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Determination of phytoplankton composition by microscopy and HPLC-derived pigment analysis in the Sea of Marmara

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Abstract

This study is an integrated overview of pigment and microscopic analysis of phytoplankton composition in the Sea of Marmara. The study was conducted from 27 sampling stations during spring (May 2017 and 2018) and summer (August 2016 and 2018). The phytoplankton community was represented mostly by diatoms and dinoflagellates, as the major groups, and also other phytoflagellate groups as shown by both techniques. Chlorophyll-*a*, chlorophyll-c_1+c_2, peridinin, 19'-butanoyloxyfucoxanthin, fucoxanthin, zeaxanthin, alloxanthin, 19'-hexanoyloxyfucoxanthin, diadinoxanthin, chlorophyll-*b* and ß-carotene concentrations were determined by High Performance Liquid Chromatography (HPLC). A total of 124 eukaryotic taxa belonging to eight algal classes were found through microscopic analysis. Sixty (60) of such taxa were dinoflagellates, 52 were diatoms and 12 taxa were other phytoflagellates. The number of diatoms and dinoflagellates, as the major groups, accounted for 90.4% of the total phytoplankton species. Chlorophyll-*a*, fucoxanthin and peridinin concentrations varied between 0.03-7.20, 0.01-5.23 and 0.01-2.14 µg L^{-1}, throughout the research period, respectively. The highest chlorophyll-*a* values were measured at stations MD26 (7.20 µg L^{-1}) and MD22 (6.61 µg L^{-1}) in May 2018, which were located in Gemlik and Bandirma bays. There was a significant correlation (r:0.87, p<0.001 n:54) between HPLC determined fucoxanthin concentrations and diatom abundances in August 2016 and 2018. Also, higher chlorophyll-c_1+c_2 concentrations revealed consistency with high diatom abundances and fucoxanthin concentrations in August 2016 and 2018. This result confirmed that diatoms are the most important carrier of fucoxanthin and that it can be used for taxonomic evaluation of the diatom community of the Sea of Marmara.

Keywords: Phytoplankton; chlorophyll-*a*; marker pigment; HPLC; Sea of Marmara.

Introduction

Phytoplankton, the primary producer of the marine food chain, is an easily detectable indicator of ecological change and is sensitive to various environmental factors (Paerl *et al*., 2007). The structure of food webs, the cycling of nutrients and the transportation of particles to the benthic system are affected by phytoplankton communities (Mendes *et al*., 2011). Due to the important role of phytoplankton, monitoring of phytoplankton composition and community structure is of major importance for understanding aquatic ecosystems (Paerl *et al*., 2003).

Traditional identification of phytoplankton via microscopy is highly dependent on personel skills and requires extensive time for sample preparation and counting (Wang *et al*., 2018). In addition, some fragile and small phytoplankton cells can be difficult to identify via microscopy, since they lack significant external morphological features (Mackey *et al*., 1996; Agirbas *et al*., 2015). Altough chlorophyll-*a* concentration has been used to estimate phytoplankton biomass in the marine environment for many years, this method cannot distinguish between the different phytoplankton groups (Wang *et al*., 2018). Alternatively, in recent years, using HPLC allows phytoplankton group characterization by the presence or absence of marker pigments (Ediger *et al*., 2001; Dandonneau & Niang, 2007; Aiken *et al*., 2009; Madhu *et al*., 2014; Chai *et al*., 2016; Oseji *et al*., 2019). Certain marker pigments are indicators of phytoplankton groups example.g. fucoxanthin, peridinin and alloxanthin are the diagnostics for diatoms, dinoflagellates and cyrptophytes, respectively (Wang *et al*., 2018). The HPLC technique allows easy and fast separation, identification of phytoplankton pigments while a large number of samples can be processed compared to microscopy (Schlüter *et al*., 2000; Agirbas *et al*., 2015). Nevertheless, using HPLC analysis without microscopic confirmation can sometimes be misleading. Thus, a combination of both

approaches has been recommended (Millie *et al*., 1993; Ediger *et al*., 2006; Seoane *et al*., 2011; Mendes *et al*., 2015; Miranda Alvarez *et al*., 2020).

The Sea of Marmara is subject to dense anthropogenic pressure (pollution) and has numerous fragile bays where frequent algal blooms occur. The phytoplankton community of the region has been widely investigated by microscopy in previous studies (Balkıs, 2003; Unsal *et al.,* 2003; Balkıs, 2004; Turkoglu *et al.,* 2004; Aktan *et al*., 2005; Okuş & Taş, 2007; Deniz & Taş, 2009; Turkoglu, 2010; Turkoglu & Erdogan, 2010; Turkoglu & Oner, 2010; Balkıs & Toklu Alıçlı, 2014). However, the phytoplankton composition of the region has not yet been identified using HPLC marker pigment analysis. Moreover, the HPLC method has been widely used in recent studies conducted in Turkish seas, and these studies have focused on the Black Sea and the Mediterranean Sea (Ediger *et al*., 2006; Eker Develi *et al*., 2012; Agirbas *et al*., 2015, 2017; Yücel *et al*., 2017; Eker Develi *et al*., 2019).

This study aims to investigate spatial and seasonal variations of phytoplankton composition in the Sea of Marmara via microscopic examination and HPLC derived pigment analyis. A specific objective was to evaluate the utility of HPLC pigment analysis for identifying phytoplankton group composition of the Sea of Marmara.

Material and Methods

General characteristics of the sampling area

The Sea of Marmara, an inland sea, is a transition zone between the Black Sea and the Mediteranean Sea (Fig. 1). It connects to the Aegean Sea through the Çanakkale Strait (Dardanelles) in the south-west and to the Black Sea via the Istanbul Strait (Bosphorus) in the north-east and forms the "Turkish Strait System" (Besiktepe *et al*., 1994). The characteristic of the system is a permanent stratification separating the Black Sea less saline upper layer (~18 psu) from the saline Mediterranean waters (~38 psu) of the lower layer (Özsoy *et al.*, 1988). The upper layer extends to depths of about 25 m and the lower layer lies below ~25 m. The interface between the two layers lies between 16 and 28 m (Sur *et al*., 2002). The southern Marmara region is typically influenced by river discharges. In addition, the industralized regions of the Izmit, Gemlik, Bandirma and Tekirdag bays are some of the most densely populated coastal regions in Turkey.

Sample collection

Four field studies were conducted at 27 sampling stations in the Sea of Marmara (Fig.1) during spring (between 22-31 May 2017 and 24-31 May 2018) and summer (between 16-26 August 2016 and 10-18 August 2018). Seawater samples were taken from the surface (0.50 m) using 5.00 L Niskin bottles mounted on a CTD rosette (SBE 911) system. For HPLC pigment analysis, seawater samples (250-1000 mL) were filtered immediately through Whatmann GF/F filters (25 mm diameter) under a gentle vacuum (< 0.70 atm) and kept frozen until the extraction procedure.

Phytoplankton enumeration

250 mL seawater samples were preserved with acidic Lugol's solution (2%) in glass bottles and kept under dark and cool (4°C) conditions until microscopic analysis (Throndsen, 1978). Sample volumes of 10-50 mL were allowed to settle for 24-48 h (Utermöhl, 1958), depending on the estimated abundance of cells, based on the sampling area. Phytoplankton cells were enumerated using a LEICA DM IL LED inverted microscope equipped with phase contrast optics. The samples were examined at appropriate magnifications (×100 to ×400) and classified into taxonomic categories such as diatoms, dinoflagellates and other phytoflagellates.

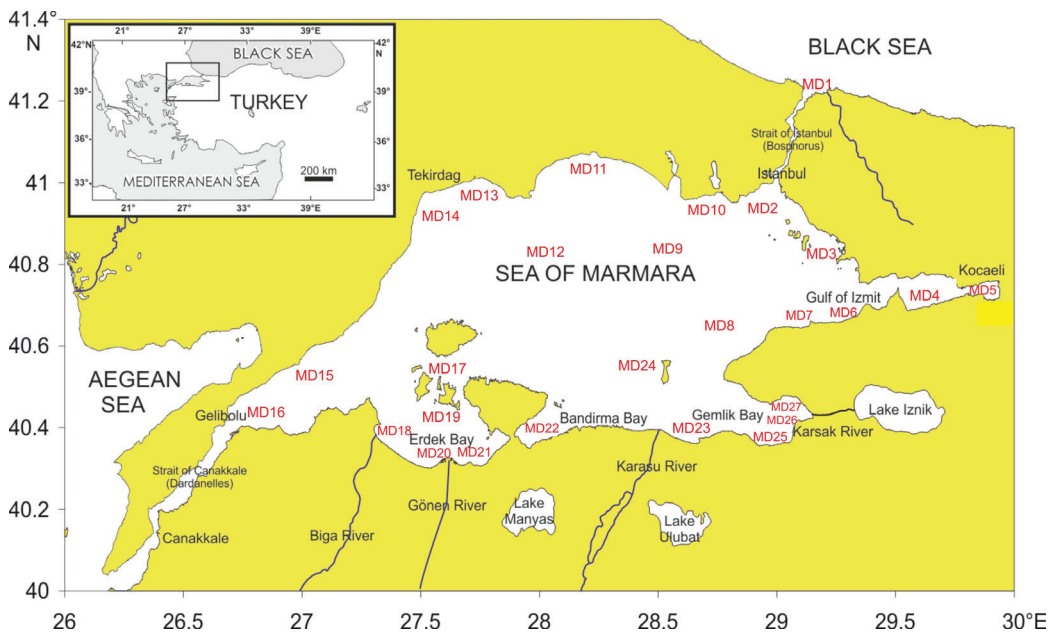

Fig. 1: Study area and sampling locations.

HPLC pigment analysis

The method chosen for this study (Barlow *et al*., 1993) is a modification of the method given in Mantoura & Llewellyn (1983). According to this procedure, the frozen filters were extracted in 5.00 mL of 90% HPLC-grade acetone, ultrasonicated for 1 min at 60 Hz and centrifuged at 3500 rpm for 10 min to remove cellular debris. A 500 µL aliquot of sample was filtered through a Millex-GS 0.22 µm disposable filter into a vial and 500 µL of 1 M ammonium acetate was added; and then 100 µL of this mixture was injected into the HPLC. The HPLC system was calibrated for each pigment using commercial standards (chlorophyll-*a*, chlorophyll-*b*, chlorophyll-c_1+c_2, peridinin, 19'-butanoyloxyfucoxanthin, fucoxanthin, zeaxanthin, alloxanthin, 19'-hexanoyloxyfucoxanthin, divinyl chlorophyll-*a*, diadinoxanthin, lutein and ß-carotene: DHI LAB, Denmark) (Fig. 2) and peaks were identified based on their retention times (Table 1). Chromatographic analyses were carried out using a Hewlett-Packard (HP) Model 1100 equipped with an inline degasser, quarternary pump, autosampler and diode-array detector. Data collection and processing of results were performed using the HP Chemstation software. Pigments were separated on a Thermo Scientific Hypersil MOS-2 C8 (150 mm × 4.6 mm, 3µ) column. The detection wavelength was set at 440 nm with a 10 nm bandwith; the reference wavelength was 750 nm with a 100 nm bandwith. The mobile phases were A: 70% methanol plus 30% 1M ammonium acetate and B:100% methanol. The flow rate was set at 1.0 mL min^{-1}. Gradient elution was programmed at 25% B, maintained for 1 min and increased to 50% over 1 min, which was maintained for 19 min. Elution was then followed by an increase to 100% B over 5 min before programming back to initial conditions over 7 min. The initial conditions were maintained for a further 7 min, resulting in a total cycle time of 39 min.

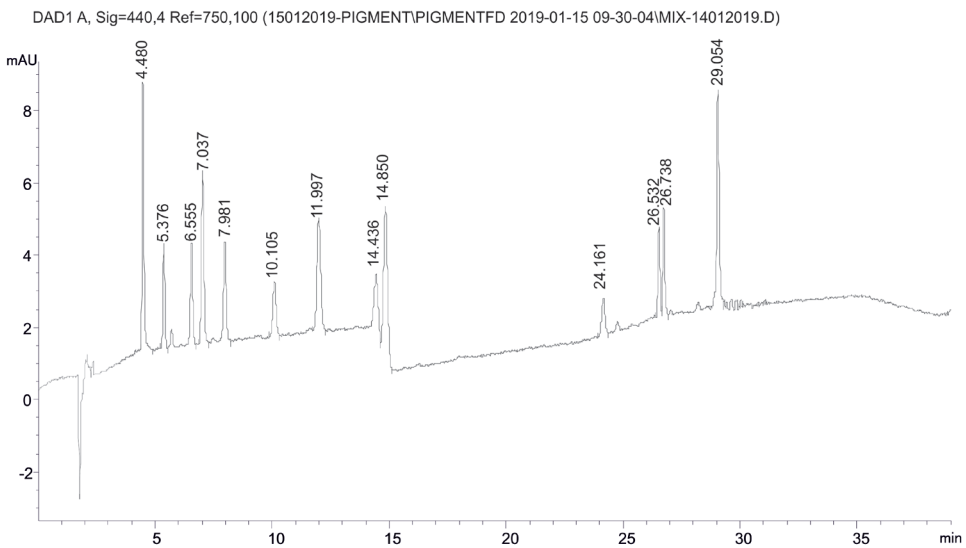

Fig. 2: HPLC chromatogram of the mixed-pigment standard.

Table 1. Peak identification and retention times of phytoplankton pigments detected in surface water samples from the Sea of Marmara.

No.	Pigment	Retention time (min)
1	chlorophyll-c_1+c_2	4.480
2	peridinin	5.376
3	19'-butanoyloxyfucoxanthin	6.555
4	fucoxanthin	7.037
5	19'-hexanoyloxyfucoxanthin	7.981
6	diadinoxanthin	10.105
7	alloxanthin	11.997
8	zeaxanthin	14.436
9	chlorophyll-*b*	24.161
10	chlorophyll-*a*	26.738
11	ß-carotene	29.054

Results

Phytoplankton composition and abundance patterns

A total of 124 eukaryotic phytoplankton taxa belonging to eight algal classes were identified in surface water samples collected during the study period. Sixty (60) of the taxa (48.4%) were dinoflagellates, 52 (42.0%) were diatoms and 12 (9.6%) were other phytoflagellates, including silicoflagellates, prymnesiophytes, euglenophytes, prasinophytes, cryptophytes and raphidophytes. The number of diatoms and dinoflagellates, as major groups, accounted for 90.4% of the total number of phytoplankton. The most diverse genera were: for diatoms, *Chaetoceros, Coscinodiscus* and *Rhizoselenia*, and for dinoflagellates, *Prorocentrum*, *Protoperidinium* and *Tripos*. The most frequent diatom species were *Proboscia alata* (Brightwell) Sundström, 1986*, Pseudo-nitzschia* sp. and *Skeletonema costatum* (Greville) Cleve, 1873, and dinoflagellate species were *Prorocentrum micans* Ehrenberg, 1834, *Prorocentrum cordatum* (Ostenfeld) J.D. Dodge, 1975, *Tripos furca* (Ehrenberg) F.Gomez, 2013, *Tripos fusus* (Ehrenberg) F.Gomez, 2013 and *Scrippsiella acuminata* (Ehrenberg) Kretschmann, Elbrächter, Zinssmeister, S.Soehner, Kirsch, Kusber & Gottschling, 2015.

Total phytoplankton abundance showed seasonal and spatial fluctuations and was generally low in August 2016 ($<10^4$ cells L^{-1}), while it was higher in May 2017, May 2018 and August 2018. Higher total phytoplankton abundances were detected at stations MD2, MD5 and MD22, which were located at the junction of the Strait of Istanbul (Bosphorus), Izmit and Bandirma bays, respectively (Fig. 3). The highest phytoplankton abundance reached 1.538×10^6 cells L^{-1} at station MD5 in August 2018 (Fig. 3C). The average contribution of diatom species to total phytoplankton abundance decreased considerably from August 2016 to May 2018 (from 91.4% to 19.7%), while the average contribution of dinoflagellates increased markedly from August 2016 to May 2018 (from 8.60% to 75.9%) (Fig. 4).

Dinoflagellates

The majority of phytoplankton taxa (60 taxa) were dinoflagellates. *Tripos*, *Protoperidinium* and *Prorocentrum* were the most diverse genera. The highest abundances of dinoflagellates were observed in May and August 2018 at station MD5 (Fig. 3C, D). Nonetheless, the dinoflagellate *Gymnodinium catenatum* H.W. Graham, 1943 was responsible for a maximum abundance of 7.34×10^5 cells L^{-1} at station MD5, in August 2018 (Fig. 3C). In May 2018, three maxima were observed in dinoflagellate abundance dominated by *P. micans*. These are 1.43×10^5, 2.32×10^5 and 2.95×10^5 cells L^{-1} at stations MD6, MD4 and MD5, respectively (Fig. 3D).

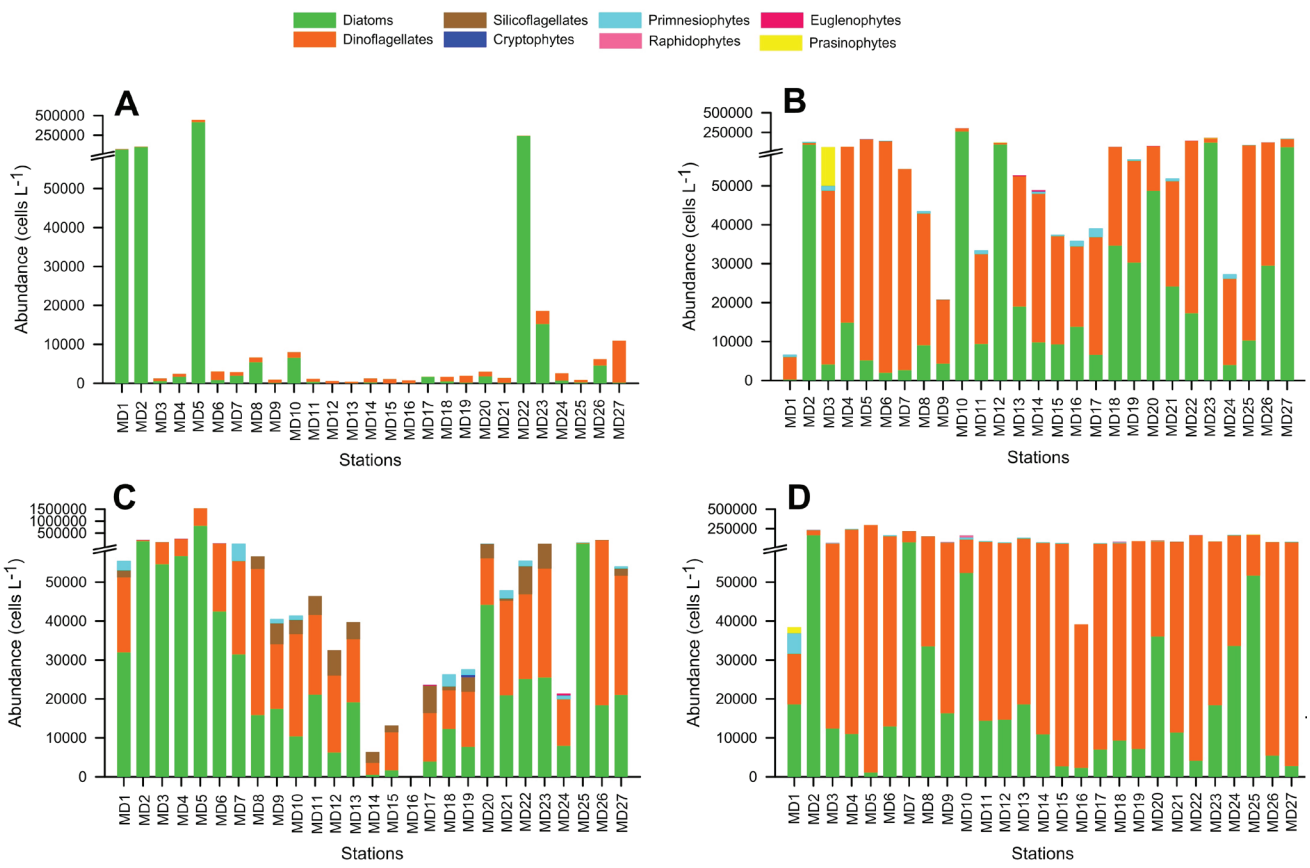

Fig. 3: Spatio-temporal variations in phytoplankton during the study period (A: August 2016, B: May 2017, C: August 2018 and D: May 2018).

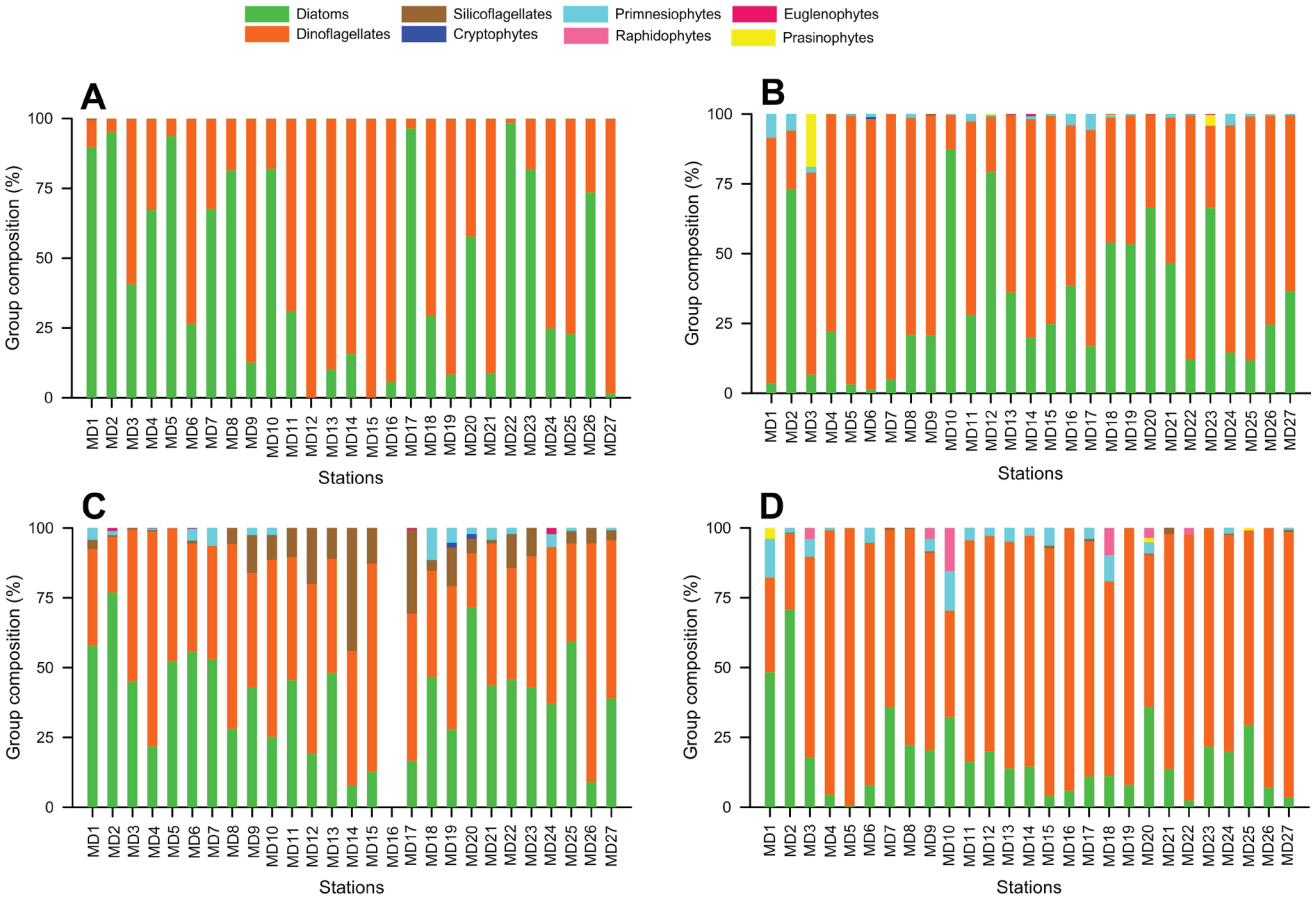

Fig. 4: Variations in the percentage of the main groups of total phytoplankton composition (A: August 2016, B: May 2017, C: August 2018 and D: May 2018).

Diatoms

Diatoms were the second largest group (52 taxa) of the phytoplankton community. The most diverse taxa were *Chaetoceros* spp. and *Coscinodiscus* spp., *S.costatum* and *Cylindrotheca closterium* (Ehrenberg) Reimann & J.C. Lewin, 1964 were the most abundant species. The highest diatom abundances were observed in August 2016 and 2018 at station MD5 (Fig. 3A, C). Maximum diatom abundance (8.04×10^5 cells L^{-1}) was observed in August 2018, dominated by *S.costatum* (4.31×10^5 cells L^{-1}) at station MD5 (Fig. 3C). *S.costatum* reached 1.12×10^5 and 2.52×10^5 cells L^{-1}, at stations MD2 and MD10, in May 2018 and May 2017, as well (Fig. 3B, D). Another diatom increase occurred at station MD5, dominated by *C. closterium* with maximum abundance of 2.60×10^5 cells L^{-1} in August 2016 (Fig. 3A).

Other phytoplankton groups

A total of 12 phytoflagellate taxa belonging to six algal classes were identified in surface water samples collected during the study period. *Emiliania huxleyi* (Lohmann) W.W.Hay & H.P.Mohler, 1967, a bloom-forming primnesiophyte, was commonly observed in low abundances except in August 2016 and its maximum abundance reached 23.0×10^3 cells L^{-1} at station MD10 in May 2018 (Fig. 3D). In May 2018, maximum abundance (25.0×10^3 cells L^{-1}) of the raphidophyte *Heterosigma akashiwo* (Y. Hada) Y. Hada ex Y. Hara & M. Chihara, 1987 was observed at station MD10 (Fig. 3D). Euglenophytes, including *Eutreptia* sp. and *Eutreptiella* sp., were rarely observed in the study area in May 2017 and August 2018 (Fig. 3B, C). The highest abundance of euglenophytes was 2.00×10^3 cells L^{-1} at station MD2, in August 2018. The prasinophyte *Pyramimonas* sp. appeared only in May 2017 and reached 12.0×10^3 cells L^{-1} at station MD3 (Fig. 3B). The contribution of silicoflagellates (1.30%) and cryptophytes (0.20%) to total phytoplankton abundance was very low during the study period (Fig. 4).

Spatial and seasonal distribution of phytoplankton pigment concentrations

Based on the obtained mix-standard chromatogram (Fig. 2), chlorophyll-*a* and ten (10) other group-specific pigments were identified in the Sea of Marmara (Table 1). Lutein and divinyl chlorophyll-*a* were not detected at any time and at any station. The chlorophyll-*a* and accessory pigment (e.g. fucoxanthin and peridinin) concentrations for all stations and all seasons are presented in Figure 5.

Chlorophyll-*a* concentrations ranged between 0.03 and 7.20 µg L^{-1} in surface waters during the study period (Fig. 5A). The overall averaged chlorophyll-*a* concentra-

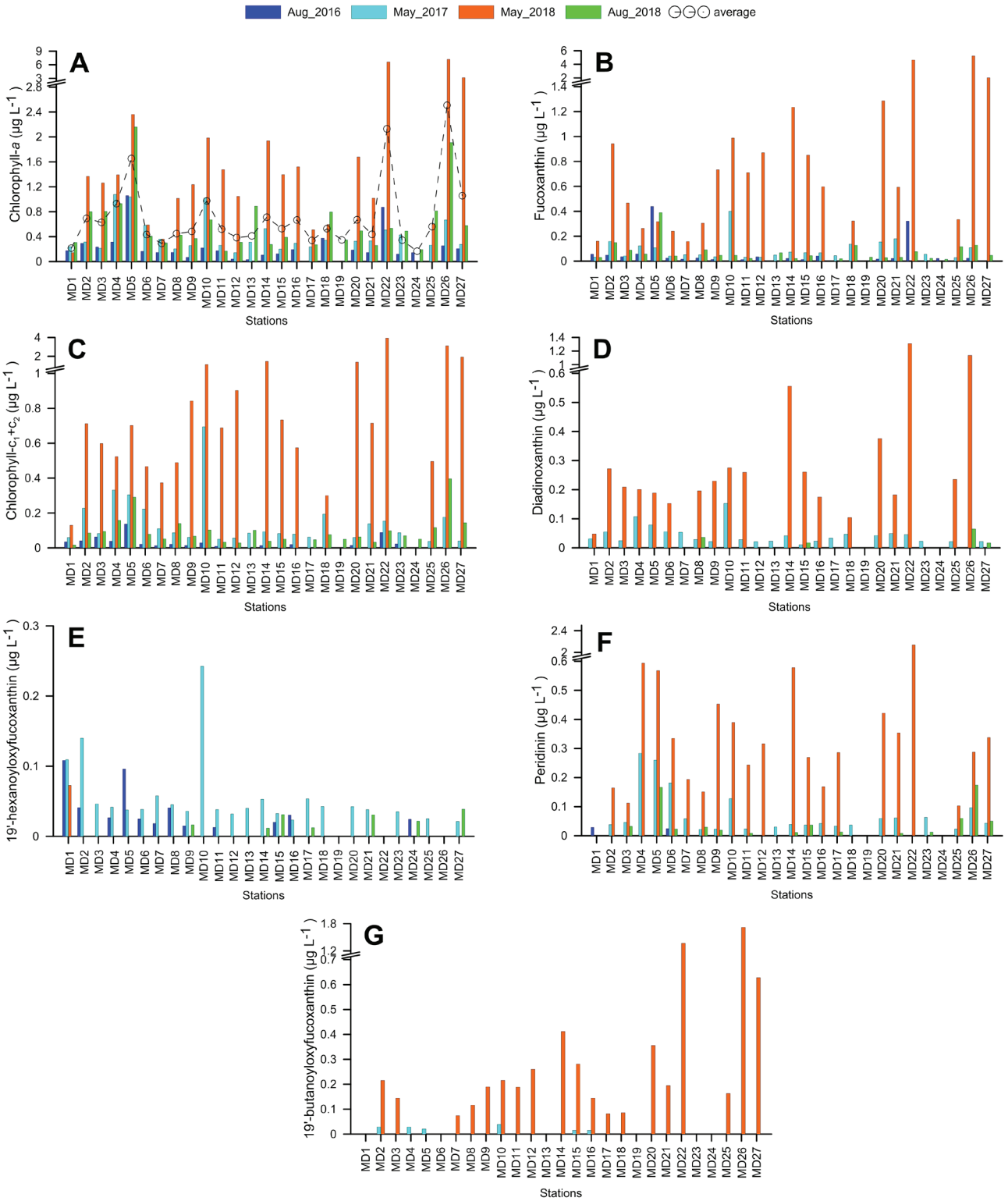

Fig. 5: Distribution of surface pigment concentrations in the Sea of Marmara.

tions were 0.24, 0.42, 1.76 and 0.61 µg L^{-1} during August 2016, May 2017, May 2018 and August 2018, respectively. Maximum chlorophyll-*a* values were observed at stations MD26 (7.20 µg L^{-1}) and MD22 (6.61 µg L^{-1}) in May 2018, which were located in Gemlik and Bandirma bays, respectively (Fig. 5A).

In addition to the chlorophyll-*a* concentrations of another two accessory pigments, namely fucoxanthin and peridinin, being the major markers of diatoms and dinoflagellates, respectively, were quantified from the study region. The concentrations of fucoxanthin, the dominant accesory pigment in May 2018, was low during other sampling periods (Fig. 5B). Its concentration in surface waters ranged from 0.01 to 5.25 µg L^{-1}. Average fucoxanthin concentrations were 0.06, 0.09, 1.06 and 0.07 µg L^{-1} during August 2016, May 2017, May 2018 and August 2018, respectively. Maximum fucoxanthin values were measured at stations MD26 (5.25 µg L^{-1}) and MD22

(4.64 µg L^{-1}) in May 2018; stations were located in Gemlik and Bandirma bays, respectively (Fig. 5B).

Peridinin concentrations were significantly low during August 2016, May 2017 and August 2018 (Fig. 5F). Its concentration in surface waters ranged from 0.01 to 2.14 µg L^{-1} and average peridinin concentration was 0.40 µg L^{-1} during May 2018. Maximum peridinin values were measured at stations MD22 (2.14 µg L^{-1}) and MD4 (0.60 µg L^{-1}) in May 2018; stations located in Bandirma Bay and the middle section of the Izmit Bay, respectively (Fig. 5F).

The third accesory pigment was 19'-hexanoyloxyfucoxanthin, mostly detected during May 2017, and its concentrations ranged between 0.02 and 0.24 µg L^{-1} (Fig. 5E). The highest 19'-hexanoyloxyfucoxanthin value was observed at station MD10 (0.24 µg L^{-1}) in May 2017; a station located at Kucukcekmece (northern part of the Sea of Marmara).

19'-butanoyloxyfucoxanthin was mostly detected during May 2018 and the average concentration was 0.36 µg L^{-1} (Fig. 5G). The highest 19'- butanoyloxyfucoxanthin value was observed at station MD26 (1.72 µg L^{-1}), which was located in Gemlik Bay. Diadinoxanthin concentrations ranged from 0.01 to 1.30 µg L^{-1} (Fig. 5D) while the highest value was measured in May 2018 at station MD22, which was located in Bandirma Bay. Chlorophyll-c_1+c_2 was mostly observed in May 2018 and concentrations varied between 0.01 and 3.94 µg L^{-1} (Fig. 5C). The highest chlorophyll-c_1+c_2 value was detected at station MD22, as well. Other accessory pigments, i.e. zeaxanthin, alloxanthin, chlorophyll-*b* and ß-carotene were detected at these stations but they were minor and not consistently present. Concentrations of these pigments ranged between 0.03-0.05, 0.03-0.06, 0.09-0.20 and 0.01-0.60 µg L^{-1}, respectively.

Discussion

The advantage of using marker pigments measured by HPLC to estimate phytoplankton group composition has been demonstrated for the Black Sea (Ediger *et al*., 2006; Eker Develi *et al*., 2012; Agirbas *et al*., 2015, 2017) and the Mediterranean Sea (Yücel *et al*., 2017; Eker Develi *et al*., 2019). To the best of our knowledge, this study, based on HPLC marker pigment analysis, is the first report for the Sea of Marmara. In contrast, the phytoplankton composition considered so far in the region (Balkıs, 2004; Turkoglu *et al.,* 2004; Aktan *et al*., 2005; Deniz & Taş, 2009; Turkoglu, 2010; Balkıs & Toklu Alıçlı, 2014) is based on microscopy analysis.

In this study, diatoms in summer (August 2016 and 2018) and dinoflagellates in spring (May 2017 and 2018) dominated the phytoplankton composition in terms of abundance, as shown by microscopy and pigment analysis. The reasons for such seasonal and regional differences, in terms of abundance can be explained; by general hydrographical conditions such as salinity, flow regime etc. and adaptation to the environment. Moreover, regional climate changes, increased temperature, industrialization and anthropogenic pressures may cause regional differences in species distribution (Özsoy *et al*., 2016). The highest phytoplankton abundance (1.538×10^6 cells L^{-1}) was found at station MD5 in August 2018, although some higher abundance values were observed throughout the investigated period. The highest chlorophyll-*a* value (7.20 µg L^{-1}) was observed at station MD26 in May 2018, a station located in Gemlik Bay. Moreover, chlorophyll-*a* values were generally higher at stations MD5, MD22, MD26 and MD27, located in Izmit, Gemlik and Bandirma bays. Industrial and wastewater discharges, anthropogenic and agricultural loads are the most important nutrient sources for the Sea of Marmara, particularly for Izmit, Gemlik and Bandirma bays (Polat & Tuğrul, 1995). Changes in environmental and hydrographic conditions increase the risk of eutrophication and determine the intensity of phytoplankton production particularly in semi-enclosed bays where water exchange with the open sea is relatively limited (Tuğrul & Morkoç, 1990). Thus, the TRIX eutrophication index (Vollenweider *et al*., 1998) is an important tool that has been used in the management of coastal regions and the analysis of the trophic status of the environment. Ediger *et al*. (2013) have reported a detailed examination of TRIX index values for the Sea of Marmara and indicated that the values have increased in the enclosed bays and close to rivers, which indicates bad trophic conditions in the enclosed bays due to human impact. As a consequence of the situations mentioned above, phytoplankton blooms has been commonly observed in the bays of the Sea of Marmara (Tas & Okus, 2004; Aktan *et al*., 2005; Tufekci *et al*., 2010; Ergul *et al*., 2015).

Chlorophyll-*a* concentrations detected by HPLC are consistent (r:0.76, $p<0.001$, n:54, using simple linear regression) with total phytoplankton abundance in August 2016 and 2018. Silva *et al*. (2008), who found a good correlation between total phytoplankton abundance and HPLC derived chlorophyll-*a* concentrations in Lisbon Bay, Portugal arrived at the same conclusion. On the contrary, no relationship was found between total phytoplankton abundance and chlorophyll-*a* in May 2017 and 2018; this situation may be due to high number of picoplanktonic species that cannot be identified by microscopy, as reported by Pérez *et al*. (2006).

As stated by Viličić *et al*. (2008) for the Adriatic Sea, the dominant marker pigment was fucoxanthin that was highly correlated with the chlorophyll-*a* values in the Sea of Marmara during August 2016 and 2018 (Fig. 6A, B). Furthermore, chlorophyll-c_1+c_2 was present and higher in August 2016 and 2018, and highly correlated with fucoxanthin (Fig. 6C, D) and chlorophyll-*a* values, when diatom species were abundant as was stated by Ediger *et al*. (2001) for Galway Bay, Ireland. Moreover, microscopy confirmed that diatoms were the most diverse and abundant microplanktonic group in August 2016 and 2018, as reported for the Black Sea (Agirbas *et al*., 2017) and the Basque coast (Bay of Biscay, northern Spain) (Seoane *et al.*, 2005) as well. HPLC- derived fucoxanthin concentrations appeared to be a good indicator for observed diatom abundances, with synchronized seasonal variations. Sig-

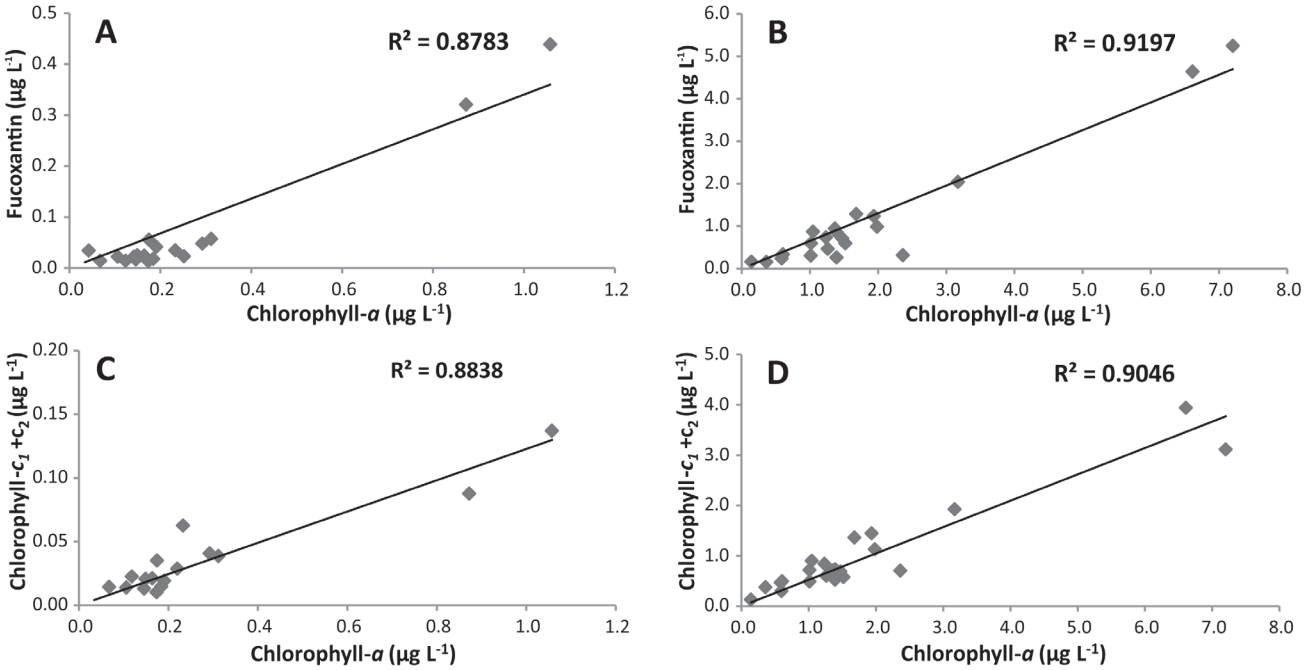

Fig. 6: Linear relationship between: i) (A-B) chlorophyll-*a* and fucoxanthin concentrations; and ii) (C-D) chlorophyll-*a* and chlorophyll-c_1+c_2 concentrations (A: August 2016, B: August 2018, C: August 2016, D: August 2018; n: 27).

nificant linear relationships were found (r:0.87, p<0.001 n:54) between diatom abundance and fucoxanthin concentration in August 2016 and 2018, and this indicated that diatoms are the most important carrier of fucoxanthin in the samples taken from the Sea of Marmara.

The highest diatom abundances have been observed during summer (August 2016 and 2018) instead of spring, as reported by Totti *et al*. (2000). On the contrary, the concentration of fucoxanthin was found to be highest in May 2018, which is in accordance with the data for the Black Sea (Agirbas *et al*., 2017). Also, it differs from the outcome of a study conducted by Ansotegui *et al*. (2003) in Urdaibai estuary (Bay of Biscay, Northern Spain) where the highest fucoxanthin concentration was found in late winter. The phytoplankton community consisted of microplanktonic diatoms, as well as nanoplanktonic dinoflagellates, prasinophytes and raphidophytes (Sieburth *et al*., 1978). The dominant marker pigment throughout the study period was fucoxanthin, with the highest values observed in May 2018. This differs from the low diatom abundances (at stations MD14, MD22, MD26 and MD27, located in Tekirdag, Bandirma and Gemlik bays, respectively) which can be considered that the peak of fucoxanthin may have originated from nanoplanktonic non-diatom species at these stations (Krivokapic *et al*., 2018).

Generally, low concentrations of peridinin were detected through HPLC analysis (Fig. 5F), although a large diversity and abundance of dinoflagellates was found by microscopic analysis (Fig. 3). Even though dinoflagellate species were abundant during May 2017 and 2018, peridinin concentrations were relatively low in the study area. On the other hand, *Protoperidinium* and *Dinophysis* were the most diverse genera of the dinoflagellate community during these periods. Thus, the low correlation (r:0.48, p<0.002, n:54) between dinoflagellate abundance and peridinin concentration in May 2017 and 2018 was due either to the dominance of heterotrophic dinoflagellate species *Protoperidinium* spp., as reported by Loret *et al.* (2000) and Krivokapić *et al.* (2018) or to the abundance of dinoflagellates such as *Dinophysis* spp., which are rich in phycobilin rather than peridinin, as reported by Takishita *et al.* (2002).

Comparative studies generally reveal a good relationship between microscopy and HPLC analysis for diatom species (especially larger ones), but the correlation is lower for dinoflagellates, raphidophytes and primnesiophytes due to the shared marker pigments. (Zapata *et al*., 2004; Eker Develi *et al*., 2012; Agirbas *et al*., 2015). Determination of cell sizes and abundances of phytoplankton species using microscopy provides highly valuable data for monitoring studies, which cannot be done by pigment analysis. Moreover, microscopy is crucial for accurate assignment of marker pigments to phytoplankton taxa, thus allowing for reliable phytoplankton composition studies. On the contrary, HPLC pigment analysis is a helpful and faster way of analyzing greater changes in the phytoplankton community with significantly less effort compared to microscopy (Silva *et al*., 2008). It may be said that, HPLC pigment analysis provides valuable information on the entire phytoplankton community, particularly regarding small-sized groups, whereas microscopy provides good taxonomic reliability for larger cells.

Environmental factors, such as salinity, temperature and nutrients, might affect the seasonal variation of phytoplankton composition. Finding the relationships be-

tween biotic and abiotic factors and phytoplankton composition requires more detailed research. On the other hand, it may be possible to establish phytoplankton distribution maps, which can deal with certain difficulties encountered while determining small-sized groups (picoplankton) in microscopic studies, and cover broader areas using the HPLC method applied in this study. This study may help future investigations to evaluate the changes in the phytoplankton composition of the Sea of Marmara. Moreover, it is recommended that microscopy combined with HPLC-derived pigment data be used in future studies to gain a better understanding of phytoplankton distribution in the Sea of Marmara.

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