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Marine Pollution Bulletin

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

Baseline

Increasing contribution of coccolithophorids to the phytoplankton in the northeastern Black Sea

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ARTICLE INFO

Keywords:

Black Sea
Blooms
Coccolithophorids
Emiliana huxleyi
Eutrophication
Phytoplankton

ABSTRACT

During 2005–2011, 418 phytoplankton samples were collected in Novorossiysk and Tuapse ports and near the resort cities of Anapa and Gelendzhik in the northeastern Black Sea. The maximal values of both abundance and biomass of phytoplankton related to high nutrient concentration, probably due to anthropogenic load, were observed at Novorossiysk (5.82×10^5 cells/l, 1.492 g/m^3); in other bays values were about three times lower. The annual cycle included two to four phytoplankton abundance peaks. *Emiliana huxleyi* was the most abundant coccolithophorid (1.15×10^5 cells/l offshore and 2.20×10^4 cells/l in bays and ports). In spring-summer it contributed up to 90% of the phytoplankton abundance offshore. The maximal abundance of *E. huxleyi* was observed offshore of Gelendzhik (up to 1.32×10^6 cells/l); the minimum was in the coastal zone in the port of Novorossiysk (on average 7.7×10^3 cells/l). Generally, the species appears to avoid eutrophic waters.

In recent years coccolithophorids (Haptophyta: Coccolithophyceae) known from the Triassic (Bown, 2005) have been the focus of investigations mainly because of their capacity to regulate the CO₂ content of the atmosphere, converting it into insoluble CaCO₃ in the form of their external skeleton composed of isolated calcareous plates (coccoliths) that after the cell's death become one of the principal components of the bottom oceanic sediments (Westbroek et al., 1989; Paasche, 2002; Riebesell, 2004; Rost and Riebesell, 2004; Engel et al., 2005). Over the past 220 years there has been a 40% increase in average coccolith biomass (Iglesias-Rodriguez et al., 2008). The oxidation products dimethylsulphide and dimethylsulfoniopropionate released by blooming coccolithophorids could affect albedo (Holligan et al., 1993; Malin and Kirst, 1997; Simó, 2001; Paasche, 2002; Van Rijssel and Gieskes, 2002). The transparent colorless covering of coccolithophorid cells is highly efficient in refracting light in the water column and makes *Emiliana huxleyi* (Lohmann) W.W. Hay et H.P. Mohler (Isochrysidales: Noelaerhabdaceae) easily visible to satellites. The oceanic areas with blooming coccolithophorids are characterized by a high albedo, a great portion of solar light and heat returning to the atmosphere instead of warming the ocean.

The importance of coccolithophorids as primary producers and calcifiers has been widely discussed in literature. Since the early 1990s many studies have dealt with calcification and coccolith formation by

coccolithophorids, response to ocean acidification, the global carbon cycle, carbonate chemistry and calcium carbonate cycling, air/sea CO₂ exchange, photosynthesis, viral susceptibility, factors controlling coccolithophorid blooms and other themes.

Emiliana huxleyi is the most abundant coccolithophorid species widely distributed throughout the oceans, except for the polar regions (Holligan et al., 1993; Winter et al., 1994; Paasche, 2002; Tyrrell and Merico, 2004; Bendif et al., 2014), among about 200 extant coccolithophorid species (Jordan and Chamberlain, 1997). In the last 100,000 years it has been numerically dominant (Bendif et al., 2016). Recently, poleward expansion of *E. huxleyi*'s geographical range has been suggested (Winter et al., 2014). Most coccolithophorid species inhabit temperate and warm oxygen rich waters poor in nutrients, with a pH of 8.00–8.05 and temperatures above 8 °C (Deflandre, 1952; Paasche, 2002).

In the late 1970s during the annual cycle of phytoplankton of the northeastern Black Sea (NEBS), the spring-summer transitional period was characterized by the replacement of a predominantly diatom taxocenosis by dinoflagellates (Zernova, 1981). Oguz and Merico (2006) determined the bloom sequence in the internal basin as diatoms-dinoflagellates-*E. huxleyi*-picophytoplankton, referring to the period of 1995–2002. Reduced eutrophication, climatic peculiarities (an anomalously hot summer and early autumn) and increasing temperatures in

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the uppermost quasi-homogeneous layer during the later years resulted in changes in the dominant phytoplankton species composition. Since the mid-1990s these occurred within “the diatoms-coccolithophorids system” (Pautova et al., 2007; Mikaelyan et al., 2011, 2013; Silkin et al., 2015). In the modern period diminishing mineral forms of nitrogen and increasing phosphate concentrations have been observed, resulting in more intense proliferations of *E. huxleyi* (Mikaelyan et al., 2011). Xu et al. (2006) and Stelmakh et al. (2009) presented evidence of mixotrophy in *E. huxleyi* during phosphorus deficiency in the water column.

Until the mid-1980s the contribution of coccolithophorids to the phytoplankton of the Black Sea was negligible (3%); their biomass was 8 µg/l. During the 1980s the mean biomass of coccolithophorids increased to 106 µg/l (Mikaelyan et al., 2011, 2013). From the mid-1990s until the present coccolithophorids frequently have dominated in both cell abundance and biomass. During the last 40 years a general tendency for an increasing contribution of coccolithophorids to phytoplankton communities coincides well with increasing phosphate concentrations in the surface layer. This is in good accordance with laboratory experimental data that indicate coccolithophorid growth in May–June is phosphate-limited (Mikaelyan et al., 2011). In addition, Mikaelyan et al. (2013) suggested the low silicate content would limit the growth of coccolithophorids.

Recently, as a result of the studies performed by the P.P. Shirshov Institute of Oceanology of the Russian Academy of Sciences, some changes in species composition and cell abundances of the phytoplankton communities have been observed. Among the reasons for these changes, physical-chemical factors related to climatic changes and anthropogenic load are suggested to be involved (Burenkov et al., 2006; Mikaelyan et al., 2006, 2011, 2013; Pautova et al., 2007; Silkin et al., 2009, 2011; Flint and Poyarkov, 2010). Since 1998 atypically warm winters have occurred. Since the mid-1990s in the Black Sea reduced nitrogen and phosphorus concentrations have been reported to be a result of the economic recession in the countries around the sea and the reduced use of fertilizers. This has caused a replacement of previously common dominant diatoms by rare and new species for the Black Sea.

Yearly blooms of *E. huxleyi* due to its rapid growth at extremely low nutrient concentrations have become usual in the NEBS. The maximal abundance (up to 6.00×10^6 cells/l) was observed in 2004–2006 after warm winters when the water column was highly stable (Mikaelyan et al., 2013, 2015). Moderate blooms (1.00×10^6 – 1.40×10^6 cells/l) occurred after cold winters (2003 and the second part of the 2006–2007 winter) and mild winters (2002 and 2008) under conditions of low stability of the water column during spring-summer. It was shown that the bottom-up flux of pycnocline waters during winter convection is the main driver of coccolithophorid blooms that occur in the open sea in May and June after cold winters (Mikaelyan et al., 2015); in the Black Sea the permanent pycnocline is located at the 60–180 m depth or deeper (Titov, 2004). In 2004–2006 the mass development (up to 9.00×10^5 cells/l) of diatoms new to the Black Sea, *Chaetoceros thronsenii* Marino, Montresor et Zingone and *C. minimus* (Levander) Marino, as well as the potentially toxic diatoms *Pseudo-nitzschia* cf. *pseudodelicatissima* (Hasle) Hasle and *P.* cf. *delicatissima* (Cleve) Heiden (up to 5.00×10^5 – 7.00×10^5 cells/l), took place. In the years of moderately blooming *E. huxleyi* (2003, 2007 and 2008) elevated abundances of the diatoms *Proboscia alata* (Brightw.) B.G. Sundström, *Chaetoceros curvisetus* Cleve and *Skeletonema costatum* (Grev.) Cleve (3.00×10^5 – 1.10×10^6 cells/l of each of these species) typical for the 1970s–1980s were observed (Pautova et al., 2011; Silkin et al., 2011).

During the anomalously warm first part of the 2006–2007 winter in late December, at about 50 nautical miles offshore of Gelendzhik, for the first time for the entire Black Sea, a winter *E. huxleyi* bloom (1.40×10^6 cells/l) was observed at 8.9 °C and homothermy down to 40-m depth. Previously, in January–February 2003, 2006 and 2007 the species was found in high abundance but not reaching bloom

concentrations in Sevastopol Bay (5.00×10^5 to 8.00×10^5 cells/l) when intense river runoff resulted in decreased salinities of 16.78–17.47. In recent years the *E. huxleyi* cell abundance in winter has reached the spring-early summer levels; it is suggested that *E. huxleyi* limited by phosphorus takes advantage of organic matter brought to the sea by rivers and from diatom metabolites (Stelmakh et al., 2009).

The purpose of this investigation was to study the details of the interannual and seasonal changes in the development of *E. huxleyi* in terms of the cell abundance and frequency of coccolithophorid blooms in the coastal zone of the NEBS and their contribution to the entire phytoplankton population in the bays of the ports of Novorossiysk and Tuapse and the resorts of Anapa and Gelendzhik and some offshore areas for comparison. This is the first study of phytoplankton performed in the bays in the NEBS; it comprised several years and three seasons (spring, summer and autumn).

On the whole, the Black Sea is a brackish deep water basin (down to 2212 m) with a limited water exchange with the Mediterranean Sea through the Bosphorus Strait and the Azov Sea through the Kerch Strait (Sorokin, 2002). In winter in the NEBS the northeasterly wind called bora is prevalent and reaches 40 m/s (Korshenko et al., 2011). This area is characterized by a narrow shelf, usually within 110 m depth (Zatsepin et al., 2011). Of the studied bays, Novorossiysk Bay is one of the largest on the northeastern coast of the Black Sea. The depths of the four studied bays can reach 40 m (Navigation Chart of the Black Sea, 1958). The coastal area is a zone of transit of fine-grained sediments due to high hydrodynamic activity.

In February (winter) the mean surface temperature is 9 °C, sometimes reaching 5–6 °C, and the upper 40-m layer is homothermous. In June–September the surface layer warms up to 25–26 °C, and the thickness of the homothermous layer can be up to 25 m in September. Additionally, in September the surface water starts to chill, resulting in the winter type of vertical distribution of temperature, with the minima in shallow water and the maxima in deeper layers. Homohalinity is observed in winter in the upper 40-m layer and in summer in the uppermost 10–15 m; salinity usually varies between 17.6 and 18.2, but in some bays (Tuapse Bay) it can drop to 11–12 or even 8.5 (Korshenko et al., 2006, 2007, 2008, 2009, 2010, 2011, 2012). At 25 m depth salinity remains almost constant (18.1–18.3) throughout the year. Density of the upper water layer is homogenous to 50 m in February–March and to 10–15 m in August–September (Krivosheya et al., 2011). A seasonal pycnocline occurs deeper to about 35 m.

In the study area the quasi-permanent Rim Current encircling the entire sea (with its fragment called the Caucasian Current) is the most important, comprising a band of 30–40 miles flowing along the coast generally to the north-west and reaching a velocity of 50–60 cm/s (Zatsepin et al., 2011). Numerous meanders and eddies lie between this current and the coastline. A homogenous upper layer to 50 m depth with a dissolved oxygen content (DO) of about 7.0 ml/l (saturation < 95% in the surface layer) occurs throughout the winter (Chasovnikov, 2011). In summer DO is approximately 5.0 ml/l (saturation up to 110–120% in the surface layer). In summer and autumn the DO maxima occur at 30–50-m depth. The annual mean pH is 8.39, varying during the year between 6.93 and 8.69 (Efimova et al., 2011; Korshenko et al., 2012).

Phytoplankton samples were collected near the ports of Novorossiysk and Tuapse and the resort cities of Anapa and Gelendzhik, with some sampling stations located offshore, during oceanographic cruises onboard small vessels of the Administration of Sea Ports of the Black Sea (Federal Marine and River Transport Agency of the Ministry of Transport of the Russian Federation) during different seasons from 2005 to 2011 (Fig. 1). Novorossiysk Bay, the largest bay, is semi-enclosed and deeply inserted into the land. The biggest dry cargo and petroleum port in southern Russia is located there, with a turnover of 127 million tons per year (Sea Ports of Russia, 2016); it is also a naval base. On the eastern coast of the bay, a shipyard, a cement plant and a petroleum terminal haven are located. The western coast is subjected to

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