Carbon-to-chlorophyll-α ratio in the phytoplankton of the Black Sea surface layer: variability and regulatory factors

LIUDMILA V. STELMAKH¹, TATIANA I. GORBUNOVA²

¹Kovalevsky Institute of Marine Biological Research, Russian Academy of Sciences, Sevastopol, Russia, e-mail: lustelm@mail.ru
²Institute of natural and technical systems, Russian Academy of Sciences, Sochi, Russia

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Abstract
Based on the research, conducted in the Black Sea during period of time from 2000 to 2011, seasonal dynamic of C:Chl a ratio and its spatial variability in nano- and microphytoplankton of surface water layer (0–0.5 m) had been analyzed. Maximum values of this parameter were observed in summer, and minimum ones – in winter. Intermediate values of C:Chl a were marked in spring and autumn. The main reasons for variability of the ratio between an organic carbon and chlorophyll a are the light, and different size of phytoplankton and its taxonomic composition. In coastal areas of the sea during summer period, when average values of light intensity in the upper mixed layer are above 20 E·m⁻²·day⁻¹, size and taxonomic composition of phytoplankton provide main influence to C:Chl a ratio.

Key words: ratio between an organic carbon and chlorophyll a, phytoplankton biomass, Black Sea.

Introduction
Phytoplankton biomass is a one of the most informative parameters, reflected state of the primary component of marine ecosystems. Deep knowledge about its variability is necessary for studying numerous aspects, related to climate and anthropogenic activity impact to the processes, occurring in the sea. Among these processes should be marked variations in nutrient regime, biochemical cycles of carbon, nitrogen, phosphorus, silicon and other elements. For evaluation of phytoplankton biomass in the surface layer many authors often used concentration of chlorophyll a, determined by the direct methods (Marañón et al. 2000; Sathyendranath et al. 2009), as well as derived from satellite information (Wang et al. 2013, Bellacicco et al. 2016). For conversion from chlorophyll a concentration to carbon phytoplankton biomass it is necessary to know the ratio value between these parameters (C:Chl a). Determination of the ratio between an organic carbon and chlorophyll a in the ocean based on direct measurements (Sathyendranath et al. 2009) and satellite information (Bellacicco et al. 2016), allows to conclude, that C:Chl a ratio is subjected to significant spatial and temporal variability. Its range is at least one order of values (20–500). It is considered, that this variability is due to phytoplankton acclimation to fast changing environmental conditions, mainly to light and nutrients (Geider 1997; MacInture et al. 2002; Bellacicco et al. 2016), and also by variations of phytoplankton species composition (Geider et al. 1997; Finenko et al. 2003; Stelmakh & Babich 2006).
From the nutrients a nitrogen provides greatest influence to the ratio between an organic carbon and chlorophyll $a$ variability, an impact from silicon and phosphorus is significantly lower (Harrison et al. 1977).

Determinations of C:Chl $a$ ratio in the Black Sea prior to our research were sparse and sporadic (Vedernikov et al. 1983; Vedernikov & Michaelyan 1989; Finenko et al. 2005). It was difficult to evaluate about variability of this parameter and its causes on the ground of that information. It is known, that in the Black Sea the majority of phytoplankton biomass ($\geq 90\%$) is consisted, as a rule, from microalgae with linear dimensions, which are above 2 µm, the species relating to nano- and microphytoplankton (Vedernikov & Michaelyan 1989; Ratkova 1989; Kopuz et al. 2012). Therefore, calculation of the ratio between an organic carbon and chlorophyll $a$ we carried out for total nano- and microphytoplankton, without taking picophytoplankton in account.

The aim of this work is to study spatial and temporal variability of C:Chl $a$ ratio in total nano- and microphytoplankton of the Black Sea surface layer and to identify the key factors regulating it.

Material and Methods

In period from 2000 to 2010 the studies were conducted once per month on 5 stations in Sevastopol area and 4 stations in South Crimea coast (Figure 1). The first of them was in the most closed bay of Sevastopol. Here the water exchange was significantly weakened in compare to other sea areas. At the same time other stations, located in the bays and open coastal zone were subjected to a stronger influence of water dynamics. In the areas, where the work was carried out, sea depth from surface to bottom was less than 20 m, and only on station 5 it reached 50 m. The work was performed in three stages: in 2000–2003, in 2006–2007, and also in 2010.

Figure 1. Location of sampling stations in the coastal waters of the Black Sea near Sevastopol and the southern coast of Crimea in 2000-2003 and 2006-2010: 1 - Sevastopol Bay, 2, 3 - Karantinnaya Bay, 4 - Kruglaya Bay, 5 - open coastal zone near Sevastopol, 6,7 - Golubaya Bay, 8, 9 - Laspi Bay.
During 2010–2011 the studies were conducted in 2 expeditions on the research vessel "Professor Vodyanitsky". The work had been carried out at 43 stations, located predominantly in North-Western part of the Black Sea, and, also near Crimea peninsula with the coordinates on the stations nets 44.00–46.50° N, 29.70–35.00° E (Figure 2).

Samples of water 2–4 L volume were collected in the surface layer of the sea (0–0.5 m) by Niskin bottles. For concentration of nano- and microphytoplankton were used nuclear filters with pore size 2 µm (made in Dubna, Russia). Concentration of phytoplankton in 20–30 times was carried out with using the reverse funnel filtration, in a closed chamber, where the phytoplankton was concentrated to 100 ml volume (Sorokin et al. 1975). Concentrated sample was divided in two parts. The first part (10 ml) was fixated by 1% formalin solution. It was used for the phytoplankton analysis and calculation its biomass. Second part (90 ml) was used for measuring chlorophyll a concentration. Samples were filtered through 0.45 µm polycarbonate filters under low vacuum (< 100 mm Hg). Filters were frozen at -20 °C and preserved there until the beginning of the treatment process. The abundance and linear dimensions of algae cells were determined in a 0.1 ml drop with three - five replications under light microscope ZEISS Primo Star. Linear measurements were converted to cell volume using various geometric formulas. Average cell volume of phytoplankton (V_{ave}) was determined as a ratio of total volume for all cells to their abundance. Phytoplankton organic carbon concentration was calculated from average cell volume for each species of diatoms and dinoflagellates using the equations presented in the work (Menden-Deuer & Lessard 2000), for coccolithophorid Emiliania huxleyi (Lohmann) W.W. Hay & H.P. Mohler, 1967 – using the equation (Montagnes et al. 1994), for other algae – using the equation (Strathmann 1967). Phytoplankton species identification was carried out using the manual (Tomas 1997).

Chlorophyll a concentration was measured in acetone extracts using fluorometric method (JGOFS Protocol 1994). The nutrient concentrations in the water were determined by the methods, described earlier (Stelmakh & Babich 2006). Information on the sea water temperature was obtained in scientific expeditions using STD probe-complex.

Illuminance levels were measured with a luxmeter U-116. The transition coefficient of illuminance in lux to the light intensity in the range of photosynthetic active radiation (PAR) was set equal to 10^4 lux = 200 µE·m^{-2}·s^{-1} (Parsons et al. 1982). The average irradiance in the limits of upper mixed layer was calculated by using equation:

\[ I_z = I_0 e^{-kz}, \]  \hspace{1cm} (1)
where $I_0$, $I_r$, $I_z$ – irradiance at the sea surface and at depth $z$ (m) respectively, E·m$^{-2}$·day$^{-1}$. Average value of the light reduction coefficient for the layer (k) was calculated from the decreasing of total irradiance in the PAR range, using the dependence (Vedernikov 1989).

For statistical treatment of the data, correlation, regression and variance analysis were carried out using the software Excel 2007 and Sigma Plot 2001 for Windows. In multiple regression equations we used only those predictors that are not correlated or weakly correlated between themselves. Map building was carried out using the program Surfer 8.

**Results**

*Seasonal variability of organic carbon to chlorophyll a ratio of phytoplankton in the coastal surface sea waters in the area of Sevastopol and the southern coast of Crimea.* Investigations, conducted in 2000–2010 in surface water layer (0–0.5 m) of Sevastopol Bay, indicated, that C:Chl $a$ values (mg C·mg Chl $a$$^{-1}$) were changing during a year approximately on one order magnitude: from 25–55 to 250–500 (Figure 3). The minimum values were obtained in the winter period when the intensity of solar radiation (4–6 E·m$^{-2}$·day$^{-1}$) and water temperature ($7–10$ °C) are lowest. At the same time, the concentrations of nitrogen (nitrate and ammonium) were relatively high. Usually the minimum values of C:Chl $a$ ratio were observed on the background of low phytoplankton biomass (<100 mg C·m$^{-3}$) and development of small diatoms Skeletonema costatum (Greville) Cleve 1873, Chaetoceros socialis H.S. Lauder 1864 and Chaetoceros curvisetus Cleve 1889. These species generated the main phytoplankton biomass. The same values of C:Chl $a$ ratio were obtained in autumn and spring, in the period preceding of diatom bloom. In other cases, in spring and autumn, intermediate values of C:Chl $a$ ratio (100–300) were observed. They were obtained on the background of different nutrient availability, and also different level of phytoplankton development. Thus, concentrations of nitrate had been varied from 0 to 8 µM, and ammonium – from 0.1 to 3 µM. Phytoplankton biomass values varied from 100 to 1100 mg C·m$^{-3}$. It is important to state, that in these periods an intensity of solar radiation was increasing approximately in 3–5 times comparing with winter. And highest C:Chl $a$ values (250–500) were recorded in the period from July to September, when phytoplankton biomass was 50–550 mg C·m$^{-3}$. Concentrations of nitrate and ammonium usually were low and did not exceed 1 µM. At the same time, maximum or close to maximum values of solar radiation intensity (40–55 E·m$^{-2}$·day$^{-1}$) and water temperature (20–28 °C) were observed. Herewith, over 50 % of nano- and microphytoplankton biomass were usually consisted of dinoflagellates Ceratium tripos (O.F. Müller) Nitzsch 1817, Tripos furca (Ehrenberg) F. Gómez 2013, Prorocentrum cordatum (Ostenfeld) J.D. Dodge 1975, Scrippsiella trochoidea (Stein) Loeblich III 1976. Similar character of C:Chl $a$ changes was marked in other bays and open coastal zone near Sevastopol (Stelmakh & Babich 2006; Stelmakh 2014). This circumstance allowed us to present the seasonal dynamics of the entire dataset in a common plot (Figure 4). It can be described as a unimodal curve with its maximum in the summer period – 234±139 (mean ± standard deviation) and minimum – in winter (56±23). Intermediate C:Chl $a$ values were observed in spring (113±90) and autumn (138±101).

**Impact of phytoplankton taxonomic composition, volume of its cells and environmental factors to variability of C:Chl $a$ ratio during a year.** As an indicator, reflecting phytoplankton taxonomic composition variability, we elected the relative share of dinoflagellates in the total biomass of nano- and microphytoplankton ($B_{Dinof}$), expressed in percentage. The size structure of total nano- and microphytoplankton can be characterized by average volume of its cells ($V_{ave}$).

The table 1 contains summarized data on the values of C:Chl $a$ ratio, relative share of dinoflagellates in the total nano- and microphytoplankton biomass, average volume of phytoplankton cells, and also some related environmental parameters in surface layer of the coastal waters of the Black Sea at different seasons of 2000–2010. The analysis of these results, obtained from all studied stations, showed, that minimum values of C:Chl $a$ ratio, observed in winter period, connected to the lowest proportion of dinoflagellates in the total phytoplankton biomass (22±21 %) and lowest values of $V_{ave}$ (2700±2000 µm$^3$). At that, minimum values of light intensity (8±5 E·m$^{-2}$·day$^{-1}$) and water temperature (9±2 °C) at the sea surface were marked. At the same time, maximum concentrations of total nitrate and ammonium in the environment were measured (5.6±4.9 µM).
CARBON-TO-CHLOROPHYLL-A RATIO IN THE PHYTOPLANKTON OF THE BLACK SEA SURFACE LAYER

Table 1. C:Chl a ratio, relative share of dinoflagellates in the total nano- and microphytoplankton biomass ($B_{Dinofl}$), average volume of phytoplankton cells ($V_{aver}$), and also some related environmental parameters in surface layer of the coastal waters of the Black Sea at different seasons of 2000–2010.

| C:Chl a, mg C·mg Chl a⁻¹ | I, E·m⁻²·day⁻¹ | T, °C | N-(NO₃+NH₄), µM | $B_{Dinofl}$, % | $V_{aver}$, µm³ |
|--------------------------|-----------------|-------|-----------------|-----------------|---------------|
| Winter (December – February), n = 65 | | | | | |
| 56±23 (25–87) | 8±5 (3–18) | 9±2 (7–12) | 5.6±4.9 (0.1–17.0) | 22±21 (0–97) | 2700±2000 (680–4800) |
| 113±90 (27–300) | 16±6 (10–30) | 15±5 (9–21) | 2.6±2.3 (0.2–11.0) | 34±25 (5–90) | 3700±2500 (1100–7500) |
| Summer (June – August), n = 69 | | | | | |
| 234±139 (35–500) | 45±15 (30–60) | 23±4 (20–28) | 1.5±1.5 (0–4.2) | 53±28 (1–96) | 15500±15000 (770–100000) |
| Autumn (September – November), n = 40 | | | | | |
| 138±101 (30–364) | 14±5 (10–28) | 16±3 (12–20) | 2.3±2.2 (0.1–5.3) | 33±24 (10–90) | 3900±2700 (1000–7200) |

Note: I – light intensity at the sea surface, T – water temperature, N – (NO₃+NH₄) – total concentration of nitrate and ammonium, upper line - average value and standard deviation, bottom line – the limits of parameters variability, n – number of determinations.

On the background of increasing solar radiation intensity from winter to summer in average up to 45 E·m⁻²·day⁻¹ and water temperature – in average up to 23 °C, there was observed decreasing of nitrogen concentration (nitrate and ammonium) approximately in 3.7 times. These processes, and also dominating in phytoplankton the largest algae, relating to dinoflagellates, were leading to rising C:Chl a from winter to summer. As a result, the average values of C:Chl a ratio in summer period were in for time higher, than in a winter. However, during summer period this parameter varied in rather wide range (from 35 to 500). For identification of the factors, regulating the variability of C:Chl a ratio, correlation analysis was performed (Table 2). A strong correlation between carbon to chlorophyll a ratio and average phytoplankton cell volume ($V_{aver}$) was observed. Correlation coefficient (R) was 0.76. A medium correlation degree was marked between C:Chl a ratio and relative share of dinoflagellates in the total biomass of nano- and microphytoplankton (R = 0.66). Value of the ratio between an organic carbon and chlorophyll a was correlated weakly with environmental parameters (nitrogen, light and temperature). Relative dinoflagellates biomass was weakly correlated with all environmental parameters, and also with $V_{aver}$. This allows us to consider two structural indicators of phytoplankton ($V_{aver}$ and $B_{Dinofl}$) as the main independent variables, determining variability of C:Chl a ratio in a summer period. Resulting that, variability of C:Chl a ratio in phytoplankton of the surface water layer was dependent in generally from average cell volume of total nano- and microphytoplankton ($V_{aver}$) and relative share of dinoflagellates in total phytoplankton biomass ($B_{Dinofl}$). As independent predictors, they determined 79% of the variability of the relationship between organic carbon and chlorophyll a:

\[
C:Chl\ a = 0.0177 \cdot V_{aver} + 1.8104 \cdot B_{Dinofl}.
\]

(2)

where determination coefficient $R^2=0.79$, standard error SE= ±61.93, Fisher test $F = 200.61$; t-test for the equation coefficients were 3 times higher than the critical values at a confidence level $p<0.0001$. Additional calculations indicated that standardized coefficient of first independent variable was 0.71, and of second one it was 2 times lower. This is confirming the leading role of phytoplankton size structure in variability of ratio between an organic carbon and chlorophyll a in a summer season. Taxonomic composition follows that. And only small share of variability (no more than 21%) is conditioned, possibly, by different nutrient availability. From the presented equation (2) it is follows, that in summer period in surface layer of the Black Sea coastal waters near Crimea increasing size of phytoplankton cells and relative share of dinoflagellates in its total biomass is accompanied by a rise in C:Chl a ratio.
Figure 3. Seasonal variability of C:Chl a ratio (mg C·mg Chl a⁻¹), phytoplankton biomass, water temperature, nitrate and ammonium concentrations in the surface water layer of Sevastopol Bay, as well as solar radiation intensity, which can reach the sea surface.

In spring and autumn periods of the year environmental conditions, taxonomic and size structure of nano- and microphytoplankton are approximately the same (table 1). Therefore, average values of C:Chl a between the indicated periods were differed slightly. Main role in the given parameter variability during the spring and autumn belongs, perhaps, to joint influence of environmental conditions (predominantly light and nutrients).
Table 2. Correlation (R) between C:Chl a ratio, average nano- and microphytoplankton cell volume (V_{avg.}), share of dinoflagellates in total nano- and microphytoplankton biomass (B_{Dinofl}) and environmental factors based the data, received in the Black Sea coastal waters in Sevastopol and South Crimea coast area at summer period.

| Parameters | C:Chl a | B_{Dinofl} | N-(NO_3+NH_4) | V_{avg.} | T | I |
|------------|---------|------------|----------------|----------|----|---|
| C:Chl a    | 1       |            |                |          |    |   |
| B_{Dinofl} | 0.66    | 1          |                |          |    |   |
| N-(NO_3+NH_4) | -0.41   | -0.37     | 1              |          |    |   |
| V_{avg.}   | 0.76    | 0.40       | 0.37           | 1        |    |   |
| T          | 0.04    | 0.36       | -0.03          | 0.014    | 1  |   |
| I          | 0.21    | 0.43       | -0.23          | 0.16     | 0.51 | 1 |

Note: I – intensity of photosynthetic active radiation at the sea surface, N-(NO_3+NH_4) – total concentration of nitrate and ammonium, T – water temperature.

Figure 4. Seasonal dynamic of monthly average values of C:Chl a ratio (mg C·mg Chl a⁻¹) and their standard deviation (vertical lines) for all stations, located in the Black Sea coastal waters (during period 2000–2010).

C:Chl a ratio in nano- and microphytoplankton of surface water layer in different areas of shallow part of the sea. Studies, performed in a warm period of the year (in August and October) indicated that in different shallow areas of the Black Sea ratio between the main phytoplankton groups (diatoms and dinoflagellates) was different. So, in August 2011, when the water temperature in the upper mixed layer was 22–24 °C, on the largest part of the studied area diatoms dominated, their fraction had exceeded 50 % of the total nano- and microphytoplankton biomass (Figure 5). Among the latter Pseudosolenia calcar-avis (Schultze) B.G.Sundström 1986, Thalassionema nitzschoideis (Grunow) Mereschkowsky 1902 and Chaetoceros curvisetus Cleve 1889 were dominating. Only in the centre of North-Western area and in Karkinitsky Bay their fraction was lower 30–40%. Here major phytoplankton biomass was consisted of dinoflagellates (genus Ceratium, Protoperidinium, Gymnodinium). Share of other taxonomic groups was not significant.

In areas, closest to the Dnieper River, lowest values of C:Chl a ratio were observed (Figure 5). They changed from 72 to 134, averaging 108 (Table 3). Here most often diatoms were dominated, and share of dinoflagellates was in average 30% (± 20 %). On the rest of the water area C:Chl a values varied from 164 to 330, averaging 217. Herewith, share of diatoms in the total nano- and microphytoplankton biomass was decreased, and share of dinoflagellates was increased to an average of 52 % (± 23 %). Rising of dinoflagellates share had not caused significant increase of average cell volume in the phytoplankton (Table 3). However, nutrient content was subjected to significant variability: phosphate – from 0.1 to 0.3 µM, total nitrate and ammonium – from 0.1 to 2.5 µM, and silicate – from 0.10 to 8.14 µM. Length of the upper mixed layer on different stations was changing in rather wide range (from 4 to 19 m). Resulting that, light intensity in the upper mixed layer was changing from 10 to 32 E·m⁻²·day⁻¹.
Figure 5. Spatial distribution of chlorophyll a, phytoplankton biomass, relative share of diatoms in the total biomass of nano- and microphytoplankton and C:Chl a ratio in the surface water layer of the Black Sea in August 2011.

Table 3. C:Chl a ratio, share of dinoflagellates (B_Dinofl.) and diatoms (B_Bacillar.) in total nano- and microphytoplankton biomass, average nano- and microphytoplankton cell volume (V_aver.) and some related parameters in surface water of shallow Black Sea areas in August 2011.

| C:Chl a, mgChl a⁻¹ | B_Dinofl. % | B_Bacillar % | V_aver. µm³ | N-(NO₃)⁺ NH₄, µM | P-PO₄, µM | Si, µM | UML, m | I_UML, E·m⁻²·day⁻¹ | n  |
|---------------------|-------------|--------------|-------------|-----------------|----------|--------|--------|-------------------|----|
| 108±22              | 30±20       | 60±25        | 12300±9000  | 0.8±0.3         | 0.2±0.1  | 3.63±3.01| 10±4   | 21±5              | 12 |
| 72–134              | 9–58        | 30–90        | 2000–32000  | 0.4–1.3         | 0.1–0.3  | 0.12–8.14| 4–19   | 16–32             |    |
| 217 ± 68            | 52±23       | 37±21        | 11400±10200 | 0.9±0.6         | 0.2±0.1  | 2.11±2.07| 10±3   | 22±5              | 10 |
| 164 – 400           | 20–91       | 5–60         | 1400–38000  | 0.1–2.5         | 0.1–0.3  | 0.10–7.42| 7–17   | 10–29             |    |

Note: upper line – average values and standard deviation, low line – limits of parameters variation, UML – upper mixed layer, I_UML – average solar radiation intensity for upper mixed layer, n – number of determinations.

Correlation analysis indicated that C:Chl a ratio was correlated with two studied parameters only. These are the relative share of dinoflagellates in total phytoplankton biomass (B_Dinofl.) and average light intensity in the upper mixed layer (I_UML). Both these variables were not correlated between themselves. As follows from the multiple regression equation, they determined 65% of the C:Chl a variability:
where \( R^2 = 0.65 \), \( SE = \pm 38.6 \), \( F = 36.95 \) with \( p < 0.0001 \); t-test for the first coefficient of equation was 5.93, and for the second one – 6.24. Standardized coefficient of equation with the first independent variable equal to 0.77, and with second one – 0.39, what reflects dominating role of taxonomic composition of phytoplankton for variability of C:Chl \( a \) ratio comparing to light. The rest of the variability of this ratio, apart from the two presented predictors, that was, probably, conditioned by different nutrient supply of phytoplankton and other unaccounted factors. As a result of C:Chl \( a \) variability in a summer period (in August), the spatial distribution of phytoplankton biomass and chlorophyll \( a \) is different (Figure 5).

The main impact of light and the taxonomic composition of the phytoplankton to spatial variability of C:Chl \( a \) ratio were revealed in the autumn too. So, in October 2010, in the surface layer of shallow North-Western part of the sea, diatoms, dinoflagellates and coccolithophores were marked (Table 4). Share of the latter was in the range from 2 to 38 %. Among coccolithophores \( E. huxleyi \) was dominating. Share of diatoms in total nano- and microphytoplankton biomass varied from 0 to 90%, and share of dinoflagellates – from 1 to 80%. Main biomass of diatoms was consisted of \( C. socialis \), \( S. costatum \), \( T. nitzschioidea \) and \( Proboscia alata \) (Brightwell, Sundström 1986. Among dinoflagellates \( Prorocentrum micans \) Ehrenberg 1834, \( P. cordatum \), \( Gymnodinium simplex \) (Lohmann) Kofoid & Swezy 1921 and \( Lingulodinium polyedra \) (F. Stein) J.D.Dodge 1989 were dominating. In this period C:Chl \( a \) ratio values were different in the studied waters (Figure 6). Lowest ratio of an organic carbon to chlorophyll \( a \) (from 25 to 58) was marked in the most productive area near Danube River. Here diatoms created the basic phytoplankton biomass, and average light intensity in upper mixed layer was minimal, in average 5 \( \text{E} \cdot \text{m}^{-2} \cdot \text{day}^{-1} \) (Table 4). In the centre of North-Western part C:Chl \( a \) ratio increased up to 120–276. Here dinoflagellates were dominating, and average light intensity in upper mixed layer was increased in over 2 times. On the rest of the water area C:Chl \( a \) ratio was within the range from 62 to 114. Increasing of the ratio between an organic carbon and chlorophyll \( a \) had been accompanied not only by light amplification in upper mixed layer, but, also, decreasing of nutrient concentration. So, minimum C:Chl \( a \) ratio values (25–58) were obtained in conditions of maximum nutrient concentration (Table 4). Average phosphate concentration was 0.5 \( \mu \text{M} \), total nitrate and ammonium concentration – 4 \( \mu \text{M} \), and silicate – 4.9 \( \mu \text{M} \). Maximum values of C:Chl \( a \) (120–276) were marked with lowest phosphate concentration (in average 0.1 \( \mu \text{M} \)), as well as concentration of mineral nitrogen forms (in average 0.5 \( \mu \text{M} \)) and silicate (in average 0.7 \( \mu \text{M} \)). Average cell volume of total nano- and microphytoplankton was changing on spatial ground insignificantly (Table 4). The water temperature in the upper mixed layer was 15–17 °C on all water areas.

**Table 4.** C:Chl \( a \), ratio, share of dinoflagellates (\( B_{\text{Dinofl.}} \)), diatoms (\( B_{\text{Bacillar.}} \)) and coccolithophores (\( B_{\text{Coccolit.}} \)) in total nano- and microphytoplankton biomass, average nano- and microphytoplankton cell volume (\( V_{\text{aver.}} \)) and some related parameters in surface waters of shallow Black Sea areas in October 2010.

| C:Chl \( a \), mgC \( \cdot \text{mgChl } a^{-1} \), B_{\text{Dinofl.}}, % | B_{\text{Bacillar.}}, % | B_{\text{Coccolit.}}, % | V_{\text{aver.}}, \mu \text{m}^3 | \text{N-(NO}_3^+, \text{NH}_4^+), \mu \text{M} | \text{P-P}_4^-, \mu \text{M} | \text{Si}, \mu \text{M} | \text{UML, m} | \text{I}_\text{UML, -} \text{E} \cdot \text{m}^{-2} \cdot \text{day}^{-1} | \n\n| 47±11 | 14±8 | 57±18 | 22±12 | 2500±1600 | 4.0±3.6 | 0.5±0.4 | 4.9±3.0 | 22±5 | 5±1 |
| (25–58) | (1–26) | (36–90) | (2–38) | (1000–7000) | (1.1–13.6) | (0.2–1.2) | (2.2–12.0) | (12–28) | (4–9) |
| n=8 |
| 87±19 | 39±27 | 43±24 | 17±13 | 2200±1400 | 1.7±0.9 | 0.3±0.2 | 3.0±2.4 | 17±7 | 10±3 |
| (62–114) | (1–74) | (6–72) | (2–32) | (1100–4500) | (0.8–3.4) | (0.1–0.7) | (0.9–6.8) | (9–27) | (4–14) |
| n=8 |
| 200±71 | 68±16 | 5±3 | 27±11 | 2400±1100 | 0.5±0.2 | 0.1±0 | 0.7±0.2 | 17±4 | 13±2 |
| (120–276) | (43–80) | (0–9) | (10–37) | (1200–5000) | (0.3–0.9) | (0.1–0.1) | (0.6–0.9) | (15–21) | (10–16) |
| n=5 |

Note: upper line – average values and standard deviation, low line – limits of parameters variation, UML – upper mixed layer, \( \text{I}_{\text{UML, -}} \) – average solar radiation intensity for upper mixed layer, \( n \) – number of determinations.

\[ C: \text{Chl } a = 1.962 \cdot B_{\text{Dinofl.}} + 4.392 \cdot I_{\text{UML, -}} \]
Following to the research results analysis, received in October, the multiple regression equation was calculated (4), where determination coefficient indicates, that 70% of C:Chl \(a\) ratio variability is caused by changing light intensity in upper mixed layer and relative share of dinoflagellates algae in total phytoplankton biomass with dominating role of the light:

\[
C:Chl\ a = 8.382 \times I_{UML} + 0.865 \times B_{Dinofl},
\]

where \(R^2 = 0.70\), \(SE = \pm 0.38.0\), \(F = 42.14\) with \(p < 0.0001\). Values of t-test for the equation coefficients were above the critical. Standardized coefficient with the first predictor was 0.50, and with the second one it decreased to 0.35, what reflects dominating role of light for variability of C:Chl \(a\) ratio comparing to taxonomic composition of phytoplankton. The rest of this ratio variability was probably conditioned by different nutrient concentration in the water. As follows from the equation (4), as far as the intensity of solar radiation in the limits of upper mixed layer and relative share of dinoflagellates in total phytoplankton biomass increase, the C:Chl \(a\) values are growing. As a result of C:Chl \(a\) variability in the autumn (in October), spatial distribution of phytoplankton biomass and chlorophyll \(a\) is different (Figure 6).

**Figure 6.** Spatial distribution of chlorophyll \(a\), phytoplankton biomass, relative share of diatoms in the total biomass of nano- and microphytoplankton and C:Chl \(a\) ratio in the surface water layer of the Black Sea in October 2010.
Phytoplankton biomass carbon is usually estimated from cell bio-volume and abundance by light microscopic measurement and flow cytometry (Wang et al. 2013). Also this parameter is calculated basing on chlorophyll a concentration which can be determined not only by the direct methods, but, as well, with remote sensing techniques (Behrenfeld et al. 2005; Sathyendranath et al. 2009; Wang et al. 2013; Bellacicco et al. 2016). However the relationship between phytoplankton carbon biomass and chlorophyll a concentration is usually non-linear. As a result, C:Chl a ratio is not constant (Finko et al. 2003; Stelmakh & Babich 2006; Wang et al. 2013). This means that with the same phytoplankton biomass, the concentration of chlorophyll a can vary significantly. The carbon:chlorophyll a ratio is a sensitive indicator of physiological state of microalgae, reflecting specific content of chlorophyll a in cells. Its variability in response to changes in environmental conditions contributes to the optimization of phytoplankton growth (Geider et al. 1997). Experiments on the microalgae cultures have shown that the main factor, determining the variability of C:Chl a ratio is light. If the light intensity increases, the C:Chl a values are growing. At the same time, temperature and nutrients have the opposite effect (Geider et al. 1997; Finko et al. 2003). It was indicated, that with optimal for the algae growth light and temperature conditions, as well as with sufficient amount of nutrients, C:Chl a ratio for dinoflagellates in 2–3 times higher, than it is for diatoms (Banse 1982; Geider et al. 1997; Finko et al. 2003). Moreover, large algae species can have higher values of this ratio comparing to the small ones (Finkel 2001). In the sea with conditions of joint influence of environmental factors and periodical changing of phytoplankton species, role of the individual factors will be, possibly, varying.

As a rule, diatoms and dinoflagellates consist of the main biomass of the Black Sea phytoplankton (Vedernikov, 1989; Vedernikov & Michaelyan, 1989; Polikarpov et al. 2003; Stelmakh & Babich 2006; Stelmakh et al. 2013). Only randomly coccolithophorid E. huxleyi can dominate (Stelmakh & Georgieva 2014). The ratio between these groups of algae is undergoing through regular temporal and spatial variability (Polikarpov et al. 2003; Stelmakh et al. 2013). This is caused by the joint effect of environmental factors and biotic interactions in the plankton. When the environmental parameters (light, temperature, nutrients) and grazing rate of phytoplankton by zooplankton are changing, gradual restructuring of the species and size structure of phytoplankton are occurring. Its biomass, the specific pigment content in algae cells and their functional characteristics are changing. We believe, that not only environmental conditions (first of all the light), but also taxonomic composition of phytoplankton and its size structure can affect variability of C:Chl a ratio in the studied waters during a year. The amplitude of its seasonal variability (by monthly average values) in the studied coastal waters near Sevastopol and Crimea had reached 6 times. Similar situation had been observed for surface waters phytoplankton in the English Channel (Llewellyn et al. 2005), where during several years (from 1999 to 2002) monthly measurements of chlorophyll a concentrations were conducted and evaluated phytoplankton biomass in the carbon units, calculated by the volume of algae cells. Here the seasonal variability amplitude of this ratio was 4–5 times. Based on the satellite measurements analysis, similar results were obtained on seasonal variability of C:Chl a in surface waters of the equatorial regions of the Atlantic and Pacific Oceans (Behrenfeld et al. 2005), as well as Mediterranean Sea (Bellacicco et al. 2016).

In the coastal Black Sea waters lowest average values of C:Chl a ratio were received in winter. It was due to low level of solar radiation, rather high concentration of nutrients and dominance of diatoms in the phytoplankton. When light is low, phytoplankton need to increase chlorophyll a to capture the light more efficiently. Therefore, the C:Chl a ratio is minimal. During the entire winter period variability of C:Chl a ratio was regulated predominantly by light, because, within low light intensities the linear relationship between these parameters was observed (Geider et al. 1997, Finko et al. 2003). Based on results, performed on the microalgae cultures (Geider et al. 1997), it can be assumed that the influence of the light on this ratio was intensified by low temperature and relatively high mineral nitrogen concentration. Highest average values of C:Chl a ratio were observed in summer, when solar radiation intensity near the sea surface was maximal and nutrients were limiting. In this period the ratio between an organic carbon and chlorophyll a had been changing in wide range. However, the role of light in this variability is, perhaps, insignificant. It is known that if the intensity of the photosynthetic active radiation is over 20–25 E·m⁻²·day⁻¹, C:Chl ratio is poorly dependent from it (Geider et al. 1997; Finko et al. 2003). In a summer period intensity of solar radiation at the sea surface is above these values, and in the upper mixed layer, which was no more than 5 m in the studied waters, it only slightly decreased. In these conditions variability of carbon:chlorophyll a ratio
was dependent preliminary from the average phytoplankton cell volume. Relative dinoflagellates share in total phytoplankton biomass was second parameter by its importance. Influence of the nutrients was, perhaps, weak. Finally, in spring and autum C:Chl a ratio values were determined by complex impact of environmental factors, and also, possibly, were depending on phytoplankton taxonomic composition.

Studies of the seasonal variability of C:Chl a ratio in phytoplankton of deep water area of the Black Sea were not performed previously. The vision of it was obtained on the basis of the ratio calculations from the simultaneous measurements of the total nano- and microphytoplankton biomass and chlorophyll a concentration for different months during period of time from 1988 to 2013 (Stelmakh 2014). Resulting reconstruction of this parameter variability during a year for the deep water sea area, it is shown, that maximal average values (173±36) were observed in summer period, and the minimal ones (49±22) were obtained in a winter. Intermediate values were marked in spring and autumn (90±43 and 130±40 respectively). It is possible to conclude, that character of C:Chl a ratio seasonal variability in total nano- and microphytoplankton for shallow and deep water areas of the sea is identical.

The data presented here shows, that in summer and autumn periods in the surface layer of different areas of Black Sea C:Chl a ratio is not equal. In its spatial variability light pays an important role. Solar radiation intensity, which reaches the sea surface, is changing over the sea water area insignificantly. However, the length of the upper mixed layer was not the equal in different parts of the sea. Therefore average values of solar radiation intensity in this layer were varying over the water area. For instance, in October 2010 dominating role of light in C:Chl a ratio variability is a due to the fact, that average values $I_{\text{UML}}$ on different stations were ranged from 4 to 16 E·m$^{-2}$·day$^{-1}$. With such light intensity values the ration between an organic carbon and chlorophyll, as it was stated above, connected to light by linear dependency. Second by its importance parameter, influencing the C:Chl a ratio variability in October, was relative share of dinoflagellates in total biomass of phytoplankton. However, role of the last parameter in carbon:chlorophyll a ratio variability was predominant in August 2011. At the same time, light influenced less than in October 2010. That is conditioned by the circumstance, that in a summer average values of light intensity in upper mixed layer in some cases were high and went beyond its linear relationship with C:Chl a ratio. Following that, in a summer, as well as in autumn in the studied surface waters, this ratio variability was conditioned mainly by two parameters: average value of light intensity in upper mixed layer and relative dinoflagellates share in total phytoplankton biomass. Nutrient impact to variability of ratio between an organic carbon and chlorophyll a perhaps, was insignificant.

In the Black Sea large variability of abiotic factors are not always observed, as well as chlorophyll concentration, phytoplankton biomass and its taxonomic composition. As we stated previously, in a beginning of warm season (in May 2013) there was observed intensive development of *E. huxleyi* and its domination in phytoplankton by biomass in North-Western part of the sea and near Crimea (Stelmakh & Georgieva 2014). Average light intensity in the upper mixed layer was in the range from 16 to 32 E·m$^{-2}$·day$^{-1}$. Nitrate and phosphate concentrations were varying from 0.1 to 0.3 µM. Concentrations of ammonium were significantly higher, than those of nitrate (0.5–1.9 µM). Chlorophyll a concentration was extremely low on the entire water area, and, as a rule, had not exceeded 0.10–0.18 mg·m$^{-3}$. In these conditions C:Chl a ratio values varied in fairly narrow limits – from 61 to 153, averaging 104.

### Conclusion

Character of seasonal C:Chl a ratio variability in nano- and microphytoplankton in surface layer of coastal and deep water sea areas is identical and appeared as a unimodal curve with a maximum in summer and minimum in winter. During most of the year major impact to variability of this ratio is caused by abiotic environmental conditions and, primarily, by light. However, during a summer in coastal surface waters, where light influence to the C:Chl a is insignificant, its variability is determined by two structural phytoplankton parameters (average cell volume of total nano- and microphytoplankton, and, also relative share of dinoflagellates in its biomass). In shallow water sea areas in the warm period of the year (in August and October) C:Chl a ratio values in nano- and microphytoplankton of surface water layer are differ by space in limits of 5–10 times. Main variability share of this parameter is conditioned, as a rule, by variability of average values of light intensity in upper mixed layer, and also different ratio between dinoflagellates and diatoms in total nano- and microphytoplankton biomass. As a result, spatial distribution of phytoplankton biomass and chlorophyll a is different. The presented results not only adds insights on the acclimation
mechanisms of phytoplankton to the environmental conditions, but also emphasizes the importance of using variable C:Chl $a$ ratio to estimate carbon phytoplankton biomass on chlorophyll $a$ concentration. Phytoplankton biomass is an important element of the monitoring studies that are needed to assess the current state of the Black Sea ecosystem.

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