Journal of Marine Systems 94 (2012) 18–32

Contents lists available at SciVerse ScienceDirect

Journal of Marine Systems

journal homepage: www.elsevier.com/locate/jmarsys

Phytoplankton taxonomy based on CHEMTAX and microscopy in the northwestern Black Sea

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ARTICLE INFO

Article history:
Received 29 July 2011
Received in revised form 3 October 2011
Accepted 7 October 2011
Available online 19 October 2011

Keywords:
Phytoplankton carbon biomass
Chlorophylls
Emiliania huxleyi
CHEMTAX
HPLC
Pigments
Black Sea

ABSTRACT

Abundance and carbon biomass of different phytoplankton groups obtained by microscopy were compared with taxonomy derived from pigment measurements and CHEMTAX analysis of samples collected in June 2006 in the NW Black Sea. The diatom *Chaetoceros curvisetus* was dominant in terms of carbon biomass based on cell volume at inshore stations, while the coccolithophore *Emiliania huxleyi* was prevalent at offshore. *Emiliania huxleyi* reached bloom abundance of 3.3×10^6 cells L^{-1}. The chlorophyll *a* (chl *a*) concentration within phytoplankton groups as allocated by CHEMTAX was in agreement with microscopy derived carbon biomasses of the taxonomic groups diatoms, dinoflagellates and cryptophytes only. Carbon biomass of less abundant phytoplankton taxa (cyanophytes, euglenophytes and chlorophytes) did not correlate with group-specific chl *a*. It was not possible to detect *E. huxleyi* bloom by CHEMTAX analysis probably due to much higher biomass of other species containing 19'-hexanoyloxyfucoxanthin. Nutrient concentrations were generally high in the waters where diatom and dinoflagellates dominated the community but low in the area of *E. huxleyi* bloom. A good correlation between total carbon biomass of phytoplankton and chl *a* was found and the estimated C:chl *a* ratio of phytoplankton varied between 36 and 256 (in average 124 ± 50).

1. Introduction

The ecosystem of the Black Sea, which is one of the world's largest inland water bodies, has been subjected to multiple pressures since 1960s. Increasing nutrient loads of rivers, especially of the Danube River, contributed substantially to the eutrophication process of this enclosed basin (Cociasu and Popa, 2004; Cociasu et al., 1996; Humborg et al., 1997). While increased nutrient concentrations have caused an increase in bloom frequencies and abundances of many phytoplankton species, retention of silicate in the dams constructed across the Danube River has induced a change in phytoplankton composition in favour of coccolithophores and dinoflagellates rather than diatoms after 1970s (Bodeanu et al., 1998; Bologa et al., 1995; Cociasu et al., 1996; Humborg et al., 1997; Moncheva and Krastev, 1997). Intense blooms of *Emiliania huxleyi*, which are suggested to play an important role in carbondioxide drawdown from the atmosphere (Buitenhuis et al., 2001; Suykens et al., 2010; van der Wal et al., 1995), during early summer months became a common phenomenon in the Black Sea after 1980s (Bodeanu et al., 1998; Cokacar et al., 2004; Mikaelyan et al., 2005; Moncheva et al., 2001; Pautova et al., 2007; Stoica and Herndl, 2007).

Tracking changes in phytoplankton taxonomic structure and carbon biomass is highly relevant for assessment of recent ecological status. HPLC technique is advantageous in the large scale-mapping of pigments in the world oceans (Llewellyn et al., 2005; Mantoura and Llewellyn, 1983; Tester et al., 1995; Vidussi et al., 2001). In addition, this technique allows to detect and identify microscopically overlooked or nondescript small phytoplankton species, based on their pigment content (Ansotegui et al., 2003; Antajan et al., 2004; Garibotti et al., 2003). However, the usage of HPLC analysis alone without microscopy for determining taxonomic composition and biomass of phytoplankton has not been recommended so far due to the ambiguous character of some marker pigments (i.e. their being shared by several phytoplankton groups) and changes in cellular pigment contents due to light and temperature variations, nutrient availability, distinct growth phases, as well as from the preclusion of phycobiliprotein quantification through HPLC (Havskum et al., 2004; Irigoien et al., 2004; Llewellyn et al., 2005). Although chl *a* could be used as a proxy of phytoplankton biomass, quantification of carbon biomass is essential to calculate phytoplankton growth rates, to study aquatic food webs and to model the flux of organic matter in marine ecosystems (Behrenfeld et al., 2005; Garibotti et al., 2003). Chl *a* values obtained with HPLC method can be converted to carbon using C:chl *a* ratios, taking into account variations in this ratio with changing irradiance, temperature, nutrient concentrations and growth phases. Based on

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doi:10.1016/j.jmarsys.2011.10.005

marker pigments measured with HPLC, chl *a* is attributed to corresponding phytoplankton groups employing the statistical software CHEMTAX (Llewellyn et al., 2005; Mackey et al., 1996; Schlüter et al., 2000). This program has been widely used in different regions of the world oceans to study phytoplankton composition (Havskum et al., 2004; Irigoien et al., 2004; Llewellyn et al., 2005; Schlüter et al., 2000). In some investigations CHEMTAX estimates were in agreement with microscopic results for major phytoplankton groups (Eker-Develi et al., 2008; Llewellyn et al., 2005; Wright et al., 1996) while not in some others (Havskum et al., 2004; Irigoien et al., 2004; Lewitus et al., 2005; Lionard et al., 2008).

In the present study CHEMTAX program was applied for the first time in the Black Sea to analyze data from a summer period that coincided with a coccolithophore bloom, aiming at the comparison of results with microscopy derived carbon biomasses of phytoplankton classes. In addition, relationships among species composition of phytoplankton, nutrients, water stratification and salinity were investigated.

2. Material and methods

Between 2nd and 17th of June 2006, 93 stations were sampled in the northwestern Black Sea onboard the RV 'Akademik' from the Institute of Oceanology in Varna, Bulgaria (Fig. 1). Surface sampling of phytoplankton and pigments were performed at 30 and 93 stations by a sampling bottle, while nutrient sampling was carried out at 60 stations by General Oceanic Go-Flo Rosette bottles.

2.1. Physicochemical measurements

Vertical profiles of temperature and salinity were obtained by a Seabird-19 CTD down to a depth of ~30 m.

2.2. Nutrient analyses

Nutrient (PO_4–P, P_{org}, NO_3–N, NO_2–N, NH_4 and Si) samples were collected in 100-mL polyethelene bottles and kept frozen (−20 °C) for a few weeks until their analysis by standard spectrophotometric methods (Grasshoff et al., 1983). Detection limits of PO_4–P, NO_3–N, NO_2–N, NH_4 and Si were 0.05 μM, 0.02 μM, 0.02 μM, 0.05 μM and 0.05 μM respectively.

2.3. Phytoplankton analysis

Water samples for phytoplankton identification were collected in 250 mL amber glass bottles to which a buffered formaldehyde solution was added onboard to obtain a final concentration of 2%. In order to prepare samples for microscopic analysis, the sedimentation method was used (Eker-Develi et al., 2006a). Samples were kept immobile 2 weeks in the laboratory and concentrated from a total volume of 250 ml to 20–50 ml by siphoning the supernatant using thin curved tubes. The micro- and nanophytoplankton species were counted in a Sedgewick-Rafter counting chamber under a phase-contrast binocular microscope (with ×100–×200 magnification). Approximately 400 cells were counted in each sample. Phytoplankton species from the following 8 taxonomic groups were: Diatoms (Diat), dinoflagellates (Dino), cryptophytes (Crypto), coccolithophores (Cocco), chlorophytes (Chloro), euglenophytes (Eugleno), prasinophytes (Prasino), cyanophytes (Cyano), and small flagellates (sFlag) (Table 1). The sFlag group may include prymnesiophytes, cryptophytes, chrysophytes, prasinophytes and raphydophytes in the size range ~2 to 20 μm.

The volume (V) of each cell was calculated based on morphometric measurements of the corresponding geometric shapes (Kovala and Larrance, 1966; Olenina et al., 2006). One μm^3 V was assumed equivalent to 1 pg wet weight (Wasmund et al., 1998). Wet weight of the phototrophic ciliate *Mesodinium rubrum* Lohmann (= *Myrionecta rubra*Jankowski) was included in cryptophyte wet weight as in previous studies by Eker-Develi et al. (2008) and Schlüter and Møhlenberg (2003). C biomasses of cells throughout the text were calculated from the measured V by the equations of Menden-Deuer and Lessard (2000) as follows: for diatoms (Eq. 1), for diatoms >3000 μm^3 (Eq. 2), for dinoflagellates (Eq. 3), for coccolithophores (Eq. 4), for chlorophytes, euglenophytes and prasinophytes (Eq. 5), for small flagellates, cryptophytes and cyanophytes (Eq. 6).

$$\log C = -0.541 + 0.811(\log V) \quad (1)$$

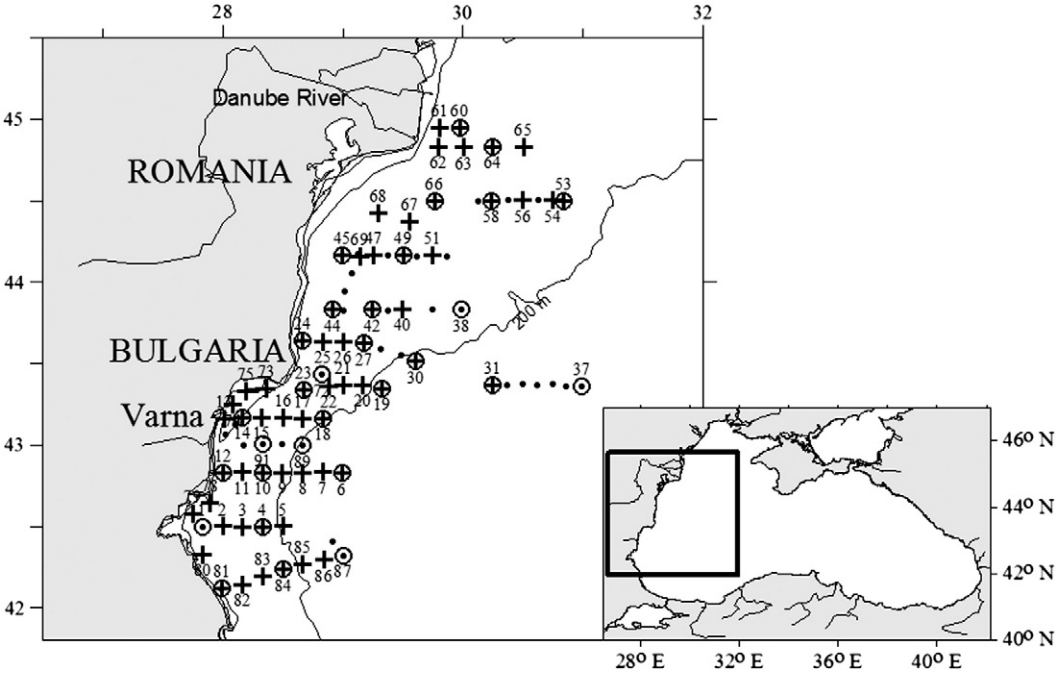

Fig. 1. Sampling stations in the western Black Sea in June 2006(● Pigment, ⊙: Pigment + Phyto, +:Pigment + Nutrient, ⊕:Pigment + Phyto + Nutrient).