MARINE BIOLOGY

Coccolithophorids in the Black Sea: Their Interannual and Long-Term Changes

A. S. Mikaelyan, V. A. Silkin, and L. A. Pautova

Shirshov Institute of Oceanology, Russian Academy of Sciences, Moscow, 117997 Russia

E-mail: mikaelyan@ocean.ru

Received December 5, 2008; in final form, February 19, 2009

Abstract—A five-year study of the interannual changes observed in May–June during the mass reproduction of coccolithophorids was carried out in the northeastern part of the Black Sea. The long-term dynamics were analyzed using the data on the phytoplankton collected during the last 40 years. The fraction of coccolithophorids represents either 20 or 60% of the total biomass of the algae and does not depend on either the previous winter conditions or the nitrogen content and the N : P ratio in the surface water layer. Our studies have revealed the dependency between the phosphate content and the size of the coccolythophorids' fraction in the total phytoplankton biomass. The long-term population dynamics includes three periods. Until the mideighties, the coccolythophorid fraction in the Black Sea phytoplankton was insignificant (3%). The average biomass was equal to 8 µg/l. In the late eighties, the average biomass increased up to 106 µg/l. Since the mid-nineties, coccolythophorids often prevail in the number and mass among the other phytoplankton species. The general tendency for the growth of the coccolythophorid fraction in the phytoplankton cenoses coincides with the increase in the phosphate concentration in the near-surface water observed within the last 40 years. This fact corresponds to the experimental results, which demonstrate that the coccolythophorid development during May–June is phosphate-limited.

DOI: 10.1134/S0001437011010127

INTRODUCTION

The coccolythophorids of the Black Sea are represented mainly by one common species *Emiliania huxleyi*. This alga was observed in both the shelf and open sea regions [1, 2, 4, 8, 17, 25, 26, 36, 48]. The early development of *E. huxleyi* up to the bloom level (millions of cells per liter) represents a rather rare phenomenon. The mass reproduction of this species in the Black Sea was first observed in 1951, when its population increased up to 0.85×10^6 cells per liter [8]. In the succeeding years, an increased population of this alga was observed in the spring and early summer seasons [2, 25, 26]. However, these population outbreaks were described as rather short and local. A population outbreak of *E. huxleyi* was also registered in November of 1993 [17].

In recent years, a conclusion about the regular population outbreaks of coccolythophorids in the early summer season has been reached based on the satellite data [22, 23]. The mass reproduction of coccolythophorids in May–June was also registered in the course of field observations [11, 35]. In this study, we considered the reasons for this phenomenon and the time of its appearance. The material necessary for the study of the long-term changes was provided by our own observations carried out in recent years and taken from the Black Sea phytoplankton database, which contains data collected over 40 years [7]. We also discuss the features of the interannual variations observed during the May–June period of the mass reproduction of coccolythophorids.

The stratification of the near-surface water layer, the high illumination, and the low phosphate concentrations are considered to be the main factors providing for the development of coccolythophorids [43, 46]. Initially, it was supposed that, due to the very efficient phosphatases, low phosphate concentrations do not limit the development of coccolythophorids in a nitrate-limited medium [44]. However, preliminary experiments unexpectedly showed that, in the case of the Black Sea coccolythophorids, phosphates represent a first-order limiting factor [35]. In this study, we present the results of our experiments performed during the mass reproduction season and devoted to the influence of different food additives on the development of coccolythophorids and the importance of this factor in the interannual and long-term population dynamics.

MATERIALS AND METHODS

Field studies. The experimental material was collected during eight voyages of the *Akvanavt* research vessel in the northeastern part of the Black Sea. All the works were carried out mainly along the transect line directed from the coastline near Gelendzhik to the sea

Table 1. Quantitative characteristics of the collected material

Year	Date of the survey	Number of stations	Number of samples
2002	May 18−20	24	80
	May 24−30	18	18
2004	June 11−16	24	24
	June 20−24	28	88
2005	June 9−13	38	84
	June 24−28	19	30
2006	May 24−28	20	83
2007	June 10−13	13	34
Total		184	441

Table 2. Experimental scheme of the addition of biogenic elements

Variant	Nitrates (N)	Phosphates (P)
1	−	−
2	+	−
3	−	+
4	+	+

center (60−90 miles from the shore) and along the transect lines directed along the coast line and located between Gelendzhik and Sochi. During all five years of the studies, the material was collected in the May−June period. The total number of sampling stations was 184 (Table 1). At all the stations, we took samples from the sea's surface. In the case of 46 stations, we also took vertical series of samples.

The sampling of the phytoplankton was carried out using a SeaBird bathometer probe to a depth of 10−100 m depending on the sea depth. The vertical series included 6−8 samples. The sampling depth depended on the vertical water mass's structure as determined by the hydrophysical probing; we also took additional water samples from the same depths to carry out a hydrochemical analysis.

In the case of the samples collected along the coastline−open water transects, the number of phytoplankton cells was calculated in concentrates obtained by the filtration of 2−5 l of water through inverse filtering chambers equipped with 1-μm nuclear filters [16]. Using 1% formaldehyde, the obtained concentrates were fixed up to the final concentration (1%). In the case of some samples collected along the transects parallel to the coastline, we determined only the number of coccolythophorids. Unfiltered 100-ml samples were fixed using the above-mentioned procedure. Both types of samples were treated in the coastal laboratory within 1−2 months.

The identification of the species and the calculation of the cells were carried out using an Ergoval light microscope (Karl Zeiss, Jena) at 16×10 and 16×40 magnifications. The nano- and microplankton cells were calculated using 0.05-ml Nageotte and 1-ml Naumann counting chambers. The small flagellates (2−4, 4−6, and 6−8 μm fractions), picoplankton (1−2 μm fraction), and coccolythes were calculated using a Fuchs−Rosenthal counting chamber. The biomass was calculated using a volumetric method (a cell was equated with a corresponding geometrical figure (a cylinder, sphere, or ellipsoid of rotation)) and expressed in green weight units.

To analyze the long-term changes in the coccolythophorids, we used the Black Sea phytoplankton database containing the data collected from 1968 to 2007 [7]. To analyze the changes in the hydrochemical parameters, we used a database created within the framework of the NATO TU-BLACK project [29] and the data collected by the Laboratory of Hydrochemistry of the Southern Division of the Institute of Oceanology of the Russian Academy of Sciences (IO RAS).

Experimental studies. The influence of nitrate- and phosphate-containing additives on the productivity of a natural phytoplankton cenosis was studied in May−June of 2005 and 2006 in the coastal laboratory of the Southern Division of the IO RAS located in Gelendzhik. Samples were collected from the sea's surface (0−0.5 m) in both coastal waters (middle shelf, 50-m depth) and in the open sea (50 miles from the coast). The collected samples were bottled with their simultaneous filtration through two layers of mill gauze no. 36 to remove the zooplankton. The volume of each bottle was 5 liters. To determine the initial species composition of the natural phytoplankton, two liters of unfiltered water were concentrated using inverse filtering chambers. The experiments were carried out using 0.5-l Erlenmeyer bottles; the cultural medium's volume was 200 ml. The amount of nitrate and phosphate additives was 12−14 and 1 μM, respectively. The experiments were organized in accordance with the complete factorial scheme [3] using a thermoluminostat. The cultural medium's temperature corresponded to the water temperature at the sea's surface. The incident light intensity was maintained at the PAR level equal to 58−61 $\mu M/m^2$. The day/night period was 16 : 8. In all the experiments, we used a periodic (accumulative) cultivation mode.

All the experiments were arranged using the same scheme (Table 2). Each experimental variant was repeated 2−3 times. The statistical treatment of the obtained results was carried out using the 5% significance level. The result of each experiment can be presented as a regression equation that generalizes all the variants:

$$\text{Nst} = \text{No} + k_1\text{N} + k_2\text{P} + k_3\text{NP},$$

where Nst is the number of cells in the stationary phase of the enrichment culture; No is the average number

Table 3. Abiotic characteristics of the environmental conditions during the period of the investigations (the average values and variation ranges)

Year	2002	2004	2005	2006	2007
*Wind speed, m/s	4.53	4.52	3.88	3.66	3.78
**Air temperature, °C	5.46	6.88	6.24	4.8	5.92
Period	May 24–30	June 20–24	June 9–13	May 24–28	June 19
***Water temperature, °C	18.75	22.65	19.69	18.35	24.68
PO_4, μM	0.8 (0–2.5)	0.3 (0–0.8)	0.66 (0.3–1.7)	0.51 (0.15–1.9)	0
Si , μM	4.82 (3–7.6)	6 (3–10)	7.9 (6–9)	6.8 (3.6–11.7)	53.2 (50.2–57.8)
NO_3, μM	–	0.15 (0.3–0.32)	0.19 (0.16–0.24)	1.5 (0.5–2.4)	2.86 (2.2–3.2)
NO_2, μM	0.024 (0–0.2)	0.02 (0–0.06)	0.056 (0.05–0.06)	0.035 (0.17–0.56)	0.03 (0.02–0.04)
NH_4, μM	0.49 (0.1–1.0)	0.25 (0.1–0.4)	0.32 (0.21–0.43)	0.36 (0.22–0.54)	0.44 (0.42–0.46)
Total N, μM	–	0.44	0.56	1.9	3.33
N : P	–	1.5	0.85	3.7	>100

Notes: The table includes the average concentrations of the biogenic elements in the water layer of 0–15 m deep.
       * The average winter wind speed (December–February).
      ** The average winter air temperature (December–February).
     *** The average surface water temperature in the period of the investigation.

of cells in the stationary phase calculated for all the variants; and k_1, k_2, and k_3 are coefficients reflecting the significance of the experimental variants with the addition of biogenic elements (nitrogen (N), phosphorus (P), and their combination (NP)) [14]. The coefficients were calculated as the difference between the average biomasses of the variants with and without any added elements. If this difference is uncertain, then the coefficient is considered to be zero, and the corresponding term of the equation is omitted. Therefore, the higher the coefficient, the higher the alga growth limitation in the variant.

The number of alga cells was calculated every day right after the withdrawal of some cultural liquid; we used a 0.05-ml Nageotte counting chamber. During the calculations, we took into account all the systematic and size groups of algae, except for the picoplankton fractions (1–2 μm).

RESULTS

Field observations. We registered significant differences in the hydrological and hydrochemical situations of the different years studied (Table 3). The coldest winter seasons preceding the observations were registered in 2002 and 2006, and the most windy winter seasons took place in 2002 and 2004. The water temperature in May–June differed due to the seasonal trend. The beginning of the summer season in 2005 was significantly colder than in 2004 and 2007.

All the studied periods can be clearly divided into the years with high (2002, 2005, and 2006) and low (2004 and 2007) phosphate contents. In the case of

nitrogen and silicon, we did not observe such a division (see Table 3).

For all the studied periods, the most part of the coccolythophorid population with rare exceptions was located in the near-surface water layer (10–15 m deep). *Emiliania huxley* dominated among the coccolythophorids; the magnitude of its population was 90–99% of the total number of algae in this group. In the different years, the number of coccolythophorids in the near-surface water layer varied from 0.004 to 8.2 × 10^6 cells/l (Table 4). Even in the case of population outbreaks, the fraction of coccolythophorids in the total phytoplankton did not exceed 15%. In the different years, the biomass of the coccolithophorids varied from 0.2 to 1460 μg/ml; the mean value varied from 60 to 350 μg/l (Table 4). The maximum fraction of coccolythophorids in the total phytoplankton biomass was registered in 2002, 2005, and 2006; the mean value of this parameter was 56–69%. Thus, three of the five years studied (2002, 2005, and 2006) were characterized by the domination of coccolythophorids among the other phytoplankton species. In 2004 and 2007, the percentage of this species in the total alga biomass was 20%. It is interesting that the maximum number and biomass of coccolythophorids was observed in 2004, when the significance of this species in the phytoplankton was the lowest.

The contribution of coccolythophorids to the total alga biomass was not connected with the preceding winter conditions, the content of silicon or nitrogen in the near-surface water layer, or the N : P ratio. At the same time, we observed a clear relation between the

Table 4. The number and mass of the coccolythophorids and their percentage in the surface phytoplankton (0−15 m layer; the average values and variation ranges)

Year	Date of the survey	Population		Biomass	
		Cells, 10^3/l	% of the total number of phytoplankton cells	μg/l	% of the total biomass
2002	May 18−20	382 (6−1682)	39* (0.1−97)	58 (0.2−147)	56 (2.6−90)
	May 24−30	1368 (48−3840)	40* (0.3−98)	246 (9−691)	65 (12−95)
2004	June 11−16	1307 (64−8241)	3 (0.1−19)	236 (12−1486)	20 (2−64)
	June 20−24	419 (4−1152)	10 (0.1−97)	82 (0.1 −208)	20 (0.1−75)
2005	June 9−13	764 (274−1637)	4 (1−10)	146 (54−302)	57 (31−76)
	June 24−28	1780 (940−2740)	14 (3−41)	348 (194−610)	69 (35−93)
2006	May 24−28	1702 (93−4428)	12 (3−29)	351 (17−825)	67 (30-89)
2007	June 10−13	663 (150−1320)	5 (3−61)	119 (27−238)	20 (3−61)

* The picoplankton species were not taken into account.

role of this alga in the phytoplankton cenosis and the phosphate content (Fig. 1). During the years when the phosphate content was 0.5−0.8 μM, the fraction of coccolythophorids made up 56−69% of the total phytoplankton biomass; in the case of a low phosphate concentration (0−0.3 μM), it was about 20%.

To analyze the long-term dynamics of the coccolythophorid population in the Black Sea, we used the Black Sea phytoplankton database [7]. We took into account the results of the field studies carried out in recent years [11, 35]. Since the population outbreaks of coccolythophorids regularly occur in the May−June period, we used the data obtained only for these months to compare the different years. We did not restrict the region of our observations, since earlier it

Fig. 1. Percentage of coccolythophorids in the total phytoplankton biomass and the average concentration of phosphates (μM) in the 15-m near-surface water layer in different years (sea depth > 50 m).

was shown that the population outbreaks of this species in the Black Sea occur equally in both the shelf and open water. The water layer's depth was limited by 10 m, since during a "bloom" period, the most part of the population is located at this level. After the corresponding data filtration, we include into our analysis 790 samples collected at 274 stations.

The total number of field studies performed between 1968 and 2007 during the May−June period was 14 (Fig. 2). The average biomass of the coccolythophorids in the near-surface water layer varied from 0.1 to 362 μg/l. The total number of coccolythophorid cells varied from 1 to 1780×10^3 cells per liter, and their percentage in the total phytoplankton biomass also varied in a wide range (0.1−70%).

Concerning the long-term dynamics, we registered three periods differing in the level of the quantitative development of the coccolythophorids. Until the eighties, the role of these algae in the Black Sea phytoplankton was insignificant. Their average biomass was 8 μ/l, and they formed about 3% of the total phytoplankton biomass. In the eighties, the average biomass of the coccolythophorids increased to 106 μg/ml, and their role became more significant. Since the nineties, coccolythophorids have determined the phytoplankton's structure and often dominate in their number and biomass. During this period, the average biomass of this alga species and its contribution to the total biomass were equal to 227 μg/l and 42%, respectively.

In spite of the large volume of material (790 samples), the time distribution of the data was nonuniform. The time interval between some data was equal to 7−9 years. Thus, to confirm the trend, one had to use any additional data. The development of coccolythophorids in the earlier years can be tracked via the
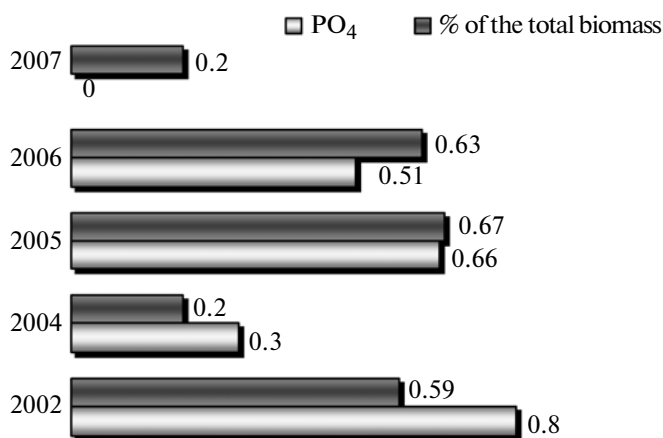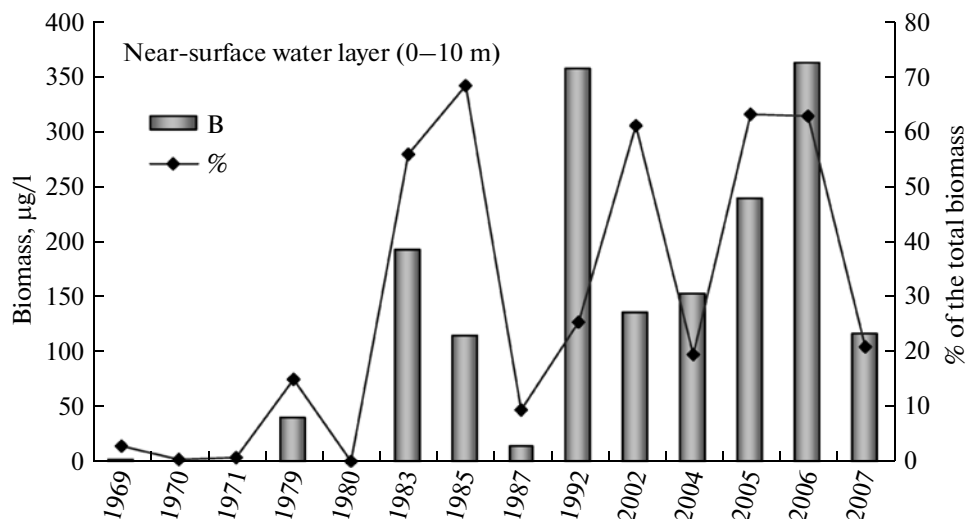
Fig. 2. Long-term changes in the coccolythophorid biomass (B) in the near-surface water and its percentage in the total phytoplankton biomass.
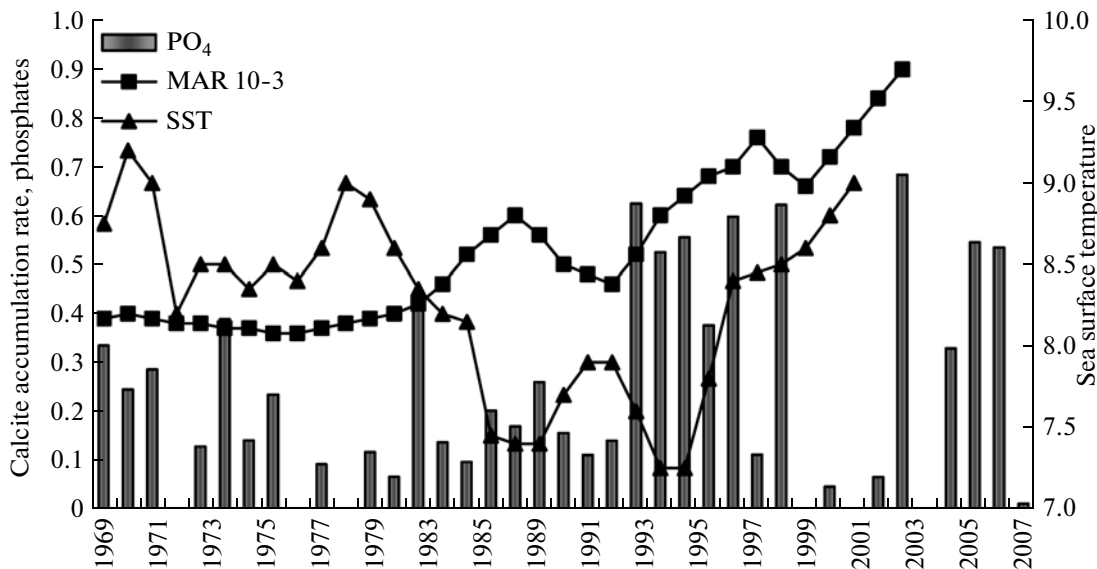

Fig. 3. Interannual changes in the average concentration of phosphates (PO_4, μM) in the near-surface water, the mean rate of the calcite accumulation in the sediments (MAR, relative units) [31], and the average sea surface temperature (SST, °C) obtained for the open sea in the December–March period [39].

rate of the calcite accumulation in the sediments. Actually, the calcite accumulation in the seabed is provided by the accumulation of coccolythes in the form of dead cells or fecal pellets. Such an investigation was carried out using samples collected in the western part of the sea at a depth of 450 m [31]. Starting from the seventies and until now, the calcite accumulation rate has a clearly positive trend (Fig. 3). During this period, the calcite flow to the seabed increased by three times. The changes in the concentration of phosphates (1504 measurements) measured during last 40 years also

demonstrate a trend of an increase of the phosphate content. Until 1990, the average phosphate concentration was equal to 0.21 μM, and then it increased up to 0.4 μM. The analysis of the winter water temperatures showed the presence of a "cold" 10-year period starting from the early eighties up to the mid-nineties. During this time, the average water temperature in December–March was equal to 7–8°C, whereas, in the previous and subsequent years, it was equal to 8–9°C.

Experimental studies. In all four experiments, phosphorus was found to be the main limiting biogenic

Table 5. Regression equations describing the influence of the nitrogen- and phosphorus-containing additives on the size of the coccolythophorid population (Nst) during the spring–summer periods of 2005–2006

No.	Date	Regression equation	Confidence interval	Population
1	May 25, 2005	Nst = 20.76 + 12.34N + 16.3P + 13.2NP	7.93	Shelf
2	May 19, 2006	Nst = 15.56 + 6.84P	4.75	Shelf
3	June 6, 2006	Nst = 15.9 − 3.53N + 15.5P	3.31	Shelf
4	May 26, 2006	Nst = 21.15 + 7.55P	2.2	Open sea

element (Table 5), having the maximum coefficient values in the regression equations. Experiments 2 and 4 did not reveal any influence of the nitrogen-containing additives on the growth of the coccolythophorids. In the case of experiment 1, these additives demonstrated the least influence, whereas, in the experiment 3, they caused a statistically significant reduction in the coccolythophorid population.

DISCUSSION

During the spring and early summer, we revealed significant interannual differences in the development of the coccolythophorid population. The average number and biomass of this alga changed almost by 4 times (see Table 4). The quantitative changes in the Black Sea phytoplankton are connected with the winter convection intensity. In the winter season, the central area of the Black Sea provides favorable conditions for the formation of a surface water layer with heightened concentrations of oxygen and nutrients [10]. To the end of the winter, some surface water is transformed to the cold intermediate water, determining the renewal of the cold intermediate layer (CIL) of water. Then the "new" cold intermediate water is distributed through the whole basin area [18]. The CIL water renewal became maximal in the case of the extremely cold winter and is almost absent in the case of a very warm winter [9]. The biogenic elements raised from the deep in the winter determine the phytoplankton development during the whole vegetation season. For example, the maximum phytoplankton biomass was observed in the summer of 1993 after the extremely cold winter [34].

In our case, we did not observe any dependence of the coccolythophorid population size on the average air temperature and the average wind speed in the winter. We also did not reveal any influence of these parameters on the percentage of coccolythophorids in the total phytoplankton biomass. The maximal value of the total phytoplankton biomass was registered after the "warm" winter of 2004 (up to 1 mg/l). In spite of the rather large number of coccolythophorids (up to 8×10^6 cells/l), their contribution to the total phytoplankton biomass did not exceed 20%. After the "cold" winter of 2006, the total phytoplankton biomass did not exceed 0.5 mg/l and was represented mainly by coccolythophorids (67%). The obtained picture of the spring growth of the coccolythophorid population does not correspond to the previous studies of the interannual changes in the total Black Sea phytoplankton. According to many authors, a cold winter usually causes a more intensive spring "bloom" and a heightened number of plankton algae during the next vegetative season [5, 6, 12, 13, 34].

Experiments with natural shelf and open sea populations showed that the development of the coccolythophorid population is phosphorus-limited (Table 5). Similar results were obtained in 2004 for the open sea populations [35]. One should note that these results were obtained for the May–June period. In the case of the western part of the Black Sea, the specific phytoplankton division rate is determined by the winter concentrations of nitrates and the summer concentrations of ammonium [15]. In the case of May–June of 2001, when dinoflagellates and coccolythophorids represented more than 90% of the phytoplankton biomass in the western part of the Black Sea, the nitrogen concentration was the main limiting factor for their growth and development [51]. Thus, one can conclude that either the limiting factors are spatially divided or interannual changeability takes place. It is also possible that the limiting factors can vary in the course of a year; such a situation was observed in North American bays [33].

Initially, it was considered that a low silicon concentration and a high N : P ratio provide an advantage for coccolythophorids compared to diatoms [24]. The ability of coccolythophorids for active growth at low phosphorus concentrations was considered to be one of the basic traits providing the high competitive ability of this alga species; this fact was reflected in the early mathematical models of their development [19, 46]. However, now there is much evidence that the real situation is quite opposite; i.e., coccolythophorids have some competitive advantage in the case of a low nitrogen content [32, 47].

During our studies, the N : P ratio averaged for the whole water area was lower than the Redfield ratio

(16 : 1), except for the data obtained for 2007; therefore, the nitrogen concentration should serve as a growth-limiting factor for the phytoplankton development. Similar results were obtained for the enclosed Golubaya bay in May and June [50]. However, in June 2004, when the nitrogen content was relatively low (N : P = 1.5), the biomass of the coccolythophorids was about the same as in June 2007 (N : P > 100). The percentage of coccolythophorids in the total phytoplankton biomass did not depend on the N : P ratio (Tables 3 and 4). It seems that the N : P ratio is not a significant condition for the "blooming" of coccolythophorids in the early summer and does not have any significant influence on the intensity of their growth.

The studied years can be clearly divided into the periods with the low (<20%) and high (>55%) contents of coccolythophorids in the total phytoplankton biomass. This division clearly correlates with the phosphate content in the near-surface water layer (Fig. 1). Thus, the development of coccolythophorids in the northwestern part of the Black Sea in the early summer is determined rather by the absolute phosphate concentration than by the N : P ratio. For example, in the case of a nitrogen shortage (the low N : P ratio in 2005 and 2006), one can expect that the growth of the coccolythophorids would be limited by this biogenic element. However, the main limiting factor was the phosphate content. The phosphorous-limited growth of the coccolythophorids was observed in different years in all our experiments (Table 5) and also in the earlier experiments [35]. This result was very surprising, since earlier the dominating growth of coccolythophorids was considered to be connected with a very low phosphate semisaturation constant and, therefore, a low need for phosphates [44]. One can suppose that the Black Sea coccolythophorids differ in this characteristic from those inhabiting other regions of the World Ocean.

During May–June, the phosphate concentration in the near-surface mixed water layer is determined mainly by the regeneration cycle connected with the ecosystem's functioning in the spring. Therefore, there should not be any direct dependence of the coccolythophorids' growth on the abiotic conditions of the previous period (the average winter air temperature, the convection depth, the spring stratification conditions, etc.). To understand and forecast the interannual changes, one should take into account the events occurring in the Black Sea ecosystem prior the blooming of the coccolythophorids.

The observed long-term changes in the Black Sea phytoplankton are unique for this region. Some long-term observations of the shelf water have been performed near the Bulgarian and Romanian shores [20, 36–38, 49]; however, in these cases, the authors investigated the problem of the periodic "blooming" in this region rather than the population dynamics of the coccolythophorids, whose long-term trends were studied only in model experiments [41].

Before the mid-eighties, the contribution of coccolythophorids to the biomass of the Black Sea phytoplankton was rather insignificant (Fig. 2). A similar picture is traced in other publications. Population outbreaks of these algae species were observed occasionally in different seasons and did not have any systematic character [1, 2, 8, 11]. Since the mid-eighties, the coccolythophorid fraction of the Black Sea phytoplankton significantly increased during the late spring and early summer seasons (Fig. 2); this phenomenon was also observed by other investigators [25, 26]. At the same time, some single population outbreaks were observed during other seasons [17]. After the mid-nineties, the mass "blooming" of coccolythophorids in May–June became a regular phenomenon, which is confirmed by the satellite observations [22, 23]. Thus, the obtained picture of the long-term changes in the Black Sea coccolythophorid population agrees with the results of independent studies.

The long-term trend of the increase of the coccolythophorid fraction in the Black Sea phytoplankton agrees with the increase in the calcite sedimentation rate observed in the Black Sea during the last decades [31]. However, the reasons for this trend are still unclear. Possibly, the growth of the coccolythophorid population corresponds to the global tendency for the mass development of these algae in many regions of the World Ocean [28]. There is an hypothesis that it is somehow connected with the increase in the atmospheric CO_2 concentration or with the temperature increase. At the same time, the growth of the CO_2 concentration increases the water's acidity and, therefore, reduces the rate of the cell membranes' calcification [42]. On the other hand, the growth of the CO_2 concentration in the water increases the growth rate of the phytoplankton, including coccolythophorids [45]. As a result, the influence of the increase in the water's *Emiliania huxleyi* concentration on the development of coccolythophorids remains unclear [27]. The study of the influence of bicarbonates on the growth of *Emiliania huxleyi* performed in 2004 by V.A. Silkin (unpublished data) showed that this factor is able to limit the cell growth at concentrations exceeding 10×10^6 cells/l; at the same time, during the whole observation period, the maximum magnitude of the sea populations of this alga did not exceed 8.2×10^6 cells/l. Therefore, the development of the coccolythophorids is not determined by the state of the Black Sea's carbonate system.

During the last 40 years, there were three periods in the Black Sea's climate. The "first warm" period lasted until the early eighties, it was followed by the "cold" period up to the mid-nineties, and the "second warm" period has lasted from the mid-nineties until now [40]. These periods are also traced by some other parameters such as the average winter temperature and

the temperature of the surface and CIL water (Fig. 3). It is interesting that these periods correspond to the observed differences in the development of the coccolythophorid population. The cold period was accompanied by the sharp growth of this population and its percentage in the Black Sea phytoplankton during May–June. In ten years, the population and biomass of the coccolythophorids increased by 2–3 orders; during this period, the total phytoplankton biomass was also maximal [4, 34]. However, after the beginning of the second warm period, the coccolythophorids maintained their large fraction in the total phytoplankton biomass. Possibly, this fact evidences that the phytoplankton cenosis underwent a so-called regime shift and did not return to the state observed before the cold period. The observations of the long-term changes of the different abiotic, hydrochemical, and biological parameters confirmed that such a regime shift took place in the whole Black Sea ecosystem [40].

The cold period coincided with a series of extraordinary events that influenced the functioning of the Black Sea ecosystem. The introduction and development of the comb jelly *Mnemiopsis leidyi* occurred in the end of eighties and resulted in a sharp reduction of the zooplankton populations and, therefore, could have promoted the growth of the coccolythophorid population [30]. In the same period, the volume of biogenic elements transferred to the Black Sea by the rivers sharply decreased [21], which could have reduced the amount of biogenic elements in the central part of the sea; changed their ratio; and, therefore, probably influenced the coccolythophorid population. However, the first signs of the growth of this population appeared several years before 1989–1990 (see Fig. 2); this fact indicates there is not any cause–effect relation between these events. At the same time, the growth of the coccolythophorid population coincides only with the cold period of the early eighties (Fig. 3). It was the time when the population of these algae became noticeable in May–June (Fig. 2). During this period, the first peak of the calcite accumulation rate was observed (Fig. 3). It seems that, even if it is impossible to trace the influence of the cold (but not extremely cold) winter periods within the scale of the interannual changes, this factor became obvious on the decade scale. One can only suppose it somehow provides a significant content of phosphates in the near-surface water layer to the beginning of the May–June period, when the coccolythophorid population outbreak starts. The dependence of the coccolythophorid population's development on this factor was experimentally proved. In addition, the general tendency of the long-term changes in the phosphate concentration in the surface water during this period coincides with the coccolythophorids' growth trend. However, the mechanism of this relation still remains unclear.

REFERENCES

1. E. V. Belogorskaya and G. M. Kondrat'eva, "Distribution of Phytoplankton in the Black Sea," in *Studies of Phytoplankton of the Black and Aral Seas* (Naukova dumka, Kiev, 1965), pp. 36–69 [in Russian].

2. L. V. Georgieva, "Phytocene Species Composition and Dynamics," in *Plankton of the Black Sea* (Naukova dumka, Kiev, 1993), pp. 31–55 [in Russian].

3. V. N. Maksimov, *Multifactor Experiment in Biology* (Nauka, Moscow, 1980) [in Russian].

4. V. I. Man'kovskii, V. L. Vladimirov, E. I. Afonin, et al., "Multiannual Variability of Water Transparency in the Black Sea and Factors Determining Its Significant Decrease in Late 1980s and Early 1990s," Preprint (Morsk. Gidrofizich. Inst., Sevastopol, 1996).

5. G. P. Mashtakova, "Multiannual Dynamics of the Planktonic Community of the Eastern Part of the Black Sea," in *Oceanological and Fishery Studies of the Black Sea* (Nauka, Moscow, 1985), pp. 52–65 [in Russian].

6. G. P. Mashtakova and M. I. Roukhiyainen, "Seasonal Dynamics of Phytoplankton," in *Fundamentals of Biological Productivity of the Black Sea* (Naukova dumka, Kiev, 1979), pp. 85–88 [in Russian].

7. A. S. Mikaelyan, L. A. Pautova, L. V. Georgieva, and V. Yu. D'yakonov, "Database on Phytoplankton of the Black Sea," Okeanologiya **47** (3), 477–480 (2007) [Oceanology **47** (3), 445–448 (2007)].

8. N. V. Morozova-Vodyanitskaya and E. V. Belogorskaya, "The Significance of Coccolithophores, Especially Pontosphaera, in the Plankton of the Black Sea," Tr. Sev. Biol. Stantsii **9**, 14–21 (1957).

9. I. M. Ovchinnikov, L. V. Moskalenko, Yu. I. Popov, and V. V. Serditenko, "Some Characteristics of Winter Hydrological Structure in the Black Sea," in *Changes of the Ecosystem in the Black Sea,* Ed. by M. E. Vinogradov (Nauka, Moscow, 1991) [in Russian].

10. I. M. Ovchinnikov and Yu. I. Popov, "Formation of Cold Intermediate Layer in the Black Sea under Conditions of Extreme Winter," Tr. Gos. Okeanogr. Inst., 132–151 (1990).

11. L. A. Pautova, A. S. Mikaelyan, and V. A. Silkin, "Structure of Plankton Phytocoenoses in the Shelf Waters of the Northeastern Black Sea during the *Emiliania huxleyi* Bloom in 2002–2005," Okeanologiya **47** (3), 408–417 (2007) [Oceanology **47** (3), 377–385 (2007)].

12. M. I. Roukhiyainen, "Seasonal Phytoplankton Dynamics in the Black Sea," in *Marine Biology* (Naukova dumka, Kiev, 1973), No. 28, pp. 150–161 [in Russian].

13. M. I. Senicheva, "Seasonal Dynamics of Abundance, Biomass, and Productivity of Phytoplankton of Sevastopol Bay," in *Marine Ecology* (Naukova dumka, Kiev, 1980), No. 1, pp. 3–11 [in Russian].

14. V. A. Silkin and K. M. Khailov, *Bioecological Monitoring Mechanisms in Aquaculture* (Nauka, Leningrad, 1988) [in Russian].

15. L. V. Stel'makh, V. I. Gubanov, and I. I. Babich, "Seasonal Changes in the Growth Rate and Limitation of Phytoplankton near Sevastopol," Morsk. Ekol. Zh. **3** (4), 55–73 (2004).

16. I. N. Sukhanova, "Concentration of Phytoplankton in Samples," in *Modern Methods of Monitoring of Quantitative Distribution of Marine Plankton* (Nauka, Moscow, 1983), pp. 97–105 [in Russian].

17. I. N. Sukhanova, "Phenomenon of Coccolithophorid Bloom Early in Autumn in the Black Sea," Dokl. Akad. Nauk **340** (2), 256–259 (1995).

18. V. G. Yakubenko and S. E. Esaulov, "Spatiotemporal Variability in Central Parts of Cyclonic Eddies in the Black Sea," in *Ecosystem of Open Waters of the Black Sea in Winter*, Ed. by M. E. Vinogradov (IO RAN, Moscow, 1992) [in Russian].

19. D. L. Aksnes, J. K. Egge, R. Rosland, and B. R. Heimdal, "Representation of *Emiliania huxleyi* in Simulation Models. A First Approach," SARSIA **79**, 291–300 (1994).

20. N. Bodeanu, S. Moncheva, G. Ruta, and L. Popa, "Long-Term Evolution of the Algal Blooms in Romanian and Bulgarian Black Sea Waters," Cercetari Marine, INCDM. Constanta **31**, 37–55 (1998).

21. A. Cociasu and L. Popa, "Significant Changes in Danube Nutrient Loads and Their Impact on the Romanian Black Sea Coastal Waters," Cercetari Marine, INCDM. Constanta **35**, 25–37 (2004).

22. T. Cokacar, N. Kubilay, and T. Oguz, "Structure of *Emiliania Huxley* Blooms in the Black Sea Surface Waters as Detected by SeaWIFS Imagery," Geophys. Res. Lett. **28** (24), 4607–4610 (2001).

23. T. Cokacar, T. Oguz, and N. Kubilay, "Satellite-Detected Early Summer Coccolithophore Blooms and Their Interannual Variability in the Black Sea," Deep-Sea Res. **51** (8), 1017–1031 (2004).

24. J. K. Egge and D. L. Aksnes, "Silicate As Regulating Nutrient in Phytoplankton Competition," Mar. Ecol.: Proc. Ser. **83**, 281–289 (1992).

25. E. Eker, L. Georgieva, L. Senichkina, and A. E. Kideys, "Phytoplankton Distribution in the Western and Eastern Black Sea in Spring and Autumn 1995, ICES," J. Mar. Sci. **56**, 15–62 (1999).

26. E. Eker-Deverli and A. E. Kideys, "Distribution of Phytoplankton in the Southern Black Sea in Summer 1996, Spring and Autumn 1998," J. Mar. Systems **39**, 203–211 (2003).

27. A. Engel, I. Zondervan, K. Aerts, et al., "Testing the Direct Effect of CO_2 Concentration on a Bloom of the Coccolithophorid *Emiliania huxleyi* in Mesocosm Experiments," Limnol. Oceanogr. **50** (2), 493–507 (2005).

28. M. D. Iglesias-Rodriguez, C. W. Brown, S. C. Doney, et al., "Representing Key Phytoplankton Functional Groups in Ocean Carbon Cycle Models: Coccolithophorids," Global Biogeochem. Cycles **16**, 1100 (2002).

29. L. I. Ivanov, S. Konovalov, V. Melnikov, A. Mikaelyan, et al., *Nato TU-BLACK Sea Project Ecosystem Modeling as a Management Tool for the Black Sea, Symposium on Scientific Results*, Ed. by L. Ivanov and T. Oguz (Kluwer Acad. Publ., Dordrecht, 1998), pp. 11–37.

30. A. V. Kovalev, A. D. Gubanova, and A. E. Kideys, et al., *Ecosystem Modeling as a Management Tool for the Black Sea. NATO Science Partnership Sub-Ser.*, Ed. by L. Ivanov and T. Oguz (Kluwer Acad. Publ., Dordrecht, 1998), Vol. 2, pp. 221–234.

31. G. V. Laptev and O. V. Voitsekhovitch, "Geochronological Reconstruction of Fallout Radionuclides 137Cs and 241Am Accumulation in Abessal Sediment of the Black Sea," in *Black Sea Ecosystem 2005 and Beyond. Abstracts of 1st Biannual Scientific Conference* (Istanbul, 2006), pp. 45–46.

32. E. J. Lessard, A. Merico, and T. Tyrell, "Nitrate:Phosphate Ratios and *Emiliania huxleyi* Blooms," Limnol. Oceanogr. **50**, 1020–1024 (2005).

33. T. C. Malone, D. J. Conley, T. R. Fisher, et al., "Scales of Nutrient-Limited Phytoplankton Productivity in Chesapeake Bay," Estuaries **19**, 371–385 (1996).

34. A. S. Mikaelyan, *Sensitivity of Semi-Enclosed and Enclosed Seas to Anthropogenic and Climatic Changes*, Ed. by E. Ozsoy and A. Mikaelyan (Kluwer Acad. Publ., Dordrecht, 1997), pp. 105–116.

35. A. S. Mikaelyan, L. A. Pautova, S. I. Pogosyan, and I. N. Sukhanova, "Summer Bloom of Coccolithophorids in the Northeastern Black Sea," Oceanology **45** (Suppl. 1), S127–S138 (2005).

36. S. Moncheva, O. Gotsis-Skretas, K. Pagou, and A. Krastev, "Phytoplankton Blooms in Black Sea and Mediterranean Ecosystems Subjected to Anthropogenic Eutrophication: Similarities and Differences," Estuar. Coastal Shelf Sci. **53**, 281–295 (2001a).

37. S. Moncheva and A. Krastev, *Sensitivity of Semi-Enclosed and Enclosed Seas to Anthropogenic and Climatic Changes*, Ed. by E. Ozsoy and A. Mikaelyan (Kluwer Acad. Publ., Dordrecht, 1997), Vol. 27, pp. 79–94.

38. S. Moncheva, V. Doncheva, and L. Kamburska, "On the Long-Term Response of Harmful Algal Blooms to the Evolution of Eutrophication off the Bulgarian Black Sea Coast: Are the Recent Changes a Sign of Recovery of the Ecosystem—The Uncertainties," in *Proceedings of IX International Conference on "Harmful Algal Blooms,"* Ed. by G. M. Hallegraff et al. (Hobart, Tasmania (UNESCO-IOC, Paris), 2001b), pp. 177–182.

39. T. Oguz, "Black Sea Ecosystem Response to Climatic Variations," Oceanography **18** (2), 122–133 (2005).

40. T. Oguz and D. Gilbert, "Abrupt Transitions of the Top-Down Controlled Black Sea Pelagic Ecosystem during 1960–2000: Evidence for Regime-Shifts under Strong Fishery Exploitation and Nutrient Enrichment Modulated by Climate-Induced Variations," Deep-Sea Res. I **54**, 220–242 (2007).

41. T. Oguz and A. Merico, "Factors Controlling the Summer *Emiliania huxleyi* Bloom in the Black Sea: A Modeling Study," J. Mar. Systems **59**, 173–188 (2006).

42. J. C. Orr, V. J. Fabry, O. Aumont, et al., "Anthropogenic Acidification over the Twenty-First Century and Its Impact on Calcifying Organisms," Nature **437** (2005) (2005).

43. E. Paasche, "A Review of the Coccolithophorid *Emiliania huxleyi* (Prymnesiophyceae), with Particular Ref-

erence to Growth, Coccolith Formation, and Calcification–Photosynthesis Interactions," Phycologia **40** (6), 503–529 (2002).

44. R. Reigman, A. A. M. Noordeloos, and G. C. Cadee, "Phaeocystis Blooms and Eutrophication of the Continental Coastal Zones of the North Sea," Mar. Biol. (Berlin) **112**, 479– 484 (1992).

45. U. Riebesell, "Effects of CO_2 Enrichment on Marine Phytoplankton," J. Oceanogr. **60** (4), 719–729 (2004).

46. T. Tyrrel and A. H. Taylor, "A Modeling Study of *Emiliania Huxley* in the NE Atlantic," J. Mar. Syst. **9**, 83–112 (1996).

47. T. Tyrrell and A. Merico, *Coccolithophores—From Molecular Processes to Global Impact*, Ed. by H. R. Thierstein and J. R. Youngs (Springer, 2004), pp. 75–97.

48. Z. Uysal, A. E. Kideys, L. Senichkina, et al., *Ecosystem Modeling and Management Tool for the Black Sea*, Ed. by L. I. Ivanov and T. Oguz (Kluwer Acad. Publ., Dordrecht, 1998), Vol. 1, pp. 151–162.

49. V. Velikova and V. Mihneva, "High and Low Energy Ecosystem Structure in Terms of Regime Shifts: Examples from the Western Black Sea," in "Large Scale Disturbances (Regime Shifts) and Recovery in Aquatic Ecosystems: Challenges for Managements towards Sustainability," UNESCO-Roste/BAS Workshop on Regime Shifts, Varna, June 14–16, 2005), Ed. by V. Velikova and N. Chipev (Varina, 2005), pp. 42–57.

50. E. V. Yakushev, V. S. Arkhipkin, E. A. Antipova, et al., "Seasonal and Interannual Variability of Hydrology and Nutrients in the Northeastern Black Sea," Chem. Ecol. **23** (1), 29–41 (2007).

51. A. Yilmaz, Y. Coban-Yildiz, F. Telli-Karakoc, and A. Bologa, "Surface and Mid-Water Sources of Organic Carbon by Photoautotrophic and Chemoautrophic Production in the Black Sea," Deep-Sea Res. II **53**, 1988–2004 (2006).