EMILIANIA HUXLEYI BLOOMS IN THE BLACK SEA: INFLUENCE OF ABIOTIC AND BIOTIC FACTORS

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Abstract

Stelmakh L.V., Gorbunova T.I., 2018: *Emiliania huxleyi* blooms in the Black Sea: influence of abiotic and biotic factors. – Botanica, 24(2): 172–184.

The studies were conducted in the Black Sea in deep and shallow water areas in October 2010 and May 2013. The main abiotic and biotic factors, which control spring and autumn coccolithophorid *Emiliania huxleyi* blooms, were identified. During late May this phenomenon was observed under optimal light and temperature conditions, and also optimal ratio between mineral forms of nitrogen and phosphorus (N/P) in the water. Biotic variables (the net growth rate of phytoplankton and relative dinoflagellates share in its total biomass) determined the uneven distribution of *E. huxleyi* within a bloom. In October, water temperature was almost 4ºC lower compared to that in May, and solar radiation intensity decreased approximately by 2–3 times. However, as a result of seasonal adaptation to light and temperature, *E. huxleyi* abundance reached blooming level. In that period, the variability of N/P ratio in the water and the relative share of diatoms in total biomass of phytoplankton played a major role in the uneven distribution of this coccolithophorid within the studied area. In the areas with low water salinity, a bloom was not developing. Within the main part of the studied water area, the major source of nitrogen was ammonium, which was favourable for the growth of *E. huxleyi* and dinoflagellates, but limited the growth of diatoms.

Keywords: Black Sea, *Emiliania huxleyi* bloom, microzooplankton grazing, nutrients, phytoplankton growth rate.

INTRODUCTION

Coccolithophorids are an abundant, ubiquitous component of marine phytoplankton assemblages which cells typically are surrounded by several layers of calcium carbonate plates, the coccoliths (Gafar et al., 2018). Coccolithophorids are important in the cycling of carbon and sulphur, and affect the optical and heat-absorbing characteristics of surface waters during bloom events (Balch et al., 1991; Gafar et al., 2018). The most abundant species among them is small coccolithophorid *Emiliania huxleyi* (Lohmann) W.W. Hay & H.P. Mohler. The blooms of *E. huxleyi* produce the "bright water" phenomenon (in satellite images) due to the light-scattering of the huge amount of coccoliths or of "milky sea" (due to the turbid waters), as observed from the sea surface. These blooms have significant environmental impacts, consisting of increasing water albedo, large fluxes of calcium carbonate on the water surfaces, and a decrease in light and heat depth penetration (Tyrrell & Merico, 2004). Blooms of *Emiliania huxleyi* could act as an important source of dimethylsulfoniopropionate (DMSP) in water (Balch et al., 1991).

In different areas of the World Ocean, mass development of this species is often recorded. *E. huxley* bloom has been noticed in the North Atlantic Ocean (Balch et al., 1991; Raitsos et al., 2006), the Mediterranean Sea (Oviedo et al., 2015), the Black Sea (Stelmakh et al., 2009; Oguz & Merico, 2006; Mikaelyan et al., 2011; Stelmakh & Georgieva, 2014), and also in the Bering Sea (Olson & Strom, 2002). It is commonly accepted that abundance of *E. huxleyi*, which reaches 1×10^6 cells·L^{-1}, corresponds to the bloom level (Balch et al., 1991; Mikaelyan et al., 2011).

In the Black Sea, mass developments of this coccolithophorid have been registered in different months from April to October. However, its main maximum is observed, as a rule, at the end of May – June (Oguz & Merico, 2006; Stelmakh et al., 2009; Mikaelyan et al., 2011; Stelmakh & Georgieva, 2014). Among the main factors that control their intensive development, the light, temperature and nutrients (Oguz & Merico, 2006; Stelmakh et al., 2009; Mikaelyan et al., 2011) as well as microzooplankton grazing (Stelmakh & Georgieva, 2014) were specified. Based on these published data, we are not able to answer some important questions. Firstly, how do biotic and abiotic factors correlate during *E. huxleyi* blooms in spring and autumn? Secondly, why does this species of coccolithophorids dominate in the phytoplankton during a spring bloom not just in abundance, but also in biomass, and in autumn the main biomass consists of dinoflagellates and diatoms? It is, therefore, possible to suggest that the role of abiotic and biotic factors in regulation of the spring and autumn blooms is not the same.

Therefore, the goal of this work was to identify the role of abiotic and biotic environmental factors in the formation of spring and autumn blooms of *E. huxleyi* in the Black Sea.

MATERIALS AND METHODS

Study area

Two research cruises were conducted in the Black Sea during *E. huxleyi* blooms: 10–21 October 2010 and 22–30 May 2013, both on the R. V. "Professor Vodyanitsky". The research was conducted at 38 stations located predominantly in the western part of the Black Sea (Fig. 1). The investigation area included both shallow water areas (≤ 100 m) and deep water areas (≥1000 m). In May 2013, at some stations (№ 7, 9, 14, 18, 35), the experiments were conducted in two replications.

Water samples of 12–15 L volume were collected, as a rule, from the surface layer (~ 0.5 m depth) using Niskin bottles. The exception was four stations (two in each expedition), where the water samples were collected not only from the surface layer, but, in addition, from 3–5 horizons within the photosynthetic zone. Selection of the horizons was carried out taking into account the water temperature vertical distribution. The samples were collected from the upper mixed layer, in the thermocline and deeper, near the photosynthetic zone base.

During the study period, the concentration of chlorophyll *a*, phytoplankton specific growth rate,
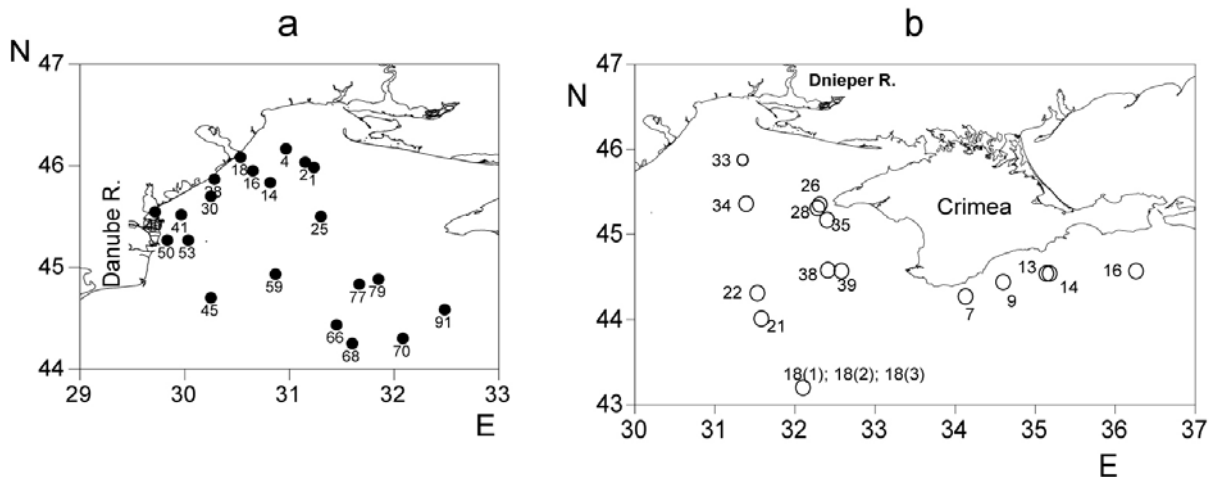

Fig. 1. Location of sampling stations in the Black Sea in October 2010 (a) and in May 2013 (b)

specific rate of microzooplankton grazing on it, species composition and nano- and microphytoplankton biomass were determined. Water temperature and nutrient concentrations (nitrate, ammonium, phosphate and silicon) as well as solar radiation intensity during the whole day were measured.

Phytoplankton structural-functional characteristics and abiotic parameters

For determination of phytoplankton abundance, biomass and species composition, ~3–4 L samples of sea water was concentrated under nuclepore membranes (1 μm pore size; the product of the Institute of Nuclear Researches, Dubna, Russia) in the inverse filtering funnel (SOROKIN et al., 1975). The samples were condensed to 50 mL and were fixed with neutralized 40% formaldehyde (1% final concentration in the sample). The abundance and linear dimensions of algae cells were determined in 0.1 mL drop, placed into Nageotte counting chamber, with 3–5 replications using a light microscope ZEISS Primo Star. Linear measurements were converted to cell volume using various geometric formulas (SENICHKINA, 1978). Phytoplankton organic carbon concentration was calculated from the average cell volume for each species of diatoms and dinoflagellates using the equations presented in the work (MENDEN-DEUER & LESSARD, 2000), for coccolithophorid *E. huxley* – using the equation of Montagnes (MONTAGNES et al., 1994), and for other algae – using the equation of Strathmann (STRATHMANN, 1967). Phytoplankton species identification was carried out using the manual of TOMAS (1997).

Chlorophyll *a* concentration (Chl *a*) was measured in acetone extracts employing the fluorimetric method (JGOFS protocol, 1994). Sea water was filtered through Whatman GF/F filters. After filtration filters were placed in 90% acetone (5 mL) and chlorophyll *a* was extracted for 24 h at 4 ºC in the dark. The acetone extracts were centrifuged, and their fluorescence determined before and after acidification in a fluorometer (excitation 440 to 480 nm, emission > 665 nm), which was calibrated with pure chlorophyll-*a* (Sigma Chemical Co). The precision of these measurements was high, with a relative standard deviation of 5%. The calculations were performed by the standard equation, presented in the work (JGOFS protocol, 1994).

The phytoplankton growth rate and microzooplankton grazing were calculated with application of the dilution method (LANDRY & HASSET, 1982). Using this method, one should bear in mind three key points. Firstly, phytoplankton loss from grazing by microzooplankton linearly correlates with phytoplankton concentration and decreases with increasing dilution factor. Secondly, phytoplankton growth rate does not depend on the degree of dilution. Thirdly, specific growth rate of microalgae can be described by exponential function. Additional amounts of nutrients were not added to the original samples, as this procedure could inhibit the growth and feeding activity of microzooplankton (LANDRY & HASSET, 1982). About 3–4 L of sea water samples was filtered through 200 μm mesh to remove mesozooplankton. In order to have filtrate clear from suspended particles, 6–8 L of the initial sample was filtered through Whatman GF/F fiberglass filters (47 mm in diameter) under low pressure (< 0.1 atm) that prevented destruction of algal cells and their penetration into the filtrate. Initial sample was diluted with the filtrate so that to have a series of samples with reducing dilution factor (DF) of 1.0, 0.75, 0.50, 0.25 and 0.1 in two replications. Factor 1.0 was typical of the original undiluted sample, whereas factor 0.1 – of a tenfold dilution. After preparation, the samples were poured into 1 L polycarbonate bottles, which were rinsed with 10% hydrochloric acid and distilled water and placed for a daily exposition into a flow-through incubator. The incubator was placed on deck to provide exposition under natural light and at a temperature 1–3°C warmer or cooler than seawater temperature. Initial samples and the samples after daily exposition were filtered through Whatman GF/F fiberglass filters (47 mm in diameter). After filtration, the filters were placed into 90% acetone. As soon as pigments were extracted, chlorophyll *a* was measured using the fluorometric method.

The initial concentration of Chl *a* was determined only for the undiluted samples, while for the diluted samples it was recalculated according to the dilution factor (DF). The observed daily phytoplankton growth rate for each of five dilution treatments (μ_{DF}) was calculated as:

$$\mu_{DF} = \ln(Chla_{final}/ Chla_{initial}),$$

where $Chla_{initial}$ and $Chla_{final}$ – initial chlorophyll *a* concentration and after 24 hours of exposure (mg·m⁻³).

The linear regression equations were calculated to estimate the interrelations between the observed phytoplankton growth rate (μ_{DF}) and the dilution factor (DF) as:

$$\mu_{DF} = -g \cdot DF + \mu,$$

where μ – is true phytoplankton growth rate (day^{-1}) and g – the zooplankton grazing rate (day^{-1}).

Nitrate concentration was measured by reducing it to nitrites using copper-plated cadmium with following determination by single "colour reagent", ammonium – utilizing the Grasshoff-Johansen test, phosphates – by Morphy and Riley, and silicate – by the blue silica-molybdenum complex (SAPOŽNIKOV, 1988).

Information regarding the sea water temperature was obtained in scientific expeditions using a STD probe-complex. The average values of solar radiation intensity in the upper mixed layer were calculated on the basis of its values near the sea surface, and also thickness of the upper mixed layer and water transparency (STELMAKH & GORBUNOVA, 2018).

Data analysis

For identification of the factors determining variability of *E. huxleyi* abundance in the Black Sea during the periods of its bloom, linear correlation analyses as well as dispersion and regression analyses were performed. The pair coefficients of Pearson's correlation between the *E. huxleyi* abundance, on the one hand, and the abiotic and biotic parameters, on the other, were determined. Among the abiotic parameters were the thickness of the upper mixed layer, water temperature and salinity, concentrations of nitrates, ammonium, phosphates and silicon in the surface layer; among the biotic – the chlorophyll *a* concentration and phytoplankton biomass, true and net specific growth rate of phytoplankton and specific rate of microzooplankton grazing on phytoplankton. In the preparation of multiple linear regression equations, only the independent variables, which were most correlated with the abundance of the coccolithophorid, were used. Statistical treatment of the data was carried out using the software Excel 2007 and Sigma Plot 2001 (in the ANOVA package) for Windows. Map building was carried out using the program Surfer 8. The graphs were performed using the programme Grapher 3.

RESULTS

Autumn period

Development conditions

In October 2010, in the western part of the Black Sea, the upper mixed layer was varying within the water area from 9 to 28 m (Table 1). Water temperature in UML ranged from 13.50 to 17.29ºC, salinity in UML was 16.62–17.94‰, and the average values for the two sea areas were not significantly different. In the central part of the north-western area of the Sea (shallow water area) and in the deep water area, concentrations of the main nutrients were not high. The nitrate concentrations were bellow 0.4 µM, ammonium – bellow 1 µM, silicate – bellow 1.5 µM, and phosphate – bellow 0.2 µM. Only in the coastal areas located near the Danube River mouth, the concentrations of nutrients increased several times (Fig. 2). The atomic ratio between the mineral nitrogen (nitrate plus ammonium) and phosphate (N/P) varied from 3.9 to 14.1, and on average was 9.63 in deep water areas and 5.88 – in shallow water areas (Table 1). The solar radiation intensity, reaching the sea surface, was 21–27 E·m^{-2}·day^{-1}. An average value of this parameter for the upper mixed layer was more variable and located within the range from 3 to 15 E·m^{-2}·day^{-1}.

Phytoplankton structure

In the surface waters of the western part of the Black Sea, there was a significant heterogeneity ob-
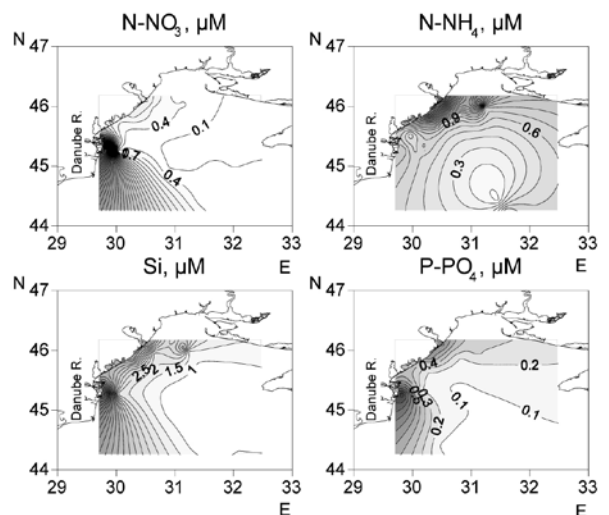

Fig. 2. Distribution of nutrients in surface layer of the Black Sea in October 2010

Table 1. Abiotic variables at the sampling stations in the Black Sea in October 2010: thickness of upper mixed layer (UML), temperature (T), salinity (S) and N/P ratio in the surface layer (0–0.5 m), solar radiation intensity near the surface (I_0) and its average values for upper mixed layer (I_{UML})

No station	Depth, m	UML, m	T, ºC	S, ‰	N/P	$I_0(I_{ULM})$, E.m^{-2}.day^{-1}
Western part of the sea, open-sea waters						
66	950	21	16.48	17.90	5.59	22(9)
68	1110	21	16.55	17.92	14.07	22(9)
91	1347	18	16.73	17.87	10.59	25(10)
70	1475	15	15.04	17.94	8.25	27(14)
Mean ± SE	1221± 232	19 ± 3	16.2 ± 0.78	17.91 ± 0.03	9.63 ± 3.60	24 ± 2(11 ± 2)
Western part of the sea, near-shore waters						
28	9	9	14.65	16.88	5.24	21(9)
18	10	10	14.77	17.61	5.49	24(13)
40	12	12	13.96	17.86	5.90	23(5)
14	15	15	16.88	17.77	7.53	24(11)
16	16	15	15.86	17.43	5.37	24(12)
30	16	16	16.46	17.52	7.38	21(7)
1	22	22	16.08	17.86	4.11	23(8)
41	22	22	15.84	16.90	4.03	23(3)
53	24	27	15.83	16.62	3.92	21(4)
25	25	23	16.60	17.64	6.53	23(4)
4	27	25	14.80	17.67	4.54	23(6)
2	30	25	14.80	17.83	6.08	23(6)
50	30	21	13.50	17.96	11.51	21(4)
45	31	28	17.29	17.85	5.85	23(4)
59	64	21	15.27	17.83	3.63	21(8)
79	64	12	16.38	17.78	6.22	27(15)
77	65	19	16.37	17.80	6.55	27(11)
Mean ± SE	28 ± 18	19 ± 6	15.61 ± 1.06	17.58 ± 0.40	5.88 ± 1.87	23 ± 2(8 ± 4)

N/P – ration between total inorganic nitrogen and phosphate, SE – standard deviation.

served in the chlorophyll *a* distribution and phytoplankton biomass in October 2010 (Fig. 3). The maximum values of this parameter were recorded in coastal waters near the Danube River mouth. There the chlorophyll concentration was above 1 mg·m^{-3}, and phytoplankton biomass was above 50 mg C·m^{-3}. In the remaining studied water area, these values were 3–5 times lower. In the zones of maximum biomass, the diatoms *Skeletonema costatum* (Greville) Cleve, *Chaetoceros socialis* H.S. Lauder, *Proboscia alata* (Brightw.) Sundstrőm, *Thalassionema nitzschoides* (Grunow) Mereschkowsky dominated. Their share in total phytoplankton biomass varied from 50 to 90%. In the major part of the study area, the specific biomass of diatoms was significantly below 40% of its total value. There dinoflagellates dominated, and their biomass made up 50–80% of the total biomass of phytoplankton. Among these, most common were *Prorocentrum cordatum* (Ostenfeld) J.D. Dodge,
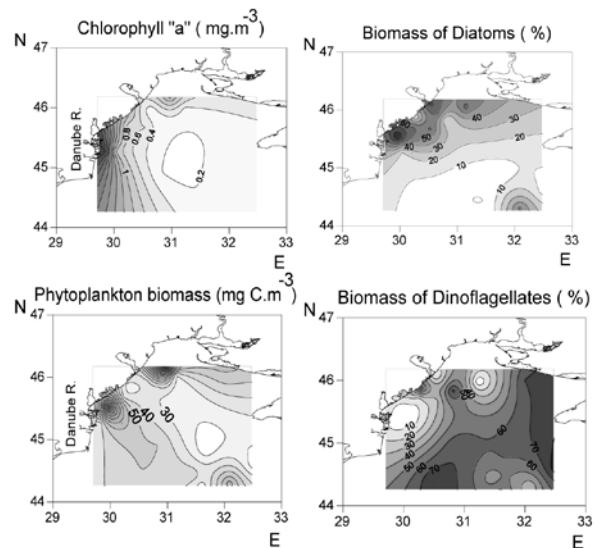

Fig. 3. Distribution of chlorophyll *a* concentration, total biomass of phytoplankton, diatom and dinoflagellate biomass in surface layer of the Black Sea in October 2010

Prorocentrum micans Ehrenberg and *Gymnodinium simplex* (Lohmann) Kofoid & Swezy. Most common coccolithophorids were *E. huxleyi*, *Syracosphaera* sp., *Calyptrosphaera* sp., *Coccolithus* sp. However, the first species of algae dominated by the abundance and biomass, while the share of others was 3–10% by abundance and 10–25% by biomass.

In the major part of the research area, the abundance of *E. huxleyi* was $1–3 \times 10^6$ cells·L^{-1}, what corresponds to the bloom level (Fig. 4). Only in the area of the Danube River runoff and in the eastern part of the Sea, its abundance was below 1×10^6 cells·L^{-1}. The cell diameter was 8–10 μm. All cells were covered with coccoliths. Within the limits of photosynthetic zone, its maximum value was observed in the layer of 0–5 m (Fig. 5).
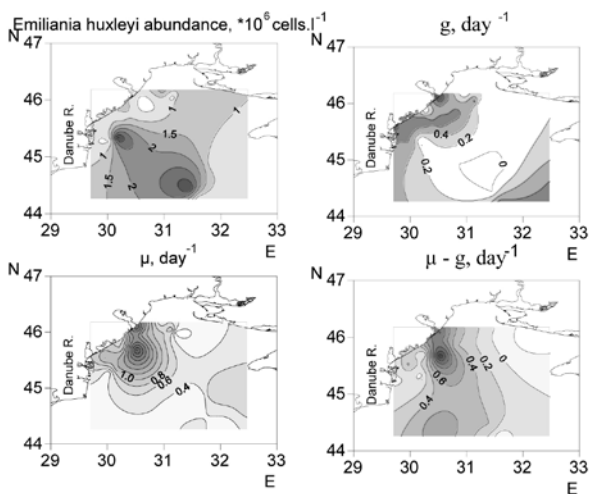
Functional parameters

Under these conditions, the specific phytoplankton growth rate in the studied water area differed by order: from 0.20 to 2.47 day^{-1} (Fig. 4). Maximum values (2.23–2.47 day^{-1}) were observed at some distance from the Dnieper River runoff, where the main biomass of phytoplankton (more than 50%) was formed by small forms of diatoms. As the distance from this area increased, the specific growth rate of phytoplankton decreased to 0.20–0.40 day^{-1}.

Specific rate of microzooplankton grazing on

Fig. 4. Distribution of *Emiliania huxleyi* abundance, phytoplankton growth rate (μ), microzooplankton grazing (g) of phytoplankton and net phytoplankton growth rate (μ – g) in surface layer of the Black Sea in October 2010
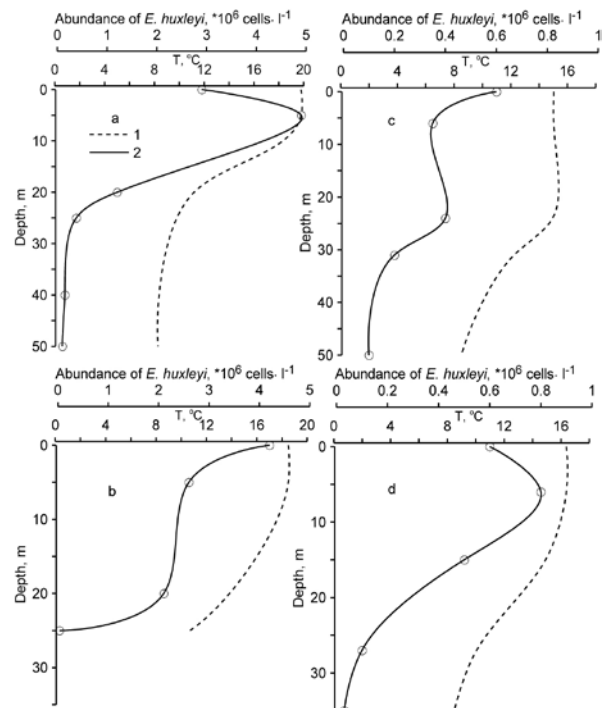

Fig. 5. Distribution of *Emiliania huxleyi* in photosynthetic zone of the Black Sea in May 2013 (a, b) and in October 2010 (c, d); a, c – open part of the Sea; b, d – coastal waters (line 1 – temperature, line 2 – *E. huxleyi* abundance)

Table 2. Multiple regression equations reflecting the relationship of *Emiliania huxleyi* abundance (N$_{Emil.}$) with environmental factors as well as their statistical parameters

SE	F	p	SE$_1$	t$_1$	p$_1$	SE$_2$	t$_2$	p$_2$	SE$_3$	t$_3$	p$_3$
October 2010 N$_{Emil.}$ = 0.95*S − 0.22*N/P − 0.02*B$_{Diatom.}$, R^2= 0.56, n = 21											
0.69	7.28	0.002	0.46	2.04	0.05	0.06	−3.32	0.003	0.005	−3.47	0.003
May 2013 N$_{Emil.}$ = 2.01*(μ−g) + 0.05*B$_{Dinofl.}$, R^2= 0.55, n = 23											
0.76	7.32	0.002	0.70	2.86	0.01	0.01	2.73	0.01	–	–	–

SE – standard error of the equation, F – Fisher criterion, p – the level of significance, SE$_1$, SE$_2$, SE$_3$ – standard error of the equation coefficients, t$_1$, t$_2$, t$_3$ – Student criterion for the coefficients, p$_1$, p$_2$, p$_3$ – the level of significance for the coefficients.

phytoplankton varied from 0 to 0.8 day^{-1} (Fig. 4). Its maximum values most often, spatially corresponded to the maximum values of the specific phytoplankton growth rate in the zones, where the diatoms dominated. The last ones, probably, were most favourable food for the microzoplankton during that period. Minimum values of microzooplankton grazing were recorded in the central part of the studied water area, where abundance of *E. huxleyi* was greater than 1 × 10^6 cells·L^{-1}, the main phytoplankton biomass was formed by dinoflagellates.

Net phytoplankton growth rate ($\mu - g$) was within the rate from –0.20 to 1.20 day^{-1}. The highest values of this parameter (over 0.4 day^{-1}) were observed most often in the *E. huxleyi* bloom zone. At the same time, both low and high values of the net growth rate were identified in the remaining water area.

Factors that determine bloom

Based on the performed linear correlation analysis between abundance of coccolithophorid *E. huxleyi* and all the presented abiotic and biotic parameters, it was determined that abundance of this species cells weakly correlated with all the studied variables. The most significant Pearson's pair correlation coefficients (R = 0.35–0.40) were obtained between *E. huxleyi* abundance and such independent variables as water salinity (S), N/P ratio and relative share of diatoms in total biomass of phytoplankton (B$_{Diatom}$). On this basis, the multiple linear regression equation was calculated (Table 2). As follows from the equation, the main share of variability of *E. huxleyi* abundance in autumn period is associated with these independent variables (R^2 = 0.56). The presented statistical parameters indicate reliability of the equation and its coefficients.

Spring period

Development conditions

At the end of May 2013, in the western and eastern areas of the Black Sea, a well-pronounced seasonal thermocline was formed (Table 3). Water tem-

Table 3. Abiotic variables at the sampling stations in the Black Sea in May 2013: thickness of upper mixed layer (UML), temperature (T), salinity (S) and N/P ratio in the surface layer (0–0.5 m), solar radiation intensity near the surface (I$_0$) and its average values for upper mixed layer (I$_{UML}$)

| No station	Depth, m	UML, m	T, ºC	S, %	N/P	I$_0$ (I$_{ULM}$), E .m^{-2} day^{-1}
Eastern part of the sea, near-shore waters						
7	80	4	19.40	17.92	7.07	45 (32)
9	80	4	21.01	17.78	4.61	45 (30)
13	52	8	20.29	17.90	6.75	44 (23)
14	35	6	19.22	17.79	5.61	44 (26)
16	32	9	20.30	17.44	3.30	44 (22)
Mean ± SE	56 ± 23	6 ± 2	20.04 ± 0.73	17.71 ± 0.76	5.47 ± 1.39	44 ± 1(27 ± 4)
Western part of the sea, open-sea waters						
18(1)	2000	8	19.84	18.49	3.08	44 (27)
18(2)	2000	8	19.60	18.53	5.30	44 (27)
18(3)	2000	8	19.77	18.54	7.85	44 (27)
21	1500	9	19.07	18.13	3.03	40 (22)
22	1000	10	19.20	17.92	2.60	40 (19)
Mean ± SE	1700 ± 447	9 ± 1	19.50 ± 0.34	18.3 ± 20.28	4.37 ± 1.98	42 ± 2(24 ± 4)
Western part of the sea, near-shore waters						
26	30	15	18.50	18.18	4.59	41 (19)
28	27	5	19.01	18.15	6.29	41 (19)
33	81	12	19.14	15.51	5.09	44 (20)
34	41	5	20.54	16.30	7.07	42 (28)
35	39	19	19.70	18.05	4.41	47 (18)
38	100	10	19.10	17.90	6.93	47 (24)
39	98	16	19.87	17.91	3.47	47 (24)
Mean ± SE	59 ± 32	12 ± 5	19.41 ± 0.67	17.43 ± 1.07	5.41 ± 1.38	44 ± 3(22 ± 4)
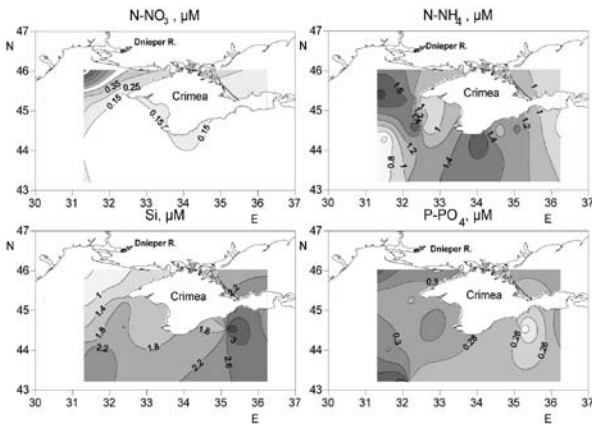
Fig. 6. Distribution of nutrients in surface layer of the Black Sea in May 2013

perature in UML layer did not depend on the research area and it was in the range of 18.50–21.01ºC. Water salinity in UML layer was, as a rule, 17.44–18.54%. Only in the north-western part of the Sea, near the drain of the Dnieper River, it decreased to 15.51–16.30%. In the surface layer (0–0.5 m), nitrate concentrations were low throughout the entire study area (Fig. 6), averaging 0.19 μM (± 0.07 μM). Average ammonium concentrations were approximately six times higher, and silicate concentrations – almost 10 times higher. Phosphate concentrations varied within a rather narrow range (0.27–0.37 μM). Atomic ratio between mineral nitrogen (nitrates plus ammonium) and phosphate (N/P) varied from 2.6 to 7.9. Solar radiation intensity, which reached the Sea surface, varied from 40 to 47 E·m^{-2}·day^{-1}. Average value of this parameter for the upper mixed layer was 19–32 E·m^{-2}·day^{-1} (Table 3).

Phytoplankton structure

During this period, chlorophyll *a* concentration and phytoplankton biomass in the surface layer, as a rule, were characterized by extremely low values (Fig. 7). Exceptions were the two stations located in close proximity to the drain of the Dnieper River (stations 33 and 34). There chlorophyll *a* concentrations were 1.10 and 0.33 mg·m^{-3}, phytoplankton biomass was 89 and 50.47 mg C·m^{-3}, respectively, and diatoms were dominating, mainly the small species *Cyclotella caspia* Grunow. The main phytoplankton biomass was formed by coccolithophores and small species of dinoflagellates, which linear dimensions were bellow 20 μm. Dinoflagellates were represented predominantly by *Scrippsiella trochoidea* (Stein) Loeblich

III and the representatives of the genus *Gymnodinium* F.Stein. Among coccolithophorids, *E. huxleyi* dominated (95–98% of this group biomass). Its cell diameter was 5–6 μm. All cells were covered with coccoliths. Almost everywhere the abundance of this species exceeded 1 × 10^6 cells·L^{-1} (Fig. 8), what corresponds to an initial stage of bloom. An exception was the area, where salinity was reduced (station 33 and 34). There the abundance of *E. huxleyi* cells was decreasing to 0.27 × 10^6 and 0.53 × 10^6 cells·L^{-1}, respectively. Obviously, in most of the water area, biomass of this species exceeded 50%, and in particular cases it reached 67–69% of the total biomass of phytoplankton. That can be derived as evidence of the initial stage of its spring-summer bloom, because at the peak of bloom, share of this species takes even larger part of the phytoplankton biomass. The analysis of *E. huxleyi* distribution in the photosynthetic zone indicated that in deep water as well as in shallow areas of the Sea, its maximum abundance was observed in the upper mixed layer (0–10 m). At the same time, near lower boundary of the photosynthetic zone, this species cell abundance was at a minimum (Fig. 5).

Functional parameters

True specific growth rate of phytoplankton (μ), reflecting its biomass reproduction rate, usually was rather high (Fig. 8). Its values in 95% of the cases were within the range 0.97–1.44 day^{-1}. Poor variability of phytoplankton growth rate in the studied water area was accompanied by significant change in mi-
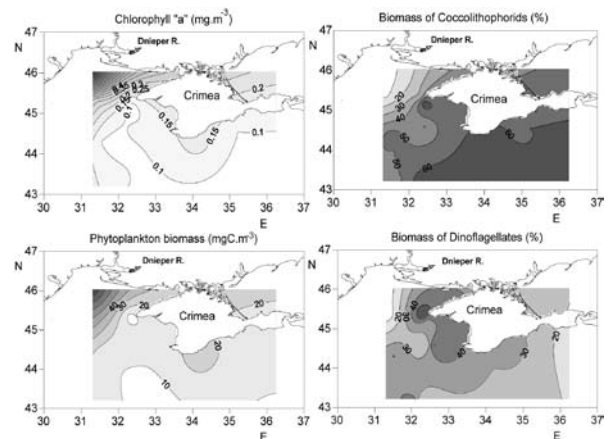

Fig. 7. Distribution of chlorophyll *a* concentration, total biomass of phytoplankton, coccolithophorid and dinoflagellate biomass in surface layer of the Black Sea in May 2013
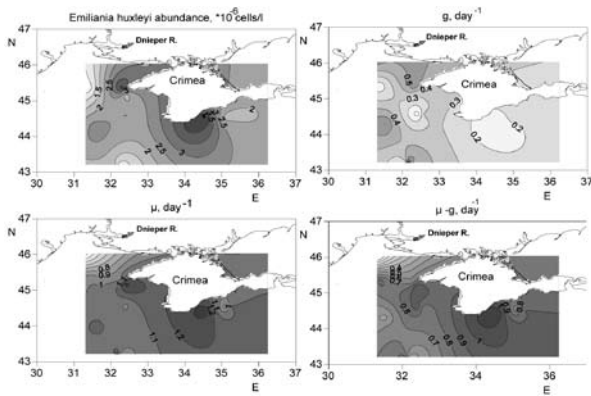
Fig. 8. Distribution of *Emiliania huxleyi* abundance, phytoplankton growth rate (μ), microzooplankton grazing (g) of phytoplankton and net phytoplankton growth rate (μ – g) in surface layer of the Black Sea in May 2013

crozooplankton grazing of phytoplankton (g). This parameter varied from 0.04 to 0.93 day^{-1}, and on average was 0.36 day^{-1} (± 0.21 day^{-1}). In order to identify abiotic and biotic factors influencing the specific grazing rate, the correlation analysis was carried out. As follows from Table 4, the correlation coefficient between value g and relative dinoflagellate biomass was the highest (r = 0.75). This indicates that as the relative dinoflagellate biomass increased, the specific rate of microzooplankton grazing on phytoplankton was raising. Net phytoplankton growth rate (μ g) was sufficiently high on almost all the studied water area, varying from 0.46 to 0.94 day^{-1}. This stimulated further increase of the phytoplankton abundance and biomass. Only at one station located near the drain of the Dnieper River (station 33), where low phytoplankton specific growth rate (μ = 0.27 day^{-1}) was observed, this parameter was negative (-0.27 day^{-1}).

Factors that determine bloom

The results of the linear correlation analysis indicated that the highest Pearson's correlation coefficients (R = 0.61–0.64) were obtained between *E. huxleyi* abundance and such independent variables as net specific growth rate (μ–g), and also relative share of dinoflagellates in the total phytoplankton biomass ($B_{Dinofl.}$). As follows from the multiple linear regression equation (Table 2), the main share of variability of *E. huxleyi* abundance in the spring period was associated with these identified independent variables ($R^2 = 0.55$). The presented statistical parameters indicate reliability of the equation and its coefficients.

DISCUSSION

Regular studies conducted by us during several years in the Black Sea coastal waters (predominantly in bays) indicated that coccolithophorid *E. huxleyi* is present in phytoplankton almost throughout the year. The maximum of its development is observed, as a rule, twice a year: in May – June and in October – November (STELMAKH et al., 2009). The studies carried out in the Black Sea, using the satellite data confirm the presence of these maximums (OGUZ & MERICO, 2006). However, what are the main factors that determine *E. huxleyi* bloom in late spring and autumn can only be detected by direct observations, which include the measurement of the complex of abiotic and biotic parameters. Such studies were conducted by us in the Black Sea in October 2010 and May 2013.

The spring-summer bloom is the main as it is the longest and covers a huge area of the Black Sea

Table 4. Correlation (R) between microzooplankton grazing of phytoplankton (g), structural parameters of phytoplankton, temperature and salinity of waters in the surface layer of the Black Sea in May 2013*

Parameters	g	B_{Diatom}, %	B_{Dinofl}, %	B_{Coccol}, %	B	T	S
g	1.00						
B_{Diatom}, %	-0.38	1.00					
B_{Dinofl}, %	0.75	-0.57	1.00				
B_{Coccol}, %	0.12	-0.80	-0.01	1.00			
B	-0.44	0.64	-0.59	-0.40	1.00		
T	-0.45	0.16	-0.34	0.10	0.63	1.00	
S	0.47	-0.73	0.56	0.51	-0.77	-0.36	1.00

* g – microzooplankton grazing of phytoplankton; B_{Diatom} – relative share of diatoms in total phytoplankton biomass; B_{Dinofl} – relative share of dinoflagellates in total phytoplankton biomass; B_{Coccol} – relative share of coccolithophorid in total phytoplankton biomass; B – total phytoplankton biomass; T – temperature; S – salinity.

(Oguz & Merico, 2006). Probably, in different areas of the Sea the most favourable abiotic conditions for *E. huxleyi* growth occurred at the end of spring – beginning of summer. At this time, the water temperature is optimal not only for growth of this coccolithophorid, but also for the process of its cells calcifications, as it has been confirmed by the laboratory studies (Feng et al., 2017).

At the end of May 2013, in the studied areas of the Black Sea, the average values of solar radiation intensity for the upper mixed layer did not go beyond the light optimum of growth, established in the experiments on the culture of this species (Harris et al., 2005). During the study period, the nutrients concentrations, probably, did not limit the phytoplankton growth. At the same time, the N/P ratio was 3–4 times lower than the Redfield ratio, what is most favourable for the development of a cocolithophorids bloom (Mikaelyan et al., 2011). The ammonium concentration was several times higher than the nitrate's one, and on average reached 1.24 µM. With such concentration this nutrient inhibits the nitrate uptake by phytoplankton (Varella, Harrison, 1999). Therefore, possibly, ammonium was the main source of nitrogen for phytoplankton growth in the western part of the Sea in May 2013. Under these conditions, the coccolithophorid *E. huxleyi*, perhaps, grew at a maximum rate (1.03–1.44 day^{-1}). These values correspond to the maximum growth rates, which have been obtained in the laboratory experiments on *E. huxleyi* (Langer et al., 2009; Feng et al., 2017). Water salinity in the bloom zone localized at some distance from the Dnieper River flow was optimal and did not limit this coccolithophorid development.

Against the background of optimal abiotic conditions, the intensity of *E. huxleyi* development in its bloom zone was not the same as it was determined by variability of the biota properties. Among biotic environmental factors that influence phytoplankton development level, the most important one is the microzooplankton grazing on phytoplankton. At the same time, as is known, microzooplankton feeds on the *E. huxleyi* poorly (Strom et al., 2003), what is favourable for its growth. Relatively high values of phytoplankton specific growth rate (µ) and low values of specific grazing rate (g) in May 2013 provided a physiological basis for the phytoplankton biomass increment and *E. huxleyi* bloom development. Not only the high net growth rate of phytoplankton (µ–g), but also rises of the dinoflagellates relative share in the total biomass of phytoplankton contribute to the increase in this coccolithophorid abundance. Perhaps, the dinoflagellates provided a stimulating effect on the *E. huxleyi* development.

In May 2013, in the largest part of the water area surveyed, the diatoms contribution to the total biomass of phytoplankton was not high. This can prove a presence of unfavourable environmental conditions for their development. What is the limitation factor for the growth of diatoms? The water temperature and average values of solar radiation intensity in UML, as a rule, do not go beyond the optimum limits for diatom development. It has been proved by the experiments on the cultures of some species of this algae group (Shoman & Akimov, 2013) and the data that confirm their intensive development in the western part of the Black Sea during the summer period (Stelmakh & Gorbunova, 2018). Silicate and phosphate concentrations were high enough and, probably, did not limit the diatoms growth in the studied areas of the Black Sea. However, nitrate concentration was low. Simultaneously, specifically nitrate is necessary for intensive development of diatoms, while other groups of algae can develop well on both nitrate and ammonium (Glibert et al., 2016).

At the end of October 2010, in the western area of the Black Sea, the final stage of autumn *E. huxleyi* bloom was registered. After 10–12 days, the degradation of this bloom was observed (Stelmakh et al., 2013). In the area of the Danube River runoff, where the Sea salinity decreased, the *E. huxleyi* share was not large, but outside the desalinated area it increased significantly. However, *E. huxleyi* development there was uneven, what was predominantly determined by variability of N/P ratio. Maximum abundance of this species was observed with N/P ratio values, which were approximately three times lower than the Redfield ratio.

Significant decrease in solar radiation intensity and water temperature in October were not limiting an intensive *E. huxleyi* development, what is determined by seasonal adaptation of this species and the phytoplankton in general to light and temperature conditions. Under decreasing light intensity and temperature, the maximum values of photosynthesis and the microalgae growth rate as well as their light satu-

ration are known to decrease naturally (Finenko et al., 2002; Shoman & Akimov, 2013). In the zone of *E. huxleyi* bloom, weak microzooplankton grazing on phytoplankton (g = 0–0.2 day^{-1}) contributed to the dinoflagellates and coccolithophorids growth. The decreased microzooplankton grazing, probably, was caused by the fact that on the final stage of *E. huxleyi* bloom, it can release into the environment significant amount of dimethylsulfoniopropionate (DMSP), which inhibits the microzooplankton activity (Strom et al., 2003). Probably, it was one of the main reasons of dinoflagellates domination by biomass. At the same time, in the *E. huxleyi* bloom zone development, the diatom growth was limited by extremely low concentration of nitrate and silicate. Only in the western part of the study area, very close to the coast, the nutrients did not limit the growth of diatoms, which dominated by their biomass and reduced the *E. huxleyi* development level. This has been determined, possibly, by inhibitory influence of diatoms on *E. huxley* growth (Vasconcelos et al., 2002).

CONCLUSION

Development of the coccolithophorid *E. huxleyi* bloom in the studied regions of the Black Sea in May and October was controlled by a combined effect of several abiotic factors such as light, temperature, salinity and nutrients, as well as biotic factors such as net phytoplankton specific growth rate and share of diatoms and dinoflagellates in the total biomass of phytoplankton. In May, the most favourable abiotic conditions for this coccolithophorid were observed. Under these conditions, uneven distribution of *E. huxleyi* was controlled predominantly by the phytoplankton net growth rate and the relative dinoflagellates share in the total biomass of phytoplankton. Increase of the values of these parameters caused a rise in the abundance of this coccolithophorid. Simultaneously, the dinoflagellate growth was limited by increased microzooplankton grazing on it, and diatoms growth – by deficiency of nitrate. As a result, *E. huxleyi* often represented the main share of phytoplankton biomass. In October, this coccolithophorid bloom was registered under conditions when phytoplankton adapted to low values of temperature and solar radiation intensity. In low water salinity zone, the bloom was not observed. In the rest of the water area, the N/P ration in the water as well as relative share of diatoms in the total biomass of phytoplankton played the main role in *E. huxleyi* abundance regulation. The decrease of these parameters led to the increase in *E. huxleyi* abundance. In the zone of its bloom, where the phytoplankton was consumed by microzooplankton extremely weakly, the main phytoplankton biomass was formed by dinoflagellates. Low nitrate and silicate concentrations in this zone, perhaps, were a reason for a weak diatom growth.

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EMILIANIA HUXLEYI „ŽYDĖJIMAI" JUODOJOJE JŪROJE: ABIOTINIŲ IR BIOTINIŲ VEIKSNIŲ ĮTAKA

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Santrauka

Tyrimai buvo vykdomi Juodosios jūros skirtingo gylio zonose 2010 m. spalio ir 2013 m. gegužės mėnesiais. Nustatyti pagrindiniai abiotiniai ir biotiniai veiksniai, kurie kontroliuoja kokolitoforidų *Emiliania huxleyi* pavasario ir rudens „žydėjimus". Gegužės pabaigoje *E. huxleyi* „žydėjo" esant būdingoms rūšiai šviesos ir temperatūros sąlygoms bei esant optimaliam azoto ir fosforo (N/P) santykiui vandenyje. Biotiniai veiksniai, tokie kaip fitoplanktono augimo greitis ir šarvadumblių santykis biomasėje, lėmė nevienodą *E. huxleyi* pasiskirstymą „žydėjimo" zonose. Spalį vandens temperatūra buvo 4 °C žemesnė lyginant su geguže, o šviesos intensyvumas sumažėjo maždaug 2–3 kartus. Tačiau rūšis prisitaikė prie sezoninių šviesos ir temperatūros pokyčių ir jos gausumas pasiekė „žydėjimo" lygį. Šiuo laikotarpiu kokolitoforido gausumo pasiskirstymą lėmė N/P santykis vandenyje ir titnagdumblių santykis fitoplanktono biomasėje. *E. huxleyi* „nežydėjo" mažo druskingumo zonose. Tirtose Juodosios jūros zonose amonis buvo pagrindinis azoto šaltinis, tai buvo palanku vystytis *E. huxleyi* ir šarvadumbliams, tačiau ribojo titnagdumblių gausumą.