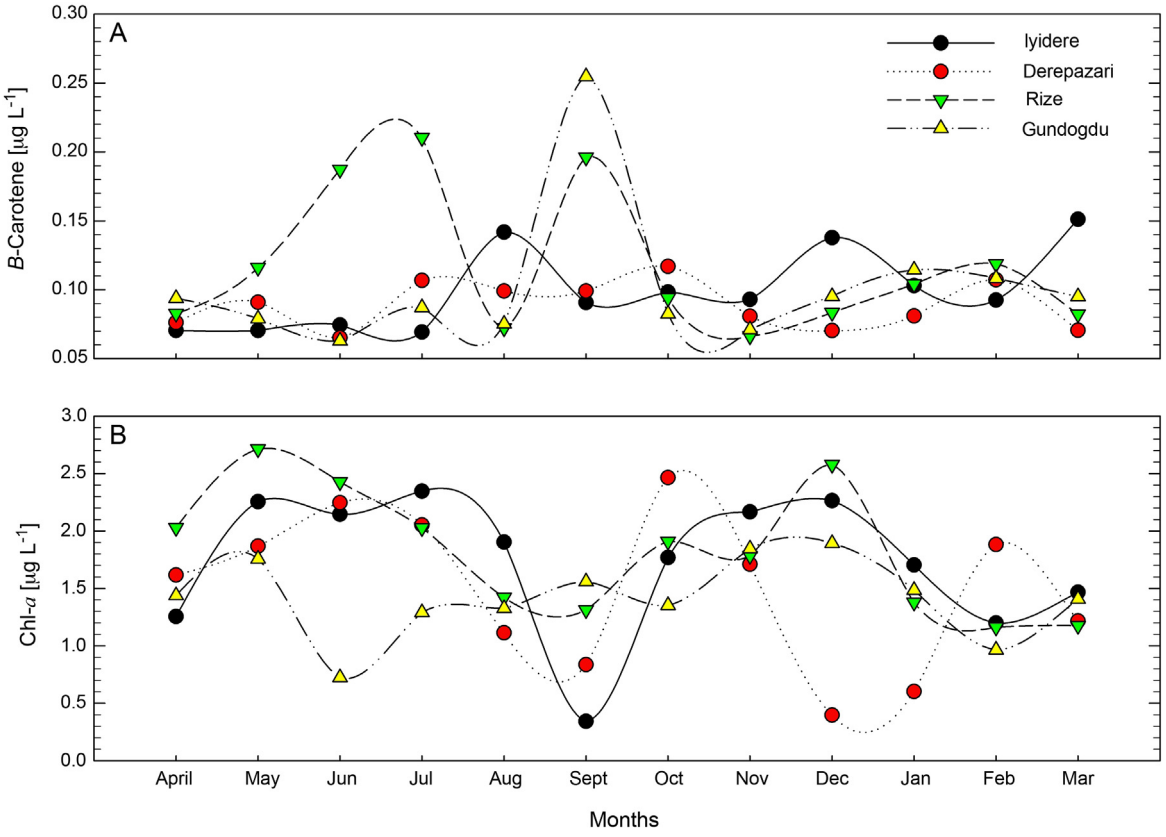

Figure 9 Monthly variation of β-Carotene and Chl-*a* along the stations.

Derepazari (Fig. 14). However, the correlation between marker pigments and group specific carbon biomass was generally higher than the correlation between total phytoplankton carbon and Chl *a* throughout the study period.

4. Discussion

The results obtained from the present study provide a description of dynamics of major phytoplankton groups, diatom to dinoflagellate ratio, pigment composition and nutrient concentration along SE coastal waters (Rize) of the Black Sea. Overall, diatoms and dinoflagellates made substantial contribution to total phytoplankton biomass (in terms of Phyto-C). Contribution of other phytoplankton groups to Phyto-C was generally low due to their small size (Fig. 10). In terms of species number, dinoflagellates were found to be the dominant group along the stations in agreement with previous studies (e.g. Agirbas et al., 2015; Mikaelyan et al., 2013) reported from the Black Sea.

Phytoplankton community composition and their ratio impact the pelagic ecosystem throughout changes in carbon export, food web dynamics, and availability of nutrient to higher trophic levels (e.g. Edwards and Richardson, 2004; Koeller et al., 2009; Nagata et al., 1996; Pedersen et al., 1999; Platt et al., 2003), eutrophication (Micheli, 1999) and climate change (Leterme et al., 2006; Richardson and Schoeman, 2004). Diatoms and dinoflagellates are major components of the phytoplankton community in the Black Sea throughout the year (Georgieva, 1993; Morozova-Vodyanitskaya, 1957; Pautova et al., 2007; Senicheva, 2000). However in summer, small flagellates often play significant role in phytoplankton especially in the open waters, where they reach to 80% of the total phytoplankton biomass (Sukhanova et al., 1991). Our findings agree with recent studies which have revealed that the phytoplankton community composition of the Black Sea has shifted substantially during the last three decades due to drastic changes (i.e. eutrophication) in the Black Sea ecosystems, and is numerically dominated by dinoflagellates and coccolithophores (Eker-Develi and Kideys, 2003; Kideys, 1994; Mikaelyan et al., 2011, 2013; Moncheva and Krastev, 1997; Uysal et al., 1998).

Eutrophication caused changes in the phytoplankton abundance, biomass, community composition and bloom timing in the Black Sea ecosystem in particular NW shelf. However, the Black Sea has shown some signs of recovery (e.g. an increase in the proportion of diatoms in the phytoplankton community, a decrease in the number of monospecific algal blooms, a decrease in phytoplankton biomass, etc.) in recent years (Bodeanu et al., 2004; McQuatters-Gollop et al., 2008). Decline in influx of nutrient supply (mainly silicate) from river runoff due to the dams constructed across rivers (e.g. River Danube) also changed phytoplankton community composition in favour of coccolithophores and dinoflagellates rather than diatoms after the 1970s (Bodeanu et al., 1998; Bologa et al., 1995; Cociasu et al., 1996; Humborg et al., 1997; Moncheva and Krastev, 1997). During the period of eutrophication, biomass of dinoflagellates

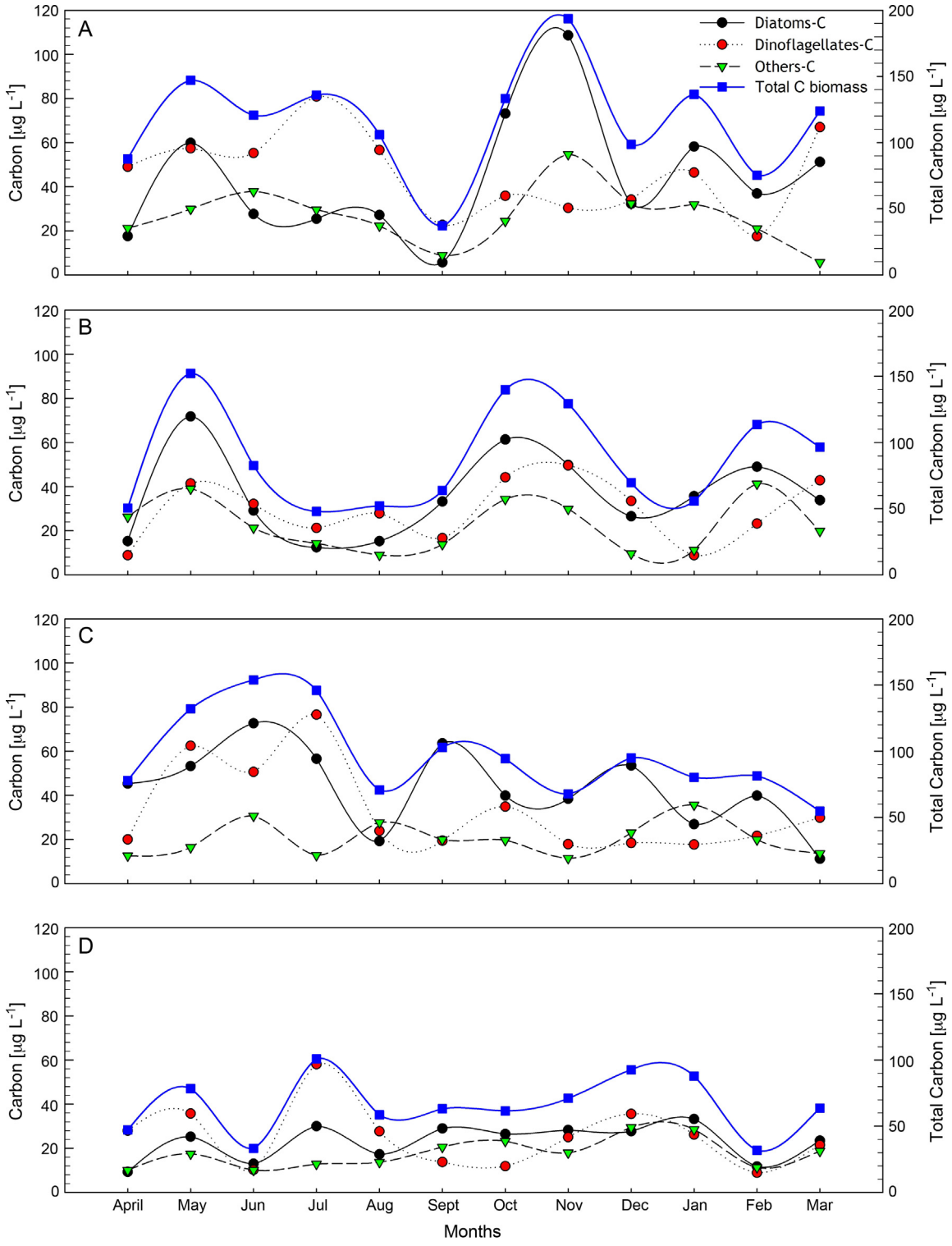

Figure 10 Monthly variation of phytoplankton carbon along the stations [A: Iyidere station; B: Derepazari station; C: Rize station; D: Gundogdu station].

increased notably from 1.3 ± 3.2 g m^{-2} (1969—1983) to 7.9 ± 1.6 g m^{-2} (1984—1995) for the water column. Concurrently, biomass of diatoms increased from 1.8 ± 1.9 g m^{-2} to 8.1 ± 1.0 g m^{-2}, respectively. Similarly, biomass of coccolithophores increased substantially from 0.05 ± 0.05 g m^{-2} to 1.9 ± 0.9 g m^{-2} for the same period (Mikaelyan et al., 2013). After the 1980s, contribution of diatoms to total phytoplankton biomass decreased gradually from 92.3% (1960—1970) to 62.2% (1983—1988) off Romania, and conversely the proportion of dinoflagellates increased from 7.6% to 30.9% for the same periods (Bodeanu, 1989). Correspondingly, the species number and abundance of dinoflagellates

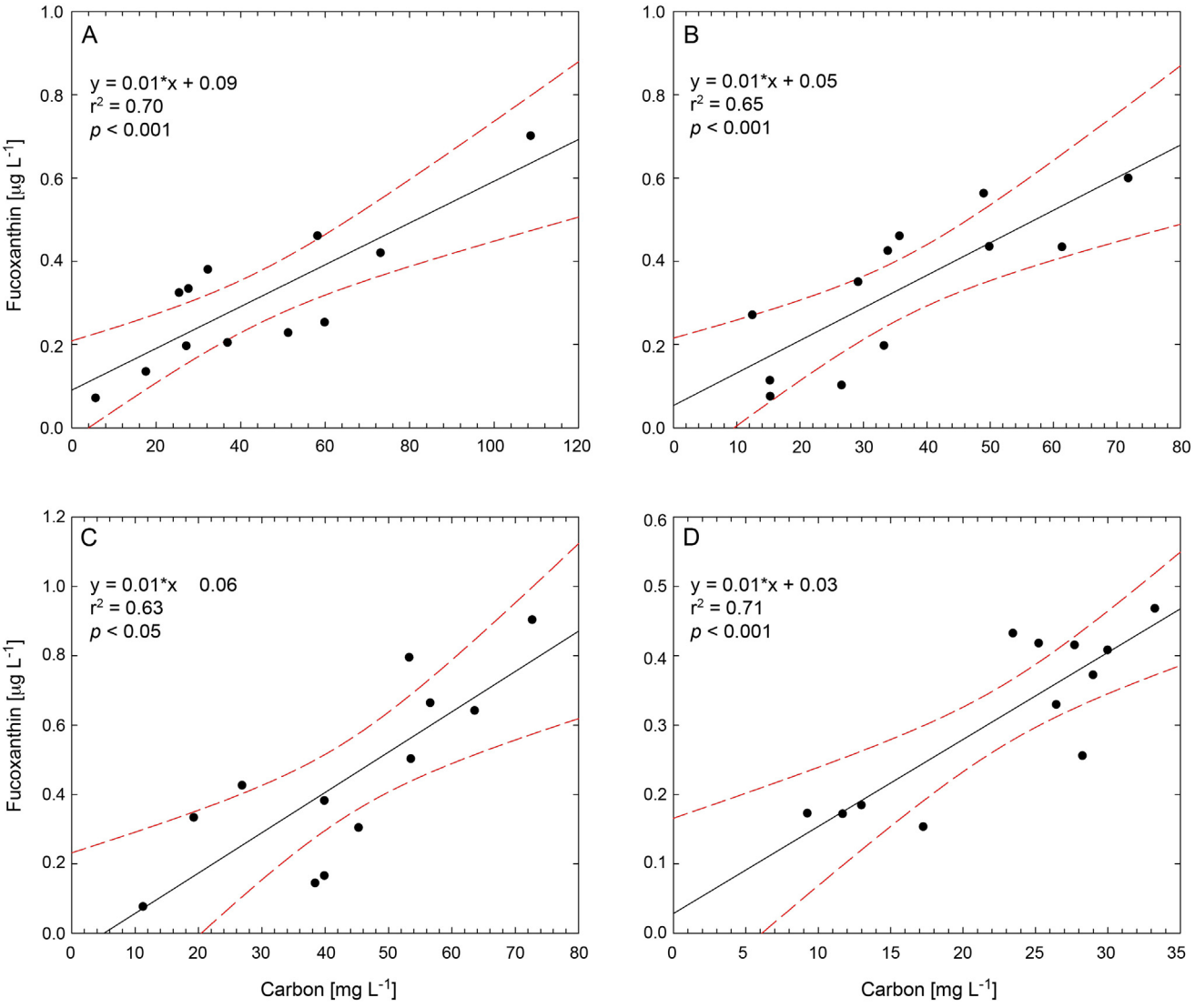

Figure 11 Linear relationship between Diatom-C and fucoxanthin with 95% confidence intervals [A: Iyidere station; B: Derepazari station; C: Rize station; D: Gundogdu station].

increased, and they became the dominant group especially during the summer periods (Agirbas et al., 2015; Bodeanu, 1989; Bologa, 1986; Ediger et al., 2006; Eker-Develi and Kideys, 2003; Ivanov, 1965; Mikaelyan et al., 2013; Moncheva et al., 2001; Uysal and Sur, 1995; Zaitsev and Alexandrov, 1997). Similarly, a high contribution of dinoflagellates to phytoplankton species number was observed in the present study, which concurs with recent trends reported from the Black Sea. In the present study, temporal variations were observed in diatom to dinoflagellate ratio (based on abundance), which was the highest in the late spring and autumn. During those periods, the phytoplankton were mainly dominated by diatoms.

The qualitative changes in community structure of phytoplankton can be related with the changes in nutrient concentration. In general, nitrogen and phosphorus lead to an increase in phytoplankton. However, diatoms also require silica for their shells in addition to these nutrients (Escaravage et al., 1999). Increase of abundance of coccolithophorids during recent decades in the Black Sea was governed by PO_4-P concentrations (Mikaelyan et al., 2013). In the present study, nutrient concentrations varied throughout the study period, but not statistically significant (One-way ANOVA, $p > 0.05$). In general, high nutrient concentrations recorded during vertical mixing periods leaded to high diatom abundance in the present study. However, low nutrient concentrations were observed during stratified periods in which dinoflagellates and coccolithophores were dominant along the stations. The ratio of nutrients (N:P and Si:N) changed along the stations with statistically significant differences (One-way ANOVA, $p < 0.05$). Depending on phytoplankton activity, the ratio of N to P fluctuated over the time, whereas the highest ratios were generally recorded during mixing periods. The ratio of Si to N along the stations increased during mixing periods. Declining SiO_2-Si did not affect diatoms while probably it limited growth of silicoflagellates (Mikaelyan et al., 2013). Experiments carried out in the open waters of the Black Sea also revealed that silicate was not a limiting nutrient of phytoplankton growth in natural populations (Yilmaz et al., 2006). Chai et al. (2016) reported that

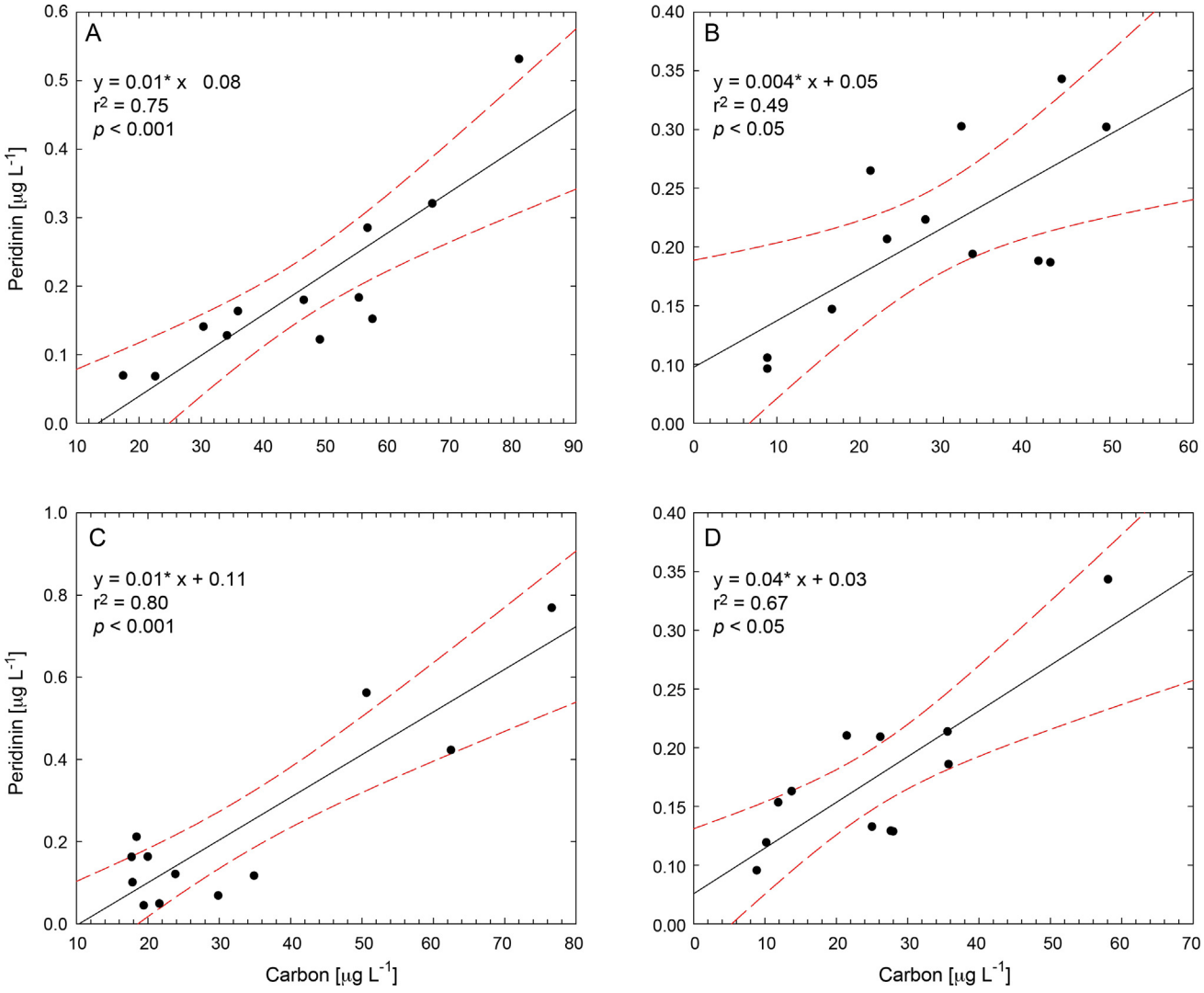

Figure 12 Linear relationship between dinoflagellates-C and peridinin with 95% confidence intervals [A: Iyidere station; B: Derepazari station; C: Rize station; D: Gundogdu station].

high N:P ratio suggested a potential phosphorus limitation in their study. They also found that nutrients were the most important environmental factors which affect the distribution of phytoplankton in the Pearl River estuary. In many cases, high N to P ratio results in predominance of diatoms while the lower ratio often is favourable for dinoflagellates (Aiken et al., 2009; Heil et al., 2007; Hodgkiss and Ho, 1997; Malviya et al., 2016; Uitz et al., 2006). Moreover, low N to P ratio was favourable for coccolithophores whereas high N to P ratio was much convenient for diatoms (Silkin et al., 2014). In general, diatoms are more abundant in nutrient-enriched waters and during the onset of the spring bloom. After the spring bloom, depletion of nitrate and silicate causes dominancy of small sized groups and coccolithophores in the upper part of the water column (Barlow et al., 2004; Gibb et al., 2000; Llewellyn and Gibb, 2000). The observed negative correlation between diatoms and nitrate in the present study probably explains the dominance of diatoms due to nitrogen preference of diatoms during spring and autumn periods where extensive mixing and nutrient input take place in SE coasts of the Black Sea. Especially dinoflagellates (stress-tolerant) and flagellates are adapted to survival in low nutrient stable waters (Holligan, 1987; Margalef, 1978). Similarly, an increase in microphytoplankton but decrease in nanophytoplankton and picophytoplankton was reported from the Pearl River estuary with high nutrient concentration (Chai et al., 2016). On the other hand, dinoflagellates were found to be the most abundant group in the regions where were exposed to anthropogenic influence and generally more abundant in the warmer part of the year along the eastern Adriatic coast (Bužančić et al., 2016). Therefore, they can be used as an indicator for anthropogenic pressures.

Phytoplankton community and their ratios influence the energy transfer through the food-web (Beaugrand, 2003; Beaugrand and Reid, 2003). Especially, large celled phytoplankton (e.g. diatoms) are believed to be responsible for the majority of carbon export. Moreover, a positive correlation between diatoms and the growth of anchovy was also reported (Cury et al., 2008). A strong correlation was reported between diatom availability and the anchovy catch in the Humboldt ecosystem (Jackson et al., 2011). Therefore, shifts in phytoplankton community composition resulted in

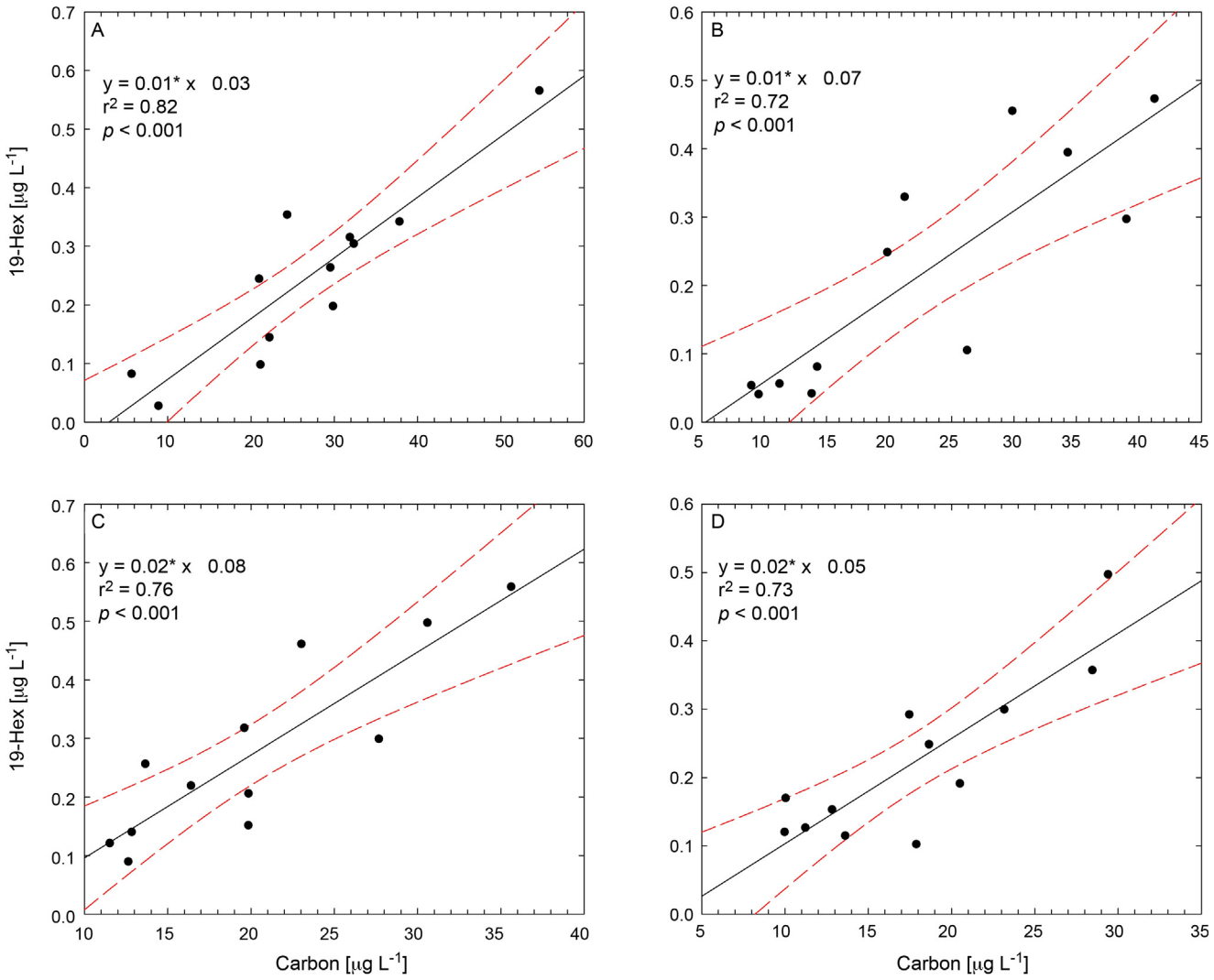

Figure 13 Linear relationship between other groups-C and 19-hexanoyloxyfucoxanthin with 95% confidence intervals [A: Iyidere station; B: Derepazari station; C: Rize station; D: Gundogdu station].

alteration of maximum sustainable yield in fishery (Henson et al., 2010). Thus, comprehensive monitoring on phytoplankton community composition is required to ensure sustainable management of fisheries resources (IOCCG, 2014).

HPLC-based pigment analysis provides the information about phytoplankton assemblages, and their physiological status in addition to microscopic enumeration. During the study period, Chl *a* and diagnostic pigments were significantly correlated with Phyto-C (see Figs. 11—13). In general, the correlation between 19′-Hex and other groups was stronger than the correlations between fucoxanthin and diatoms, and peridinin and dinoflagellates. This is likely due to *E. huxleyi*, which consisted of majority of other phytoplankton groups along the stations. Moreover, it should be noted that some dinoflagellate species also contain 19′-Hex (Eker-Develi et al., 2012). Similarly, high correlation between 19-Hex concentrations and Dinoflagellate-C was also reported from the north-western Black Sea (Eker-Develi et al., 2012). Using diagnostic pigments obtained from HPLC to infer phytoplankton groups is still problematic because some major pigments such as fucoxanthin (indicator for diatoms) may also be found in some flagellates (Vidussi et al., 2001). Therefore, pigment-based estimation of phytoplankton groups does not completely reflect phytoplankton community composition (Brewin et al., 2010). However, a good correlation between pigments and Phyto-C was obtained in the present study which provided a more comprehensive picture of the phytoplankton community composition for the study area, and also suggested that microscopic investigation should be accompanied by HPLC-based pigment analysis.

5. Conclusion

We examined spatio-temporal changes and composition of surface phytoplankton community, diatom to dinoflagellate ratio and pigment compositions with nutrient data in the SE Black Sea from April 2013 to March 2014. The overall picture helps us to better understand how the spatio-temporal distribution of phytoplankton groups and pigment profiles can influence ecosystem structure and functioning as well as to illustrate the patchy nature of phytoplankton distribution in

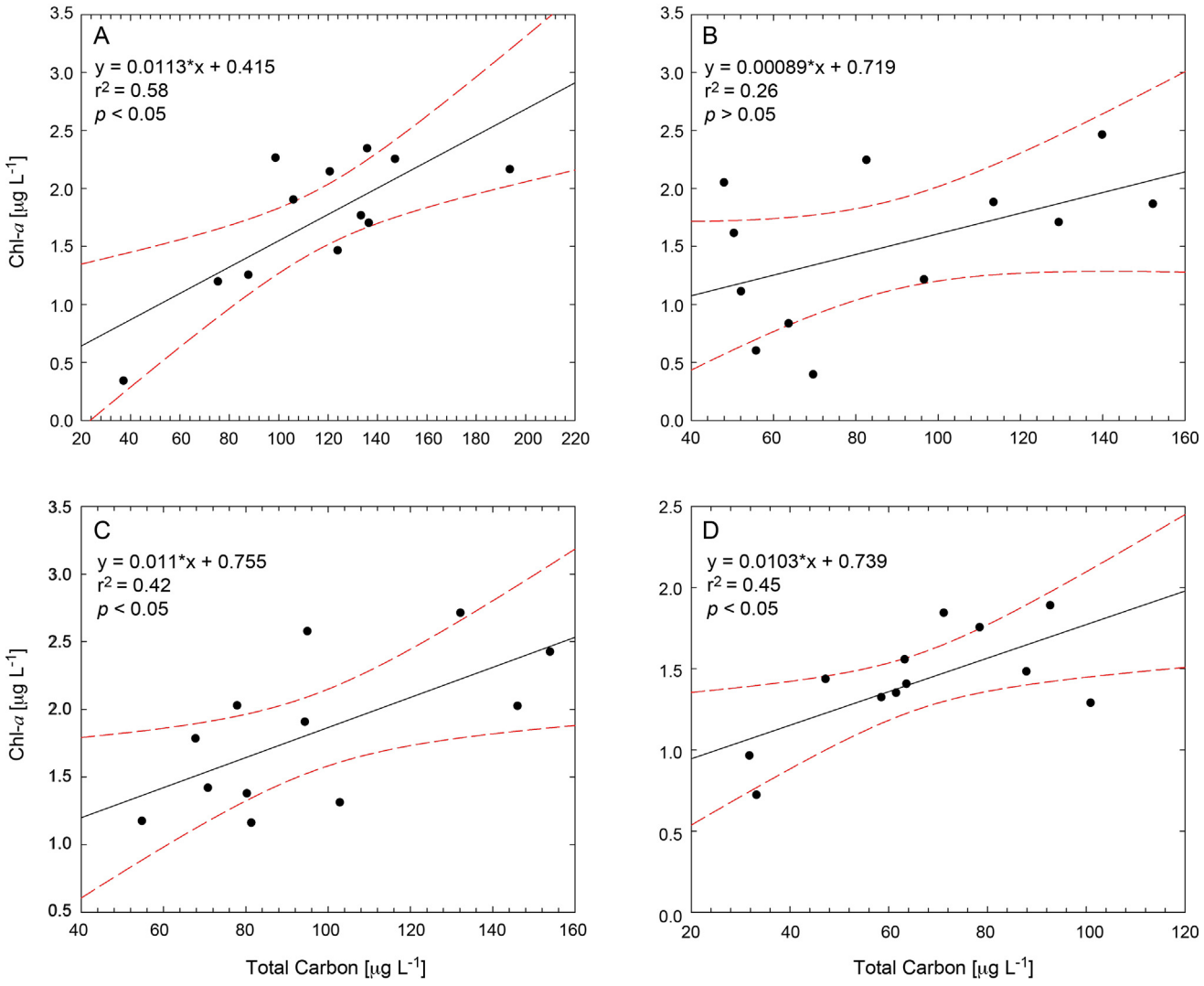

Figure 14 Linear relationship between total phytoplankton carbon and Chl-*a* with 95% confidence intervals [A: Iyidere station; B: Derepazari station; C: Rize station; D: Gundogdu station].

the SE Black Sea. Many of photosynthetic pigments have strong chemotaxonomic associations that may be exploited to map the oceanographic abundance and composition of the phytoplankton community (Gibb et al., 2001). Moreover, regressions of diagnostic pigments with some bio-minerals (e.g. Chl *a*, Fuco and 19-Hex. with POC, SiO_2 and $CaCO_3$) revealed the spatial relationships and hence the potential of pigments as proxy bio-mineral markers (Gibb et al., 2001).

Statistically significant changes in Phyto-C, phytoplankton composition, diatom to dinoflagellate ratio and pigment composition among the stations were observed. These changes are likely due to fluctuation in nutrient concentration and other environmental factors. We also observed statistically significant correlation among diagnostic pigments, group specific Phyto-C, and key nutrients, which has provided key information for the Black Sea ecosystem. These findings also support that spatio-temporal distribution of phytoplankton community is closely related to its environmental condition (e.g. nutrients, light, turbidity, etc.) as reported by Bužančić et al. (2016) and Chai et al. (2016). Results indicated that diatom to dinoflagellate ratios and pigment composition varied throughout the year. Diatoms were the main group in terms of abundance and Phyto-C along the stations. Depending on the abundance and Phyto-C biomass, the spring phytoplankton bloom is greater than in other seasons and is dominated mostly by diatoms and followed by dinoflagellates. In general, Phyto-C values are in good agreement with pigment data for large-celled diatoms; however, the agreement is poorer for small phytoplankton groups (e.g. dinoflagellates, prymnesiophytes, picoplankton, etc.) due to ambiguous and shared marker pigments (Agirbas et al., 2015; Eker-Develi et al., 2008, 2012; Irigoien et al., 2004; Jeffrey and Vesk, 1997; Wright et al., 1996; Zapata et al., 2004). Besides, the comparison of HPLC-based pigment analysis with microscopy can be difficult, because both methods measure different parameters and use different units (Higgins et al., 2011). Hence, pigment-based chemotaxonomy and microscopic observation are complementary procedures. In the present work, microscopy and HPLC-based pigment analysis revealed nearly similar picture of phytoplankton community composition in the SE Black Sea. More importantly, HPLC-based pigment analysis provided extra

information for smaller groups (e.g. pico and nano-phytoplankton) rather than microscopy. Additionally, the obtained results confirm that HPLC-based pigment approach can be used for monitoring studies along the SE Black Sea.

We believe that the present study will illuminate the relationship between pigments and taxonomic classes of phytoplankton with environmental parameters for the study area. Besides, such studies are also important for remote sensing studies especially optically different land locked seas. Using satellite data in the coastal regions of the Black Sea however can be problematic because of the high CDOM absorption, which can cause errors in Chl *a* (Oguz and Ediger, 2006). Enclosed seas (e.g. Baltic Sea, Black Sea, etc.) have undergone more dramatic changes in physics and biology than the open oceans (Anadón et al., 2007). Hence, monitoring studies have a vital importance for the Black Sea ecosystem.

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