Oceanology, Vol. 45, Suppl. 1, 2005, pp. S127–S138.

MARINE BIOLOGY

Summer Bloom of Coccolithophorids in the Northeastern Black Sea

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Received April 19, 2005

Abstract—On the basis of the observations performed in June–July 2004, the main parameters of the coccolithophorid blooming are described. *Emiliania huxleyi* was the dominant species. Its maximum abundance amounted to 1.5–6.0 × 10^6 cells l^{-1}. High concentrations occurred throughout the entire area studied (up to 70 miles from the shore). The main share of the population was confined to a thin upper mixed layer. The population of this alga is characterized by two types of cells: calcified (with coccoliths) and naked (lacking coccoliths). Cells of the latter type were mainly observed under the seasonal pycnocline layer, while those of the former type prevailed in the surface layer. The coccolithophorid biomass in the surface layer averaged 180 mg/m^3 throughout the study area. Experiments aimed at determining the dark adaptation of phytoplankton revealed no inhibition of photosynthesis in the upper water layers under coccolithophorid domination. Experimental data indicate a dependence of the *Emiliania huxleyi* population development on the phosphorus concentrations.

INTRODUCTION

Coccolithophorids are of particular interest because they consume $CaCO_3$ from water for construction of coccoliths, in addition to fixation of atmospheric CO_2 when constructing their cells, which results in elevated CO_2 withdrawal from water and, in particular cases of mass development, in the transformation of the carbonate system [31]. In the Black Sea, coccolithophorids are largely represented by the species *Emiliania huxleyi.* This species was recorded in waters both over the shelf and in the open sea [1–4, 7, 19, 20, 29, 37].

Previously, *Emiliania huxleyi* blooms (1.0 × 10^6 cells l^{-1}) were rare. The first bloom of this species in the Black Sea was observed in 1951 [4]. At that time, the maximum abundance was 0.85 × 10^6 cells l^{-1}. Later on, its high abundance was also observed in the spring and early summer seasons [2, 19, 20]. These blooms were, however, described as short-term and local events. A mass development of *Emiliania huxleyi* was observed in November 1993 [7].

The regular coccolithophorid blooming during the early summer season was recently inferred from the analysis of long-term satellite observations [16, 17]. Areas of mass development occupied up to 40–60% of the sea. Unfortunately, these data were not accompanied by field observations. The species composition and abundances of coccolithophorids during this period remain unknown so far. Taking into consideration the significance of this phenomenon for the ecosystem of the Black Sea and carbon cycle, special studies of coccolithophorid development in the early summer were carried out in the northeastern part of this basin.

MATERIALS AND METHODS

The materials for this study were collected during three cruises of the R/V *Akvanavt.* Most of the samples were obtained during cruise 62 on June 21–26, 2004, in the northeastern part of the sea. Figure 1 shows location of stations. In total, samples were collected at 50 stations along two transects oriented from the shore toward the open sea. The first (northern) transect extends over 70 miles from the town of Gelendzhik and the second (southern) transect runs over 50 miles from Sochi. Samples from the surface waters were taken at all the stations. Five and three vertical sample series were collected along the first and second transects, respectively.

Two weeks before the main phase of operations on cruise 61 of the R/V *Akvanavt*, samples were taken from the sea surface along practically the same two transects on June 12–17 (Fig. 2). Seven additional phytoplankton samples were taken along the transect extending from Tuapse on June 14–15 on cruise 66 of the same vessel (Fig. 2).

Phytoplankton samples were taken using Rosette bottle samplers mounted on a SeaBird CTD probe down to depths of 30–100 m depending on the sea depth. Vertical series consisted of 6–8 samples. Sampling depths were determined on the basis of data obtained during the preliminary hydrophysical sounding. Water samples for the hydrochemical analysis were collected from the same depths.

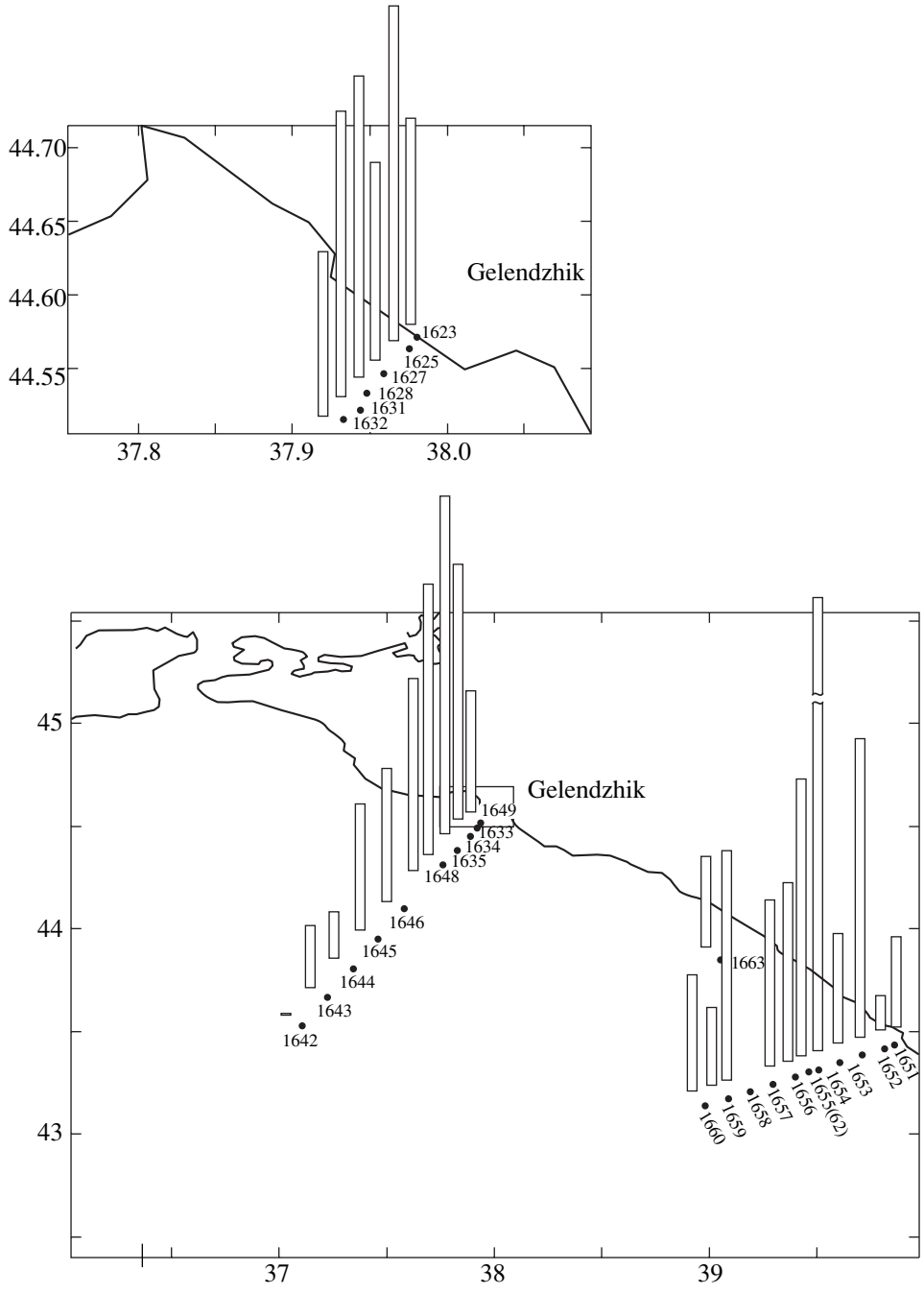

Fig. 1. Coccolithophorid abundance at the sea surface in the period of June 21–June 25 (1 cm in the height of the rectangle corresponds to 150×10^3 cells l^{-1}).

Concentrates of the samples collected during cruise 62 of the R/V *Akvanavt* for phytoplankton cell counting were obtained by filtration of 2 to 5 l of water in chambers of reverse filtration through nuclepore filters with a pore size of 1 μm [8]. Concentrates were fixed by neutral formaldehyde up to a final concentration of 1%. In the samples collected on cruises 61 and 66, only the number of coccolithophorids was determined. Nonfiltered samples 100 ml in volume were fixed in the same manner. Both kinds of samples were treated at the onshore laboratory for one to two months.

Identification of species and counting of cells were carried out in aqueous preparations under a *Ergoval* (Karl Zeiss, Jena) light microscope with magnifications of 16×10 and 16×40. Naujotte and Naumann counting chambers with volumes of 0.05 and 1.0 ml, respectively, were used for counting nanno- and microphytoplankton cells. Small flagellates (fractions 2–4, 4–6,

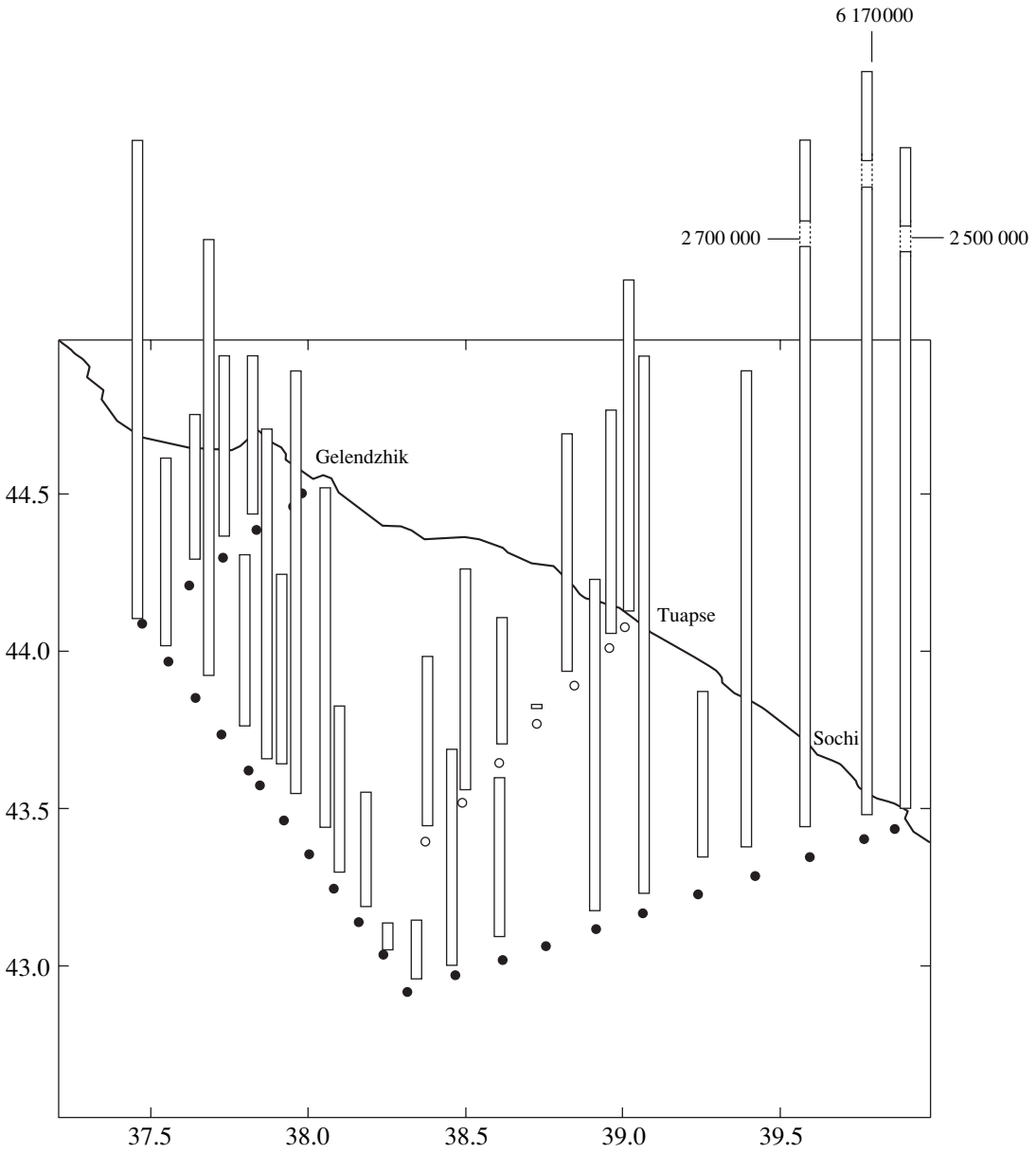

Fig. 2. Coccolithophorid abundance at the sea surface (1 cm in the height of the rectangle corresponds to 125×10^3 cells l^{-1}). The closed and open circles designate the stations observed during cruises 61 (June 12–17) and 66 (July 14–15), respectively.

and 6–8 μm) and coccoliths were counted in a Fuchs–Rosenthal chamber. The relative error was approximately 20%.

The biomass was calculated in terms of the raw weight by the method of geometric similarity equating cells to shape-corresponding figures (cylinder, sphere, rotation ellipsoid). Manuals [21, 23, 34, 41] were used for taxa identification. Coccolithophorids were identified under a scanning electron microscope (SEM).

In order to estimate the efficiency of the primary photosynthesizing processes and adaptation characteristics of the photosynthetic system of phytoplankton, fluorescence of chlorophyll *a* was measured on cruises 61 and 62. The measurement of the ratio between the fluorescence intensity of chlorophyll *a* under exciting light (Fm) saturating photosynthesis and conditions harmless to the photosynthesizing system (Fo) makes it possible to determine the efficiency of the primary photosynthesis processes (Fv/Fm), which is calculated using the formula (Fm – Fo)/Fm = Fv/Fm [6]. The fluorescence intensity values (Fo) correspond with a high correlation coefficient to the total content of pigments. The higher the Fv/Fm value, the more efficient the cell system under given illumination conditions.

These parameters (Fo and Fv/Fm) were determined in bottle samples using an onboard fluorimeter. In addition, fluorescence parameters of phytoplankton chloro-

phyll were continuously measured in the surface layer with an interval of 1 min during the extent of the entire cruise using a flow-through fluorimeter. Each every-minute value resulted from averaging of 400 measurements.

In order to estimate factors limiting the growth of coccolithophorids, an experiment was carried out with the natural population of these algae. Water was taken at station 1662 from a depth of 1.5 m (water temperature 23°C). To eliminate the factor of grazing by mesoplankton, water was filtered through a no. 36 sieve with a mesh size of 180 μm. Additional aliquots 4 l in volume were placed into 5-l transparent plastic containers. Each container received 400 ml of water taken from two different layers. In one variant of the experiment (three replications), water with a high concentration of nitrates was taken from a depth of 115 m, and in another experimental series (three replications), water with a high content of phosphates was taken from a depth of 155 m. The reference containers (two replications) took filtered water from a depth of 1.5 m. Before adding to the experimental containers deep water, it was heated to 20°C. Water was exposed for 3 days under conditions corresponding to the regime of a "northern window." Phytoplankton cells were counted (up to 200–400 cells) in line with the traditional procedure.

RESULTS

Abiotic Environments

According to the hydrophysical studies, the upper boundary of the cold intermediate layer (CIL) during the cruise was located at depths of 25–30 m in the northern transect and of 40–50 m in the southern one. This layer was only 50–60 m thick in the central part of the sea and was 90–100 m thick in the area of the Main Black Sea (Rim) Current (BSRC).

The midstream of the BSRC was located at some distance from the shore. The maximum dynamical current velocities were observed at a distance of 20 and 25 miles from the shore in the northern and southern transects, respectively. The respective dynamical velocities in the current mainstream were 20 and 30 m/s.

During cruise 61, the thickness of the upper mixed layer ranged from 6 m over the shelf to 15 m in the open sea. The water temperature at the surface was 17–18°C. During the subsequent period of cruise 61 and throughout cruise 62, the weather was sunny and calm. The upper mixed layer was intensely heated, which resulted in its stratification. The temperature sharply decreased downward. A particularly sharp gradient was observed in the upper 5 m. During cruise 62, the temperature of the surface waters increased from 20 to 24°C.

Along the southern transect, a thin surface layer was freshened. In the upper 4-m-thick layer, the salinity varied from 15 to 17‰, while in the northern transect, the salinity was never below 17‰ even at the shallowest water stations.

In the upper 20 m, the phosphate content ranged from 0.02 to 0.05 μmol in the open sea and from 0.04 to 0.8 μmol at near-shore stations. Nitrites in this layer were practically missing. The nitrate content varied from 0.2 to 0.4 μmol in the open sea and from 0.3 to 0.6 μmol at near-shore stations. The ammonia nitrogen concentration in the layer 0–20 m averaged 0.2–0.5 μmol.

Species Composition of Phytoplankton

In total, 132 species and varieties of unicellular algae representing seven taxonomic groups were registered in the phytocoenosis. Dinoflagellates were dominant (68 species) with the genus *Protoperidinium* being the most diverse (11 species). Diatoms (46 species) were largely characterized by their littoral–benthic forms (11 species, 24%), which occurred only in the shelf waters. Among planktonic diatoms, the genus *Chaetoceros* was the most diverse (8 species); its representatives were the most abundant along the southern transect. The taxonomic compositions of phytoplankton assemblages in both transects were similar.

The seven most abundant coccolithophorid species were identified under a scanning electron microscope. *Emiliania huxleyi* (Lochmann) Hay et Mohler, 1967 was the most common among coccolithophorids. Its cells and coccoliths varied in size from 6 to 12 μm and from 3 to 4 μm, respectively. The other species were *Syracosphaera ossa* (Lecal) Loeblich and Tappan, *Syracosphaera* sensu *dilatata* Jordan *et al.*, 1993, *Syracosphaera* sensu *exique* Okada and McIntyre, 1977, *Calciosolenia brasiliensis* (Lochman, 1919) Young. n. comb., *Acanthoica* sensu *quattrospina* Lochman, 1903, and *Helladosphaera* sensu *cornifera* (Schiller, 1913) Kamptner, 1937.

Under a light microscope, *Emiliania huxleyi*, which was dominant, was readily identifiable among coccolithophorids. In addition, several types of coccolithophorid cells were also distinguishable, but unidentifiable at a species level, because species identification of coccolithophorids is based on the structure of coccoliths, which are indiscernible under a light microscope. The highest diversity of coccolithophorids, *E. huxleyi* and nonidentified forms included, was recorded along the northern transect in the open sea (8 "species," station 1642, depth 75 m).

Two types of cells occur among the *E. huxleyi* population: calcified (with coccoliths) and so-called "naked" cells (lacking coccoliths). The first type of cells includes, in turn, two simultaneously occurring varieties conventionally named as "form 1" and "form 2." In form 2, structural elements of the coccosphere (envelope consisting of coccoliths that surrounds the cell) are poorly distinguishable under a light microscope. In contrast to the latter, form 1 bears distinct coccoliths and looks like a typical *E. huxleyi* illustrated in all the manuals available. Form 2 was always dominant in the

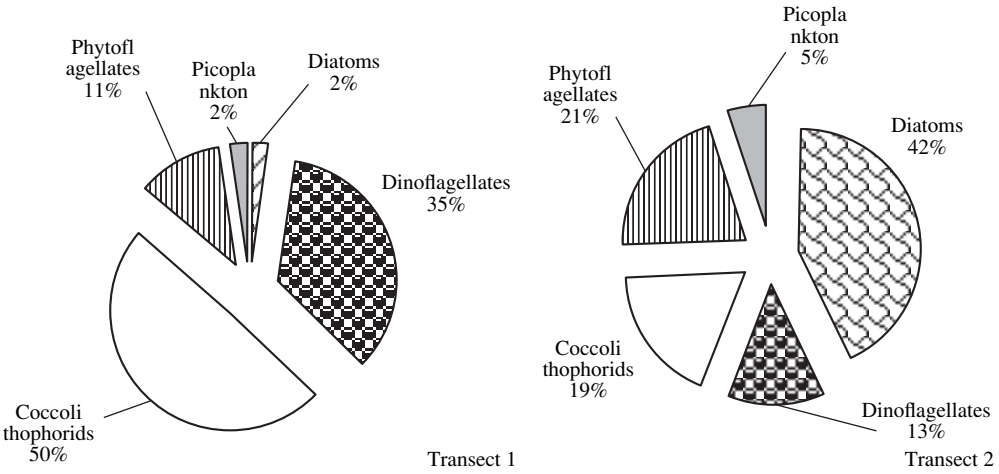

Fig. 3. Proportions of different taxonomic groups in the phytoplankton biomass at the sea surface.

E. huxleyi population, whereas the share of form 1 in the upper mixed layer was insignificant (2–4%); their abundance increased with depth up to 4–5%.

The abundance of *E. huxleyi* cells lacking a coccosphere ("naked" cells 4–7 μm across, with compact cell wall membranes) increased in the waters located under the seasonal density jump layer throughout the entire examined depth interval. Their share in the population was 50–70%. In the surface layer, this proportion between the calcified and "naked" cells was noted only at station 1642, where the total abundance of *E. huxleyi* was very low (0.009×10^6 cells l^{-1}).

Quantitative Variations

In terms of abundance, picophytoplankton dominated throughout the entire study region. Its share in the total abundance varied from 85 to 99%. Small flagellates comprised 1–10% of the population.

The northern and southern transects differed in the quantitative characteristics of the phytoplankton community: the total phytoplankton biomass ranged from 80 to 615 mg/m^3 (382 mg/m^3 on the average) and from 84 to 2385 mg/m^3 (1162 mg/m^3 on the average), respectively. The total biomass calculated for a 1-m^2 water column varied from 5.9 to 16.9 g/m^2 in both transects.

Coccolithophorids provided the bulk of the phytoplankton biomass (up to 50%) in the northern transect (Fig. 3). The share of dinoflagellates and diatoms in total biomass was 35 and 2%, respectively. Along the southern transect, the dominant role belonged to diatoms. They provided 42% of the total phytoplankton biomass, while dinoflagellates and coccolithophorids constituted 21 and 19%, respectively.

High contents of coccolithophorids were observed throughout the entire study region. Their abundances along both transects were nearly identical. The biomass averaged 194 and 177 mg/m^3 in the northern and southern transects, respectively. The total phytoplankton biomass was substantially higher along the southern transect owing to the intense development of diatoms. The abundance of coccolithophorids varied from 0.001×10^6 to 1.5×10^6 cells l^{-1} (Fig. 1). Through most of the region under consideration, their abundance exceeded 0.5×10^6 cells l^{-1}, approaching the blooming level (1.0×10^6 cells l^{-1}). Because of the small sizes of coccolithophorid cells, their biomass was low, varying from 60 to 440 mg/m^3.

Two weeks earlier, during cruise 61, coccolithophorid abundance in the surface layer was slightly higher along the southern transect, where it varied from 0.064×10^6 to 6.17×10^6 cells l^{-1} (Fig. 2). As during cruise 62, its maximal values were observed in the southern transect. Two weeks after cruise 62, coccolithophorid abundance in the surface layer remained high, ranging from 0.009×10^6 to 0.566×10^6 cells l^{-1} (cruise 66).

During the entire survey period, high concentrations of coccolithophorids were observed in the near-shore zone, shoals included. For example, at a station located at a depth of 10 m (Fig. 1, station 1623), their abundance in the surface layer was 0.55×10^6 cells l^{-1}. Both transects demonstrated a tendency of seaward decrease in the coccolithophorid abundance. The minimum abundance (0.009×10^6 cells l^{-1}) was recorded at the most remote station of the northern transect (Fig. 1, station 1642).

Water samples contained numerous single coccoliths belonging to *E. huxleyi.* Their abundance was two orders of magnitude higher than that of the cells and varied from 10×10^6 to 140×10^6 ind. l^{-1}. The maximal abundance of coccoliths (325×10^6 ind. l^{-1}) was noted at the station characterized by the maximum abundance of cells (6.17×10^6 cells l^{-1}); i.e, they demonstrated a positive correlation (Fig. 4).

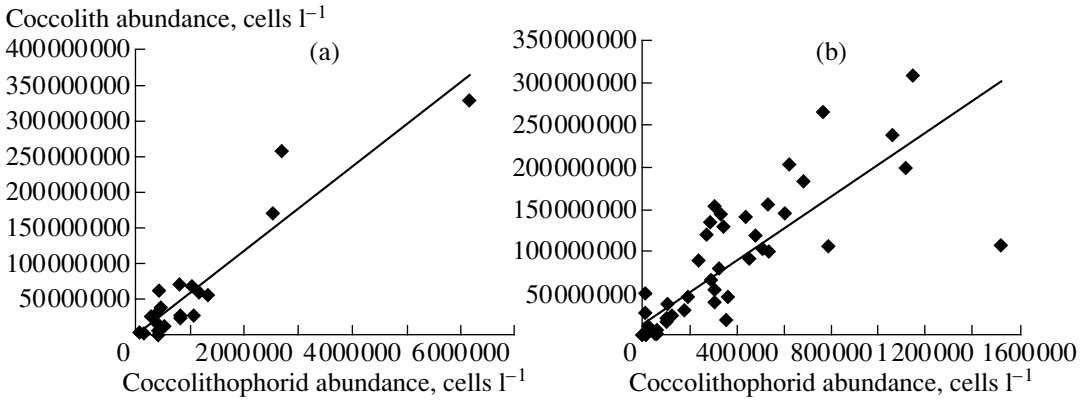

Fig. 4. Proportions of coccoliths and cells in the samples: (a) cruise 61; (b) cruise 62. The straight lines show the trends.

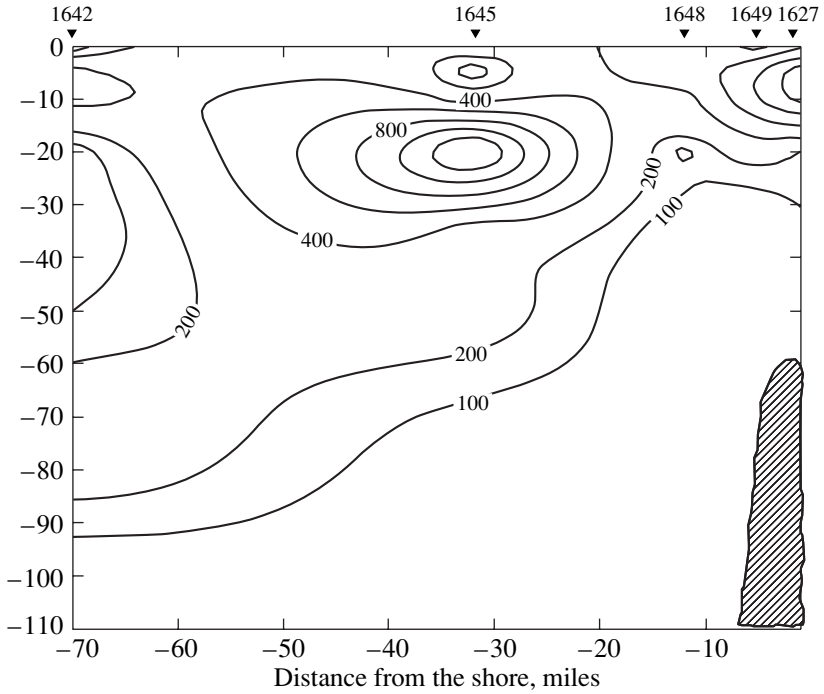

Fig. 5. Vertical distribution of the coccolithophorid abundance along the northern transect, $N \times 10^3$ cells l^{-1}.

Vertical Distribution

The vertical distribution of phytoplankton was studied at eight stations. Because of the strong stratification, the core of the *E. huxleyi* population (form 2) occurred mainly in the 10-m upper layer along both the northern and southern transects (Figs. 5, 6). Under these conditions, the maximum abundances of *E. huxleyi* in the shelf zone were recorded at shallow depths (1.1×10^6 cells l^{-1}, station 1627, depths 5 and 10 m). Seaward, its maximum abundances were observed both at the surface (0.52×10^6 cells l^{-1}, station 1648, depth 0 m; 1.5×10^6 cells l^{-1}, station 1662, depth 0 m) and in the upper mixed (0.48×10^5 cells l^{-1}, station 1642, depth 5 m) layers. Only in the BSRC area (northern transect) was the maximum of the *E. huxleyi* abundance (1.1×10^6 cells l^{-1}) noted at a depth of 20 m at a temperature of 13°C (maximum temperature gradient).

Form 1 of *E. huxleyi* occurred mainly in the lower cold-water layers along the northern transect with the maximum abundances observed in the BSRC waters (0.065×10^6 cells l^{-1}, station 1645, depth 20 m). The elevated concentrations of this form were recorded in the near-bottom waters of the shelf zone (0.004×10^6 cells l^{-1}, station 1627, depth 40 m) and near the upper boundary of the main pycnocline in the open sea (0.0024×10^6 cells l^{-1}, station 1642, depth 75 m). Along the southern transect, form 1 occurred only occasionally. Single individuals were found only in the surface

layer (0–1.5 m) at station 1662 with a very strong thermocline.

In June 2004, a significant abundance of *Coronosphaera meiditerranea* was also observed in addition to *E. huxleyi.* This alga was recorded throughout the entire study region with maximum abundances along the northern transect at a depth of 20 m both in the open sea and over the slope (0.13×10^6 cells l^{-1}, station 1642; 0.11×10^6 cells l^{-1}, station 1648). A significant amount was also noted in the surface shelf waters (station 1651). Along the southern transects, its abundance did not exceed 0.2×10^6 cells l^{-1} (depth interval 30–40 m).

State of the Phytoplankton Photosynthetic System

The study of the fluorescence intensity (Fo) distribution over the water column carried out on cruise 61 shows that its maximum values are mainly confined to a depth of 10 m in the shelf zone and of approximately 30 m in the open sea. The photosynthetic efficiency of phytoplankton Fv/Fm was maximal (0.65–0.70) at the same depths, decreasing toward the surface and at depths greater than 50 m. During the daytime, the Fv/Fm value at the surface varied from 0.1 to 0.25. During the nighttime, this parameter increased up to 0.5–0.6. Similar studies repeated on cruise 62 provided similar results.

In order to estimate the photoinhibition degree of the photosynthetic system in algae, experiments with dark adaptation of cells were carried out. The photoinhibition value was obtained by measuring the increase in the fluorescence intensity of chlorophyll Fo under a prolonged (3 h) adaptation of algae in the dark. Figure 7 illustrates the variations in the fluorescence intensity of chlorophyll depending on the incubation period in the dark estimated for the phytoplankton samples taken from the surface layer during the daytime at stations 1625 and 1644. At station 1625, no changes in the fluorescence intensity were observed during the entire exposure period. In contrast, at station 1644, Fo increased from 1 to 1.35 after 3 h.

Fluorescence parameters of the phytoplankton chlorophyll were measured while the ship was moving near stations 1625 and 1644 (Fig. 8). The measurements were performed in the daytime under maximal insolation. The fluorescence intensity Fo was higher between stations 1644 and 1645, where it was approximately equal to 1. Between stations 1623 and 1628, this parameter was substantially lower, varying from 0.5 to 0.7. At the same time, the efficiency values of the primary photosynthesis processes were, in contrast, notably higher between these stations as compared with those measured between stations 1644 and 1645 (0.18 ± 0.025 versus 0.12 ± 0.025).

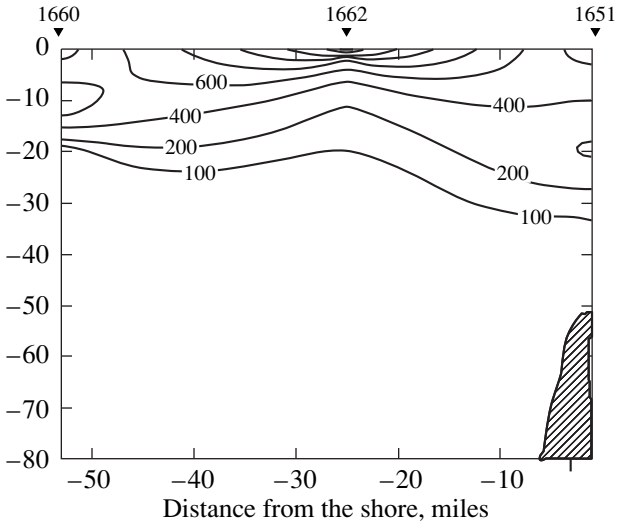

Fig. 6. Vertical distribution of the coccolithophorid abundance along the southern transect, $N \times 10^3$ cells l^{-1}.

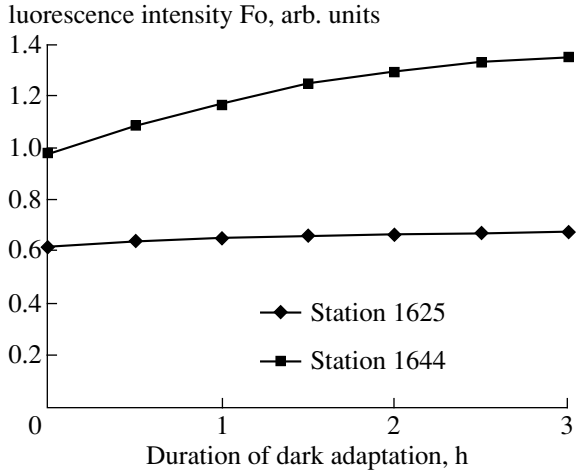

Fig. 7. Variations in the chlorophyll fluorescence intensity (Fo) during phytoplankton dark adaptation. Samples were taken from the surface layer at stations 1625 and 1644 at 2 : 00 p.m. and 1 : 00 p.m., respectively.

Experimental Studies

The experiment carried out with addition of deep seawater to the containers with the surface population showed that, after 72 h, the abundance of *E. huxleyi* in the reference containers decreased by only 8% (table). Similar results were also obtained for containers where water with enhanced nitrogen content was added: the abundance of the species decreased by 23% on the average. In the containers with an elevated phosphorus content, the coccolithophorid abundance increased by a factor of 1.79 on the average (at a 5% significance level) by the end of the experiment; i.e., it was substantially higher as compared with the initial one. The growth rate of the cells remained, however, relatively low. The specific growth rate averaged 0.2 per day and the population doubling time was 3 days.

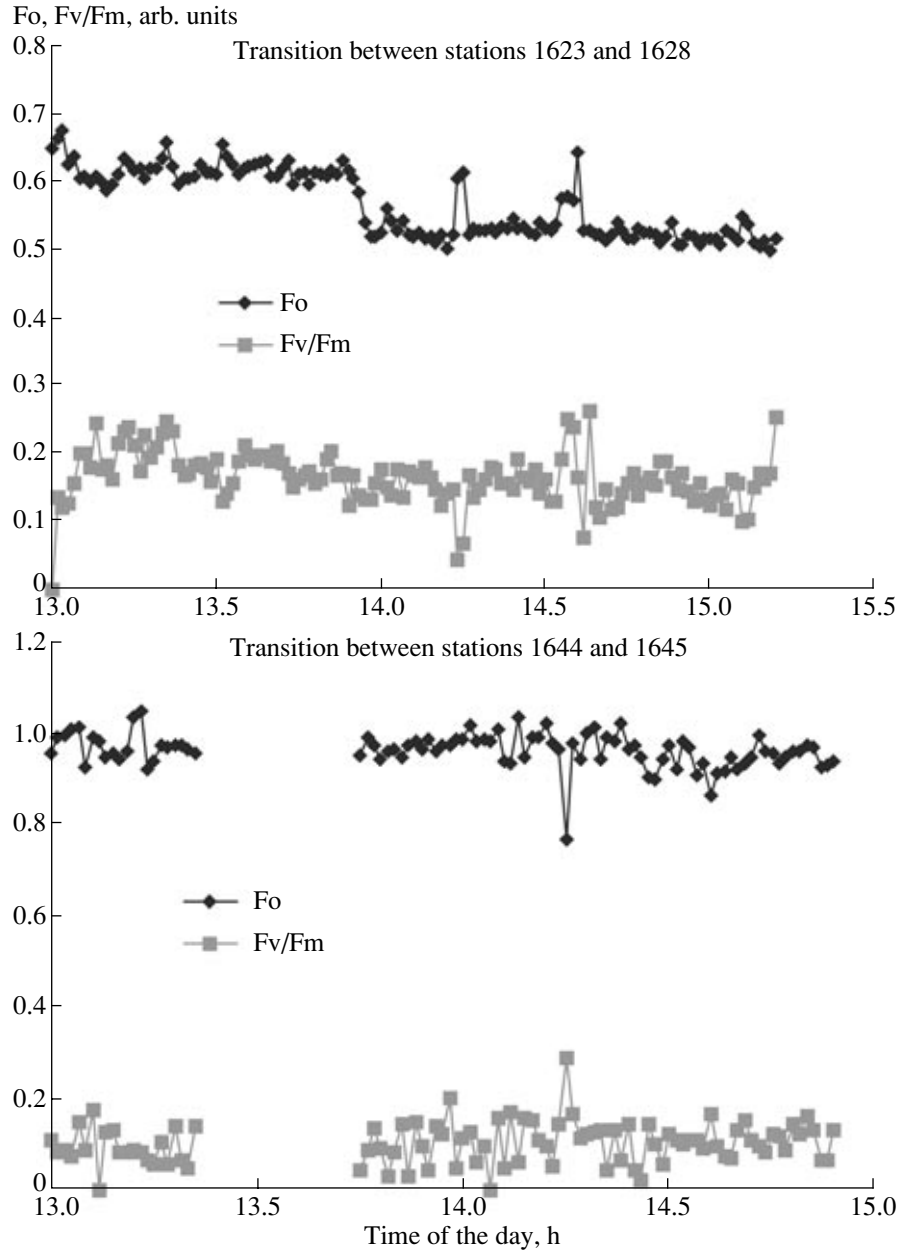

Fig. 8. Values of fluorescence (Fo) and variable chlorophyll fluorescence (Fv/Fm) in the surface waters at transitions between stations 1623 and 1628 and stations 1644 and 1645. In the period from 1:20 pm to 1:45 pm, observations were stopped.

DISCUSSION

Morozova-Vodyanitskaya and Belogorskaya [4] determined 18 coccolithophorid species under a light microscope. Identification of coccolithophorids at the species level is possible, however, only under a scanning electron microscope, which has not practically been used for the study of samples from the Black Sea. This method was first applied when examining samples collected in the autumn of 1993, when only five species were identified [7]. The same species are present in our samples as well: *Emiliania huxleyi* (Lochman) Hay et Mohler 1967; *Syracosphaera ossa* (Lecal) Loeblich and Tappan; *Syrocosphaera* sensu *dilatata* Jordan *et al*., 1993; *Syracosphaera* sensu *exique* Okada and McIntyre, 1977; *Calciosolenia brasiliensis* (Lochman, 1919) Young. n. comb. In addition, the samples contained *Acanthoica* sensu *quattrospina* Lochman, 1903, which was previously identified in the spring population [4], and *Helladosphaera* sensu *cornifera* (Schiller, 1913) Kamptner, 1937, which was found for the first time in the Black Sea.

Coccolithophorid abundances of $0.5–6.1 \times 10^6$ cells l^{-1} recorded in our samples correspond to their average contents of $1.0 \times 10^6–10 \times 10^6$ cells l^{-1} frequently cited

Variations in the coccolithophorid abundance (N, cells l^{-1}) in the experiment on the influence of the concentrations of nutrients on the algae growth

Container	Calculated concentration of main nutrients	N_0 +/– σ	N_t +/– σ	N_t/N_0	N_t/N_0 (average)
1 Nitrogen	NO_3—0.45 μmol	694500 +/– 35000	632100 ± 43108	0.910151	0.77
2 Nitrogen	PO_4—0.01 μmol		577300 ± 43515	0.831246	
3 Nitrogen			391700 ± 33588	0.564003	
1 Phosphorus	NO_3—0.2 μmol		1068000 ± 59703	1.537797	1.79
2 Phosphorus	PO_4—0.6 μmol		1287000 ± 66907	1.853132	
3 Phosphorus			1377600 ± 68034	1.983585	
1 Reference control	NO_3—0.2 μmol		618240 ± 41307	0.890194	0.92
2 Reference control	PO_4—0.01 μmol		656640 ± 44678	0.945486	

Note: N_0 is the initial concentration, N_t is the terminal concentration, and σ is the mean square deviation.

in publications [9, 18, 26, 36, 39]. Sometimes, coccolithophorid concentrations in natural environments can reach 100×10^6 cells l^{-1} [11, 13]. In cultures and experiments with mesocosms, *E. huxleyi* abundance can be as high as 300×10^6 cells l^{-1} [18].

The usual concentration of *E. huxleyi* cells in the Black Sea ranges from 1×10^4 to 1×10^5 cells l^{-1}. Such abundances are characteristic of almost all its areas, the open sea included [2, 10, 20, 29]. Satellite observations record a mass development of coccolithophorids throughout the entire Black Sea in May–June [16, 17]. Since 1997, this phenomenon has annually been observed in the Black Sea. At this time, up to 70% of the sea area is involved into bloom. Nevertheless, direct measurements of the cell abundance during blooms of *E. huxleyi* are extremely rare. Its outbursts in the summer season were recorded in both near-shore and open-sea waters [4]. In February 1951, coccolithophorid abundance in the waters over the northwestern shelf was 0.9×10^6 cells l^{-1} [10]. A mass development of coccolithophorids was noted in November 1993, when their abundance in the surface layer varied from 0.3×10^6 to 0.7×10^6 cells l^{-1} [7]. Data on two outbursts in *E. huxleyi* development in 1990 and 1992, which occurred during the summer season (more exact data are unavailable), are cited for the northwestern part of the sea. The population density at selected levels was as high as 4.0×10^6 cells l^{-1} at that time [3]. The maximum coccolithophorid abundance of 6.17×10^6 cells l^{-1} recorded during our studies is unique for the Black Sea.

The role of coccolithophorids in the phytocoenoses during their mass development (1.0×10^6 to 10.0×10^6 cells l^{-1}) is significant, although, because of the small cell size and low content of chlorophyll *a* [31], their share in the total phytoplankton biomass is usually below 50%. In the Bering Sea, the contribution of coccolithophorids to the total algae biomass during their bloom was 41–57% [9]. In Norwegian fjords, the share of coccolithophorids in the total chlorophyll *a* content was estimated to be 30% [26]. During our studies, they constituted 20–50% of the total biomass. The maximum abundance of algae along the southern transect was as high as 1.5×10^6 cells l^{-1} (or 19%). The contribution of coccolithophorids to the total biomass of phytoplankton was probably higher two weeks before the commencement of the main studies, when their abundance varied from 2×10^6 to 6×10^6 cells l^{-1}.

The spatial distribution of coccolithophorids showed a distinct tendency toward an increase in the content of cells in the near-shore waters and in the BSRC area (Fig. 1, 33). A high population density of 0.55×10^6 cells l^{-1} was noted at a depth of 10 m at the shallowest water station 1623 (Fig. 1). Moreover, samples taken from a pier (5 m) two weeks before the main investigations contained 2.4×10^6 cells l^{-1}. This is inconsistent with the earlier opinion based on the analysis of satellite images that coccolithophorid blooms occur only in the offshore areas of the Black Sea over a short period of 2–3 weeks [16]. Our observations that were carried out for 1.5 months show that the alga abundance during this entire period was high. In the middle of June, it amounted to 6×10^6 cells l^{-1}. At the end of June and in July, it decreased slightly, although it remained at a rather high level of 0.5×10^6 to 1.5×10^6 cells l^{-1}. The bloom maximum itself continues for probably two to three weeks, but high abundances of coccolithophorids are observed during a substantially longer period. Such development patterns of coccolithophorids in the Black Sea are confirmed by advanced satellite observations [17].

Termination of blooming is indirectly evidenced by the abundance of single coccoliths in the water. It is known that, at the stage of growth in cultures and at the beginning of the bloom, the ratio between the alga cells and coccoliths is 1 : 10–35 [12, 28]. At the end of the blooming period, this ratio increases to a few hundred [23, 38]. During cruise 61, in the middle of June, this parameter averaged 1 : 57 (Fig. 4). Two weeks later, the ratio became 1 : 460. This suggests a cessation of blooming, destruction of cells, and increase in the

amount of single coccoliths in water. It should be noted, however, that this could be explained by a partial destruction of *E. huxleyi* cells on nuclepore filters in cruise 62. For example, the abundances of coccolithophorids at station 1662 determined for the surface layer in native water and in concentrates were 1.5×10^6 and 2.2×10^6 cells l^{-1}, respectively.

The vertical distribution of *Emiliania huxleyi* is determined by the hydrological situation and ecophysiology of the species. Its blooms usually occur in the surface layer under conditions of strong seasonal stratification and calm weather [9, 24, 26]. In our case, this phenomenon was also observed under calm clear weather conditions. All the abundance peaks but one (station 1645, 20 m) were recorded in the layer 0–10 m (Fig. 6); the maximum population was recorded at the sea surface (station 1662, 0 m).

In the southern transect, the salinity in the thin upper layer was 1.0–1.5‰ lower than that in the northern one. It is known that different *Emiliania huxleyi* ecotypes are adapted to a wide salinity spectrum, 11–41‰ [31]. Oceanic and near-shore clones cannot grow under salinities below 16 and 11‰, respectively [40]. Thus, an insignificant salinity decrease should be harmless for the development of coccolithophorids at the sea surface along the southern transect. It should be noted that high concentrations of *Emiliania huxleyi* (0.9×10^6 cells l^{-1}) were noted in the northwestern part of the Black Sea characterized by substantially lower salinity values as compared with the remaining basin [10].

Some peaks of *Emiliania huxleyi* abundance were recorded below the upper mixed layer (Fig. 6). They are probably explained by the sinking of calcified cells during blooming. The sinking rate of cells 5 μm in size is approximately 0.5 m/day during the final stage of the culture growth [27]. Taking into consideration the larger sizes of the cells of the Black Sea *Emiliania huxleyi* population (6–7 μm), one can assume their higher sinking rate during cessation of the blooming. The abundance peak recorded at a depth of 20 m (station 1645) is most likely explained by this phenomenon. The maximum density gradient was also confined to this depth. The deep abundance maximum at station 1642 (0.3×10^6 cells l^{-1}, 75 m) can be related to the cell sinking as well. If this is the case, blooming at the surface should have occurred a few months before our observations, i.e., in February–March, which was previously noted for the Black Sea [1]. It cannot be ruled out, however, that this deep maximum was formed by hydrological processes.

The co-occurrence of the calcified and naked forms in the *Emiliania huxleyi* population is well known. They are observed both in cultures [31] and under natural conditions [15]. In the Black Sea, naked cells were mostly confined to the pycnocline layer and the underlying waters. A similar distribution of naked cells was observed in the Bering Sea [9]. As in our materials, the population of *Emiliania huxleyi* there was dominated by calcified cells. Naked forms were dominant only in selected areas, where their share increased up to 60%. Similar values (up to 70%) were also observed in the Black Sea below the seasonal pycnocline. It should be noted that the assessment of the number of naked cells under a light microscope is difficult because they are almost indistinguishable from other Prymnesiophyceae [31].

The co-occurrence of two forms of cells in the same population has never been noted before. These two forms are so particular that, under a light microscope, they were initially identified as different species. Such differences between the cells of this species can be explained by the different degree of coccolith calcification [22]. It can be assumed that the poorly visible coccosphere of form 2 had smaller coccoliths as compared with form 1. The latter was subordinate in the population and was mainly confined to the layer below the seasonal pycnocline. An analysis of SEM images shows, however, that coccoliths varied in size from 3 to 4 μm both at the surface and in the deeper layers, i.e., they were identical in this respect. The differences visible under a light microscope were probably related to the different position of coccoliths in a cell and to its internal structure.

The elevated content of naked cells in deep layers is probably explained by the calcification processes. According to some data, a lower temperature decreases the calcification degree of the cells [31]. Nevertheless, this process largely depends on illumination. Under a low illumination, the calcification rate decreases and the number of coccoliths in the cells also decreases [12]. In addition, the calcification rate increases under P limitation [32, 33]. In deep layers, all three factors (low illumination, low temperature, and relatively high P content) act in parallel, resulting in a substantial increase in the number of naked cells in the population.

The entire observation period was characterized by cloudless weather conditions with a high surface insolation up to 800 μmol quanta/m^2 s. Such a high illumination caused a significant decrease in the efficiency of photosynthesis Fv/Fm, which indicates its photoinhibition in the upper water layers. Some experiments on dark adaptation of cells support this observation (Fig. 7, station 1644), although algae sampled at another station (Fig. 7, station 1625) showed no dark adaptation, which implies a lack of photoinhibition. The measurements carried out with a flow-through fluorimeter near these stations confirm these observations. In the area of station 1644, photoinhibition was stronger than near station 1625, which was evident from the differences in the Fv/Fm values (Fig. 8). This can be explained by the differences in the taxonomic compositions of the populations at the stations under consideration. As is well known, coccolithophorids are practically insensitive to photoinhibition. It is observed only under extremely high illumination of 1500–2500 μmol quanta/m^2 s [30]. At station 1625, coccolithophorids provided over 75%

of the biomass, whereas at station 1644, they constituted only 45% of the biomass. This probably explains the lack of dark adaptation in phytoplankton at station 1625.

The vertical profiles of fluorescence Fo and efficiency of the primary phothosynthesis processes Fv/Fm are similar, which points to the steady-state phase in the development of the phytoplankton community [5]. This is also evident from the fact that, during three weeks of observations (cruises 61 and 62), the phytoplankton community showed no significant changes. The fluorescence parameter Fo accords well with the phytoplankton abundance [6]. In our observations, no correlation between Fo and the number of coccolithophorids was found. As is well known, the quantum output of the chlorophyll fluorescence in haptophytic algae is a factor of 2 lower than in diatoms and phytoflagellates [14]. Therefore, it is probable that the significant contribution of coccolithophorids to phytoplankton biomass did not affect the chlorophyll fluorescence.

The mass development of coccolithophorids was observed under both phosphorus- and nitrogen-limited conditions [26]. The experiment carried out with a natural *Emiliania huxleyi* population showed that the growth of cells occurred in the containers with an elevated phosphate content (table). The specific growth rate of algae was only 0.2, while the maximum value of this parameter determined for *Emiliania huxleyi* at 20°C was 0.9 [31]. A growth rate that low was determined by the low illumination during the experiment, which permitted to keep the initial concentration of nutrients for a long time. The results obtained suggest a P limitation of the population growth. The data available indicate high competition abilities of coccolithophorids in phosphate consumption. The half-saturation constant of the phosphate consumption kinetics is extremely low (<0.001 μmol PO_4) [35]. At the same time, the content of phosphates in the reference containers during our experiments exceeded this value by an order of magnitude. It is likely that the *Emiliania huxleyi* population in the Black Sea differs in this parameter from oceanic ecotypes. Unfortunately, a single experiment prevents one from concluding to what extent P limitation of the *E. huxleyi* growth is typical in terms of its temporal and spatial development.

ACKNOWLEDGMENTS

We are grateful to M.V. Flint for the idea of this study and his help in performing of the study, A.Yu. Skirta and O.I. Podymov for the hydrological and hydrochemical data, and V.I. Burenkov and S.G. Poyarkov for phytoplankton sampling during cruises 61 and 66. This study was partly supported by the Russian Foundation for Basic Research, project nos. 02-05-64844, 03-04-48040, and 05-05-79074.

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