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# Estimation of phytoplankton biomass using HPLC pigment analysis in the southwestern Black Sea

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**Abstract**

The phytoplankton population of the southwestern Black Sea in May 2001 was studied by taxonomic analysis using microscopic examination and by pigment analyses using high-performance liquid chromatography (HPLC). Pigment data, which identified phytoplankton assemblages dominated by dinoflagellates, diatoms and coccolithophores in May 2001, were compared to phytoplankton cell counts and biomass. There were significant ($p<0.002$–$0.01$, $r=0.56$–$0.67$) relationships between the taxon-specific pigment concentrations and the taxon-specific cell numbers during this sampling period. The ratios of chlorophyll-*a* to the dominant accessory pigments calculated by multiple linear regressions were 1.2 (chlorophyll-*a*: peridinin) in dinoflagellates, 1.8 (chlorophyll-*a*: fucoxanthin) in diatoms, and 2.66 (chlorophyll-*a*: 19′-hexonoyloxyfucoxanthin) in coccolithophores. HPLC-determined chlorophyll-*a* biomass correlated well with the sum of the group-specific pigment biomass ($p<0.001$, $r^2=0.95$). The phytoplankton assemblage as revealed by the microscopic and HPLC analyses was thus made up of common Black Sea groups showing that HPLC pigment analysis can be used to quantify phytoplankton assemblages in the Black Sea based on simple ratios.


*Keywords:* Phytoplankton composition; Microscopy; Photosynthetic pigments; Chemotaxonomy; Black Sea

## 1. Introduction

Phytoplankton communities play a crucial role in marine ecosystems, affecting nutrient cycling, the structure and efficiency of the food web, and the flux of particles to deep waters (Smith and Sakshaug, 1990). Thus, estimation of both phytoplankton composition and biomass has a major importance to understanding the structure and dynamics of pelagic ecosystems. Microscopic examination, the classical method to study phytoplankton, involves identification and estimation of cell abundance and biomass (Utermohl, 1958; Booth, 1993). Alternatively, analysis of water samples using HPLC allows phytoplankton characterization by chemotaxonomic study of photosynthetic pigments. Total phytoplankton standing stock is estimated as chlorophyll-*a* concentration, and algal classes are identified from the presence of marker pigments. Furthermore, the biomass of each

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taxon is calculated as a proportion of total chl-*a* using chl-*a*:marker pigment ratios (Wright and Jeffrey, 1987; Millie et al., 1993).

The characteristic signatures of pigments for identification of phytoplankton groups have been summarized in various papers (Jeffrey and Hallegraeff, 1987; Gieskes, 1991; Millie et al., 1993), but taxonomic identification from pigment signatures is not yet a straightforward task. The sole use of pigment signatures without concurrent microscopic verification sometimes could even be misleading (Gieskes, 1991; Millie et al., 1993). On the other hand, microscopic studies are often plagued by problems such as poor fixation or small size, making identification difficult. Thus a combination of both approaches has been recommended (Hallegraeff, 1981; Jeffrey and Hallegraeff, 1987; Tester et al., 1995). However, recent field studies have tended to rely mostly on pigment chemo-taxonomy using the HPLC analysis mainly because of shorter analysis time (Everitt et al., 1990; Barlow, et al., 1993; Letelier et al., 1993; Tester et al., 1995; Roy et al., 1996; Peeken, 1997).

The Black Sea provides an ideal environment to study pigment characterization in the euphotic zone. Two distinct periods of enhanced primary production, a spring bloom of diatoms and a fall bloom of the coccolithophore *Emiliania huxleyi*, characterize the annual cycle of primary productivity in the oxic portion of the euphotic zone (Honjo et al., 1987). Recently, additional summer blooms have frequently been observed in both the coastal and open waters (Hay et al., 1990; Sur et al., 1996). Production of algal pigments and their removal by oxic metabolic processes are restricted to the upper 60–100 m of the euphotic zone by the presence of permanent $H_2S$ chemocline at greater depths (Murray et al., 1989). Repeta and Simpson (1991) showed that the distribution of pigments were significantly different in the oxic and chemocline layers.

The upper layer of the Black Sea is dominated by a meandering Rim-Current system cyclonically encircling the basin, creating a cyclonic gyre within the eastern and western parts of the interior, and additional anticyclonic eddies along the Rim Current (Oguz et al., 1993). The Black Sea has a very steep and narrow continental shelf along its southwestern coast.

We report here the comparative distribution of pigments with respect to the abundance and biomass of phytoplankton groups sampled from the surface to the base of the euphotic zone in the southwestern Black Sea during a R/V *Knorr* cruise in May 2001. Samples from different regions of the southwestern Black Sea, e.g., continental shelf and shelf break regions on the Rim Current, transient layer between the main gyre, and the Rim current and in the gyre, enabled us to evaluate phytoplankton pigment composition in the entire southwestern Black Sea.

Analyses of both optical properties and pigment composition provide important information for future remote-sensing studies to differentiate major phytoplankton pigments and consequently major taxonomic groups of algae. Therefore, studies relating to the pigment characterization of phytoplankton groups will have significant importance for the Black Sea region for both for rapid phytoplankton quantification and improvement of remote-sensing algorithms.

The main aim of this study was to evaluate the utility of HPLC-based pigment analyses for detecting phytoplankton composition of the southwestern Black Sea. A specific objective was to establish whether pigment ratios could be used to quantify phytoplankton communities in this sea. A successful application of the HPLC method for these objectives might have significant consequences (e.g., in monitoring studies) for evaluating rapid and large-scale (e.g., mapping) phytoplankton characteristics in the Black Sea.

## 2. Material methods

Sampling was performed between 24 and 31 May 2001 (Table 1) at six stations in the southwestern Black Sea (Fig. 1). The sampling locations were selected by examining real-time CTD measurements and by considering the previously known circulation of the basin (Oguz et al., 1993). Hydrographic, transmissometry and fluorometry measurements were performed using a Seabird CTD. All core cruise data are available on the web site at www.ocean.washington.edu/cruises/Knorr2001.

Water samples for pigment characterization and microscopic analyses were taken from the surface and 36%, 22%, 8%, 1–3% and 0.1% surface light depths (Table 1) using 10-l Niskin bottles mounted on a Rosette. At some stations additional pigment samples were obtained at the fluorescence maximum after examination of the in situ fluorometer profiles. At each depth, a 1-l sub-sample was preserved with buffered formaldehyde (2.5% final concentration)

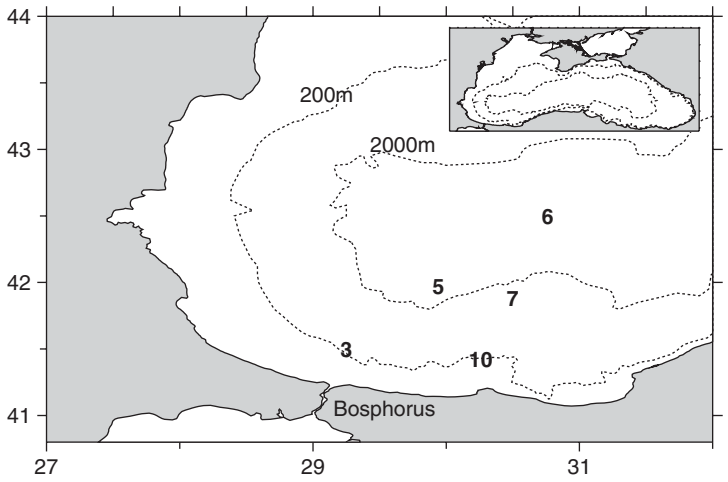


Fig. 1. Station locations (Station 10 Rim Current, which is not shown here, is very close to Station 10 Shelf, see Table 1).

for microscopic analysis after the cruise. Phytoplankton was enumerated using a modified Utermohl's technique, counting samples after sedimentation using a phase contrast inverted microscope (Hasle, 1978). A sedimentation method was used that enabled us to evaluate the contribution of small phytoplankton (as small as 6 μm). Each cell identified was individually measured for calculating its biomass from different number of morphometric measurements (i.e. diameter, length and width; Senichkina, 1986a, b; Hillebrand et al., 1999), assuming a volume of 1 μm equals to 1 pg. An additional 1–2 l, of water were filtered through a 25-mm Whatman GF/F filter and immediately frozen by placing the filter in liquid nitrogen until the HPLC analysis.

In the laboratory, the frozen filters were extracted in 5 ml 90% HPLC-grade acetone, ultrasonicated for 30 s and centrifuged to remove cellular debris. The method chosen for this study (Barlow et al., 1993) is a modification of the reverse-phase method described in Mantoura and Llewellyn (1983). Pigment analysis was carried out with a HPLC system (Agilent 1100 Products) using a CI8 column. The mobile phase consisted of a binary eluant system with solvent A (80% methanol and 20% 1 M ammonium acetate) and solvent B (60% methanol and 40% acetone). A linear gradient at a flow rate of 1 ml/min was run from 0% to 100% B for 10 min and followed by an isocratic stop at 100% B for 7.5 min. A second gradient of 2.5 min was used to return to initial condition of 100% solvent A. A 500-μl aliquot of sample was mixed with 500 μl of 1 M ammonium acetate and then a 100 μl from this mixture were injected into the HPLC. The HPLC system was calibrated for each pigment with commercial standards (chlorophyll-*a*, *b:* Sigma Chemicals; carotenoids and chlorophyll *c:* VKI Water Quality Institute, Denmark). Pigments were identified by injecting samples of phytoplankton reference cultures whose pigment composition has been documented in the literature (Mantoura and Llewellyn, 1983; Barlow et al., 1993; Jeffrey et al., 1997).

## 3. Results

### 3.1. Hydrographic data

The vertical distributions of temperature, salinity and density at the locations we studied are given in Fig. 2. The permanent pycnocline formed at intermediate depths (i.e. 100–175 m) is principally controlled by a salinity gradient due to continuous intrusion of saltier Mediterranean waters into the SW Black Sea via the Bosphorus undercurrent. The cold water formed during winter mixing (seen at ~50 m) was topped by warmer water at the surface, during May 2001. The surface temperature and salinity ranged between 15 and 18 °C and 17–18‰, respectively (Fig. 2 and Table 1). The lower salinity values were generally observed in the rim current and peripheral regions. During May 2001, waters of the cold intermediate layer (CIL, having typical

Table 1
Some oceanographic parameters of the investigated area

| Date | Station (region) | Latitude | Longitude | Sampling depths for phytoplankton (m) | Sampling depths for pigment (m) | Total depth of water column (m) | Thickness of EZ (m) | SST (°C) | SSS (‰) |
|---|---|---|---|---|---|---|---|---|---|
| 30 May | St. 10 (shelf) | 41 25.45N | 030 15.79E | 1, 11, 18, 35 | 1, 6, 11, 35 | 100 | 18 | 17.87 | 16.88 |
| 31 May | St. l0D (rim current) | 41 27.46N | 030 15.74E | — | 1, 4, 5, 7, 11, 20, 35, 49 | 410 | 18 | 18.05 | 17.58 |
| 24 May | St.3 (rim current) | 41 30.00N | 029 15.02E | 1, 6, 11, 14, 18 | 1, 6, 11, 14, 30, 60 | 320 | 16 | 15.78 | 17.87 |
| 25 May | St.5 (periphery) | 41 57.94N | 029 56.64E | 1, 9, 20, 25, 35 | 1, 4, 13, 20, 25, 35 | 2100 | 20 | 15.34 | 17.29 |
| 29 May | St.7 (periphery) | 41 52.86N | 030 30.09E | 1, 10, 15, 20 | 1, 10, 15, 20, 67 | 300 | 20 | 17.25 | 17.16 |
| 26 May | St.6 (gyre) | 42 30.03N | 030 45.91E | 1, 13, 35 | 1, 13, 35 | 2000 | 20 | 15.22 | 18.07 |

Euphotic zone (EZ) thickness is defined surface light intensity reaches. SST: sea surface temperature, SSS: sea surface salinity.

temperatures of 6–7 °C) were warmer than usual (~8 °C, Fig. 2). Below the CIL, the temperature gradually rose from 8 to 9 °C. The permanent halocline and lower boundary of oxic waters separating the deep water from the surface waters was situated at around $\sigma_\theta = 16.2$, below which the density increased with depth (Fig. 2). The EZ (euphotic zone depth, 1% of the surface light) extended only to depths of 16–20 m (Table 1).

### 3.2. Phytoplankton

Based on either abundance or biomass dominance, there were three distinct groups of phytoplankton represented in the phytoplankton samples during May 2001. Of the 62 species identified, 81% were dinoflagellates, 18% diatoms and 1% coccolithophores. The contribution of these groups to the total phytoplankton (both in terms of abundance and biomass) in the surface and water column is shown in Fig. 3A–D. *Exuviella cordata*, *Gyrodinium fusiforme,* and *Gymnodinium splendens* were the main dinoflagellates species, the contribution of these three species to the total biomass and abundance ranging from 0.18–97% and 2–23%, respectively. *Cerataulina bergonii* and *Pseudonitschia delicatissima* were the main diatoms and their contribution as a percentage of the total biomass and abundance ranged between 0.07–0.44 and 1–5.5. *Emiliania huxleyi* was the only coccolithophore species in the study area, and its contribution varied between 0.1–11% in terms of biomass and 9–81% in terms of abundance.

The contribution of dinoflagellates (as a group) to the total biomass and abundance in the surface layer varied between 97–99% and 41–87%, respectively, in the surface layer (Fig. 3A and B). Diatom contribution to the total phytoplankton was 0.2–2.6% and 0.4–9.2% in terms of biomass and abundance, respectively (Fig. 3A and B). The coccolithophorid (entirely made up by *Emiliania huxleyi)* were the third important group in the area. Although their contribution to the total biomass was low (2%) (Fig. 3A), it made up as high as 57% of the total abundance in the surface layer (Fig. 3B).

The contribution of these three groups to the total biomass and abundance of phytoplankton in the water column varied between 89–98% and 15–63% for dinoflagellates, 0.6–0.9% and 4.6–9% for diatoms, and 0.2–9.5% and 30–80% for coccolithophores, respectively (Fig. 3C and D).

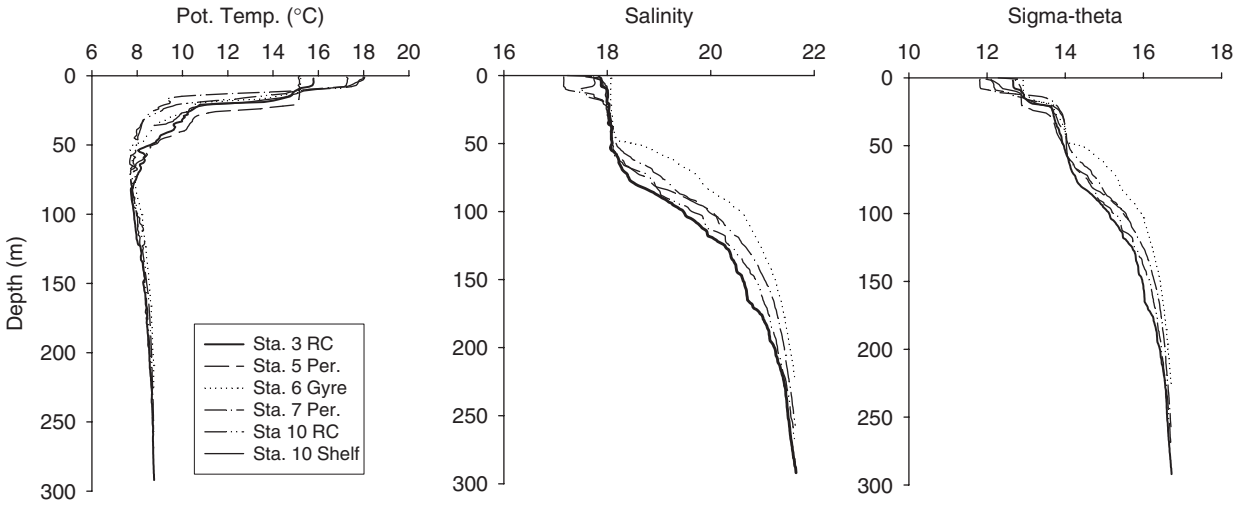


Fig. 2. Vertical profiles of hydrographic parameters.

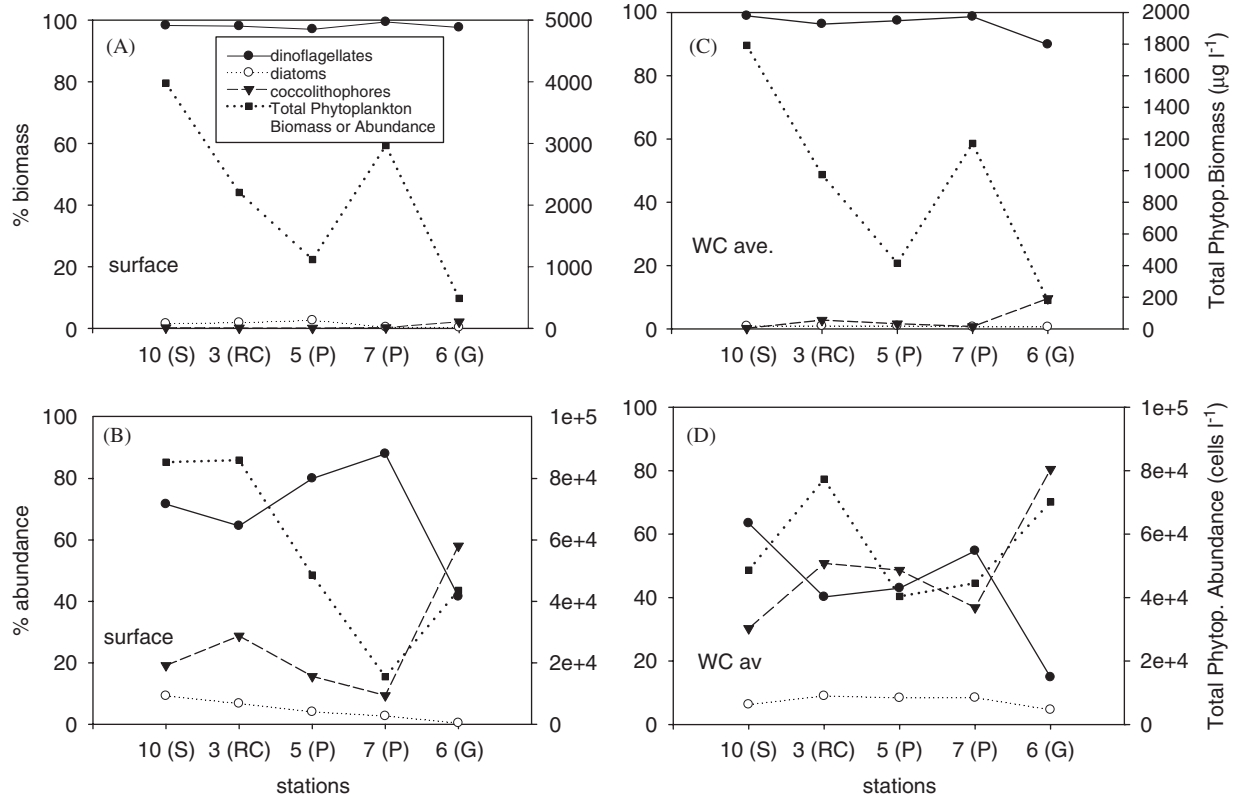


Fig. 3. (A) Contribution of different phytoplankton groups to the total phytoplankton biomass in the surface waters. (B) Contribution of different phytoplankton groups to the total phytoplankton abundance in the surface waters. (C) Contribution of different phytoplankton groups to the total phytoplankton biomass in the water-column (WC ave.: water-column average). (D) Contribution of different phytoplankton groups to the total phytoplankton abundance in the water-column (WC ave.: water-column average).

Surface and water-column averaged biomass values were highest in the shelf station (St. l0S) (Fig. 3A–C). Maximum abundances were observed at stations 10 and 3 for the surface waters and at the Rim current (sta. 3) and gyre region (sta. 6) for the water column (Fig. 3D).

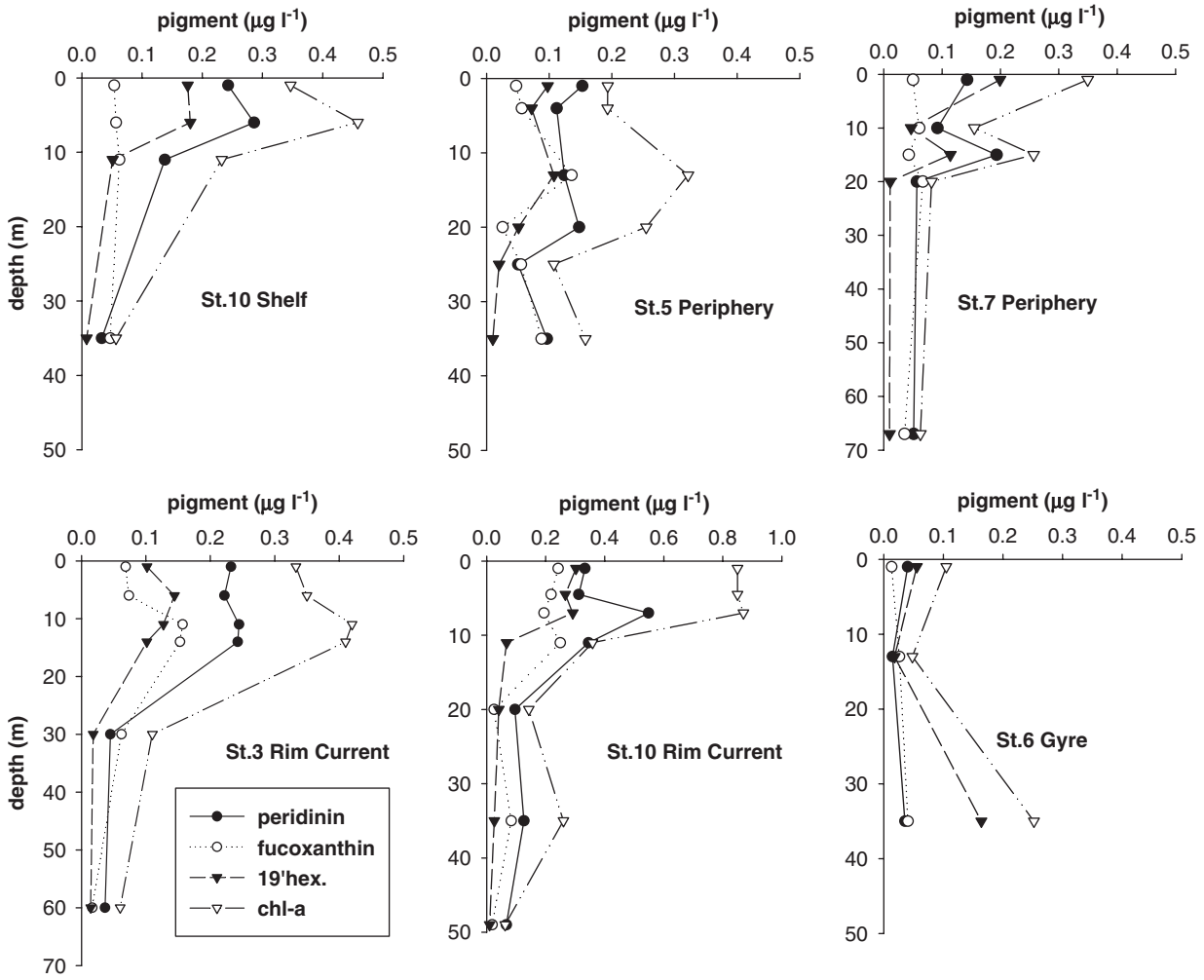


Fig. 4. Vertical profiles of pigment concentrations (for the clarity chl-a value was divided by 2).

### 3.3. Pigment

Similar to taxonomic composition, the major carotenoid (i.e. peridinin) was found to be due to dinoflagellates in May 2001 samples both spatially and vertically (Fig. 4 and Table 2). There was a reasonably strong correlation ($r = 0.56$; $p < 0.01$, using simple linear regression) between the biomass (as well as the abundance) of dinoflagellates and peridinin concentrations of samples (all combined) (Fig. 5). The concentration of fucoxanthin, which is the marker of diatoms (Wright and Jeffrey, 1987), was observed to be low in our study area (Fig. 4). As a result, there was no significant correlation between diatom biomass and fucoxanthin concentrations; however, a good correlation was observed between diatom cell number and fucoxanthin concentrations ($r$: 0.66 $p < 0.003$) (Fig. 5). There was also a very significant relationship between *Emiliania huxleyi* biomass and 19′-hexonoyloxyfucoxanthin (19′-hex.) concentrations ($r$: 0.67; $p < 0.002$).

Concentrations of chlorophyll-*a* and other group-specific pigments for the euphotic zone (both average and integrated values) are shown in Table 2. Concentrations of chlorophyll-*a* ranged between 0.21 and 1.7 $\mu l^{-1}$ in the surface layers (Table 2). Average chlorophyll-*a* concentrations in the euphotic zone varied from 0.15 to 1.23 $\mu g\, l^{-1}$. Maximum values for the surface and water column were observed in station 10, which was located in the Rim Current region (Table 2). Chlorophyll-*a* concentrations showed decreased from the Rim current

Table 2
Euphotic zone properties of the stations visited in May 2001

| Station (region) | Surface Chl-*a* ($\mu g\,l^{-1}$) | Thickness of EZ (m) | EZ integrated ($mg\,m^{-2}$) | | | | | | EZ average ($\mu g\,l^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Chl $c1+c2$ | Per. | Fuc. | 19′-hex. | Diadino. | Chl-*a* | Chl $c1+c2$ | Per. | Fuc. | 19′-hex. | Diadino. | Chl-*a* |
| St. 10 (shelf) | 0.69 | 18 | 2.04 | 2.38 | 0.57 | 1.47 | 1.56 | 7.48 | 0.12 | 0.24 | 0.11 | 0.12 | 0.14 | 0.72 |
| St. 10 (rim current) | 1.7 | 18 | 5.7 | 5.98 | 3.44 | 2.90 | 4.41 | 19.72 | 0.33 | 0.33 | 0.18 | 0.19 | 0.29 | 1.23 |
| St.3 (rim current) | 0.66 | 16 | 2.38 | 3.03 | 1.40 | 1.64 | 2.04 | 9.78 | 0.18 | 0.22 | 0.057 | 0.136 | 0.16 | 0.69 |
| St.5 (periphery) | 0.39 | 20 | 2.28 | 2.41 | 1.58 | 1.62 | 2.31 | 9.85 | 0.12 | 0.13 | 0.07 | 0.08 | 0.12 | 0.48 |
| St.7 (periphery) | 0.7 | 20 | 3.53 | 2.40 | 1.03 | 1.79 | 1.72 | 8.31 | 0.17 | 0.12 | 0.055 | 0.12 | 0.09 | 0.42 |
| St.6 (gyre) | 0.21 | 20 | 0.26 | 0.33 | 0.24 | 0.44 | 0.32 | 1.84 | 0.02 | 0.027 | 0.02 | 0.037 | 0.06 | 0.15 |

Station names are as presented in Figu thickness is defined as the depth which 1% of surface light intensity reaches. Chl $c1+c2$: chlorophyll *c* Fuc.: fucoxanthin, 19′-hex.:19′-hexonoyloxyfucoxanthin, Diadino.:diadinoxanthin, Chl-*a*: Chl.

to the central western gyre (Table 2). In addition to chl-*a*, concentrations of three other accessory pigments: peridinin, fucoxanthin and 19′-hex. being the major carotenoid of dinoflagellates, diatoms and coccolithophorids, respectively, were quantified from the stations studied (Table 2 and Fig. 4). In addition to these pigments, chlorophyll $c1+c2$ and diadinoxanthin concentrations also were quantified and given in table. Profiles of chlorophyll-*a* and accessory pigments for all stations are presented in Fig. 4 to illustrate their vertical variation in the region. Maximum chlorophyll-*a* concentrations were recorded at the first 15 m of the water column (except in Sta. 6) and there was usually a subsurface maximum. Vertical distributions of accessory carotenoids in the water column followed the general pattern of chlorophyll-*a*. Peridinin was the dominant carotenoid throughout the water column at all stations, except station 6 (Fig. 4). Its average and depth integrated values ranged from 0.03–0.33 $\mu l^{-1}$ and 0.33–5.98 $mg\,m^{-2}$, respectively (Table 2). The concentrations of 19′-hex. and fucoxanthin, ranged between 0.04–0.19 and 0.02–0.18 $\mu g\,l^{-1}$ in the water column. Average concentrations in the euphotic zone varied between 0.15–1.23 $\mu l^{-1}$ for chl-*a*, 0.03–0.33 $\mu l^{-1}$ for peridinin, 0.02–0.18 $\mu l^{-1}$ for fucoxanthin, and 0.04–0.19 $\mu l^{-1}$ for 19′-hex. The integrated chlorophyll concentration varied from 1.8 to 19.7 $mg\,m^{-2}$, with maximum concentrations observed in the rim current region.

In order to estimate the contribution of the three selected algal classes to the total phytoplankton biomass, multiple regression analysis was used to determine the ratio of chlorophyll to accessory pigment for each group. Conversion of pigment data into relative quantities of various algal groups was done by estimating the contribution by various pigment markers characteristic of different algal groups to the total chl-*a*. Thus, since dinoflagellates have a distinctive chl-*a*: peridinin ratio, the peridinin concentration observed was multiplied by this ratio and expressed relative to total chl-*a* to give their relative contributions to algal biomass and similar procedures was repeated for diatoms and coccolithophores. Thus, the total chl-*a* = constant + *a*(markerl) + *b*(marker 2) + … where *a*,*b* …. represent various chl-*a*: marker *x* ratios. This approach was used successfully in earlier studies (Gieskes and Kraay 1983; Gieskes et al., 1988; Everitt et al., 1990; Letelier et al., 1993; Roy et al., 1996 and Tester et al., 1995).

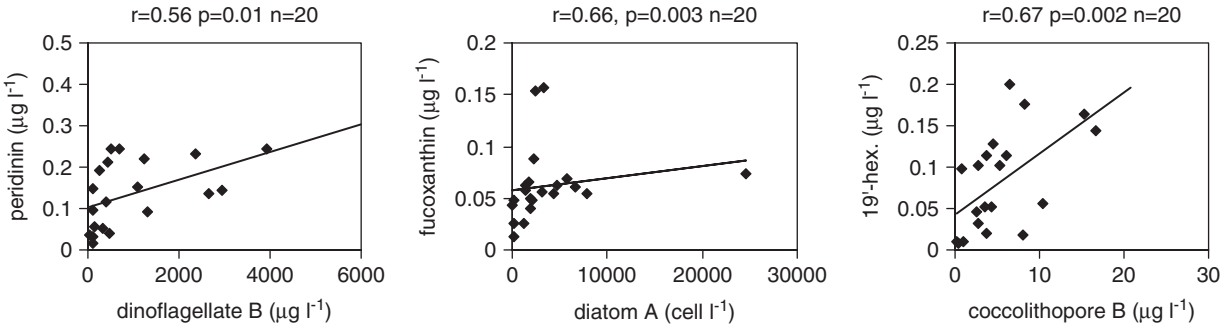


Fig. 5. Linear relationship between phytoplankton biomass (B) or abundance (A) and their marker pigments.

Our analysis produced the following equation:

$$\text{Chl-}a = -0.02 + (1.2 * \text{peridinin}) + (1.87 * \text{fucoxanthin}) + (2.66 * 19'\text{-hex.}).$$

The $r^2$ for this regression was 0.95, and all the regression coefficients were significant ($p < 0.001$).

The vertical and depth-integrated spatial distribution of chl-*a* calculated from pigment concentrations are shown in Fig. 6 and 7, respectively. Pigment-calculated (estimated) chlorophyll-*a* values were very close to those obtained from direct chl-*a* measurements. The only exception was the value at 35 m depth at Sta. 6 (Fig. 6), where *Emiliania huxleyi* reached unexpectedly high biomass ($15.25\,\mu l^{-1}$) and abundance ($135\,000\,cell\,l^{-1}$).

## 4. Discussion

The validity of using marker carotenoids and accessory chlorophylls measured by HPLC to estimate phytoplankton composition was demonstrated for the North Atlantic (Barlow et al., 1993), northern central Pacific (Letelier et al., 1993), Mediterranean Sea (Claustre, 1994 and Vidussi et al., 2000), St. Lawrance estuary (Roy et al., 1996), and Newport River estuary (Tester et al., 1995). There has not been any previous study of such pigment characterization of phytoplankton in the Black Sea. On the contrary, there have been numerous studies of phytoplankton in the Black Sea based on microscopic analysis (Moncheva et al., 1998, 2001; Eker et al., 1999; Eker-Develi and Kideys, 2003). Except those from estuaries and coastal regions near rivers, in all of these studies three phytoplankton groups (i.e. dinoflagellates, diatoms and the coccolithophorid *Emiliania hwcleyi)* dominated the phytoplankton (both in terms of abundance and biomass) similar to the present study. Although the total number of phytoplankton taxa reported from the basin is rather high (about 750 including freshwater and estuarine species, Moncheva et al., 2001), the dominating species are limited in number (around 10–15). Most of the dominant species (*Prorocentrum cordatum*, *Gyrodinium fusiforme*, *Gymnodinium splendens*, *Pseudonitzchia pseudodelicatissima*, *Emiliania hwcleyi*) found here have been reported to be in high abundance in previous studies (e.g., see Table 3 in Eker-Develi and Kideys, 2003).

To determine the quantitative contribution of the different algal groups to the total chlorophyll-*a* measured, multiple linear regression analysis can be used to determine conversion factors (Gieskes et al., 1988; Tester et al., 1995). The relative contribution of different groups to the total chlorophyll-*a* in this study was evaluated using multiple correlation analysis between diagnostic pigment concentrations and chlorophyll-*a*, peridinin, fucoxanthin and 19′-hex. They were used as indicators of dinoflagellates, diatoms and haptophytes (from which coccolithophorids being the only group present in the study area). All of these phytoplankton groups and their marker pigments were observed at all stations in this study (Figs. 3 and 4). The use of pigments as algal markers depends on how specific the marker pigments are for the various algal groups. Although most diatoms have the same pigment composition, coccolithophorids may have more than one specific pigment.

Chl-*a* biomass of diatoms was determined from fucoxanthin concentration, which is an ambiguous marker as it is also present in haptophytes (Wright and Jeffrey, 1987; Jeffrey and Vesk, 1997). Therefore, relative contributions from diatoms and haptophytes can sometimes be difficult to calculate.

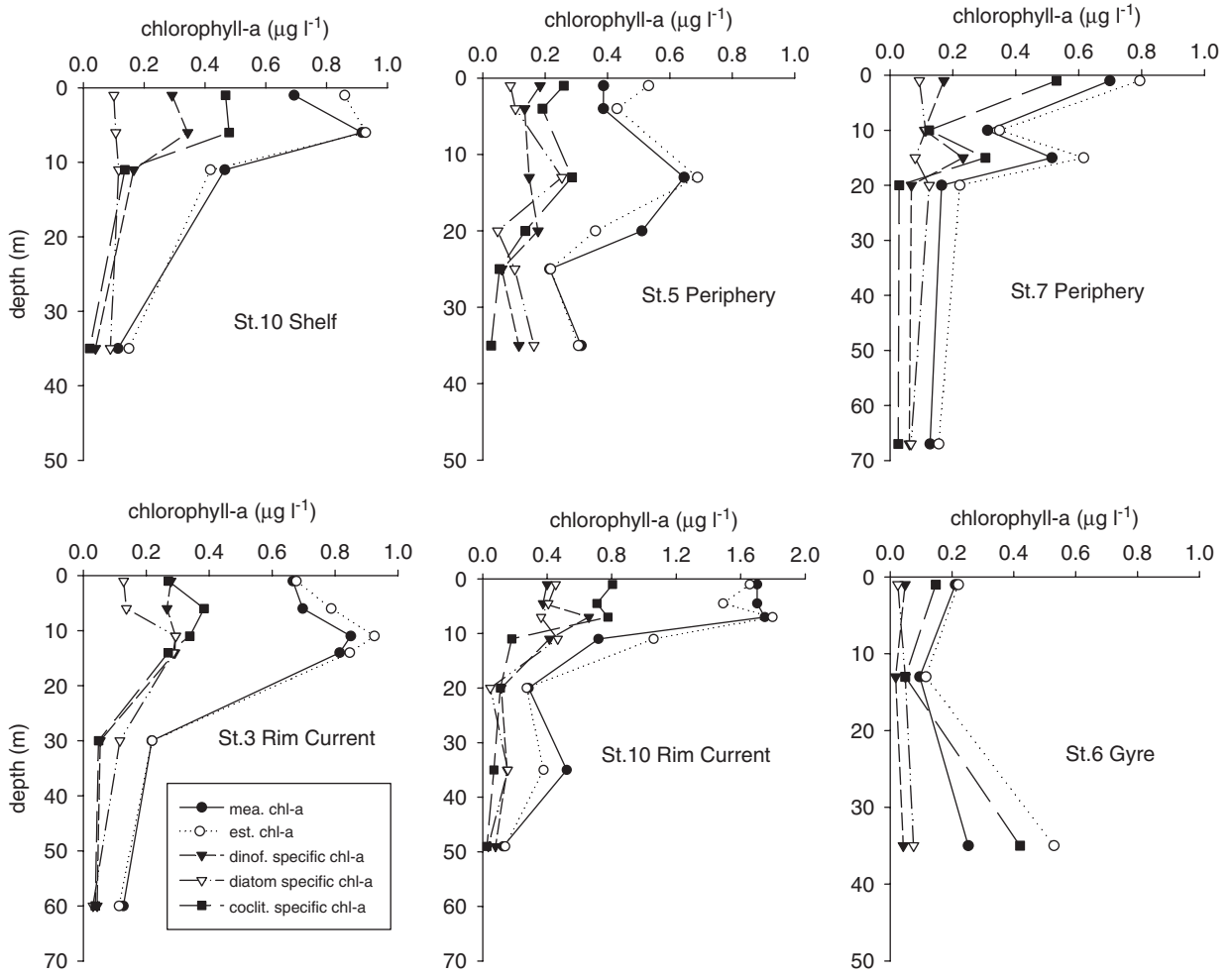


Fig. 6. Vertical distribution of Chlorophyll-*a* (measured), the group specific chl-*a* (estimated) and the sum of the group specific chl-*a* (estimated) in the study area.

In the present study, only *Emiliania huxleyi* was present from haptophyte group. The pigment content of *Emiliania huxleyi* has been studied from a wide range of localities around the world, and fucoxanthin was consistently observed as the dominant carotenoid in all coastal strains, while 19′-hex. was dominant in oceanic strains (Mantoura and Barlow, 1994; cited in Barlow et al., 1998). Stolte et al. (2000) mentioned that the 19′-hex. was the major light harvesting carotenoid in all Atlantic strains. Since the quantity of *Emiliania huxleyi* correlated well with 19′-hex. ($p<0.002$, Fig. 5) but not with fucoxantin, we suggest that the major marker of this coccolithophorid in the southwestern Black Sea is 19′-hex., which is similar to Atlantic strains. There was a significant correlation between

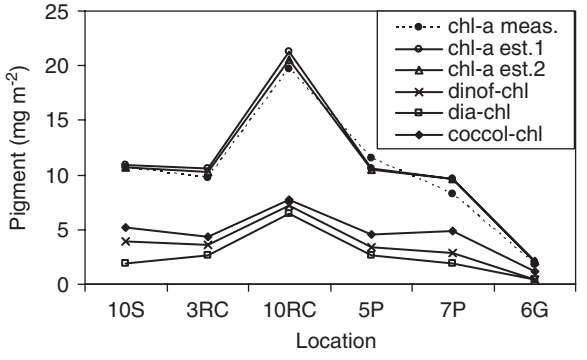


Fig. 7. Depth integrated chlorophyll-*a* (mg m$^{-2}$) (measured), depth integrated group specific chl-*a* (estimated) and sum of the group specific integrated chl-*a* along the stations (chl-*a* est.l: conversion factor was 2.66; chl-*a* est.2: conversion factor was 3.12 see Table 3).

Table 3
Chlorophyll-accessory pigment ratios (conversion factor). 19′-hex.: 19′-hexonoyloxyfucoxanthin conversion factors are calculated from multiple regression analysis in this study

| Algal class | Marker pigment | Literature range (w/w) | Conversion factor (w/w) including 35m in st. 6 | Conversion factor (w/w) excluding 35 m in st. 6 | References |
|---|---|---|---|---|---|
| Dinophyceae | Peridinin | 1.2–2.5 | 1.2 | 0.88 | Vesk and Jeffrey (1977), Burkill et al. (1987), Tester et al. (1995) |
| Bacillariophyceae | Fucoxanthin | 1.1–2.3 | 1.87 | 1.82 | Vesk and Jeffrey (1977), Tester et al. (1995), Gieskes et al. (1988) |
| Coccolithophores | 19'-hex. | 1–3.2 | 2.66 | 3.12 | Gieskes et al. (1988) |

diatom cell number and fucoxanthin concentrations (Fig. 5). This confirms that diatoms are the most important carrier of fucoxanthin in the samples from our field work in May 2001. However, there was not any significant correlation between diatom biomass with fucoxanthin concentrations. This is probably due to low fucoxanthin concentrations as well as quantification difficulties of broken large diatom cells (e.g., *Kfiizosolenk* spp.). It is worth noting that despite the fact that coccolithophores had almost always a lower biomass than dinoflagellates, due to their high pigment:chl-*a* (Table 3) and chl-*a*:cell carbon ratios, they were the major contributors to total chl-*a* in this study. This probably explains the lack of correlation between total phytoplankton biomass and chl-*a* in some previous studies (e.g., Cullen, 1982).

Multiple linear regression was found to be a suitable analysis for predicting total chlorophyll-*a* biomass throughout the May cruise. The coefficients obtained for the three different marker pigments in this study agreed relatively well with previously published values in the case of peridinin, fucoxanthin and 19′-hex. (Table 3). The relationship between marker pigment and chlorophyll-*a* varies for the different algal groups and also among species of the groups (Millie et al., 1993). In addition, the relationships can be light- and nutrient-dependent (Humphrey, 1983; Buma et al., 1991). In order to assess the validity of estimated conversion factors, estimated group-specific chlorophyll-*a* values were compared with the directly measured chlorophyll-*a* (Fig. 6) and depth-integrated chlorophyll-*a* concentrations in this study (Fig. 7). Results generally show a good agreement. The only exception was at 35 m at station 6, where estimated and directly measured chl-*a* concentrations were quite different (Fig. 6). At this depth, there was heavy dominance of *Emiliania huxleyi* (97% of total phytoplankton abundance). This depth was well below the euphotic zone (20 m corresponded to the 1% of surface light) and the reason for the high quantity of coccolithophorids here is not clear. However, it could be due to the nitrate versus phosphate requirements. It is well known that *Emiliania huxleyi* becomes more abundant at high N:P ratio (Mozetic et al., 1998). Unfortunately there were no nutrient measurements around this depth at this station (Sta. 6). This station was in the center of the western cyclonic gyre where the most intense upwelling occurred. It is interesting to note that the samples with the next highest abundance values (i.e. sta. 3 and 5) in the euphotic zone were coincident with high N:P ratios. The deviation observed in estimated chl-*a* value at this depth must be due to increased pigment levels with respect to chl-*a* as a response to decreased light intensity. When we recalculated the multiple regression equation by excluding this depth, the new ratio produced was 3.12 for the 19′-hex. (Table 3), which should be a better value for the populations of *Emiliania huxleyi* dwelling within the euphotic zone. However, this did not change the overall chl-*a* estimation (giving the same correlation coefficient value of 0.95) (Fig. 7).

Our microscopic analysis revealed changes in cell abundance, cell size, and diatom-specific composition within the area, which is highly valuable information for ecological research that cannot be obtained by pigment analysis. Thus, the choice between using the methods individually or together depends partly on the degree of taxonomic detail

needed. The agreement that we found between microscopy and chemotaxonomy suggests that pigment analysis can be used with confidence for characterization of the phytoplankton community in the southern Black Sea. This is an important finding since the pigment methodology may have a much wider utilization for a rapid and spatially large analyses with relatively much less effort compared to microscopic studies. However, still more research is needed to assess the correct application of the chemotaxonomical approach to natural phytoplankton assemblages in the study area.

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