Oceanological and Hydrobiological Studies

International Journal of Oceanography and Hydrobiology

Volume 48, No. 4, December 2019
pages (404-414)

ISSN 1730-413X
eISSN 1897-3191

# Effect of phytoplankton adaptation on the distribution of its biomass and chlorophyll *a* concentration in the surface layer of the Black Sea

by

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DOI: **10.2478/ohs-2019-0035**
Category: **Original research paper**
Received: **March 22, 2019**
Accepted: **May 15, 2019**

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## Abstract

Using the field data collected in the Black Sea in September 2005–May 2013, the authors studied the spatial variability of the ratio of organic carbon to chlorophyll *a* (C:Chl *a*) in the sea surface layer (0–1 m). The C:Chl *a* ratio is an important parameter that reflects the phytoplankton adaptation to abiotic factors. Its maximum variations occurred in September–October 2005 and October 2010 when the highest spatial variability of average light intensity and nitrogen concentration was observed in the upper mixed layer. As a result, the maps of phytoplankton biomass differed from chlorophyll maps. In August 2011, no effect of light or nitrogen on the spatial variability of the C:Chl *a* ratio was found. Changes in the contribution of dinoflagellates to the total phytoplankton biomass affected the C:Chl *a* ratio variability, which was two times lower compared to September–October 2005 and October 2010. Also, the spatial distribution of phytoplankton biomass differed from the distribution of chlorophyll *a* concentration only in some areas of the sea. In May 2013, environmental factors slightly varied across the study area and the spatial variability of the C:Chl *a* ratio was insignificant. Therefore, the map of phytoplankton biomass indicated similarities with the chlorophyll map.

**Key words:** phytoplankton biomass, nutrients, carbon to chlorophyll *a* ratio, Black Sea

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## Introduction

Phytoplankton biomass is one of the main parameters reflecting the state of the primary link within the plankton ecosystems. Documenting the spatial and temporal distributions of phytoplankton biomass is a necessary step in assessing the role of the ocean in biogeochemical cycles and in determining the long-term responses of coastal ecosystems to anthropogenic activity (Geider et al. 1997). Direct measurement of phytoplankton carbon is difficult as it is not easy to separate it from other sources of carbon such as non-living organic matter, bacteria and microzooplankton. Phytoplankton carbon biomass is usually estimated from the cell bio-volume using a light microscopy, but this approach is time-consuming (Llewellyn et al. 2005; Stelmakh & Gorbunova 2018). Alternatively, phytoplankton biomass is often inferred from chlorophyll *a*, a pigment that is common to all plankton autotrophs and can be easily measured (Wang et al. 2013).

To convert the chlorophyll *a* concentration to carbon phytoplankton biomass, it is necessary to know the ratio of these parameters (C:Chl*a*). Studies conducted in various areas of the World Ocean show that this ratio is subject to significant spatial and temporal variability (Llewellyn et al. 2005; Wang et al. 2013; Bellacicco et al. 2016). Its range is at least within one order of magnitude (25–400). The variability of the C:Chl *a* ratio results from phytoplankton adaptation (acclimation) to rapidly changing environmental conditions, mainly due to light and nutrients (Geider et al. 1997; Behrenfeld et al. 2005; Bellacicco et al. 2016). Of the nutrients, nitrogen plays a major role in the variability of the C:Chl *a* ratio (Harrison et al. 1977; Levasseur et al. 1993; Clark 2001). Under similar conditions, the C:Chl *a* ratio depends on the taxonomic affiliation of algae (Geider et al. 1997) and the size of algae cells (Finkel 2001).

In previous years, the determination of the C:Chl *a* ratio in the Black Sea was carried out occasionally (Vedernikov & Michaelyan 1989; Finenko et al. 2005). It is unclear what is the spatial variability of this parameter in the surface layer of the sea and how it affects the distribution of phytoplankton biomass and chlorophyll *a* concentration.

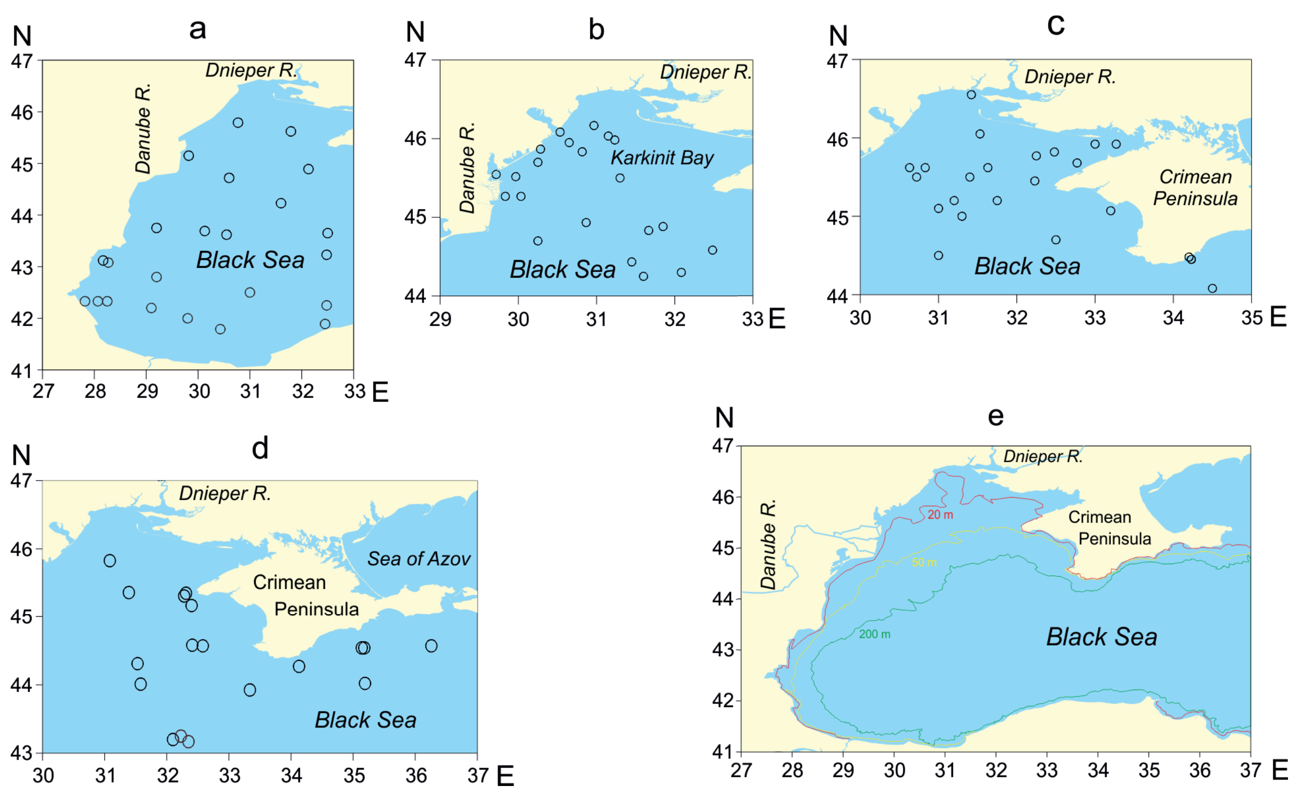



**Figure 1**

Location of the sampling sites in the Black Sea: a – September–October 2005; b – October 2010; c – August 2011; d – May 2013; e – depth map

The objective of this study was to investigate the spatial variability of the C:Chl *a* ratio and to assess its effect on the distribution of chlorophyll *a* concentration and phytoplankton biomass in the surface layer of the Black Sea.

## Materials and methods

### Study area and sampling

The research was conducted during one cruise on board the research vessel "Vladimir Parshin" in September–October 2005 (20 sampling sites) and three expeditions on board the research vessel "Professor Vodyanitsky" in October 2010 (26 sites), August 2011 (24 sites) and May 2013 (18 sites). The research was conducted in the western part of the Black Sea up to 34°E and in the eastern part – from 34°E to 37°E (Fig. 1). The study area covered both shallow (≤ 200 m) and deep water areas (> 200 m).

At each site, 3–4 l water samples were collected from the surface layer (0–1 m depth) using Niskin bottles attached to a STD rosette system.

### Phytoplankton abundance, biomass and species composition

Seawater samples of 2–3 l were concentrated in an inverse filtering funnel using track membranes with a pore size of 1 μm (Stelmakh & Georgieva 2014; Stelmakh & Gorbunova 2018). The samples were condensed to 100 ml. Concentrated samples were divided into two parts. The first part (10 ml) was fixed with 40% formaldehyde (1% final concentration in a sample). It was used for phytoplankton analysis. The second part (90 ml) was used to measure chlorophyll *a* concentration. Samples were filtered through 0.45 μm polycarbonate filters under low vacuum pressure (< 100 mm Hg). Filters were frozen at –20°C and preserved until the treatment process.

The abundance and linear dimensions of algae cells were determined in 0.1 ml drops placed into a Naujotte counting chamber, with 3–5 replications under a light microscope ZEISS Primo Star. The average cell volume for the total phytoplankton was determined as a ratio of the total volume of all cells to their abundance. Phytoplankton organic carbon concentrations were calculated from the average cell volume for each species of diatoms and dinoflagellates using the equations presented in the work of Menden-Deuer and Lessard (2000); in the case of the coccolithophorid *Emiliania huxley* (Lohmann) W.W. Hay & H.P. Mohler – using the equation provided by Montagnes et al. (1994) and in the case of other algae – using the equation provided by Strathmann (1967). Phytoplankton species identification was carried out using the identification key presented by Tomas (1997).

### Chlorophyll *a* concentration

Chlorophyll *a* was extracted from filters in 90% acetone (5 ml) in the dark at 4°C for 24 h. Acetone extracts were centrifuged and their fluorescence was determined before and after acidification using a fluorometer (excitation 440 to 480 nm, emission > 665 nm), which was calibrated with pure chlorophyll *a* (Sigma Chemical Co). The calculations were carried out according to the standard equations presented in the JGOFS Protocol (1994).

### Nitrogen concentration and hydrological parameters

Nitrate concentrations were measured by reducing them to nitrites, using copper-plated cadmium and following the determination by a single "color reagent", while ammonium concentrations were determined using the Grasshoff–Johansen test (Sapoznikov 1988). Information on the seawater temperature and salinity was obtained during the scientific expeditions using the STD rosette system.

### Solar radiation intensity

The average solar radiation intensity in the upper mixed layer was calculated on the basis of its values near the sea surface as well as the thickness of the upper mixed layer and water transparency. Luminance was measured using a light meter U-116. The coefficient of transition from the luminance to the light intensity in the range of photosynthetic active radiation (PAR) was set at $10^4$ lux = 200 μE $m^{-2}$ $s^{-1}$ (Parsons et al. 1982). The following equation was used to calculate the average solar radiation in the upper mixed layer (UML):

$$I_Z = I_0 \times e^{-k \cdot z}$$

where $I_0$ and $I_z$ – solar radiation intensity at the sea surface and at *z* (m) depth, respectively; E $m^{-2}$ $day^{-1}$. The average value of the light reduction coefficient for the layer (*k*) was calculated on the basis of the total radiation in the PAR range decreasing with depth, using the relationship described by Vedernikov (1989).

### Data analysis

To identify the factors determining the variability of the C:Chl *a* ratio in the Black Sea, a linear correlation analysis was performed. The pair coefficient of Pearson's correlation was determined between the C:Chl *a* ratio, and abiotic and biotic parameters. The abiotic parameters included solar radiation intensity in the upper mixed layer, water temperature and salinity, nitrate and ammonium concentrations, while the biotic parameters – the average phytoplankton cell volume and the contribution of dinoflagellates to the total phytoplankton biomass. The reliability assessment of differences between the mean values of the C:Chl *a* ratio, as well as abiotic and biotic parameters was performed using Student's t-test.

## Results

### Autumn 2005

#### Physical and chemical environment

In the western part of the Black Sea in late September and early October 2005, the water temperature in the upper mixed layer was 18–21°C and salinity was 15.12–18.10 PSU. The average solar radiation intensity in the upper mixed layer ($I_{UML}$) varied from 2 to 18 E m$^{-2}$ day$^{-1}$ (Fig. 2). The minimum value of $I_{UML}$ was recorded in the coastal waters, while the maximum value – in the central part of the sea. In the open part of the sea, nitrate concentrations decreased to zero and ammonium concentrations were below 0.15 μM. In the coastal areas near the Danube River drainage area and the surrounding area of the sea, nitrate concentrations increased to 1–3 μM. In these waters and in the southern part of the study area (near the Turkish coast), ammonium concentrations were above 0.20 μM (Fig. 2).

#### Phytoplankton structure and the C:Chl *a* ratio

The main phytoplankton biomass was formed by diatoms (Table 1). The smallest species *Skeletonema* costatum (Greville) Cleve, *Cerataulina pelagica* (Cleve) Hendey and the largest species *Proboscia alata* (Brightwell) Sundström, *Pseudosolenia calcar-avis* (Schultze) Sundström dominated.

The carbon to chlorophyll *a* ratio was 31–293. Its lowest values (31–105) were obtained near the Turkish coast and near the Danube River drainage area (Fig. 2). In the remaining water areas, the ratio was 119–293.

#### Factors controlling the variability of the C:Chl *a* ratio

We divided our data into two groups. Low values of the C:Chl *a* ratio were represented in the first group and high values in the second group (Table 1). As a result, low values of the C:Chl *a* ratio (52 ± 9) were accompanied by minimal $I_{UML}$ (on average 5 E m$^{-2}$ day$^{-1}$). Nitrate concentrations reached on average 1.15 μM. Ammonium concentrations were on average 0.15 μM. The average phytoplankton cell volume was 6200 μm$^3$. High values of the C:Chl *a* ratio (200 ± 23) were observed under other conditions. The light intensity in UML increased on average to 11.8 E m$^{-2}$ day$^{-1}$ and nitrate concentrations decreased on average to 0.12 μM. The average ammonium concentrations were not significantly different from the above average values ($p > 0.05$). The largest species of diatoms dominated in the phytoplankton and the average phytoplankton cell volume increased to 34 300 μm$^3$.

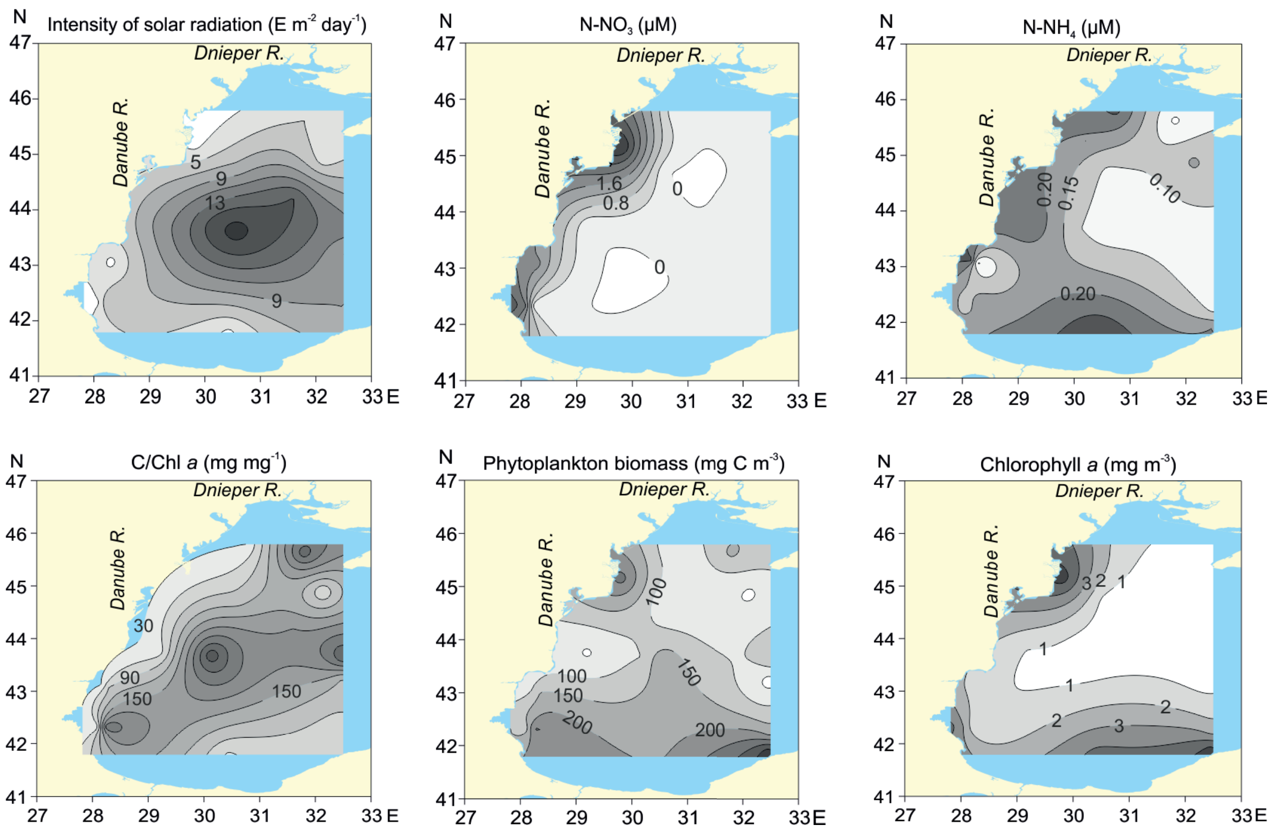
The highest value of Pearson's pair correlation coefficient (r = 0.79) for the study area was determined between the C:Chl *a* ratio and the average solar radiation intensity in the upper mixed layer ($I_{UML}$). Its lower value (r = –0.52) was recorded between the

**Table 1**

C:Chl *a* ratio, contribution of dinoflagellates ($B_{Dinofl.}$) and diatoms ($B_{Bacillar.}$) to the total phytoplankton biomass, the average volume of their cells ($V_{aver.}$), and some abiotic parameters of the surface layer of the Black Sea

| C:Chl *a* (mg mg$^{-1}$) | $B_{Dinofl.}$ [$B_{Bacillar.}$] (%) | $V_{aver}$ (μm$^3$) | N-$NO_3$ [N-$NH_4$] (μM) | $I_{UML}$ (E m$^{-2}$ day$^{-1}$) |
|---|---|---|---|---|
| September–October 2005 (n = 20) | | | | |
| 52 ± 9 | 38 ± 4 [60 ± 6] | 6200 ± 1060 | 1.15 ± 0.30 [0.15 ± 0.02] | 5.0 ± 1.0 |
| 200 ± 23 | 17 ± 2 [72 ± 6] | 34 300 ± 4800 | 0.12 ± 0.02 [0.11 ± 0.03] | 11.8 ± 1.4 |
| October 2010 (n = 26) | | | | |
| 60 ± 9 | 19 ± 6 [57 ± 8] | 1950 ± 500 | 1.16 ± 0.26 [1.19 ± 0.20] | 5.0 ± 0.5 |
| 171 ± 23 | 50 ± 11 [14 ± 10] | 2170 ± 620 | 0.32 ± 0.05 [0.57 ± 0.12] | 11.0 ± 0.4 |
| August 2011 (n = 24) | | | | |
| 107 ± 8 | 30 ± 6 [61 ± 4] | 1500 ± 100 | 0.11 ± 0.01 [1.07 ± 0.15] | 16.0 ± 0.7 |
| 214 ± 22 | 55 ± 8 [45 ± 5] | 4600 ± 600 | 0.11 ± 0.03 [0.57 ± 0.01] | 27.0 ± 0.5 |
| May 2013 (n = 18) | | | | |
| 104 ± 9 | 34 ± 4 [16 ± 6] | 140 ± 16 | 0.18 ± 0.02 [1.20 ± 0.10] | 24.0 ± 1.2 |

Note: The table shows the average values of the parameters and their standard error, n – the number of measurements, $I_{UML}$ – the average value of the solar radiation intensity in the upper mixed layer




## Figure 2

Average intensity of solar radiation in the upper mixed layer, nitrate and ammonium concentration, C:Chl *a* ratio, phytoplankton biomass and chlorophyll *a* concentration in the surface layer of the Black Sea in September–October 2005

C:Chl *a* ratio and the total nitrate and ammonium concentration. The independent variables, light and nitrogen, were not correlated with each other. The other variables (temperature and salinity) weakly correlated with the C:Chl *a* ratio ($r < 0.3$).

#### Distribution of phytoplankton biomass and chlorophyll *a*

High values of phytoplankton biomass (> 100–200 mg C m$^{-3}$) were observed in the area adjacent to the Danube River drainage area, near the Turkish shores and in the open part of the sea (Fig. 2). In other areas, these values were several times lower. High concentrations of chlorophyll *a* (2–4 mg m$^{-3}$) were observed only in the coastal areas. In the open part of the sea, this parameter was below 1 mg m$^{-3}$. As a result, the spatial distribution of phytoplankton biomass and chlorophyll *a* was not similar.

### Autumn 2010

#### Physical and chemical environment

In the north-western part of the Black Sea, the water temperature in the upper layers varied in October 2010 from 13.50 to 17.29°C, while salinity in UML was 16.62–17.94 PSU. The lowest values of $I_{UML}$ (3–8 E m$^{-2}$ day$^{-1}$) were obtained near the Dnieper River and the Danube River drainage area (Fig. 3), while in the remaining part of the study area, $I_{UML}$ increased to 9–14.5 E m$^{-2}$ day$^{-1}$. Nitrate concentrations were below 0.4 μM in the largest part of the study area and they increased to 1–4 μM only within the Danube River drainage area (Fig. 3). Ammonium concentrations were above 0.4 μM in the largest part of the study area and decreased only in the center of the north-western part of the sea.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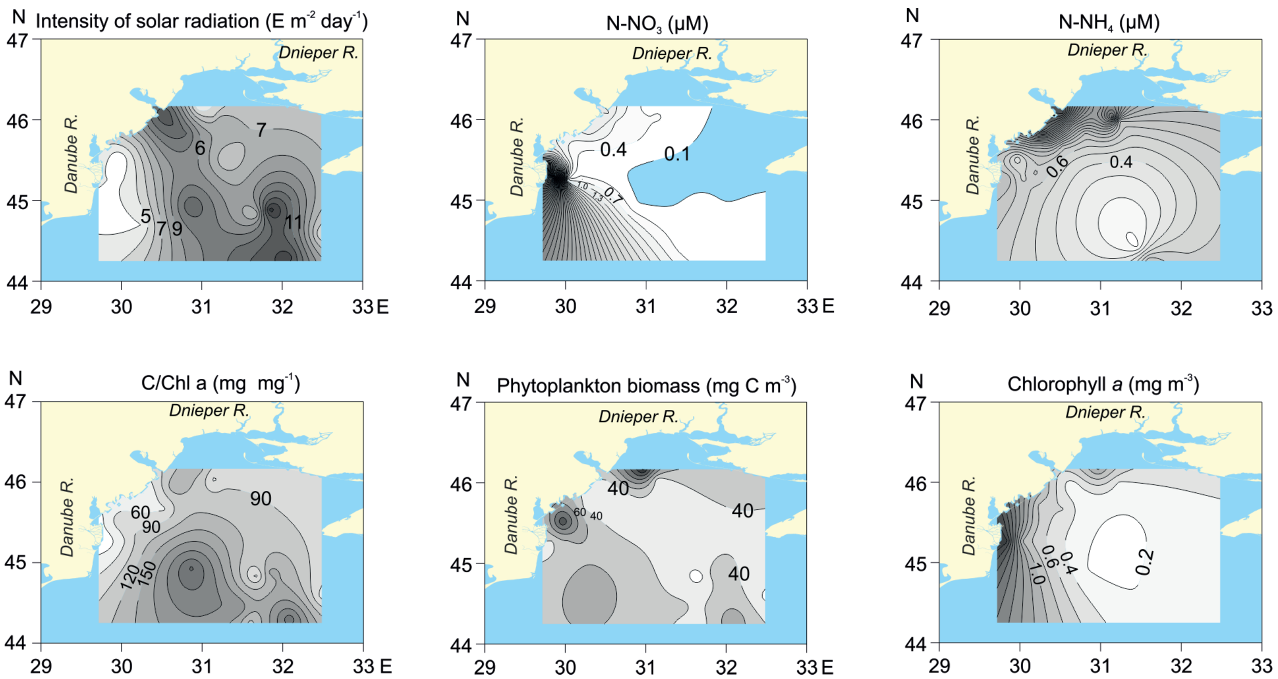


**Figure 3**

Average intensity of solar radiation in the upper mixed layer, nitrate and ammonium concentration, C:Chl *a* ratio, phytoplankton biomass and chlorophyll *a* concentration in the surface layer of the Black Sea in October 2010

**Phytoplankton structure and the C:Chl *a* ratio**

The main phytoplankton biomass was formed by diatoms and dinoflagellates. Diatom species included *S. costatum, Chaetoceros socialis* (Laud.) and *Chaetoceros curvisetus* (Cl.). Dinoflagellates were represented by *Prorocentrum cordatum* (Ostf.) Dodge, *Scrippsiella trochoidea* (Stein) Balechand and *Prorocentrum micans* Ehrenberg.

In these conditions, the lowest values of the organic carbon to chlorophyll *a* ratio (25–96) were observed near the Danube River and the Dnieper River drainage area. In the central part of the north-western region, the C:Chl *a* ratio was approximately 3–4 times higher (Fig. 3).

**Factors controlling the variability of the C:Chl *a* ratio**

Table 1 shows that low values of the C:Chl *a* ratio (60 ± 9) are accompanied by the lowest $I_{UML}$ (on average 5 E m$^{-2}$ day$^{-1}$). Nitrate concentrations reached on average 1.16 µM and ammonium concentrations – 1.19 µM. The average phytoplankton cell volume was 1950 µm$^3$. The contribution of dinoflagellates to the total phytoplankton biomass was on average 19% and of diatoms –57% on average. High values of the C:Chl *a* ratio (171 ± 23) were observed under other conditions. The light intensity in UML increased on average to 11 E m$^{-2}$ day$^{-1}$. Nitrate concentrations decreased on average to 0.32 µM and ammonium concentrations – to 0.57 µM. The average phytoplankton cell volume was 2170 µm$^3$. The contribution of dinoflagellates to the total phytoplankton biomass reached on average 50%, while the contribution of diatoms decreased on average to 14%.

For the entire study area, the highest value of Pearson's pair correlation coefficient (r = 0.67) was between the C:Chl *a* ratio and $I_{UML}$. Its lower value (r = –0.50) was between the C:Chl *a* ratio and the total concentration of nitrate and ammonium in the environment. Both independent variables ($I_{UML}$ and nitrogen) did not correlate with each other. The other variables (temperature and salinity) weakly correlated with the C:Chl *a* ratio (r < 0.3).

**Distribution of phytoplankton biomass and chlorophyll *a***

The minimum and maximum values of the phytoplankton biomass and chlorophyll *a* concentration in the study area differed by a factor of ten (Fig. 3). The maximum phytoplankton biomass (> 40 mg C m$^{-3}$) was recorded near the Danube River and the Dnieper River drainage area as well as in the south of the study area. In other regions, it decreased several times, while high chlorophyll *a* concentrations (> 1 mg m$^{-3}$) were recorded only near the Danube River

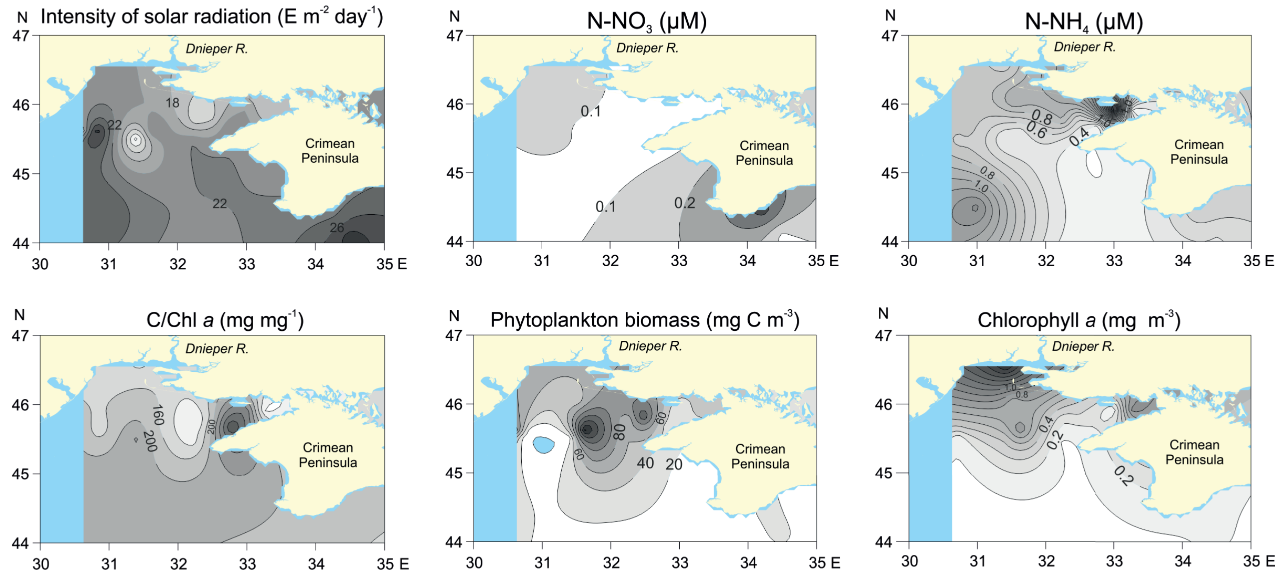


**Figure 4**

Average intensity of solar radiation in the upper mixed layer, nitrate and ammonium concentration, C:Chl *a* ratio, phytoplankton biomass and chlorophyll *a* concentration in the surface layer of the Black Sea in August 2011

drainage area. Accordingly, the spatial distribution of phytoplankton biomass significantly differed from the distribution of chlorophyll *a* concentration.

## Summer 2011

### Physical and chemical environment

In August 2011, the studies were conducted in the north-western part of the sea and close to the Crimean Peninsula coast. Water temperature in UML was 23–24°C and salinity was 17.42–18.54 PSU. In general, values of $I_{UML}$ were above 20 E m$^{-2}$ day$^{-1}$ (Fig. 4). Nitrate concentrations were low and did not exceed 0.1–0.3 μM. Ammonium concentrations were several times higher and reached 0.4–1 μM (Fig. 4).

### Phytoplankton structure and the C:Chl *a* ratio

Diatoms *Chaetoceros affinis* (Lauder), *Chaetoceros curvisetus* (Cl.) and *C. pelagica* formed the main phytoplankton biomass near the Dnieper River drainage area. The contribution of dinoflagellates [*Scrippsiella trochoidea* (Stein) Balech, *P. micans, P. cordatum, Ceratium furca* (Ehr.) Clap. Et Lachm] was small (5–30% of the total biomass). The lowest values of the C:Chl *a* ratio were observed in this area, ranging from 77 to 134 (Fig. 4). In other regions of the sea, the contribution of diatoms was below 50% and of dinoflagellates increased to 45–85%. The C:Chl *a* ratio reached 190–240.

### Factors controlling the variability of the C:Chl *a* ratio

Table 1 shows that when the C:Chl *a* ratio was low (107 ± 8), $I_{UML}$ was on average 16 E m$^{-2}$ day$^{-1}$. Nitrate concentrations decreased on average to 0.11 μM. Ammonium concentrations were on average 1.07 μM. The average contribution of dinoflagellates to the total phytoplankton biomass was 30% and of diatoms – 61%. The average phytoplankton cell volume was 1500 μm$^3$. High values of the C:Chl *a* ratio (214 ± 22) were observed under other conditions. The average light intensity in UML increased to 27 E m$^{-2}$day$^{-1}$ and nitrate concentrations did not change. Ammonia concentrations were on average 0.57 μM. The contribution of dinoflagellates increased on average to 55% and that of diatoms decreased on average to 45%. The average phytoplankton cell volume was 4600 μm$^3$.

The highest pair Pearson's correlation coefficient (r = 0.66) was determined between the C:Chl *a* ratio and the contribution of dinoflagellates to the total phytoplankton biomass. This ratio weakly correlated with abiotic parameters (r = 0.10–0.28).

### Distribution of phytoplankton biomass and chlorophyll *a*

The phytoplankton biomass reached the highest values (40–80 mg C m$^{-3}$) between the Dnieper River drainage area and the entrance to the Karkinite Bay. In other parts of the study area, it decreased several times. High concentrations of chlorophyll *a* (≥ 0.8 mg m$^{-3}$) were measured only near the Dnieper River

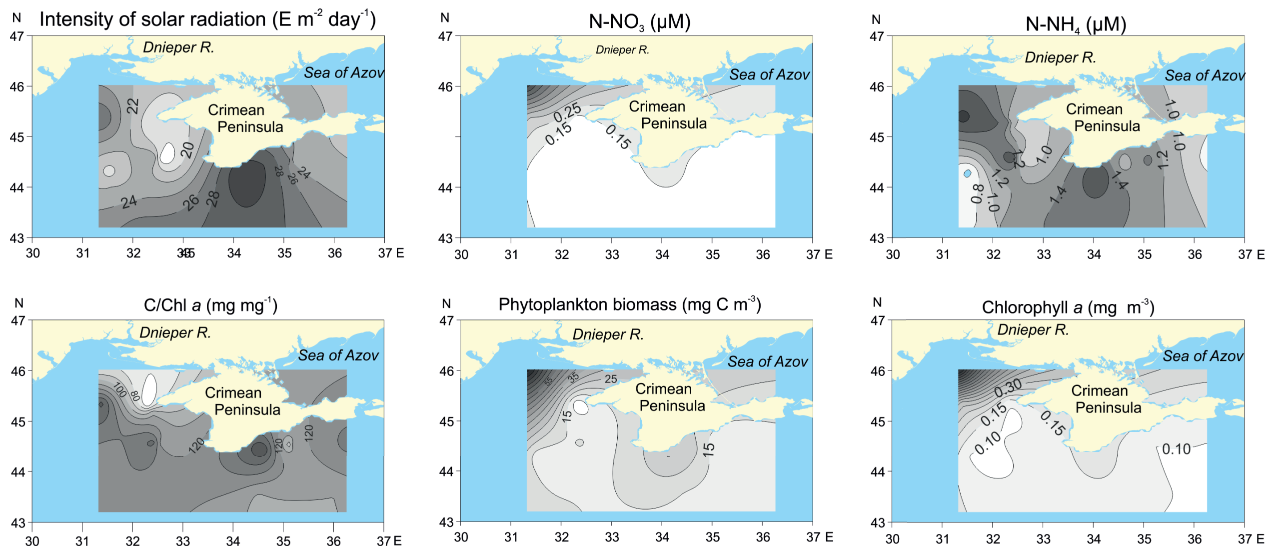


**Figure 5**

Average intensity of solar radiation in the upper mixed layer, nitrate and ammonium concentration, C:Chl *a* ratio, phytoplankton biomass and chlorophyll *a* concentration in the surface layer of the Black Sea in May 2013

drainage area. In the remaining part of the study area, it decreased to 0.1–0.7 mg $m^{-3}$.

### Spring 2013

#### Physical and chemical environment

In May 2013, the water temperature in UML of the study area was 19–21°C and salinity was 16.30–18.53 PSU. Values of $I_{UML}$ were 16–32 E $m^{-2}$ $day^{-1}$ (Fig. 5). Nitrate concentrations varied from 0.1 to 0.3 μM and ammonium concentrations reached 0.5–1.9 μM.

#### Phytoplankton structure and the C:Chl *a* ratio

The initial stage of the coccolithophorid *E. huxleyi* bloom was observed. Its contribution to the total phytoplankton biomass varied from 5% to 69% (on average 50%). The biomass of dinoflagellates was on average 34% (Table 1) and was represented by a small species [*P. cordatum* and *Gymnodonium simplex* (Lohmann) Kofoid & Swezy]. A small part of the biomass (on average 16%) was represented by diatoms *Chaetoceros socialis* H.S. Lauder, *Cyclotella caspia* Grunow 1878 and *S. costatum*. The average phytoplankton cell volume varied from 95 to 250 μ$m^3$ (Table 1). The C:Chl *a* ratio varied from 61 to 153 (Fig. 5).

#### Distribution of phytoplankton biomass and chlorophyll *a*

The phytoplankton biomass reached the highest values (> 50 mg C $m^{-3}$) near the Dnieper River drainage area. In this region, the chlorophyll *a* concentration reached the highest value (> 0.5 mg $m^{-3}$). In other areas, the phytoplankton biomass and chlorophyll *a* concentrations were low.

## Discussion

As evidenced by the laboratory studies, the complex effects of light, nutrients and temperature lead to variations in the carbon to chlorophyll *a* ratio in phytoplankton cells (Geider et al. 1997). Dynamic models of phytoplankton acclimation predict the relationships between the C:Chl *a* ratio and environmental factors (Geider et al. 1997; Wang et al. 2009). For example, the dynamic model based on laboratory experiments (Geider et al. 1997) shows that this ratio decreases from high to low light under constant temperature and nutrient-replete conditions, a phenomenon called "photoacclimation". Linear relationships between light and the C:Chl *a* ratio are observed only for light intensities that do not exceed 20 E $m^{-2}$ $day^{-1}$. With a further increase in light intensity, its effect on the variability of this ratio is insignificant (Geider at al. 1997; Llewellyn et al. 2005). The C:Chl *a* ratio increases with decreasing temperature and increasing nutrient stress. With low temperature variability (within the range of 2–3 degrees), the C:Chl *a* ratio does not change (Strzepek & Price 2000; Stramski et al. 2002).

It can be assumed that the greatest temporal and spatial differences in the C:Chl *a* ratio are to be expected in the conditions of maximum variability of environmental factors. For example, the carbon to chlorophyll *a* ratio in the phytoplankton of surface waters in the English Channel varied 8–10 times in 1999–2002 (Llewellyn et al. 2005). According to satellite data, the C:Chl *a* ratio in the phytoplankton of the surface waters in the Black Sea western cyclonic gyre varied 5 times in 1999 and 2003 (Finenko et al. 2018). These studies indicate that light is primarily responsible for the seasonal variability of the phytoplankton C:Chl *a* ratio and, as a result, the maximum and minimum phytoplankton biomass and chlorophyll *a* concentration often do not coincide during the annual cycle.

Comparative analyses of satellite data demonstrated that in the upwelling regions, the phytoplankton biomass in the surface water was considerably higher in the equatorial Pacific than in the equatorial Atlantic. However, the chlorophyll *a* concentration was much higher in the equatorial Atlantic than in the equatorial Pacific (Wang et al. 2013). These differences reflect larger C:Chl *a* ratios in the equatorial Pacific exposed to strong iron limitation.

Our study demonstrated that in the surface layer of the Black Sea, a high degree of spatial variability in the C:Chl *a* ratio was observed in September–October 2005 and October 2010. The maximum and minimum C:Chl *a* ratio varied 10 times. Light and nitrogen were the most important environmental factors that defined this variability. The average values of light intensity in UML were within the limits where the C:Chl *a* ratio was related to light by a linear relationship. Nitrogen concentrations varied over a wide range. As a result, the maps of phytoplankton biomass differed from those of chlorophyll.

No effect of light and nitrogen on the spatial variability of the C:Chl *a* ratio was observed in the surface layer of the Black Sea in August 2011. Usually, the average light intensity in the upper mixed layer was above 20 E $m^{-2}$ $day^{-1}$, which goes beyond the linear relationship between light and the C:Chl *a* ratio (Geider at al. 1997; Llewellyn et al. 2005). The nitrate concentrations were low everywhere and ammonium concentrations slightly varied. The correlation between the C:Chl *a* ratio and nitrogen was not observed ($r < 0.30$). However, the highest correlation coefficient ($r = 0.66$) was obtained between the C:Chl *a* ratio and the contribution of dinoflagellates to the total phytoplankton biomass. Similar results were obtained for the total nano- and microphytoplankton occurring in the surface layer of the tropical Atlantic Ocean (Stelmakh & Lobanova 1993). Therefore, at high values of light intensity and low variability of nutrientsin the surface layer, the C:Chl *a* ratio depends on the taxonomic composition of phytoplankton. According to our data, the spatial variability of the C:Chl *a* ratio in the surface layer of the Black Sea was two times lower in August 2011 than in September–October 2005 and October 2010. As a result, the distribution of phytoplankton biomass and chlorophyll *a* concentration differed only in some areas of the Black Sea.

In the regions with low amplitude variability in environmental factors, the C:Chl *a* ratio varies insignificantly. For example, the equatorial upwelling of the Central Pacific is a region with low amplitude variability in mixing depths, surface light and nutrient availability. Satellite data showed that the C:Chl *a* ratio varied slightly in this region in 1998–2002 and the seasonal variability in the phytoplankton biomass and chlorophyll *a* was the same (Behrenfeld et al. 2005).

The satellite-based data for the Northwest Atlantic Ocean indicate that the C:Chl *a* ratio varied slightly across the study area in May 1997–2006. As a result, the maps of phytoplankton carbon show similarities with the chlorophyll maps (Sathyendranath et al. 2009). According to our data, environmental factors in the surface layer of the Black Sea varied slightly across the study region in May 2013. Spatial variability of the C:Chl *a* ratio was insignificant. Therefore, the map of phytoplankton biomass indicated similarities with the chlorophyll map.

## Conclusion

The C:Chl *a* ratio is an important parameter reflecting the adaptation processes in phytoplankton. Our results indicate the spatial variability of this parameter in the Black Sea surface layer in the May–October period. The maximum variability was observed in September–October 2005 and October 2010, when the highest spatial variability of average light intensity and nitrogen concentration was observed in the upper mixed layer. Therefore, the maps of phytoplankton biomass differed from the chlorophyll maps.

No effect of light and nitrogen on the spatial variability of the C:Chl *a* ratio was found in August 2011. Changes in the contribution of dinoflagellates to the total phytoplankton biomass affected the C:Chl *a* ratio variability, which was two times lower compared to September–October 2005 and October 2010. Also, the distribution of phytoplankton biomass and chlorophyll *a* concentration differed only in some areas of the sea.

In May 2013, the environmental factors slightly varied across the study area and the spatial variability of the C:Chl *a* ratio was low. Therefore, the map of phytoplankton biomass shows similarities with the chlorophyll map.

## Acknowledgements

The work was conducted within the framework of the state assignment IMBR RAN No. AAAA-A18-118021490093-4 ("Functional, metabolic and toxicological aspects of the existence of aquatic organisms and their populations in habitats with different physical and chemical regime") and "Analysis of the mechanism of integrated biological indicators of aquatic ecosystems condition application in the regions of recreational and tourism specialization" (No. 0012-2014-0018). The authors express their sincere gratitude to the employees of IMBI RAN Rodionova N.Yu., Babich I.I. and Kovaliova I.V. for their assistance in this research.

## References

Behrenfeld, M.J., Boss, E., Siegel, D.A. & Shea, D.M. (2005). Carbon-based ocean productivity and phytoplankton physiology from space. *Global Biogeochemical Cycles* 19. GB 1006. DOI: 10.1029/2004GB002299.

Bellacicco, M., Volpe, G, Colella, S., Pitarch, J. & Santoleri, R. (2016). Influence of photoacclimation on the phytoplankton seasonal cycle in the Mediterranean Sea as seen by satellite. *Remote Sensing of Environment*. 184: 595–604. DOI: 10.1016/j.rse.2016.08.004.

Clark, D.R. (2001). Growth rate relationships to physiological indices of nutrient status in marine diatoms. *J. Phycol.* 37: 249–256. DOI: 10.1046/j.1529-8817.2001.037002249.x.

Finenko, Z.Z., Churilova, T.Y. & Li, R.I. (2005). Vertical distribution of chlorophyll and fluorescence in the Black Sea. *Marine Ecological Journal* 4(1): 15–45. (In Russian). https://repository.marine-research.org/handle/299011/783.

Finenko, Z.Z., Kovalyova, I.V. & Suslin, V.V. (2018). A new approach to estimate phytoplankton biomass and its variability in the Black Sea surface water layer based on satellite data. *Advances in Current Biology* 138(3): 294–307 (In Russian). DOI: 10.7868/S0042132418030079.

Finkel, Z.V. (2001). Light absorption and size scaling of light-limited metabolism in marine diatoms. *Limnol. Oceanogr.* 46: 86–94. DOI: 10.4319/lo.2001.46.1.0086.

Geider, R.J., MacIntyre, H.L. & Kana, T.M. (1997). Dynamic model of phytoplankton growth and acclimation: responses of the balanced growth rate and the chlorophyll *a*: carbon ratio to light, nutrient limitation and temperature. *Mar. Ecol. Progress Series* 148: 187–200. DOI: 10.3354/meps148187.

Harrison, P.J., Conway, H.L., Holmes, R.W. & Davis, C.O. (1977). Marine diatoms grown in chemostats under silicate or ammonium limitation. III. Cellular chemical composition and morphology of *Chaetoceros debilis*, *Skeletonema costatum* and *Thallassiosira gravid*. *Mar. Biol.* 43: 19–31. DOI: 10.1007/BF00392568.

JGOFS Protocols (1994). *Protocols for the Joint Global Ocean Flux Study (JGOFS) Core Measurements*. *Manual and Guides.* 29. DOI: 11329/220.

Levasseur, M., Thompson, P.A. & Harrison, P.J. (1993). Physiological acclimation of marine phytoplankton to different nitrogen sources. *J. Phycol*. 29: 587–595. DOI: 10.1111/j.0022-3646.1993.00587.x.

Llewellyn, C.A., Fishwick, J.R. & Blackford, J.C. (2005). Phytoplankton community assemblage in the English Channel: a comparison using chlorophyll *a* derived from HPLC-CHEMTAX and carbon derived from microscopy cell counts. *J. Plankton Res.* 27: 103–119. DOI: 10.1093/plankt/fbh158.

Menden-Deuer, S. & Lessard, E.J. (2000). Carbon to volume relationships for dinoflagellates, diatoms and other protist plankton. *Limnol. Oceanogr*. 45: 569–579. DOI: 10.4319/lo.2000.45.3.

Montagnes, D.J., Berges, J.A., Harrison, P.J. & Taylor, F.J.R. (1994). Estimating carbon, nitrogen, protein and chlorophyll *a* from volume in marine phytoplankton. *Limnol. Oceanogr*. 39: 1044–1060. DOI: 10.4319/lo.1994.39.5.1044.

Parsons, T.R., Takahashi, M. & Hargrave, B. (1982). *Biological oceanography*. Moskow: Light and food industry. (In Russian).

Sapozhnikov, V.V. (1988). *Methods of hydrochemical determinations of main nutrient elements*. Moscow. (In Russian).

Sathyendranath, S., Stuart, V., Nair, A., Oka, K., Nakane, T. et al. (2009). Carbon-to-chlorophyll ratio and growth rate of phytoplankton in the sea. *Mar. Ecol. Progress Series* 383: 73–84. DOI: 10.3354/meps07998.

Stelmakh, L.V. & Lobanova, R.A. (1993). The carbon-chlorophyll *a* ratio in phytoplankton of surface waters of Eastern tropical Atlantic. *Marine Ecology* 43: 15–20 (In Russian), https://repository.marine-research.org/handle/299011/2077.

Stelmakh, L.V. & Georgieva, E.Yu. (2014). Microzooplankton: the trophic role and involvement in the phytoplankton loss and bloom-formation in the Black Sea. *Turkish Journal of Fisheries and Aquatic Sciences* 14(5): 955–964. DOI: 10.4194/1303-2712-v14_4_15.

Stelmakh, L. & Gorbunova, T. (2018). *Emiliania huxleyi* blooms in the Black Sea: influence of abiotic and biotic factors. *Botanica* 24(2):172–184. DOI: 10.2478/botlit-2018-0017.

Stramski, D.A., Sciandra, A. & Claustre, H. (2002). Effects of temperature, nitrogen, and light limitation on the optical properties of the marine diatom *Thalassiosira pseudonana*. *Limnol. Oceanogr.* 47: 392–403. DOI: 10.4319/

lo.2002.47.2.0392.

Strathmann, R.R. (1967). Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. *Limnol. Oceanogr.* 12: 411–418. DOI: 10.4319/lo.1967.12.3.0411.

Strzepek, F.R. & Price, N.M. (2000). Influence of irradiance and temperature on the iron content of the marine diatom *Thalassiosira weissflogii* (Bacillariophyceae). *Mar. Ecol. Prog. Ser.* 206: 107–117. DOI: 10.3354/meps206107.

Tomas, C.R. (1997). *Identifying Marine Diatoms and Dinoflagellates.* New York: Academic Press.

Vedernikov, V.I. (1989). Primary production and chlorophyll in the Black Sea during summer-autumn period. *Structure and production characteristics of the plankton communities of the Black Sea.* Moscow: Science: 65–83 (In Russian).

Vedernikov, V.I. & Mikaelian, A.S. (1989). Structural and functional characteristics of different size groups of the Black Sea phytoplankton. *Structure and production characteristics of the plankton communities of the Black Sea.* Moscow: Science: 84–105. (In Russian).

Wang, X.J., Behrenfeld, M., Borgne, R.Le., Murtugudde, R. & Boss, E. (2009). Regulation of phytoplankton carbon to chlorophyll ratio by light, nutrients and temperature in the equatorial Pacific Ocean: a basin-scale model. *Biogeosciences* 6: 391–404.

Wang, X., Murtugudde, R., Hackert, E. & Maraňón, E. (2013). Phytoplankton carbon and chlorophyll distributions in the equatorial Pacific and Atlantic: A basin-scale comparative study. *Journal of Marine Systems* 109–110: 138–148. DOI: 10.1016 /j.jmarsys.2012.03.004.