Assessment of phytoplankton group composition in the Golden Horn Estuary (Sea of Marmara, Turkey) determined with pigments measured by HPLC-CHEMTAX analyses and microscopy

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Abstract

Phytoplankton group composition determined by microscopy was compared with high performance liquid chromatography (HPLC) derived from pigment signatures in surface water samples taken bi-weekly and monthly between October 2018 and September 2019 in the Golden Horn Estuary (Sea of Marmara). A total of 80 eukaryotic phytoplankton taxa belonging to eight algal classes were identified in surface water during the study period. Forty-three taxa (54%) were diatoms, 29 taxa (36%) were dinoflagellates and eight taxa (10%) were other phytoflagellates. The average contribution of diatoms to total phytoplankton abundance decreased considerably (41 to 25%), while the average contribution of dinoflagellates and other phytoflagellates increased markedly (39 to 75%) from the lower to the middle estuary. Chlorophyll-a and seven other group-specific pigments, including fucoxanthin, peridinin, chlorophyll-c1 + c2, alloxanthin, 19′-hexanoyloxyfucoxanthin, 19′-butanoyloxyfucoxanthin and divinyl chlorophyll-a were identified in the study area. The relative contribution of the major phytoplankton groups to chlorophyll-a was estimated on three different initial ratio matrices by CHEMTAX. The results obtained were compared with those from microscopic examination. It was concluded that the CHEMTAX method was not accurate enough to characterize the phytoplankton community in the Golden Horn Estuary ecosystem and microscopic analysis was essential to determine the major contributing species to chlorophyll-a.

Introduction

Phytoplankton is generally constituted of complex communities and their diversity and dynamics are highly variable. Information about the combined effects of various environmental variables is necessary in food chain studies and ecosystem modelling as well as for explaining eutrophication and harmful algal bloom events (Wänstrand & Snoeijis, 2006). Thus, distribution of phytoplankton species provides crucial data for environmental monitoring studies. As these species can change in a very short time, regular investigation of phytoplankton composition requires substantial resources for sampling and skilled staff for microscopy (Hillebrand et al., 1999).

The identification of phytoplankton through microscopic analysis is time-consuming and needs a high level of taxonomic expertise. There is also the risk of missing smaller phytoplankton groups (like picoplankton, <2 μm in size) in traditional microscopic analysis (Naik et al., 2011). An alternative or supplementary method for monitoring and identifying phytoplankton community structures is to determine their pigment signatures by using high-performance liquid chromatography (HPLC) (Wright & Jeffrey, 2006). This technique is faster and more reproducible than microscopy and independent of subjective consideration (Wänstrand & Snoeijis, 2006). Moreover, this technique can be more sensitive for identifying pico- and nanoplankton or species broken by sample fixation, which can be difficult to identify with microscopic analysis (Jeffrey & Vesk, 1997). HPLC analysis also allows us to characterize the physiological condition of phytoplankton, while counts can determine whether organisms are dead or alive (Millie et al., 1993). Furthermore, the composition of phytoplankton communities can be determined using HPLC derived pigment data by the means of pigment ratios (Higgins et al., 2011). One of the most up to date techniques used to do this is the statistical software CHEMTAX.

CHEMTAX software uses pigments/chlorophyll-a ratios to characterize algal classes (Mackey et al., 1996). This software has been widely used in different regions of the world oceans to characterize phytoplankton community structure (Schlüter et al., 2000; Havskum et al., 2004; Eker-Develi et al., 2008; Kozlowski et al., 2011; Araujoa et al., 2017). In some studies, CHEMTAX estimates were in agreement with microscopy for major phytoplankton groups (Wright et al., 1996; Llewellyn et al., 2005) while not in some others (Irigoien et al., 2004; Lionard et al., 2008). However, the application of the CHEMTAX software to coastal and estuarine regions, is limited by the lack of known pigment ratios (Wright et al., 1996; Anstotegui et al., 2003; Eker-Develi et al., 2012).
Estuaries are known as highly productive ecosystems, and they are transition zones between river and marine environments. Many studies of phytoplankton composition have been performed in estuaries around the Mediterranean Sea (Trigueros & Orive, 2001; Burić et al., 2007; Barbosa et al., 2010). Recently, studies have focused on phytoplankton communities of the Golden Horn Estuary (GHE) by microscopic analysis including the variations in phytoplankton composition (Tas et al., 2009; Dursun & Tas, 2019), planktonic diatom composition (Tas, 2017; Tas & Hernández-Becerril, 2017), and algal blooms and potentially harmful species (Tas & Okus, 2011; Tas, 2015, 2019; Tas & Yılmaz, 2015; Dursun et al., 2016; Tas & Lundholm, 2017). However, no study has been performed in the GHE using HPLC, despite HPLC pigment signatures being widely used in Turkish seas (Ediger et al., 2006; Eker-Develi et al., 2012; Agirbas et al., 2017; Yücel, 2017).

The primary aim of this study was to investigate variations in phytoplankton group composition by microscopic and HPLC pigment analysis alongside associated environmental factors. A specific objective was to test the usefulness of CHEMTAX analysis for determining variations in phytoplankton group composition in the GHE for the first time.

**Materials and methods**

**Study area and sampling strategy**

The Golden Horn Estuary (GHE) is located in the north-east of the Sea of Marmara, extending in a north-west–south-east direction, ~7.5 km long and up to 700 m wide. The study area was divided into three sections based on hydrographic and bathymetric features; lower estuary (LE), middle estuary (ME) and upper estuary (UE). The maximum depth is 40 m in the LE, it decreases rapidly to 14 m in the ME and to 4 m in the UE due to high concentrations of suspended particulate material (SPM) originating from two streams, the Alibey and Kağıthane. Three stations were chosen along the study area; ST1 interacts strongly with the Strait of Istanbul (Bosphorus) and represents the LE, and ST2 and ST3 represent the ME, with an intermediate marine influence, where a bridge (Atatürk Bridge), operating on buoys, limits the upper layer circulation (Figure 1). The LE is characterized by two-layered stratification: with less saline (~18) Black Sea water above and highly saline (~38) Mediterranean water below (Ünlülata et al., 1990). The upper layer extends to depths of about 25 m and the lower layer lies below ~25 m (Sur et al., 2002).

The sampling period lasted for one year, from October 2018 to September 2019. Surface water samples were taken at monthly (between October and March, from June to September) and biweekly (April and May) periods from the three stations, representing the LE and ME (Figure 1). Temperature, salinity, dissolved oxygen (DO) and pH of surface water were measured using a multi-parameter probe (YSI Professional Pro Plus) from the sea surface, and water transparency was measured using a Secchi disc with 30 cm diameter. No environmental data could be measured in January due to failure of the multi-parameter probe. Seawater samples were taken from the surface (0.5 m) using 5 l Niskin bottles. For HPLC pigment analysis, surface water samples
(80–700 ml) were taken and filtered immediately onto Whatmann GF/F filters (25 mm diameter) under low vacuum (<0.7 atm) and kept frozen until extraction.

**Phytoplankton analysis**

For identification and enumeration of phytoplankton species, surface water samples were taken into 250 ml bottles and fixed with acidic Lugol’s solution (2%) (Throndsen, 1978). Sub-samples (10–50 ml) were allowed to settle in sedimentation chambers for 24–48 h (Utermöhl, 1958). Phytoplankton cells were counted using a Leica DM IL LED inverted microscope equipped with phase contrast optics. Samples were examined at appropriate magnifications (100× to 400×) and placed into taxonomic categories such as diatoms, dinoflagellates and other phytoflagellates. Cell enumeration was generally performed on two or more transects, counting at least 300 cells in each sample and group abundances were calculated as cells per litre.

**HPLC pigment analysis**

The method chosen for this study (Barlow et al., 1993) is a modification of the method given by Mantoura & Llewellyn (1983). According to this procedure, the frozen filters were extracted in 5 ml of 90% 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 filter into a vial and 500 μl of 1 M ammonium acetate was added; and 100 μl was injected into the HPLC Agilent 1100 series system (Agilent Technologies, Hewlett-Packard, Waldbronn, DE).

The HPLC system was calibrated for each of the pigment standards (chlorophyll-a, chlorophyll-b, chlorophyll-c₁ + c₂, peridinin, alloxanthin, 19′-butanoyloxyfucoxanthin, 19′-hexanoyloxyfucoxanthin, zeaxanthin, divinyl chlorophyll-a, fucoxanthin, diadinoxanthin, lutein and β-carotene: DHI LAB, Denmark) and peaks were identified based on their retention times (Figure 2, Table 1). Chromatographic analyses were carried out using a Hewlett-Packard (HP) 1100 equipped with an inline degasser, quaternary pump, autosampler and diode-array detector; data collection and processing of chromatograms were done using the Chemstation software. Pigments were separated on a Thermo Scientific Hypersil MOS-2 C8 (150 mm × 4.6 mm, 3 μm) column. Detection wavelength was set at 440 nm with a 10 nm bandwith; the reference wavelength was 750 nm with a 100 nm bandwith. The flow rate was set at 1.0 ml min⁻¹. The mobile phases were A: 70% methanol plus 30% 1 M ammonium acetate and B:100% methanol. Gradient elution was designed at 25% B, lasted for 1 min and increased to 50% over 1 min, which was applied for 19 min. Elution was then resumed by increase to 100% B over 5 min before programming back to first conditions over 7 min. First conditions were applied for a further 7 min, resulting in a total analysis time of 39 min.

**CHEMTAX analysis**

On the basis of the measured pigment concentrations, the phytoplankton community composition was estimated using the
CHEMTAX software, as described by Mackey \textit{et al.} (1996). CHEMTAX is a matrix-factorization software that uses factor analysis and a steepest descent algorithm to determine the best fit to the data with a given initial ratio matrix of pigment ratios (Mackey \textit{et al.}, 1996). Using an iterative process for a given initial ratio matrix, the software optimizes the pigment ratios for each group and applies the final ratio to the total chlorophyll-\textit{a} in each sample to determine the proportion of chlorophyll-\textit{a} concentration attributed to each phytoplankton group in the community. An important step to correctly estimate the contribution of different algal classes to total chlorophyll-\textit{a} by CHEMTAX is the selection of the correct accessory pigment: chlorophyll-\textit{a} ratios (Henriksen \textit{et al.}, 2002; Rodriguez \textit{et al.}, 2002). Therefore, pigment ratios to be used in CHEMTAX should come from the major phytoplankton species native to the area from which the samples were obtained (Mackey \textit{et al.}, 1996; Lewitus \textit{et al.}, 2005).

To determine the most appropriate input ratios for our measured concentration of marker pigments data set, 60 further pigment ratio tables were generated by multiplying each cell of initial input ratio by a randomly determined factor \textit{F}. Each of the 60 ratio matrices was used and then the best 10\% of results were chosen to calculate the average of the abundance estimates (Wright & Jeffrey, 2006). Besides the partial chlorophyll-\textit{a} attributed to each phytoplankton group, an additional output is a new matrix of pigment: chlorophyll-\textit{a} ratios resulting from the best fit. For each sample, 10 successive CHEMTAX runs were performed using the output pigment: chlorophyll-\textit{a} ratio matrix of each run as input for the consequent run in order to have those ratios stabilize toward their most probable values (Latasa, 2007) (Supplementary Table S1). The three different input ratio matrices of pigment: chlorophyll-\textit{a} were tested and matrices were based on pigment ratios published in the literature for oceanic (Mackey \textit{et al.}, 1996) and estuarine species (Schlüter \textit{et al.}, 2000; Lewitus \textit{et al.}, 2005). The results are output in terms of absolute amounts (\textmu{g} l\textsuperscript{-1}) of chlorophyll-\textit{a} attributed to each phytoplankton group (Supplementary Figure S1), and as a relative amount (percentage) of chlorophyll-\textit{a} (Figure 8) in a sample.

\textit{Data analysis}

The relationships between environmental factors, pigment types and concentrations and phytoplankton abundances were analysed by Pearson’s product-moment correlation coefficients, following transformations to natural logarithms using Statistica 8.0 software. The relationships between phytoplankton cell counts obtained by microscopy and respective pigments were examined by regression analysis.

\textit{Results}

\textit{Hydrography}

During the study period (16 October 2018–11 September 2019), sea surface temperature (SST) showed seasonal fluctuations, varying between 7.2°C (February) and 24.7°C (July) (Figure 3). From November to May, values were consistently below 15°C. Salinity during the sampling period ranged from 8.7 (May, ST3) to 17.4 psu (April, ST1). Salinity values were generally higher at ST1 between October and May, and slightly decreased at ST2 and ST3 (Figure 3). Secchi disc depths decreased significantly from ST1 to ST3 and the highest value was measured as 7.5 m at ST1 (November), with a minimum of 1.1 m at ST3 (January) (Figure 3). DO values showed frequent variations between 5.25 (October, ST3) and 11.81 mg l\textsuperscript{-1} (May, ST2), and were generally high at ST1. The pH values varied between 7.97 (March, ST3) and 9.05 (August, ST3) and showed no pattern over the sampling period (Figure 3).

\textit{Phytoplankton composition}

A total of 80 eukaryotic phytoplankton taxa belonging to eight algal classes were identified in surface water samples collected.
during the study period (Table 2). Forty-three taxa (54%) were diatoms, 29 taxa (36%) were dinoflagellates and eight taxa (10%) were other phytoflagellates, including silicoflagellates, raphidophytes, cryptomonads, chrysophytes, prasinophytes and euglenophytes. The number of diatoms and dinoflagellates, as the major groups, accounted for 90% of the total number of phytoplankton. The most diverse genera were Chaetoceros and Rhizosolenia within diatoms, and Proterocladonidium, Procorcentrum and Tripos within dinoflagellates. The most frequent species observed during the study period were Pseudo-nitzschia spp., Skeletonema spp. and Chaetoceros curvisetus from diatoms; Procorcentrum micans, Triops furca, T. fusus from dinoflagellates; Plagiopsmelis prolonga from cryptomonads and Heterosigma akashiwo from raphidophytes (Table 2).

Total phytoplankton abundance showed seasonal and spatial fluctuations and was relatively low (49 × 10³ to 350 × 10³ cells l⁻¹) between October and March, while it was high (11 × 10³ to 30,000 × 10³ cells l⁻¹) between April and July (Figure 6). The highest cell abundance (~27,500 × 10³ cells l⁻¹) was detected at ST2 in June (Figure 4). 87% of the cell abundances were lower than 10⁶ cells l⁻¹, while 7% were between 10⁵ and 10⁶ cells l⁻¹, and 4% were between 10⁴ and 10⁵ cells l⁻¹. Only 2% of cell abundances were higher than 10⁶ cells l⁻¹ (Table 2).

Group composition based on cell abundance varied between the sampling months of the study period. Diatoms were clearly abundant between April and May, while cryptomonads and raphidophytes were more abundant from June to September at ST2 and ST3 (Figure 5). The average contribution of diatom abundance to total phytoplankton decreased from ST1 to ST3 (41 to 25%), while the average contribution of dinoflagellate and other phytoflagellate abundances increased (59 to 75%) (Figure 6).

**Diatoms**

The majority of phytoplankton taxa (43 taxa) were diatoms. The most diverse genera were Chaetoceros and Rhizosolenia; and the most abundant species were Pseudo-nitzschia spp., Skeletonema spp. and Chaetoceros curvisetus. In general, diatom abundance displayed marked seasonal differences and their highest abundance was observed between April and May in the study area (Figure 5). The maximum diatom abundance (~11,000 × 10³ cells l⁻¹) was found in May, dominated by Skeletonema spp. at ST3 (~10,750 × 10³ cells l⁻¹) (Figure 5). Skeletonema spp. reached ~8100 × 10³ cells l⁻¹ at ST2 in May, as well (Figure 5). Other common diatoms were Pseudo-nitzschia spp. and Chaetoceros curvisetus in the study region with a highest abundance of 520 × 10³ cells l⁻¹ at ST2 in May, as well (Figure 5). The same species reached 34 × 10³ cells l⁻¹ at ST3 in June, as well. Other dinoflagellate species, Procorcentrum scutel-lum and Heterocapsa triquetra reached 17 × 10³ cells l⁻¹ in December and 16 × 10³ cells l⁻¹ in June, respectively (Figure 5).

Dinoflagellates abundance was weakly positively correlated with DO (P < 0.05), while no correlation was found between salinity, temperature and dinoflagellate abundance in the study period (Table 3). The mean annual contribution of dinoflagellates to total phytoplankton abundance was 6.5% (Figure 6).

**Other phytoflagellates**

A total of eight phytoflagellate taxa belonging to six algal classes were observed in surface water collected during this work. Plogioselcis prolomega (Cryptophyceae), Heterosigma akashiwo (Raphidophyceae), Apedinella sp. (Chrysophyceae) and Eutreptiella sp. ( Euglenophyceae) were the most common species in the study area. Plogioselcis prolomega, a bloom-forming cryptomonad, was frequently observed and its highest abundance reached 540 × 10³ cells l⁻¹ at ST3 in August (Figure 5). Cryptomonad abundance was positively correlated with salinity (P < 0.05) and pH (P < 0.01) during the study period (Table 3). The mean annual contribution of cryptomonads to total phytoplankton abundance was 34.6% (Figure 6). Another blooming species was Heterosigma akashiwo, and it was commonly observed from June to September. A bloom of H. akashiwo occurred at ST2 in June with maximum abundance of 27,000 × 10³ cells l⁻¹ (Figure 5). Raphidophyte abundance was positively correlated with temperature (P < 0.05, Table 3). The mean annual contribution of raphidophytes to total phytoplankton abundance was 20.5% (Figure 6). Euglenophytes were generally observed between March and September (Figure 5). The highest abundance of Eutreptiella sp. was found as 432 × 10³ cells l⁻¹ at ST2 and ST3 in April. Euglenophyte abundance was negatively correlated with Secchi depth (P < 0.05) and positively correlated with DO (P < 0.05) during the study period (Table 3). Apedinella sp. appeared only in April and reached 1100 × 10³ cells l⁻¹ at station ST1 and ST2 (Figure 5). Chrysophyte abundance was positively correlated with salinity (P < 0.05, Table 3). The mean annual contributions of chrysophytes, euglenophytes, silicoflagellates and prasinophytes to the total phytoplankton abundance were 4.5, 1.8, 0.6 and 0.1%, respectively (Figure 6).

**Distribution of marker pigments**

Chlorophyll-α and nine other marker pigments were identified in the Golden Horn Estuary (Table 1). Lutein, zeaxanthin and chlorophyll-b were not detected at any time and station. Chlorophyll-α and marker pigment (e.g. fucoxanthin and peridinin) concentrations for all stations and all sampling dates are presented in Figures 4 and 7. Chlorophyll-α concentrations ranged between 0.12 and 19.49 µg l⁻¹ in surface waters during the study period (Figure 4). The highest chlorophyll-α concentrations (19.49 and 14.24 µg l⁻¹) were observed at ST3 and ST2 during July. Chlorophyll-α values were generally lower (<1.5 µg l⁻¹) from October to June, and increased markedly between July and September (Figure 4). In addition to chlorophyll-α, concentrations of two other marker pigments, fucoxanthin and peridinin, being the major markers of diatoms and dinoflagellates, respectively, were identified at the study region. The concentration of fucoxanthin, which was notably high (>0.15 µg l⁻¹) from April to September, was observed to be low (<0.05 µg l⁻¹) between October and March (Figure 7). Its concentration in surface water varied between 0.05 and 3.41 µg l⁻¹. Fucoxanthin concentrations were generally low (<0.98 µg l⁻¹) at ST1, and the maximum fucoxanthin values were measured at ST2 (3.41 µg l⁻¹) in June and at ST3 (2.47 µg l⁻¹) in July, respectively (Figure 7).

Peridinin concentrations were low from October to May (0.06–0.30 µg l⁻¹), and values were consistently below 0.30 µg l⁻¹.
Table 2. List of phytoplankton taxa identified during the study period and the groups of abundance based on the mean cell number

| Taxa                                | Oct. | Nov. | Dec. | Jan. | Feb. | Mar. | Apr-I | Apr-II | May-I | May-II | June | July | Aug. | Sep. |
|-------------------------------------|------|------|------|------|------|------|-------|--------|-------|--------|------|------|------|-----|
| Bacillariophyceae                   |      |      |      |      |      |      |       |        |       |        |      |      |      |     |
| Actinocyclus sp.                    | –    | –    | –    | –    | A    | –    | –     | –      | –     | –      | –    | –    | –    | –   |
| Aulacoseira granulata               | –    | –    | –    | A    | –    | –    | –     | –      | –     | –      | –    | –    | –    | –   |
| Asterionellopsis glacialis          | –    | –    | A    | –    | –    | –    | –     | –      | –     | –      | –    | –    | –    | –   |
| Cerataulina pelagica                | A    | –    | –    | –    | –    | –    | –     | –      | –     | –      | –    | –    | –    | –   |
| Chaetoceros decipiens               | –    | A    | –    | –    | –    | –    | –     | B      | –     | –      | –    | –    | –    | –   |
| Chaetoceros diadema                 | A    | –    | –    | –    | –    | –    | –     | –      | –     | –      | –    | –    | –    | –   |
| Chaetoceros didymus                 | –    | –    | –    | –    | –    | –    | –     | –      | –     | –      | –    | –    | –    | –   |
| Chaetoceros socialis                | –    | –    | –    | –    | –    | –    | –     | –      | –     | –      | –    | –    | –    | –   |
| Chaetoceros tortissimus             | A    | –    | –    | –    | –    | –    | –     | –      | –     | –      | –    | –    | –    | –   |
| Coscinodiscus sp.                   | A    | A    | A    | A    | A    | –    | –     | –      | –     | –      | –    | –    | –    | –   |
| Cylindrotheca closterum             | C    | A    | –    | –    | –    | –    | –     | –      | –     | –      | –    | –    | –    | –   |
| Dactylosolen fragilissimus          | –    | –    | –    | –    | A    | –    | –     | –      | –     | –      | –    | –    | –    | –   |
| Ditylum brightwellii                | –    | –    | A    | A    | –    | –    | –     | –      | –     | –      | –    | –    | –    | –   |
| Guinardia delicatula                | A    | –    | A    | B    | –    | –    | A     | –      | B     | –      | –    | –    | –    | –   |
| Guinardia striata                   | –    | –    | –    | –    | –    | –    | –     | –      | –     | –      | –    | –    | –    | –   |
| Hemiaulus hauckii                   | A    | –    | –    | –    | –    | –    | –     | –      | –     | –      | –    | –    | –    | –   |
| Lauderia annulata                   | A    | –    | –    | –    | –    | –    | –     | –      | –     | –      | –    | –    | –    | –   |
| Leptocylindrus danicus              | –    | –    | B    | A    | B    | A    | –     | –      | –     | –      | –    | –    | –    | –   |
| Leptocylindrus minimus              | A    | –    | –    | –    | B    | –    | –     | –      | –     | –      | –    | –    | –    | –   |
| Licmophora sp.                      | –    | –    | –    | –    | –    | –    | –     | –      | –     | –      | –    | –    | –    | –   |
| Melosira moniligeria                | A    | –    | –    | –    | –    | –    | –     | –      | –     | –      | –    | –    | –    | –   |
| Meuniera membranacea                | –    | –    | –    | –    | A    | –    | –     | –      | –     | –      | –    | –    | –    | –   |
| Navicula sp.                        | A    | –    | –    | –    | –    | –    | –     | –      | –     | –      | –    | –    | –    | –   |
| Nitzschia longissima                 | A    | A    | A    | A    | –    | A    | A     | A      | –     | –      | –    | –    | –    | –   |
| Species                     | Nitzschia sp. | Pleuriscia normanii | Pleuriscia sp. | Proboscia alata | Pseudo-nitzchias sp. | Pseudosolenia calcis-avis | Rhizosolenia hebetata | Rhizosolenia setigera | Skeletonema sp. | Stephanosolenia nitzschioidea | Thalassionema nitzschioidea | Thalassiosira rotula | Dinophyceae |
|----------------------------|---------------|---------------------|---------------|-----------------|---------------------|---------------------------|----------------------|--------------------|----------------|--------------------------------|----------------------------|-------------------------|----------------|
| Dinophysis acuminata       |               |                     |               |                 |                     |                           |                      |                    |                |                               |                            |                         |             |
| Dinophysis acuta           |               |                     |               |                 |                     |                           |                      |                    |                |                               |                            |                         |             |
| Dinophysis caudata         |               |                     |               |                 |                     |                           |                      |                    |                |                               |                            |                         |             |
| Diplopsalis lenticula      |               |                     |               |                 |                     |                           |                      |                    |                |                               |                            |                         |             |
| Gyrodinium sp.             |               |                     |               |                 |                     |                           |                      |                    |                |                               |                            |                         |             |
| Heterocapsa triquetra      |               |                     |               |                 |                     |                           |                      |                    |                |                               |                            |                         |             |
| Katodonium glaucum         |               |                     |               |                 |                     |                           |                      |                    |                |                               |                            |                         |             |
| Noctiluca scintillans      |               |                     |               |                 |                     |                           |                      |                    |                |                               |                            |                         |             |
| Oxytoxum scolopax          |               |                     |               |                 |                     |                           |                      |                    |                |                               |                            |                         |             |
| Phalacroma rotundatum      |               |                     |               |                 |                     |                           |                      |                    |                |                               |                            |                         |             |
| Polykrikos schwartzii      |               |                     |               |                 |                     |                           |                      |                    |                |                               |                            |                         |             |
| Prorocentrum cordatum      |               |                     |               |                 |                     |                           |                      |                    |                |                               |                            |                         |             |
| Prorocentrum compressum    |               |                     |               |                 |                     |                           |                      |                    |                |                               |                            |                         |             |
| Prorocentrum micans        |               |                     |               |                 |                     |                           |                      |                    |                |                               |                            |                         |             |
| Prorocentrum scutellum     |               |                     |               |                 |                     |                           |                      |                    |                |                               |                            |                         |             |
| Protoperidinium bipes      |               |                     |               |                 |                     |                           |                      |                    |                |                               |                            |                         |             |
| Protoperidinium brevipes   |               |                     |               |                 |                     |                           |                      |                    |                |                               |                            |                         |             |
| Protoperidinium conicum    |               |                     |               |                 |                     |                           |                      |                    |                |                               |                            |                         |             |
| Protoperidinium depressum  |               |                     |               |                 |                     |                           |                      |                    |                |                               |                            |                         |             |
| Protoperidinium divergens  |               |                     |               |                 |                     |                           |                      |                    |                |                               |                            |                         |             |

(Continued)
| Taxa                          | Sampling periods |
|------------------------------|------------------|
|                             | Oct. | Nov. | Dec. | Jan. | Feb. | Mar. | Apr-I | Apr-II | May-I | May-II | June | July | Aug. | Sep. |
| **Protoperidinium pellucidum** | –    | –    | –    | –    | –    | –    | –     | –      | –     | –     | –    | –    | –    | A    |
| **Protoperidinium steinii**   | A    | A    | A    | A    | –    | –    | –     | A      | –     | A     | –    | –    | –    | –    |
| **Protoperidinium sp.**       | A    | A    | A    | A    | A    | –    | –     | –      | –     | A     | –    | –    | –    | –    |
| **Scripsiella acuminata**     | –    | –    | –    | –    | –    | –    | –     | –      | A     | B     | C    | –    | –    | A    |
| **Tripos furca**              | A    | A    | A    | A    | A    | –    | –     | A      | –     | A     | B    | A    | A    | A    |
| **Tripos fusus**              | A    | A    | A    | A    | –    | –    | –     | A      | –     | A     | B    | A    | A    | –    |
| **Tripos horridus**           | –    | –    | –    | A    | –    | –    | –     | –      | –     | –     | –    | –    | –    | –    |
| **Tripos lineatus**           | –    | –    | –    | –    | –    | –    | –     | –      | –     | –     | –    | –    | –    | –    |
| **Tripos muelleri**           | –    | A    | –    | –    | –    | A    | A     | –      | –     | A     | –    | –    | –    | –    |
| **Dictyochophyceae**          |      |      |      |      |      |      |       |        |       |       |      |      |      |      |
| **Dictyocha fibula**          | –    | –    | –    | A    | –    | –    | –     | –      | –     | –     | –    | –    | –    | –    |
| **Dictyocha speculum**        | –    | A    | A    | A    | A    | –    | A     | –      | –     | –     | –    | –    | –    | –    |
| **Octactis octonaria**        | –    | A    | A    | A    | –    | –    | –     | –      | –     | –     | –    | –    | –    | –    |
| **Raphidophyceae**            |      |      |      |      |      |      |       |        |       |       |      |      |      |      |
| **Heterosigma akashiwo**      | –    | –    | –    | –    | –    | –    | –     | –      | –     | –     | E    | D    | E    | E    |
| **Cryptophyceae**             |      |      |      |      |      |      |       |        |       |       |      |      |      |      |
| **Plagioselmis prolonga**     | C    | C    | C    | C    | C    | C    | D     | C      | C     | –     | –    | D    | D    | D    |
| **Chrysophyceae**             |      |      |      |      |      |      |       |        |       |       |      |      |      |      |
| **Apedinella sp.**            | –    | –    | –    | –    | –    | –    | –     | E      | C     | –     | –    | –    | –    | –    |
| **Prasinophyceae**            |      |      |      |      |      |      |       |        |       |       |      |      |      |      |
| **Pyramimonas grossii**       | B    | –    | –    | –    | –    | –    | –     | –      | –     | –     | –    | –    | –    | –    |
| **Euglenophyceae**            |      |      |      |      |      |      |       |        |       |       |      |      |      |      |
| **Eutreptiella sp.**          | –    | –    | –    | –    | –    | A    | D     | –      | B     | D     | C    | A    | B    | B    |

The groups of abundance (cells l\(^{-1}\)) refers to the following: A = \(<10^3\); B = \(10^3\)–\(10^4\); C = \(10^4\)–\(10^5\); D = \(10^5\)–\(10^6\); E = \(>10^6\); (–), absent.
Peridinin concentrations were generally low at ST1 (<0.4 μg l⁻¹), with the highest concentration being measured at ST2 (4.66 μg l⁻¹) in June (Figure 7). Alloxanthin was mostly observed between April and July, and its concentration varied between 0.05 and 0.47 μg l⁻¹ (Figure 7). The maximum value was detected at ST2 in June. Chlorophyll-c₁+c₂ was generally observed during the study period and concentrations varied between 0.03 and 0.76 μg l⁻¹. The maximum chlorophyll-c₁+c₂ value was measured at ST2 in June (Figure 7). Diadinoxanthin concentrations changed between 0.09 and 0.36 μg l⁻¹ (Figure 7) and the maximum value was measured at ST2 in June. ß-carotene was mostly detected from October to January, and from July to September, and its concentration ranged between 0.05 and 0.31 μg l⁻¹ (Figure 7). The maximum ß-carotene values were observed at ST2 (0.31 and 0.30 μg l⁻¹) in December and January, respectively. Other accessory pigments, i.e. divinyl chlorophyll-a (0.34–1.43 μg l⁻¹), 19′-butanoyloxyfucoxanthin (0.35–10.75 μg l⁻¹) and 19′-hexanoyloxyfucoxanthin (0.07-0.66 μg l⁻¹), an indicator of prochlorophytes, chrysophytes and prymnesiophytes, were detected but they were not consistently present.

CHEMTAX derived estimations

The CHEMTAX analysis indicated that dinoflagellates were the dominant planktonic algae calculated based on Matrix 1 and Matrix 2 (Figure 8A, B). Dinoflagellates were the most dominant species at all stations except from April to July, representing 60–99% of the phytoplankton composition and contributing on average 62% of total chlorophyll-a in Matrix 1 (Figure 8A). Cryptomonads were identified as the second dominant algal group, contributing on average 35% to total chlorophyll-a and diatoms contributed on average 3% to total phytoplankton composition. The average contribution of dinoflagellate and cryptomonad abundances to total phytoplankton showed minor variations from ST1 to ST3. The calculation based on Matrix 2 showed that dinoflagellates were the dominant taxa at all stations except from April to July, accounting for 61–100% of the phytoplankton composition and contributing on average 62% of total chlorophyll-a (Figure 8B). Diatoms and cryptomonads contributed on average 30.3 and 7.7% to total chlorophyll-a, respectively. The average contribution of dinoflagellate abundance to total phytoplankton increased from ST1 to ST3 (59 to 67%), while the average contribution of diatom abundance decreased (34 to 25%).

The results based on the above two matrices (Matrix 1 and Matrix 2) substantially overestimated dinoflagellates but considerably underestimated diatoms (Figure 8A, B). During the estimation process, it was determined that as the shared pigments of diatoms and dinoflagellates, the increase or decrease in diadinoxanthin and chlorophyll-c₁+c₂ to chlorophyll-a ratio could affect the estimation of diatom composition, leading to the underestimation of diatoms and overestimation of dinoflagellates.
Based on the above results, we reduced the ratio of fucoxanthin and diadinoxanthin of diatoms in Matrix 1 and Matrix 2, and increased the ratio of chlorophyll-c1 + c2 of diatoms. Final adjustments were conducted to obtain Matrix 3, whose results were more similar than Matrix 1 and Matrix 2 to those from microscopic observations (Figure 8C). CHEMTAX analysis based on Matrix 3 identified diatoms as the dominant algal group, except in April at ST1 and ST2, and in June at ST3. Diatoms represented 68–100% of the phytoplankton composition with a contribution on average of 86% of total chlorophyll-a (Figure 8C). However, dinoflagellates and cryptomonads contributed on average 30.3% and 7.7% to total chlorophyll-a, respectively. The contribution of diatoms to the phytoplankton community showed higher values at ST1 and ST3 (88% and 87%) when compared with ST2 (83%). Dinoflagellates were more abundant at ST2 (11%), than ST1 and ST3 (8.1 and 8.5%). The relative distribution of cryptomonads increased from ST1 (3.8%) to ST3 (5%).

**Discussion**

The validity of using pigment signatures detected by HPLC to estimate phytoplankton group composition was tested for the Mediterranean Sea (Thyssen et al., 2011; Yücel, 2017) and Black Sea (Ediger et al., 2006; Eker-Develi et al., 2012; Agirbas et al., 2015, 2017). Although there have been many studies on phytoplankton in the Golden Horn Estuary (Tas et al., 2009; Tas & Okus, 2011; Tas & Yılmaz, 2015; Dursun & Tas, 2019; Tas, 2019) based on microscopic examination, the phytoplankton group composition had not yet been evaluated by HPLC pigment analysis in this region.

In this study, the highest abundances in total phytoplankton were observed between April and September, as shown by microscopic analysis. The abundance pattern displayed seasonal variations in this study that are similar to previous studies (Tas et al., 2009; Tas & Yılmaz, 2015; Dursun & Tas, 2019). Phytoplankton studies performed in estuaries show that environmental factors such as salinity, temperature, Secchi depth and adaptation to the environmental conditions play a major role in seasonal variations of phytoplankton in terms of diversity and abundance (Burić et al., 2007; Barbosa et al., 2010; Jasprica et al., 2012). Similar relationships were demonstrated in previous studies in the GHE by Tas et al. (2009, 2016) and Dursun & Tas (2019), but no clear relationships were found between the environmental factors and total phytoplankton abundance measured.

Fig. 5. Spatio-temporal variations in abundance of phytoplankton groups during the study period (dashed lines show the lowest limit of bloom density).
in this study. However, it is known that phytoplankton abundance is increased in coastal areas and estuarine ecosystems by eutrophication (Smith et al., 1999). As noted in previous studies in the GHE (Tas et al., 2009; Tas & Okus, 2011; Tas & Yilmaz, 2015), nutrient inputs might cause an increase in phytoplankton abundance in the GHE, particularly in summer and spring. The number of phytoplankton taxa observed during this study was similar to that of Dursun & Tas (2019), but lower than previous studies of the GHE by Tas et al. (2009) and Tas & Yilmaz (2015). The contribution of diatoms and dinoflagellates to the total number of species was higher (90%), and that of other phytoflagellates was relatively lower (10%) than previous studies performed by Dursun & Tas (2019) in the GHE and by Jasprica et al. (2012) in the Eastern Adriatic estuary (Neretva River). This inconsistency between studies was probably due to the differences in the total number of samples, sampling periods and frequencies. Burić et al. (2007) stated for the Zrmanja, Adriatic Sea that diatoms dominated in spring, while dinoflagellates and other phytoflagellates dominated in summer. Our results were generally consistent with Burić et al. (2007) and this indicates that phytoplankton group composition in the GHE may change rapidly depending on environmental conditions.

The highest phytoplankton abundance was found at ST2 in June, although some higher abundances were observed at all stations particularly in May. Furthermore, chlorophyll-α values were generally observed to be higher from July to September at all stations and the maximum chlorophyll-α value (19.50 μg L⁻¹) was measured at ST3 in July. In contrast to the study performed by Silva et al. (2008), who found a good relationship between total phytoplankton abundance and HPLC derived chlorophyll-α concentrations in Lisbon Bay, Portugal. The results of this study showed no relation between chlorophyll-α concentrations detected by HPLC and total phytoplankton abundance. The same conclusion was reported by Pérez et al. (2006) and this situation may be caused by the constitution of the high portion of phytoplankton by picoplanktonic species which could not be identified by microscopy. Thus, all phytoplankton groups could not be identified by microscopic analysis in the study area. Flow cytometry may provide...
additional information regarding the cell size distribution of the plankton community. In particular, the picoplankton component can be studied in great detail (Olson et al., 1990; Campbell & Vaulot, 1993; Veldhuis & Kraay, 2000). In addition to this, estimation of the abundance of the total phytoplankton community based on chlorophyll-a only can be unreliable because of the algal cells’ adaptation strategy to varying light levels of changing their pigment content (Everitt et al., 1990; Veldhuis & Kraay, 1990).

Higher divinyl chlorophyll-a values (0.34–1.43 μg l⁻¹) were measured from July to September (Figure 4) and these findings may indicate the existence of prochlorophytes, which are photosynthetic picoplankton. Gibb et al. (2000) stated that higher divinyl chlorophyll-a values were detected when the sea surface temperature (SST) values were greater than 15°C and our findings were consistent (SST >23°C, from July to September; Figure 3) with the results of that study. However, in this study, lutein,
zeaxanthin and chlorophyll-\(b\) pigments, which were commonly stated as the markers of the picoplanktonic species (Eker-Develi et al., 2012; Agirbas et al., 2015), were not detected. However, not all picoplanktonic groups (for example, \textit{Prochlorococcus} spp.) carry lutein, zeaxanthin and chlorophyll-\(b\) markers. This was highlighted by Veldhuis & Kraay (2004) who showed that \textit{Prochlorococcus} spp. has only divinyl chlorophyll-\(a\) pigment instead of chlorophyll-\(a\) and does not carry lutein and zeaxanthin.

Comparative studies generally show a good relationship between microscopy and HPLC analysis for diatom species (especially larger ones), but the correlation is lower for dinoflagellates, raphidophytes and crysophytes due to the shared marker pigments (Zapata et al., 2004; Eker-Develi et al., 2012; Agirbas et al., 2015). Comparison of fucoxanthin vs diatom abundance indicates that the relationship was not found in this study. It has been reported by Agirbas et al. (2017) for the Black Sea and by Seaone et al. (2011) for the Bay of Biscay (Basque coast, northern Spain) that diatoms were the most abundant microplanktonic group in summer, with the highest diatom abundances being observed during spring (April and May) in this study, similar to Totti’s (2000) observations for the middle Adriatic. Moreover, the concentration of fucoxanthin was found to be maximum in June and July in this study which differs from the data presented by Anstotegui et al. (2003) when the maximum fucoxanthin concentration was found in late winter in a Spanish estuary. This inconsistency might be the result of the peak of fucoxanthin possibly originating from nanoplanktonic non-diatom species in the study region (Krivokapić et al., 2018). All these findings suggest that fucoxanthin as a marker pigment did not work well for the diatom community and demonstrates the need for microscopic confirmation to fully characterize peak events in the GHE.

Generally, low concentrations of peridinin were detected with the HPLC analysis, in correspondence to the low abundance of dinoflagellates detected by microscopic analysis. The highest dinoflagellate abundance (214 × 10\(^3\) cells l\(^{-1}\)) was observed in June, in accordance with the highest peridinin concentration (4.66 \(\mu\)g l\(^{-1}\)) at the same period. The maximum dinoflagellate abundance was dominated by \textit{Scrippsiella acuminata} in the study region, which was indicated as the carrier of peridinin by several studies (Zhang et al., 2000; Wong & Wong, 2009; Islabao et al., 2016). Thus, comparison of marker pigment values and microscopic cell counts indicates that a significant relationship between peridinin and dinoflagellate abundance was observed in the study region (Table 3, \(N = 42, P < 0.01, r = 0.819\)).

Cryptomonads were one of the most frequent groups in the phytoplankton community from October to April in the GHE, as reported in other estuarine and coastal waters (Brunet & Lizon, 2003; Carreto et al., 2003; Garibotti et al., 2003; Seaone et al., 2006). However, alloxanthin, which indicates the presence of cryptocryptomonads (Jeffrey & Vesk, 1997), was mostly observed between April and July. The maximum value was detected in June and no correlation was found between alloxanthin and cryptomonad abundance (Table 3). Moreover, dinoflagellate abundance was highly correlated with alloxanthin values (Table 3, \(N = 42, P < 0.01, r = 0.729\)). A possible explanation of this situation might be the presence of the dinoflagellate genus \textit{Dinophysis}, which contains alloxanthin as a major pigment and shows a cryptomonad-like signature as described before by Meyer-Harms & Pollehne (1998) for the Baltic Sea, and by Schnepf & Elbrächter (1988) and Zapata et al. (2012) under laboratory conditions. Several species of \textit{Dinophysis} have been identified in the GHE, reaching densities of almost 1 × 10\(^3\) cells l\(^{-1}\), and the same densities were also reported for Nervion River Estuary, Spain and Japanese coastal waters (Nishitani et al., 2005; Laza-Martinez et al., 2007). Another possible explanation for this situation might be the existence of relatively large dinoflagellate species in the GHE, such as \textit{Gyrodinium spirale}, reaching densities of almost 1.5 × 10\(^3\) cells l\(^{-1}\), which also contain high proportions of alloxanthin (Kong et al., 2012). As a consequence, cryptomonad cell counts by microscopic analysis might not match exactly with alloxanthin values, which can show a wider distribution (Gieskes & Kraay, 1983; Rodriguez et al., 2002).

Dense algal blooms, except for a bloom-forming raphophyte \textit{Heterosigma akashiwo}, were not observed in the GHE during the sampling period. The first bloom of \textit{H. akashiwo} was reported by Tas & Yilmaz (2015) and after that by Dursun et al. (2016) almost at the same time in this study area. During this study, a bloom of \textit{H. akashiwo} occurred at ST2 in June. In previous studies, the values of major pigments of \textit{H. akashiwo} showed some variations. Generally, the major pigment was found to be fucoxanthin (Jeffrey & Vesk, 1997; Okumura et al., 2012), while others have
additionally reported zeaxanthin and violoxanthin (Fiksdahl et al., 1984; Rodríguez et al., 2006). However, the most abundant pigments were detected as fucoxanthin and chlorophyll-c2 + c3 for H. akashiwo in this study. Also, comparison of pigment values and microscopic cell counts indicates that a significant relationship between fucoxanthin and raphidophyte abundance was observed in the study region (Table 3, N = 42, P < 0.01, r = 0.558). Moreover, our results were supported by Butrón et al. (2012) for the Bay of Biscay and by Li et al. (2003) under culture conditions. As a derivative of fucoxanthin, 19′-butanoyloxyfucoxanthin is presented mainly in chrysophytes (Wright & Jeffrey, 2006; Kong et al., 2012). Exceptionally, a highly significant relationship (Table 3, N = 42, P < 0.01, r = 0.896) was detected between H. akashiwo abundance and 19′-butanoyloxyfucoxanthin in this study period. The highest abundance of H. akashiwo was observed at ST2 in June. Moreover, 19′-butanoyloxyfucoxanthin concentrations were only observed in June, when the raphidophytes were dominant in the GHE and maximum concentration (0.66 μg L−1) was measured at the same time. These findings indicate that 19′-butanoyloxyfucoxanthin might be the main marker pigment, while chlorophyll-c2 + c3 and fucoxanthin were accessory pigments of H. akashiwo in the GHE.

19′-hexanoyloxyfucoxanthin was reported as a major pigment signature of Emiliania huxleyi, a bloom-forming prymnesiosephyte, by Stolte et al. (2000) for all Atlantic strains of this species and by Ediger et al. (2006) for the south-western Black Sea. The pigment composition of the chrysophyte, Apedinella sp., was characterized by the predominance of 19′-hexanoyloxyfucoxanthin in this study, as reported by Daugbjerg & Henriksen (2001) under laboratory conditions. Apedinella sp. appeared only in April and reached maximum abundance at ST1 and ST2. Moreover, 19′-hexanoyloxyfucoxanthin concentrations were commonly observed in April, when chrysophytes were dominant in the GHE and maximum concentration (0.66 μg L−1) was measured at the same time. A significant relationship (Table 3, N = 42, P < 0.01, r = 0.762) was detected between Apedinella sp. abundance and 19′-hexanoyloxyfucoxanthin.

HPLC pigment analyses and CHEMTAX have been used previously as a valuable monitoring tool in estuaries for determining the absolute or relative contributions of major phytoplankton classes as determined by variations in pigment concentrations (Ansotegui et al., 2001; Paerl et al., 2003; Lewitus et al., 2005). Moreover, there is evidence in the literature that CHEMTAX analysis results were generally consistent with microscopic observations (Wright et al., 1996; Havskum et al., 2004; Llewellyn et al., 2005). However, in recent years, it has been found that one algal species cannot be accurately determined by one specific pigment and different algal groups can share the same pigments, thus affecting the accuracy of the CHEMTAX method (Zapata et al., 2012). There are species that carry ‘unambiguous’ marker pigments of a different phytoplankton group, e.g. fucoxanthin- and alloxyanthin-containing dinoflagellates or only fucoxanthin-containing prymnesiosephytes (Jeffrey & Veski, 1997; Irgiesen et al., 2004; Zapata et al., 2004). An important step to correctly estimate the contribution of different algal classes to chlorophyll-a by CHEMTAX is the selection of the appropriate accessory pigment: chlorophyll-a ratios (Henriksen et al., 2002; Rodríguez et al., 2002). Therefore, pigment ratios to be used in CHEMTAX should come from the major phytoplankton species native to the area from which the samples were obtained (Mackey et al., 1996; Lewitus et al., 2005). Three different input ratio matrices of pigment: chlorophyll-a were tested in this study and matrices were based on pigment ratios published in the literature for oceanic (Matrix 1, Mackey et al., 1996) and estuarine species (Matrix 2, Schlüter et al., 2000 and Matrix 3, Lewitus et al., 2005). As can be seen in the estimations of Matrix 1, Mackey et al.’s (1996) original matrix has required modifications for adapting to different regions – the application of CHEMTAX to estuaries needs to take a typological approach, that is, calibration with species representing the study region. It is not surprising that application of CHEMTAX calibrated with oceanic isolates to estuarine systems can lead to inaccurate predictions of phytoplankton group composition, as originally cautioned by Mackey et al. (1996). However, increase or decrease of the initial ratios of pigments of diatoms and dinoflagellates leads to a relatively accurate assessment for diatom composition (Matrix 3) but diatoms still tend to be overestimated. Moreover, CHEMTAX prediction of cryptomonads was also exceptionally poor in all initial ratio matrices (Matrices 1, 2 and 3). This is not surprising, because this group was derived from one species (Plagioselmis prolonga) and pigment composition can vary within this class.

In addition to correlations between HPLC pigment analysis and microscopy, phytoplankton classification using CHEMTAX can provide qualitative and quantitative data on the composition of phytoplankton, particularly in complex estuarine ecosystems. Gameiro et al. (2007) reported on HPLC derived pigment concentrations and CHEMTAX enabled identification of diatoms, dinoflagellates, cryptomonads, chlorophytes, euglenophytes, prasinophytes, cyanobacteria and haptophytes in the Tagus River estuary, Portugal, and highlighted the reliability of HPLC derived pigment analysis as a tool for the assessment of phytoplankton variability related to community diversity. In this study, it was found that the composition of phytoplankton community could not be accurately distinguished based on ratio of pigment from the literature, and the calculated results were significantly different from microscopic examination.

The marker pigments detected in this study constitute a high proportion of accessory pigments (fucoxanthin, peridinin, alloxyanthin, etc.) and represent, as far as we know, the first detailed description of the distribution patterns of these marker pigments in the Golden Horn Estuary. HPLC derived marker pigment analysis is a useful method for assessment of some phytoplankton groups quantitatively including fragile forms. This method has an advantage over fluorescence microscopy or flow cytometry, where measurements are made on the whole composition from picoplanktonic size to large colonies in a short time. On the other hand, based on the above results, it is suggested that CHEMTAX method cannot accurately characterize the phytoplankton community and needs to be applied carefully to evaluate phytoplankton composition of the Golden Horn Estuary. This indicates that CHEMTAX analysis should always be accompanied by a microscopic analysis due to the ambiguous character of some marker pigments. In future years, combined use of existing methods and also comparison with phytoplankton cell volumes instead of abundances, will be the most reliable way to monitor variations and dynamics of phytoplankton communities. Future investigations, with more frequent sampling periods, including all environmental variables (for example nutrients, light transparency), are needed to establish a clear understanding of HPLC derived phytoplankton pigment signatures in complex estuarine ecosystems.

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