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http://dx.doi.org/10.12681/mms.301

To cite this article:

VASILIU, D., BOICENCO, L., GOMOIU, M., LAZAR, L., & MIHAILEV, M. (2012). Temporal variation of surface
chlorophyll a in the Romanian near-shore waters. *Mediterranean Marine Science*, 13(2), 213-226. doi: http://dx.doi.org/10.12681/mms.301
Temporal variation of surface chlorophyll $a$ in the Romanian near-shore waters

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Received: 6 April 2012; Accepted: 26 June 2012; Published on line: 7 September 2012

Abstract

Chlorophyll $a$ (Chl $a$) dynamics in the near-shore waters of the NW Black Sea was investigated between 2002 and 2010 in the Mamaia Bay (north of Constanta, Romania) in relation to some physical-chemical parameters. Chl $a$ ranged from values below detection limit (0.17 µg l$^{-1}$) to 76.13 µg l$^{-1}$, and showed large temporal variability (CV = 142.3%), strongly related to the Danube’s discharges, meteorological conditions, and anthropogenic pressures.

Seasonally, Chl $a$ showed a winter/early spring maximum, sometimes followed by a stronger one in April/early May, closely linked to the Danube’s higher discharges in spring. After significantly lower concentrations in late spring/early summer, Chl $a$ exhibited its strongest maximum in summer (July–August), followed by another one in autumn (late September–October).

Interannual variation of Chl $a$ seems to be controlled by the hydrometeorological conditions in summer. Thus, the highest annual Chl $a$ means were observed in 2006 (8.56 ± 8.35 µg l$^{-1}$) and 2010 (9.20 ± 11.72 µg l$^{-1}$), when, also, the summer Chl $a$ concentrations were maximal due to the large riverine discharges. The lowest annual Chl $a$ mean was observed in 2004 (4.57 ± 9.81 µg l$^{-1}$), closely linked to minimal summer Chl $a$ resulted from a strong P limitation during summertime.

Keywords: Chlorophyll $a$, shallow coastal waters, NW Black Sea, the Danube’s discharges.

Introduction

Over the last few decades, the Romanian near-shore waters have experienced significant changes caused by important modifications of the nutrient regime of the Danube’s catchment area, seriously altering phytoplankton structure, both qualitatively and quantitatively (Lancelot et al., 2002; Bodeanu et al., 2002).

Phytoplankton community in the Romanian coastal waters has been subject to numerous studies during the last 50 years (Skolka, 1960; Bodeanu, 1987–1988; Bodeanu & Roban, 1989; Miheea, 1997; Bodeanu et al., 2004). One of the most discussed areas was the Mamaia Bay, located north of Constanta, where the anthropogenic impact related to urbanization, intensive recreational, and port activities (Bodeanu, 1993), as well as the marked influence of the Danube’s discharges which have led to enhancement of primary productivity.

Despite the numerous studies on inter- and intra-annual variations of both quantitative and qualitative composition of the phytoplankton community in the Romanian coastal waters, chlorophyll $a$ (Chl $a$), the main indicator of phytoplankton biomass and primary productivity level, has been rather sporadically discussed (Skolka, 1968; Bologa, 1977, 1978; Bologa et al., 1980, 1985; Miheea, 1988, 1997; Velikova et al., 2005; Vasiliu et al., 2010).

Relatively rare during 1960–1970, the chlorophyll $a$ measurements have been intensified since the mid-1980s, once it was included in the list of biological parameters monitored within the Romanian Monitoring Program (Miheea, 1988, 1997), and later in the list of indicators of eutrophication within the Water Framework Directive and Marine Strategy Directive.

Apart from in situ measurements, since the late 1970s, Chl $a$ datasets have been obtained from satellite images provided by CZCS (1978–1986), SeaWiFS (1997–2010), Aqua-MODIS and MERIS (both from 2002 to the present). In the open Black Sea, satellite observations have provided accurate Chl $a$ data (Blondeau et al., 2004), successfully used for reliable spatial and temporal patterns of distribution of primary productivity (Nezlin et al., 1997; Kopelevich et al., 2002; Yuneev et al., 2002; Cokacar et al., 2004). On the contrary, in the coastal waters, remotely sensed Chl $a$ is a less accurate indicator of actual chlorophyll concentrations than in the open sea due to increased turbidity and atmospheric effects. Hence, in the Romanian shelf waters, despite important information on the potential relationships with

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climate forcing and nutrient enrichment, satellite-derived Chl a seems to be significantly overestimated due to the large optical complexity of this ecosystem (Oguz & Edinger, 2006). The latter implies the necessity for regular in situ monitoring of Chl a to gain a better understanding of the Black Sea coastal ecosystem’s functioning under the conditions of highly variable environmental factors.

The main aim of this work is to investigate chlorophyll a variability, both interannual and seasonal, in the Romanian near-shore waters, and to identify the main factors controlling its dynamics.

Materials and Methods

Investigations were conducted in the Mamaia Bay, north of Constanta (Fig. 1), from January 2002 to December 2010 within the Romanian Monitoring Program framework carried out by the National Institute for Marine Research and Development “Grigore Antipa”, Constanta (NIMRD). The sampling station Cazino-Mamaia (bottom depth of 1.5 m) was chosen for the analysis of surface chlorophyll temporal variability, as this site is one of the long-term research stations in the NW Black Sea. It is considered representative for the Romanian coastal waters dynamics because it has been subjected to pronounced influence of both the Danube’s discharges and anthropogenic pressures.

Water samples for physical-chemical, chlorophyll, and phytoplankton analysis were collected weekly from the surface layer. Temperature was measured using a reversible thermometer. Salinity was measured by the titration method of Mohr-Knudsen (Grasshoff et al., 1999), immediately after collection of samples. Nutrient samples were stored frozen at -20 °C until their subsequent analysis in the laboratory. PO4-P, SiO4-Si, NO3-N and NH4-N were determined according to standard methods for seawater analysis (Grasshoff et al., 1999).

For Chl a measurements, variable volumes (0.5–2 l) of seawater were filtered through Whatman glass-fiber filters GF/F (nominal pore size 0.7 μm), immediately after sampling. The filters were preserved frozen (~20 °C) until subsequent analysis, which was performed within a month. Pigments were extracted with 90% acetone and measured by spectrophotometry, concentrations being calculated by using the SCORE-UNESCO equations (UNESCO, 1966).

Phytoplankton samples were preserved with 4% formaldehyde seawater buffered solution. Qualitative and quantitative phytoplankton determinations were performed by employing prior sedimentation method (Morozova-Vodianiskaya, 1954) to concentrate the samples. After 20 days, the samples were decanted down to 15–20 cm3. The taxonomic identification of species and counting of cells in a 0.1 ml fraction of each sample was carried out under ZEISS inverted microscopes, using a 40x objective lens for nanoplankton (less than 15–20 μm) and 10x or 20x for larger cells. The cell biovolume was calculated using relevant morphometric measurements of phytoplankton cells and approximation by corresponding geometric shapes after EDLER (1979).

Meteorological data (air temperature, wind direction, and speed) were kindly provided by ANM (National Meteorological Administration). It is important to note that the authors treated March as the winter month and September as the summer month.

Monthly and seasonally averaged data were visualized by the Ocean Data View 4 software (Schlitzer, 2006) and STATISTICA 8 software, respectively. The non-parametric Kruskal-Wallis (K-W) test was employed to test the differences between years and seasons for a given variable, because of the non-normal distribution of most of the variables considered. The K-W test is a non-parametric equivalent to one-way ANOVA by ranks, testing the null hypothesis that three or more groups all come from the same distribution. The Mann-Whitney significance test was applied to analyze the differences between every pair of groups. In order to investigate the factors influencing the temporal variability of Chl a, the relationships between physical-chemical and biological variables were tested after log transformation of all variables that did not comply with the assumption of normality. All statistical analyses were carried out to the significance level of α = 0.05.

Results

Temporal variability of surface Chl a in the Romanian near-shore waters was examined in relation to sea surface temperature (SST), salinity (SSS), inorganic nutrients and total phytoplankton biomass, and numerical abundance (Tot. B and Tot. A, respectively). The ranges, means, standard deviations, and medians of physical-chemical, chlorophyll a and phytoplankton data for the period 2002–2010 are presented in Table 1.

Fig. 1: Location of the sampling station Cazino-Mamaia.
Sea surface temperature (SST) and salinity (SSS)

SST showed no significant statistical differences between the studied years (K-W, \( p = 0.581 \)). Over the investigated period, SST showed minima in January–February, while maxima were observed in July–August (Fig. 5). The highest SSTs were observed in the summer of 2010 (25.26 ± 3.18 °C; Fig. 2), closely linked to high air temperatures recorded during July–August (25.66 ± 2.43 °C). The lowest summer SSTs were measured in 2003 and 2004 (20.96 ± 3.71 °C and 20.04 ± 3.54 °C, respectively; Fig. 2), when pronounced upwelling events occurred in the June–July period (prevailing southerly and southeasterly winds) (Mihailov et al., 2012). In the cold season, the highest SSTs (6.83 ± 1.64 °C) were measured in the warm winter of 2007 (air temperature of 5.33 ± 3.42 °C), while the lowest (1.56 ± 1.64 °C) were in the coldest winter of 2003 (air temperature of 0.48 ± 4.16 °C) (Fig. 2).

Table 1. Summary descriptive statistics for physical-chemical variables, Chl a, phytoplankton numerical abundance, and biomass during the period 2002–2010.

| Parameter       | No. samples | Min.  | Max.  | Mean  | St. dev. | Median |
|-----------------|-------------|-------|-------|-------|----------|--------|
| SST, °C         | 438         | -0.80 | 29.30 | 13.30 | 7.55     | 13.30  |
| SSS, psu        | 438         | 6.36  | 19.42 | 14.60 | 2.35     | 15.10  |
| PO4-P, µM       | 438         | 0.01  | 3.40  | 0.42  | 0.41     | 0.31   |
| SiO4-Si, µM     | 438         | 0.50  | 62.60 | 13.51 | 9.41     | 11.00  |
| NO3-N, µM       | 438         | 0.95  | 45.86 | 8.65  | 6.67     | 6.82   |
| NH4-N, µM       | 438         | 0.18  | 64.30 | 6.88  | 5.24     | 5.81   |
| Chl a, µg·l⁻¹   | 438         | 0.17  | 76.13 | 6.59  | 9.38     | 3.78   |
| Tot. B, g·m⁻³   | 438         | 0.002 | 219.4 | 3.55  | 16.22    | 0.44   |
| Tot. A, 10⁶·cells·l⁻¹ | 438   | 0.006 | 50.22 | 1.38  | 3.81     | 0.32   |

SSS exhibited large temporal variability closely linked to the Danube’s flow fluctuation and meteorological conditions. The K-W tests applied for variable SSS revealed statistically significant differences both between years (\( p < 0.0001 \)) and seasons (\( p = 0.0001 \)). The Mann-Whitney tests showed significantly lower SSSs in 2005, 2006, 2008, 2009, and 2010 compared with 2003, 2004, and 2007 (Table 2), while in terms of seasonal variation, lower SSSs were measured in spring and summer (Table 3). The lowest seasonal SSSs were observed in the summers of 2005 (12.95 ± 2.64 psu), 2006 (12.79 ± 3.75 psu) and 2010 (11.64 ± 2.14 psu, minimum for the studied period), and spring of 2009 (12.20 ± 2.4 psu) (Fig. 2).

Nutrients

Nutrient regime was characterized by a large variability (Table 1) mainly due to the Danube’s discharge fluctuations, water masses circulation, benthic nutrient recycling, and anthropogenic pressures. The K-W tests applied for the variables phosphate (\( p = 0.01 \)), silicate (\( p = 0.0001 \)), nitrate (\( p < 0.0001 \)), and ammonia (\( p < 0.0001 \)) revealed statistically significant differences between years. Phosphate, silicate, and ammonia were recorded in significantly higher concentrations in 2010 compared with most of the other investigated years, while nitrate showed higher concentrations in 2005 and 2008. It is worth pointing out significantly lower concentrations of phosphate and ammonia in 2004, and nitrate in 2009 and 2010 (Table 2).

In terms of seasonal variation, statistically significant differences were found for all nutrients analyzed (K-W, \( p < 0.0001 \), for PO4-P; K-W, \( p < 0.0001 \) for SiO4-Si; K-W, \( p < 0.0001 \) for NO3-N; K-W, \( p = 0.001 \) for NH4-N). Thus, phosphate recorded significantly higher concentrations in winter and autumn, silicate in winter, nitrate in spring, and ammonia in autumn (Table 3).
Table 2. Mann-Whitney significance tests between years in terms of Chl a, Tot. A, SSS and nutrients.

|        | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 |
|--------|------|------|------|------|------|------|------|------|------|
| Chl a  | 0.014|      |      |      |      |      |      |      |      |
| Tot. A |      |      |      |      |      |      |      |      |      |
| SSS    |      |      |      |      |      |      |      |      |      |
| PO₄-P  | <    | <    | <    | <    | <    | <    | <    | <    | <    |
| SiO₄-Si| <    | <    | <    | <    | <    | <    | <    | <    | <    |
| NO₃-N  |      |      |      |      |      |      |      |      |      |
| NH₄-N  |      |      |      |      |      |      |      |      |      |

Upper right values represent the level of significance ($p$); the absence of $p$ values shows not significant differences between years. Lower left symbols compare years in the first column with years in the first row.
Chlorophyll a, total phytoplankton numerical abundance and biomass (Tot. A and Tot. B, respectively)

Surface Chl a showed large temporal variability (CV = 142.3%), ranging from values below detection limit (0.17 µg l⁻¹) to 76.13 µg l⁻¹ during the studied period (Table 1). The K-W test applied for variable Chl a revealed statistically significant differences between years (p = 0.001). Further, the Mann-Whitney tests showed significantly higher Chl a in 2006 when compared with the rest of the investigated years (except 2002 and 2010), while lower concentrations were observed in 2004 (Table 2). Also, higher concentrations were observed in 2010 compared with 2004 and 2008 (Table 2). Total phytoplankton biomass did not exhibit notable interannual variability (K-W, p = 0.573), while significantly higher total abundances were observed in 2006, except 2002 and 2007 (Table 2).

Regarding the seasonal dynamics of Chl a, the K-W test revealed considerable differences between seasons (p < 0.0001). Thus, significantly higher concentrations were recorded in summer, while no notable differences were found between the other seasons (Table 3). Both total abundance and total biomass showed significant differences between seasons (K-W, p = 0.042 and p = 0.003, respectively); likewise with chlorophyll, significantly higher values of Tot.A and Tot.B were measured during summer (Table 3). Statistically, the positive correlations between Chl a and total abundance and biomass (r = 0.502, p < 0.0001, n = 438 and r = 0.416, p < 0.0001, n = 438

### Table 3. Mann-Whitney significance tests between seasons in terms of Chl a, Tot. A, Tot. B, SSS and nutrients.

| Season | Chl a | Tot. A | Tot. B | SSS | PO₄-P | SiO₄-Si | NO₃-N | NH₄-N |
|--------|-------|--------|--------|-----|-------|---------|-------|-------|
| Winter | <0.0001 | 0.004 | 0.001 | 0.016 | <0.0001 | <0.0001 | <0.0001 | 0.003 |
| Spring | 0.043 | 0.003 | <0.0001 | <0.0001 | 0.0004 | 0.0001 | 0.0001 | 0.001 |
| Summer | 0.015 | 0.005 | 0.001 | 0.0003 | <0.0001 | <0.0001 | 0.014 | 0.024 |
| Autumn | < | < | < | < | < | < | < | < |

Upper right values represent the level of significance (p); the absence of p values showed not significant differences between seasons

Lower left symbols compare seasons in the first column with seasons in the first row.
respectively) suggested quite similar annual cycles.

In terms of qualitative approach, the phytoplankton community was generally dominated by diatoms, except in 2003 (cyanobacteria and small flagellates accounted for about 80% as abundance) and 2007 (dinoflagellates accounted for more than 85% as biomass). Diatoms' domination was stronger in high-flow years of the Danube; in 2005, 2006, and 2010, diatoms accounted for more than 65%, both in abundance and biomass. Moreover, diatoms accounted for about half the number of species exceeding 10⁶ cells l⁻¹ during the study period (Table 4).

Seasonal cycle of Chl a showed a first maximum in winter/early spring (February–March), but this maximum was relatively moderate, except for 2003 (12.41 ± 5.04 µg l⁻¹), 2006 (9.80 ± 3.64 µg l⁻¹) and 2010 (15.10 ± 20.30 µg l⁻¹) (Fig. 4). The winter Chl a maximum corresponded to diatoms' blooms (diatoms accounted for more than 60% of total abundance), except 2002 (flagellates *Euglena gracilis* reached abundance of 2.28.10⁶ cells l⁻¹ and biomass of 4.56 g m⁻³, and corresponded to Chl a of 6.92 ± 6.99 µg l⁻¹) and 2007 (no winter bloom was observed). The highest level of Chl a in March 2010 (Fig. 4) corresponded to a strong diatoms bloom (blooming species *Skeletonema costatum* recorded abundances within 2.34.10⁶–49.4.10⁶ cells l⁻¹; Table 4), while long-lived winter blooms observed in 2003 and 2006 were associated with successive developments of diatoms and non-diatoms freshwater species. Thus, in 2003, the atypical cyanobacteria bloom observed in February (*Microcystis aeruginosa*, abundance of about 3.10⁶ cells l⁻¹; Bodeau *et al.*, 2004) was succeeded by a weaker diatoms bloom which extended until late March (assemblage dominated by *S. costatum*, maximum abundance of 1.6.10⁶ cells l⁻¹ and *Detonula confervacea*, biomasses within 1.86–2.27 g m⁻³). In 2006, the moderate diatoms bloom (*S. costatum*, abundances within 2.10⁶–4.27.10⁶ cells l⁻¹), which lasted from late February until mid-March, was followed in late March by high abundances and biomasses formed by the chlorophytes *Micractinium pusillum* (5.95.10⁶ cells l⁻¹ and 3.57 g m⁻³, respectively) and diatoms *Thalassiosira nordenskioeldii* (0.8310⁶ cells l⁻¹ and 8.02 g m⁻³, respectively). The lowest winter Chl a concentrations were measured in 2004 (3.25 ± 5.26 µg l⁻¹) and 2008 (3.69 ± 4.96 µg l⁻¹) (Figs. 3 and 4), when short-lived (order of days), but relatively strong, diatoms blooms were observed (abundances of 6.82.10⁶–10.77.10⁶ cells l⁻¹ in mid March 2004, and 11.88.10⁶–15.18.10⁶ cells l⁻¹ in early February 2008, respectively).

Sometimes, the winter Chl a maximum was followed by another one in spring (April–May) (Fig. 4). Relatively strong spring blooms were noted in the years characterized by higher river discharges in the spring, and favorable northerly and northeasterly winds, hence, lower salinity in the studied area. The low salinity measured in the springs of 2005 (13.61 ± 3.26 psu), 2007 (13.84 ± 1.79 psu) and 2009 (12.20 ± 2.4 psu) can be associated with higher spring Chl a observed in those years (Figs. 2 and 3). Except for 2007, the spring Chl a maxima (8.59 ± 9.16 µg l⁻¹ and 13.02 ± 21.07 µg l⁻¹, respectively in April) were stronger than the winter ones (4.36 ± 4.68 µg l⁻¹ and 5.44 ± 4.68 µg l⁻¹, respectively, both in February) (Fig. 4). The strongest spring maximum of Chl a (26.09 ± 33.78 µg l⁻¹) was observed in April 2007 and corresponded to very high
### Table 4. List of species exceeding 10^6 cells.l⁻¹ and their annual maximum abundances (10^6 cells.l⁻¹).

| Year | Bacillariophyta | Chlorophyta | Cryptophyta | Cyanobacteria | Eulagophyta | Dinoflagellata |
|------|-----------------|-------------|-------------|---------------|-------------|---------------|
| 2002 |                 |             |             | 3.06 (IX)     |             |               |
| 2003 |                 |             |             | 9.96 (X)      |             |               |
| 2004 |                 |             |             | 7.88 (X)      |             |               |
| 2005 |                 |             |             | 6.23 (X)      |             |               |
| 2006 |                 |             |             | 1.96 (X)      |             |               |
| 2007 |                 |             |             | 2.1 (V)       |             |               |
| 2008 |                 |             |             | 5.4 (VI)      |             |               |
| 2009 |                 |             |             | 1.28 (II)     |             |               |
| 2010 |                 |             |             | 1.2 (XII)     |             |               |

Month in which species abundance reached annual maximum is given in brackets.
abundances (6.65 \times 10^6–11.95 \times 10^6 \text{ cells l}^{-1}) and biomasses (maximal for the whole studied period, 218.05 \text{ g m}^{-3}) formed by dinoflagellates (Gymnodinium cf. aureolum was the dominant species; Table 4). Prior to the onset of this bloom, Chl \alpha was recorded in very low concentrations (3.81 \pm 3.55 \mu g.l^{-1}), most likely in connection with lower winter discharges of the Danube, but also with high SSTs measured during the winter (6.83 \pm 1.64 °C; Fig. 2), which weakens the mixing processes (Nezlin & Dyakonov, 1998). Also, high SSTs were measured during the winter of 2009 (5.03 \pm 1.91 °C; Fig. 2), when the winter Chl \alpha maximum was moderate (4.75 \pm 4.06 \mu g.l^{-1}; Fig. 3), but the large discharges of the Danube in April (monthly flow of 28.5 \text{ km}^3 according to FP6 SESAME, 2010) and prevailing northerly winds favored the onset of a stronger diatoms bloom in late April, which extended until mid-May. The high monthly mean of Chl \alpha observed in April 2010 (12.49 \pm 16.5 \mu g.l^{-1}) was due to very high concentrations measured in the first half of the month (within 7.50–37.00 \mu g.l^{-1}), corresponding to the strong and long-lived diatoms bloom starting from early March.

The quantitative parameters of phytoplankton showed a pronounced decrease in late spring/ mid-summer, closely linked to lower nutrient stock in the surface layer, and increased grazing pressure (Chu et al., 2005). Chl \alpha concentrations rarely exceeded 5.0 \mu g.l^{-1} during the May–June period; only in the early summers of 2006 and 2010, when the higher runoff from the Danube and favorable wind regime (suggested by low salinity in the area; Fig. 5) favored increased levels of Chl \alpha (8.83 \pm 6.62 \mu g.l^{-1} and 10.2 \pm 6.98 \mu g.l^{-1}, respectively; Fig. 4), corresponding to strong diatoms blooms (assemblages dominated by Cyclotella caspia, abundance of 4.95 \times 10^6 \text{ cells l}^{-1} in June 2006, and Cerataulina pelagica, abundance of 5.54 \times 10^6 \text{ cells l}^{-1} in June 2010; Table 4).

As we mentioned above, higher Chl \alpha concentrations were measured in summer; most of the times, the seasonal cycle of Chl \alpha showed an increasing trend from mid-July, and peaked in August and/or autumn (late September–October) (Fig. 4). Particular situations with low summer Chl \alpha were observed in 2004 and 2009 (seasonal averages of 3.88 \pm 1.99 \mu g.l^{-1} and 4.49 \pm 2.41 \mu g.l^{-1}, respectively; Fig. 3). In 2004, an autumn Chl \alpha maximum was observed in November (5.12–76.13 \mu g.l^{-1}), corresponding to intense, but short in duration (order of days) dinoflagellates bloom (abundance and biomass of 8.8 \times 10^6 \text{ cells l}^{-1} and 27.0 \text{ g m}^{-3}, respectively), while in 2009, there were no other Chl \alpha maxima by the end of the year (Fig. 4). In 2002, there was observed a weak Chl \alpha maximum in August (5.26 \pm 3.83 \mu g.l^{-1}), corresponding to diatoms abundances higher than 3.6 \times 10^6 \text{ cells l}^{-1} (blooming species Chaetoceros socialis; Table 4). This maximum was followed by a more pronounced one in autumn (10.02 \pm 6.10 \mu g.l^{-1}) resulted from successive strong blooms formed by diatoms (C. pelagica, abundance of 3.06 \times 10^6 \text{ cells l}^{-1}; Table 4) and colonial cyanobacteria (Microcystis orae, abundance of 300 \times 10^6 \text{ cells l}^{-1} and M. aeruginosa, abundance of 12.8 \times 10^6 \text{ cells l}^{-1} according to Bodeanu et al., 2004; Table 4). On the contrary, in 2003, the summer maximum of Chl \alpha (16.31 \pm 21.13 \mu g.l^{-1} in August), corresponding to high biomasses of dinoflagellates (>2.0 \text{ g m}^{-3}), was not followed by another one in autumn (Fig. 4). In 2005, Chl \alpha showed relatively moderate peaks in August (8.87 \pm 9.62 \mu g.l^{-1} and October (7.23 \pm 1.20 \mu g.l^{-1}), while in 2006, Chl \alpha showed the highest concentrations in July (20.62 \pm 20.54 \mu g.l^{-1}), corresponding to a strong bloom formed by diatoms Nitzschia tenuirotрис, abundance of 15.5 \times 10^6 \text{ cells l}^{-1} (Table 4). Favorable hydrological conditions in the summer of 2006 led to relatively high Chl \alpha also in August (6.24 \pm 3.20 \mu g.l^{-1}), which corresponded to an abundance of 6.84 \times 10^6 \text{ cells l}^{-1} formed by diatoms S. costatum. In autumn, a strong C.
pelagica bloom (abundance of 7.88 × 10^6 cells l^{-1}; Table 4) was observed from mid-September until late October, and led to a pronounced Chl a maximum (16.18 ± 9.23 µg l^{-1}). In 2007, relatively high Chl a in August (8.82 ± 5.94 µg l^{-1}) corresponded to dinoflagellates biomasses within 1.01–3.4 g m^{-3}, contrary to August 2008, when summer Chl a maximum (8.81 ± 7.80 µg l^{-1}) was associated with a relatively weak diatoms development (*Leptocylindrus minimus* and *N. tenuirostris* reached maximum abundance of 3.8 × 10^6 cells l^{-1}). In both years, stronger autumn Chl a maxima formed by large-size diatoms *C. pelagica* (abundance of 6.23 × 10^6 cells l^{-1} in 2007, and abundance of 1.96 × 10^6 cells l^{-1} and biomass of 12.73 g m^{-3}, respectively, in 2008; Table 4) were observed in October (Fig. 4).

The higher discharges of the Danube recorded in the summer of 2010 (mainly during June–July), along with prevailing northerly winds, led to an increased nutrient stock (Fig. 6), which favored high Chl a in July (9.70 ± 4.82 µg l^{-1}). This summer Chl a maximum corresponded to a strong bloom (assemblages dominated by diatoms *Thalassionema nitzschoides* and *Pseudo-nitzschia delicatissima*, abundance and biomass reached values of 7.67 × 10^6 cells l^{-1} and 4.32 g m^{-3}, respectively). In August 2010, the assemblages were dominated by flagellates *Eutreptia lanowii*, cyanobacteria *M. aeruginosa, Microcystis pulverea*, but they did not reach large abundances though the level of associated Chl a (18.38 ± 14.15 µg l^{-1}) was very high. Relatively high Chl a concentrations were also measured in the first half of October (4.79–12.06 µg l^{-1}), corresponding to a moderate autumn bloom formed by *C. socialis* (abundance ~ 3.8 × 10^6 cells l^{-1}; Table 4).

**Discussion**

The large fluctuations of the Danube’s discharges, as well as the sharp changes of the local climatic conditions are the main drivers controlling the temporal variability of the phytoplankton parameters (both quantitative and qualitative) in the Romanian coastal waters (Bodeanu, 1993). Significant negative correlation between Chl a and salinity (*r* = -0.346, *p* < 0.0001, *n* = 438) indirectly suggests the strong influence of the Danube’s discharges on the level of primary production (here expressed as Chl a). For instance, the highest Chl a concentrations were measured in 2006 and 2010 (annual averages of 8.56 ± 8.35 µg l^{-1} and 9.20 ± 11.72 µg l^{-1}, respectively), when
the Danube River registered high annual flows (258.7 km$^3$ and 310.0 km$^3$, respectively; according to Cociasu et al., 2009; FP6 SESAME, 2010), while the lowest Chl $a$ was observed in 2004 (4.57 ± 9.81 µg l$^{-1}$), when the salinity showed the highest annual mean (15.40 ± 1.78 psu) due to lower discharges of the Danube (Cociasu et al., 2009), but also strong upwelling events in the summer (Mihailov et al., 2012). Significant positive correlation between Chl $a$ and phosphate ($r = 0.132$, $p = 0.006$, $n = 438$) seems to confirm P limitation in the Romanian coastal waters (Cociasu et al., 1997, 2009; Yunev et al., 2007), especially as the lowest phosphate concentrations in the studied area were recorded in the summer of 2004 (0.24 ± 0.21 µM; Fig. 6), when the chlorophyll level was also minimal (3.88 ± 1.99 µg l$^{-1}$; Fig. 3).

The Romanian coastal waters generally show a winter/early spring diatoms bloom (February–March), closely related to intense mixing processes during the cold season (Bodeanu, 1987–1988; Yunev et al., 2002; Chu et al., 2005; Demidov, 2008). Besides, these processes which provide an internal supply of nutrients in the surface layer, the large fluctuations of the Danube’s discharges have a strong influence on the year-to-year variability of the winter/early spring bloom (magnitude, timing, and duration) mainly through the external input of nutrients, and also through the freshwater phytoplankton input (Bodeanu, 1987–1988). Generally, the seasonal cycle of the Danube’s discharges exhibits a maximum in April–May, and a minimum in September–October (Humborg, 1997), but in some years, a winter/late winter peak has also been noted. Significant negative correlation between Chl $a$ and SSS ($r = –0.477$, $p < 0.0001$, $n = 108$) observed in the cold season suggests the influence of the riverine discharges pulses and wind regime on the magnitude and duration of winter/early spring Chl $a$ maximum. Winters with larger runoff from the Danube, along with longer periods of prevailing northerly and northeasterly winds, showed strong and long-lived algal blooms in the Romanian shallow coastal waters. Such events were observed in 2003 and 2010, when the long period with dominant northerly and northeasterly winds (from December to March), even with relatively low intensities (less than 3 m s$^{-1}$ on average), led to strong and long-lived blooms. In 2010, the winter bloom was formed by diatoms S. costatum, which accounted for about 98% of total abundance, and corresponded to very high Chl $a$ during March–early April (within 7.27–58.47 µg l$^{-1}$). Moreover, this was the strongest winter/early spring Chl $a$ maximum along the studied period, and could also be related to the nutrient ratios ($N/P = 16.7$, $Si/N = 2.3$ and $Si/P = 33.2$) close to optimal ratios for diatoms growth (Redfield et al., 1963; Brzenziski, 1985), contrary to the rest of the investigated years ($N/P > 70.0$, $Si/N < 1.1$ and $Si/P > 50$). On the other hand, the large instability of wind regime during the cold season led to relatively sharp shifts of hydrological regime, thus favoring short-lived algal blooms. Such events were recorded in the winters of 2004 and 2008; the onset (mid-March in 2004 and early February in 2008, respectively) and extension (order of days) of these blooms were strongly linked to sharp changes in the salinity regime in the studied area (about 4.0 psu in less than a week). The influence of hydrological regime of the Danube on the winter/early spring Chl $a$ peak, through the input of freshwater phytoplankton, was well observed in 2003, when the high winter Chl $a$ corresponded to successive blooms formed by freshwater cyanobacteria and diatoms, which lasted from early February to mid-March. A similar situation was observed in 2006, when successive blooms formed by diatoms and freshwater chlorophytes were associated with relatively long period of high levels of Chl $a$ (late February–late March).

Stronger winter Chl $a$ maximum in high-flow years of the Danube was also reported by Bologa in the late 1970s, starting with the intensification of eutrophication. Relatively high winter Chl $a$ (5.74 ± 6.83 µg l$^{-1}$) was measured in 1977 (the Danube’s monthly flow in winter exceeded 23 km$^3$; Cocaisu & Popa, 1978), contrary to winters with lower runoff, which showed significantly weaker peaks of winter Chl $a$ (< 3.0 µg l$^{-1}$) (Bologa, 1977, 1978; Bologa et al., 1980). Similar to the 2000s, the winter assemblages during the late 1970s were dominated by diatoms, mainly S. costatum, which formed strong blooms (Bodeanu, 1987–1988), Winter Chl $a$ maxima, corresponding to diatoms blooms (dominant species S. costatum), were also observed in the other coastal areas along the Black Sea basin, but the influence of the Danube’s discharges generally leads to stronger peaks in the Romanian coastal waters (BSC, 2008; Demidov, 2008; Stelmakh et al., 2009, 2010).

The Danube’s increased discharges starting in March–April generally provided favorable conditions for stronger blooms in spring. During the investigated period, the spring bloom was not typical in the Romanian shallow coastal waters; Chl $a$ peaks were observed only in years with larger runoff from the Danube during springtime, and generally corresponded to diatoms development (except 2007). This is contrary to the period of intense eutrophication, when dinoflagellates dominated the spring phytoplankton community, and formed stronger blooms every year (Bodeanu, 1987–1988; Miheea, 1997). Thus, in the late 1970s, the spring Chl $a$ maxima observed in April–May (within 3.42–13.09 µg l$^{-1}$) were slightly weaker compared with the studied period (Bologa, 1978; Bologa et al., 1980), while in the 1980s and the 1990s, Chl $a$ concentrations were maximal during April–July (Miheea, 1997), mainly due to abnormal high P input from the Fertilizer Plant Navodari (northern Constanta) since the early 1980s until the mid-1990s (Cociasu et al., 1997; Miheea, 1997), and decreased grazing pressures as a result of the outbreak of Mnemiopsis population in the late 1980s (Kovalev & Piontkovski, 1998; Shiganova,
1998). Significant changes in nutrient regime after the mid-1990s resulted from the marked reduction of anthropogenic pressures in the coastal area (Lancelot et al., 2002; Cociasu et al., 2009) led to lower spring Chl $a$, but a maximum in April–May was also noted, especially in the years with larger spring runoff from the Danube (e.g. in 1997, Chl $a$ reached a relatively strong maximum of 10.64 µg l$^{-1}$ in April; Mihnea, unpublished data).

Generally, in the Black Sea shelf ecosystems, the winter-spring Chl $a$ maximum is followed by lower surface Chl $a$ in April–June, mainly as a result of nutrient depletion, as well as the increased grazing pressure (Chu et al., 2005; Demidov, 2008). Spatial-temporal extension of low Chl $a$ levels in the Romanian coastal waters is strongly influenced by the Danube’s discharges and the wind regime. Thus, shorter periods of low Chl $a$ were observed in the years with very large riverine discharges in spring (e.g. in 2006 and 2010, when the monthly flows of the Danube exceeded 30 km$^3$, according to Cociasu et al., 2009; FP6 SESAME, 2010), while in the years with reduced flows (e.g. in 2003; <18 km$^3$/month$^{-1}$; Cociasu et al., 2009) and/or dominant southerly winds in spring (e.g. in 2003 and 2004) the periods of low Chl $a$ levels were longer. Starting with June–July, Chl $a$ showed an increasing trend and peaked in July–August (Fig. 4), generally corresponding to strong diatoms blooms. This summer Chl $a$ maximum is quite common for the Black Sea shelf ecosystems (Demidov, 2008); its timing, duration, and magnitude are sensitive to local conditions and exhibit considerable interannual variability (Veder nikov & Demidov, 1993; Sur et al., 1996). Significant negative correlation between summer Chl $a$ and SSS ($r = -0.290$, $p = 0.005$, $n = 99$) suggests the marked role of the Danube’s discharges on the summer primary production in the Romanian coastal waters. On the other hand, the absence of significant correlations between salinity and nutrients in the warm season suggests that the Danube’s discharges are not solely responsible for the relatively high nutrient regime in the shallow coastal waters. Upwelling events, quite common in the coastal waters in early summer (Stan eva et al., 2010; Mihailov et al., 2012), benthic nutrients recycling processes, more intense with increasing thermal regime (Friedrich et al., 2002), as well as local anthropogenic pressures, provide relatively high nutrient stock, even in conditions of lower discharges of the Danube. However, the strongest summer Chl $a$ maxima were recorded in 2006 and 2010: the high discharges of the Danube and prevailing northerly winds favored strong diatoms blooms in mid-July. In both the years, Chl $a$ level remained quite high during the warm season; in 2006, a second strong diatoms bloom was observed in August, while in 2010, the high Chl $a$ concentrations were most likely due to the interference of pheopigments resulted from very strong Noctiluca blooms observed during summertime (Timofte, 2011), taking into account that the thrichromatic method does not differentiate between chlorophyll and pheopigments (Aminot & Rey, 2001). No algal blooms were observed in the summer of 2004 (Fig. 4), and this fact can be related to significant lower phosphate levels during July–August (Fig. 6), when many PO$_4$-P concentrations dropped below detection limit. Except for these situations, the summer Chl $a$ maxima were observed in August, and the blooming species changed from year to year closely related to the large variability of environmental conditions. Generally, diatoms dominated the summer assemblages, especially in high-flow years of the Danube (e.g. 2002, 2005, 2006, and 2010), while extensive biomasses of large-size dinoflagellates were observed in summers with lower discharges of the Danube (2003 and 2007). On the contrary, during the intense eutrophication period, the summer assemblages were mainly dominated by dinoflagellates, the diatoms contribution being much less (Bodeanu, 1987–1988; Mihnea, 1997). Prior to the onset of eutrophication, the summer Chl $a$ maximum was not observed (Skolka, 1968); it has become a typical feature in the Romanian coastal waters since the mid-1970s (Bologa, 1977, 1978; Bologa et al., 1984). While in the late 1970s the summer Chl $a$ maximum was relatively weak (Bologa, 1977, 1978), in the 1980s and 1990s, it increased considerably in magnitude (Bologa et al., 1984; Mihnea, 1997). Although significantly lower when compared with the previous period, the summer Chl $a$ remained high after 2000 (8.60 ± 9.41 µg l$^{-1}$), suggesting high enough nutrient stock for sustaining an increased primary production in the Romanian coastal waters. It is also worth mentioning the onset of summer bloom generally in August, contrary to the intense eutrophication period, in June–July. Similar to the Romanian coastal waters, well pronounced summer peaks of Chl $a$ are recorded in the eastern shelf of the Black Sea in line with the larger nutrient supply by mountain rivers during the flood period and local upwelling events (Demidov, 2008), while in the Bulgarian coastal waters there is no clear seasonal pattern, and the timing of intensive growth of phytoplankton varies irregularly (BSC, 2008).

In autumn, the increased nutrient stock resulted from the erosion of seasonal thermocline and favorable wind regime, as well as reduced zooplankton grazing pressures, led to relatively strong autumn blooms in the shelf waters (Demidov, 2008). Generally, the autumn Chl $a$ maximum was associated with high biomasses of large-size diatoms C. pelagica, which in most cases was the dominant species in the autumn assemblages. The diatoms’ dominance in autumn was also showed by significant negative correlation between autumn Chl $a$ and silicate ($r = -0.223$, $p = 0.047$, $n = 82$), which suggests increased Si consumption by diatoms. Except 2003 and 2009, when no autumn blooms were noted, the lowest autumn Chl $a$ maxima were observed in 2005 and 2010 (Fig. 4), closely linked to the absence of C. pelagica; diatoms Pseudo-nitzschia seriata (in 2005)
Conclusions

In the Romanian shallow coastal waters, surface Chl $a$ showed large temporal variability (CV = 142.3%; concentrations within 0.17–76.13 µg l$^{-1}$ during the studied period), mainly controlled by large fluctuations of the Danube’s discharges, sharp changes of local climatic factors and anthropogenic pressures.

Seasonal cycle of Chl $a$ showed a winter/early spring peak (February–March) in accordance with the normal growth cycle of diatoms, followed by a stronger one in spring (April–May), but only in the springs with large discharges of the Danube. After significantly lower concentrations in late spring/early summer (May–June), Chl $a$ exhibited its strongest maximum in summer (July–August), generally corresponding to strong diatoms blooms, followed by another one in autumn (late September–October), mainly associated with large-size diatoms blooms. Some deviations from this cycle were found in 2007, when no winter Chl $a$ peak was observed, and especially in 2009, when Chl $a$ levels remained low in the warm season, and did not show any peaks from May to the end of the year.

Year-to-year variability of Chl $a$ seems to be controlled by the hydrometeorological conditions during summer. Thus, the larger discharges of the Danube, as well as the prevailing northerly winds, led to the highest summer Chl $a$ a in 2006 and 2010, when, also, the annual Chl $a$ means were maximal (8.56 ± 8.35 µg l$^{-1}$ and 9.20 ± 11.72 µg l$^{-1}$, respectively). The lowest annual Chl $a$ mean was observed in 2004 (4.57 ± 9.81 µg l$^{-1}$), when, also, the summer Chl $a$ concentrations were minimal as a result of lower discharges of the Danube and other local physical processes which led to strong P limitation in the coastal area.

Although significantly lower than in the 1980s and 1990s, Chl $a$ level in the Romanian near-shore waters has remained quite high (6.59 ± 9.38 µg l$^{-1}$) after 2000 due to optimal nutrient regime provided through either external (the Danube River, anthropogenic activities) or internal sources (benthic recycling processes).

Acknowledgements

The authors are very grateful to Dr. Violeta Velikova for her helpful comments and recommendations. Special thanks to all our colleagues from NIMRD involved in field sampling.

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