

Original article

Main Characteristics of Phytoplankton and Assessment of Bio-Optical Properties of the Black Sea Coastal Waters in Summer 2023 during the Coccolithophore Bloom

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Abstract

Purpose. The purpose of the study is to describe the spatial distribution and structure of the phytoplankton community, and to reveal the relationships between the seawater optical characteristics and the phytoplankton abundance and biomass in the coastal waters of the Southern Coast of Crimea.

Methods and Results. The phytoplankton abundance and biomass were determined during the 127th cruise of R/V *Professor Vodyanitsky* by taking samples and their subsequent laboratory processing. Application of a semi-analytical algorithm made it possible to calculate simultaneously the phytoplankton pigment concentrations using the spectral reflectance coefficient of the sea measured from on board the ship. As for the species composition of phytoplankton, the water area under study was quite homogeneous: dinoflagellates, diatoms, and haptophytes were predominant in the phytoplankton community of the 0–10 m layer. It is shown that during the period of mass development of the haptophyte alga *Gephyrocapsa huxleyi* (Lohmann) P. Reinhardt 1972, its abundance dominated in the phytoplankton community, namely its contribution to the total abundance ranged from 30 to 70%. At the same time, the main part of wet biomass (42 to 98%) consisted of heterotrophic and mixotrophic forms of dinoflagellates. It was revealed that the phytoplankton pigment concentrations calculated using a semi-analytical algorithm correlated well with the biomass, but poorly with the cell abundance.

Conclusions. During the mass development of coccolithophores *Gephyrocapsa huxleyi*, a significant increase in light scattering accompanied by a slightly increased absorption complicates the description of the marine environment by optical methods. Under the specified conditions, assessing the phytoplankton pigment content, and later its biomass, is still possible.

Keywords: leading phytoplankton complex, *Gephyrocapsa huxleyi*, biomass, abundance, reflectance coefficient of the sea, chlorophyll *a*

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Introduction

The phytoplankton community of the Black Sea is a complex mechanism sensitive to changes in biotic and abiotic conditions, which lead to a shift in the leading species complex. Spatio-temporal changes in phytoplankton composition affect productivity and determine the marine ecosystem state. In recent decades, mass occurrences of the coccolithophore *Gephyrocapsa huxleyi* has been observed in the Black Sea phytoplankton [1–4]. The ability of coccolithophores to use mixotrophic nutrition and high growth rates result in their mass development, or “bloom”, in most of the Black Sea surface water layer during the spring-summer and autumn-winter periods [5]. The main role in the leading phytoplankton complex, both in its seasonal and interannual changes, is distributed among dinoflagellates, diatoms, and haptophytes (*Gephyrocapsa huxleyi*). Due to the peculiarities of *Gephyrocapsa huxleyi* cellular structure, during mass development they have a significant impact on the radiation balance of the water body [6, 7]. The fine calcareous suspension produced by the cells, firstly, leads to an increase in the reflectivity of the water surface, and secondly, hinders the penetration of solar energy into the underlying layers of the sea, preventing their illumination and warming.

Currently, under conditions of global climate change and significant anthropogenic impact on ecosystems, there is a need for continuous monitoring of phytoplankton using both remote sensing [8] and *in situ* measurement methods [9]. The main method for determining abundance and biomass remains sampling followed by laboratory processing. Optical methods, which are quite rapid and non-invasive, are also applied to assess bioproductivity. These include *in situ* measurements of light attenuation coefficient (transparency) profiles, fluorescence, underwater irradiance, as well as measurements of the upwelling radiance of the sea above or below the surface. In the present work, upwelling radiance measurements were used precisely because the empirical relationships obtained from them can subsequently be transferred to satellite measurements.

The aim of the work is to describe the structure and spatial distribution of the main phytoplankton characteristics obtained during the 127th cruise of R/V *Professor Vodyanitsky* (14 June – 6 July 2023), and their impact on the integrated characteristics of seawater – the spectral reflectance coefficient of the water column and the effective concentration of chlorophyll a in the first optical depth layer.

Materials and methods

Study area. This work examines data from measurements of the spectral reflectance coefficient (RC, ρ , dimensionless) of the water column and investigates the structure and spatial distribution of the main phytoplankton characteristics in the 0–10 m water layer during the summer period, obtained during the 127th cruise of the R/V *Professor Vodyanitsky*. The survey was conducted in the coastal part of the Black Sea off the coast of Crimea. The location of the completed stations is given in Fig. 1.

Sea reflectance coefficient. Measurements of the reflectance coefficient were carried out from the vessel using a spectrophotometer developed at the Department of Marine Optics and Biophysics of the Marine Hydrophysical Institute, RAS [10]. The measurement methodology is consistent with NASA protocols for subsatellite

measurements [11]. RC measurements were taken during daylight hours at a solar height angle greater than 30° in cloudless or partly cloudy weather. As a result, spectra within the range of 390–750 nm with a step of 5 nm were obtained.

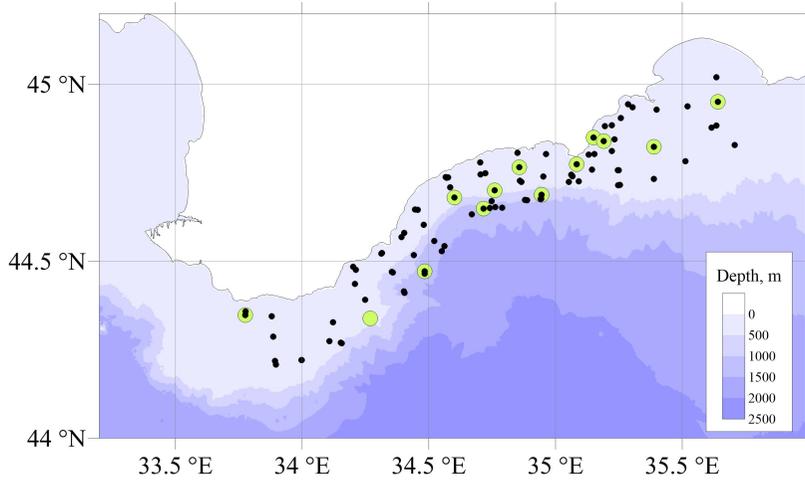


Fig. 1. Measurement stations for sea reflectance (black) and phytoplankton sampling stations (green) for determining biomass and abundance

Method for calculating chlorophyll a concentration. For further analysis, the calculated concentrations of chlorophyll *a*, absorption coefficients of non-living organic matter, and the backscattering coefficients of suspended particulate matter were used, employing a semi-analytical algorithm [10] in which the reflectance spectrum has the form:

$$\rho(\lambda) = k \frac{b_{bw}(\lambda) + b_{bp}(550)(\lambda_0/\lambda)^\nu}{a_w(\lambda) + C_{ph} a_{chl}^*(\lambda) + a_{org}(440)e^{-S(\lambda-\lambda_0)}}$$

where $k = 0.15$; $b_{bw}(\lambda)$ is the pure water backscattering coefficient [12]; $a_w(\lambda)$ is the pure water absorption coefficient [13]; $a_{chl}^*(\lambda)$ is the spectrum of specific absorption by phytoplankton pigments (normalized to chlorophyll *a* concentration) [14]; $\nu = 0.8$ is the spectral slope of the backscattering coefficient, depending on particle size¹; S is the spectral slope of the light absorption coefficient by non-living organic matter, related empirically to absorption at a wavelength of 490 nm [15]; $b_{bp}(550)$ is the particle backscattering coefficient at a wavelength of 550 nm; C_{ph} is the phytoplankton pigment concentration; $a_{org}(440)$ is the non-living organic matter absorption coefficient at a wavelength of 440 nm.

The spectral slope of the backscattering coefficient was chosen as a constant under the assumption that the main part of the mineral suspension consists of detached coccoliths, which have a fairly narrow size distribution [16].

¹ Monin, A.S., ed., 1983. *Ocean Optics. Vol. 2. Applied Ocean Optics*. Moscow: Nauka, pp. 213–233 (in Russian).

Based on the calculated parameters and the known measurement geometry, the diffuse light attenuation coefficient K_d at a wavelength of 490 nm was also obtained.

Phytoplankton abundance and biomass. Bathometric water samples (2 L) for determining the qualitative and quantitative composition of phytoplankton were collected with a bathometer from two horizons – surface and 10 m (at station 116 – from the 20 m horizon), concentrated to 50–100 mL using a reverse filtration funnel through track-etched membrane filters with a pore diameter of 1 μm (manufactured by the Joint Institute for Nuclear Research, Dubna, Russia) [17, 18] and fixed with 40% formalin to a final concentration of 2%. Identification of species composition, counting, and determination of phytoplankton cell sizes were carried out under a BRESSER BioScience Bino light microscope (at 400–1000x magnification) on special slides (laser-ruled with a strip spacing of 0.04 μm), onto which one or several drops of suspension from a thoroughly mixed test sample were applied using a dispenser pipette with a volume of 0.04 mL. To calculate the raw phytoplankton biomass, cell measurement data were used and volumes were determined based on their geometric similarity [19, 20]. Nomenclatural names of microalgae taxa are given using the online database <http://www.algaebase.org>.

For comparison with optical parameters, we used the “effective” phytoplankton biomass calculated as the depth-weighted average considering absorption by the overlying layers [21]:

$$B_{eff} = k_1 B_0 + k_2 B_{10},$$

where B_0 and B_{10} are the biomass at horizons 0 and 10 m; k_1 and k_2 are calculated for the same depths as

$$k_n = \frac{\exp(-3K_d h_n)}{\sum_n (1 + \exp(-3K_d h_n))}, n = 1 \dots, 2.$$

Here K_d is the diffuse light attenuation coefficient at a wavelength of 490 nm, calculated for each station by the semi-analytical algorithm. The weights k_1 and k_2 were, on average, 0.8 ± 0.02 and 0.13 ± 0.02 , respectively.

Results and discussion

A total of 12 stations were selected, for which simultaneous RC measurements and data on phytoplankton abundance and biomass are available. The shape of the RC spectra (with one maximum in the wavelength range of 480–500 nm (Fig. 2)) was typical for the Black Sea. The average value of the maxima was 0.036 ± 0.008 , which is overestimated compared to the normal state and, according to experimental data, is observed during the mass development of coccolithophores [10]. The increase in RC is a consequence of the increase in the concentration of fine mineral suspension (up to 2 μm) in the water, which makes the greatest contribution to backscattering, while no significant increase is observed in the long-wavelength part due to the high spectral selectivity of scattering by fine suspension¹.

The data show weak spatial variability; the standard deviation is 22% in the RC maximum region (at 490 nm). Individual outliers were observed at the boundary of the coastal and deep-water zones (Fig. 3, a). As seen in the distribution of the sea's

upwelling radiance (Fig. 3, *b*), these outliers may arise due to the inflow of individual jets of the Rim Current, carrying light-scattering impurities into the coastal zone.

Phytoplankton parameters were studied in samples taken from two horizons – 0 and 10 m.

During this period, 114 species of microalgae were identified in the surface water layer (depth 0–1.5 m) in the studied water area, 14 of which were identified only to the genus level. The phytoplankton community contained species belonging to six divisions: Miozoa (dinoflagellates), Bacillariophyta, Haptophyta, Chlorophyta, Ochrophyta, Euglenophyta. Dinoflagellates were characterized by the largest number of species (84 species), whereas other divisions were represented by significantly lower species diversity: Bacillariophyta – 17 species, Haptophyta – 9, and one or two species each from the divisions Chlorophyta, Ochrophyta, and Euglenophyta. Phytoplankton abundance varied in the range of 82.7–699.4 million cells/m³, biomass – in the range of 277.4–917.0 mg/m³ (Table). Average abundance was 423.8 ± 136.6 million cells/m³; average biomass was 696.28 ± 181.72 mg/m³. Mixotrophic and heterotrophic forms of dinoflagellates 540.05 ± 169.17 mg/m³, 77%), autotrophic forms of diatoms (72.48 ± 65.16 mg/m³, 10%), and the haptophyte alga *Gephyrocapsa huxleyi* (42 ± 26.78 mg/m³, 6%) have contributed the most to the total raw biomass.

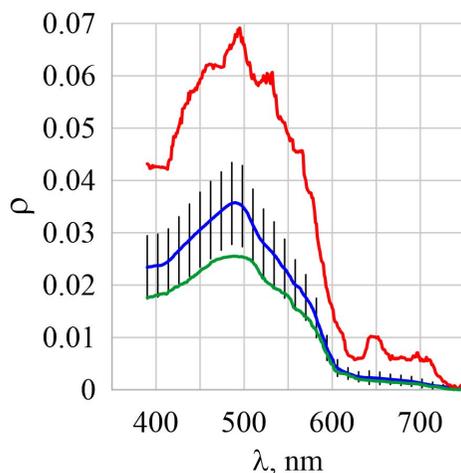


Fig. 2. Maximum (red curve), mean (blue curve), and minimum (green curve) sea reflectance spectra. Dashed lines show the standard deviation across all measurements

Abundance and biomass of main phytoplankton groups

| Divisions | Abundance $N \cdot 10^5$, cells/l | | | | Biomass B , $\mu\text{g/l}$ | | | |
|-----------------|------------------------------------|-------|-------|-------|-------------------------------|-------|-------|--------|
| | min | | max | | min | | max | |
| | at depth, m | | | | | | | |
| | 0 | | 10 | | 0 | | 10 | |
| Bacillariophyta | 5.3 | 109.7 | 10.3 | 94.0 | 8.8 | 193.9 | 9.6 | 180.2 |
| Miozoa | 38.7 | 145.5 | 49.2 | 195.3 | 183.9 | 792.2 | 174.0 | 1233.5 |
| Haptophyta | 34.3 | 475.0 | 22.2 | 508.2 | 12.1 | 135.8 | 6.4 | 101.7 |
| General | 82.7 | 699.4 | 161.8 | 716.3 | 274.3 | 916.9 | 339.4 | 1379.8 |

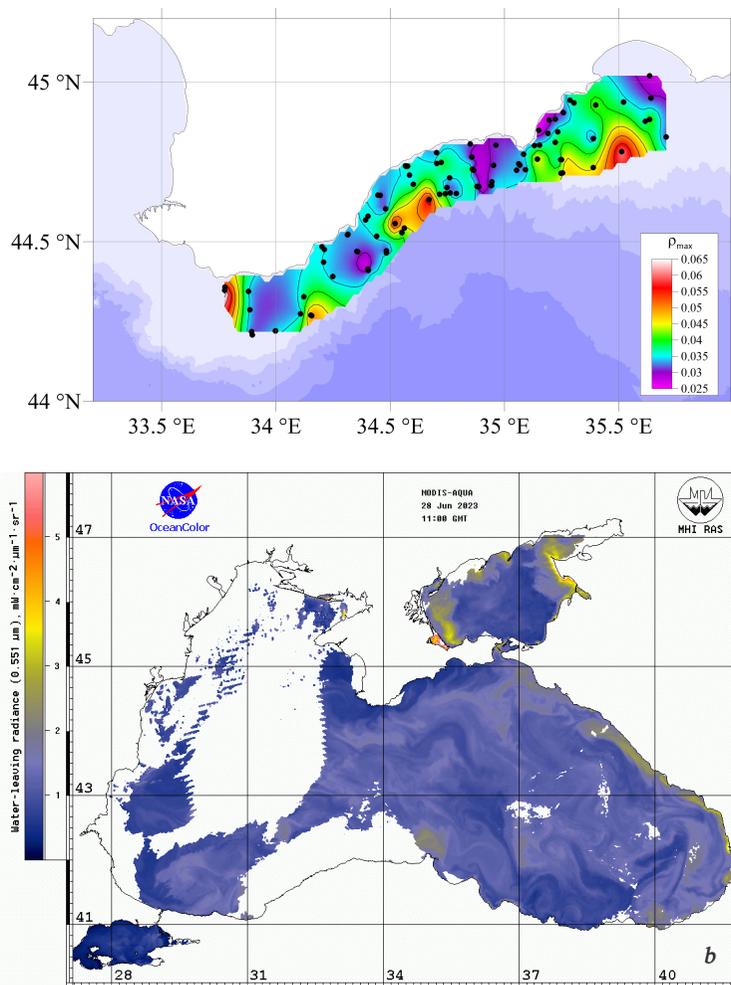


Fig. 3. Spatial distribution: *a* – maximum reflectance values (dots indicate sampled stations); *b* – water-leaving radiance at a wavelength of 555 nm (June 28, 2023; MODIS/Aqua; source: <http://dvs.net.ru/>)

As a result of the analysis, dominant species by biomass, that were present at three or more stations in the surface layer, were noted: *Diplopsalis lenticula* Bergh 1882, *Gymnodinium fuscum* (Ehrenberg) F. Stein 1878, *Phalacroma rotundatum* (Claparède & Lachmann) Kofoid & J. R. Michener 1911, *Prorocentrum balticum* (Lohmann) Loeblich III 1970, *Prorocentrum compressum* (Bailey) T. H. Abé ex J. D. Dodge 1975, *Prorocentrum micans* Ehrenberg 1834, *Protoperidinium divergens* (Ehrenberg) Balech 1974, *Scrippsiella acuminata* (Ehrenberg) Kretschmann, Elbrächter, Zinssmeister, S. Soehner, Kirsch, Kusber & Gottschling 2015, *Tripos furca* (Ehrenberg) F. Gómez 2013, *Tripos muelleri* Bory 1826, the contribution of each species at the stations was 30–107 mg/m³ (4–15%). In terms of abundance, the haptophyte alga *Gephyrocapsa huxleyi* dominated quantitatively at all stations; its abundance ranged from 33 to 471 million cells/m³, accounting for 30–70% at the stations (Fig. 4).

In the phytoplankton at the 10 m horizon, 100 species of algae were recorded, including 11 forms identified only to the genus level, from the divisions Miozoa, Bacillariophyta, Haptophyta, Chlorophyta, Ochrophyta, Euglenophyta. The greatest species diversity was observed among dinoflagellates – 74, followed by diatoms – 15, haptophytes – 7, and the rest with one or two species each. Abundance ranged from 161.8 to 716 million cells/m³, biomass – from 339.4 to 1379.8 mg/m³.

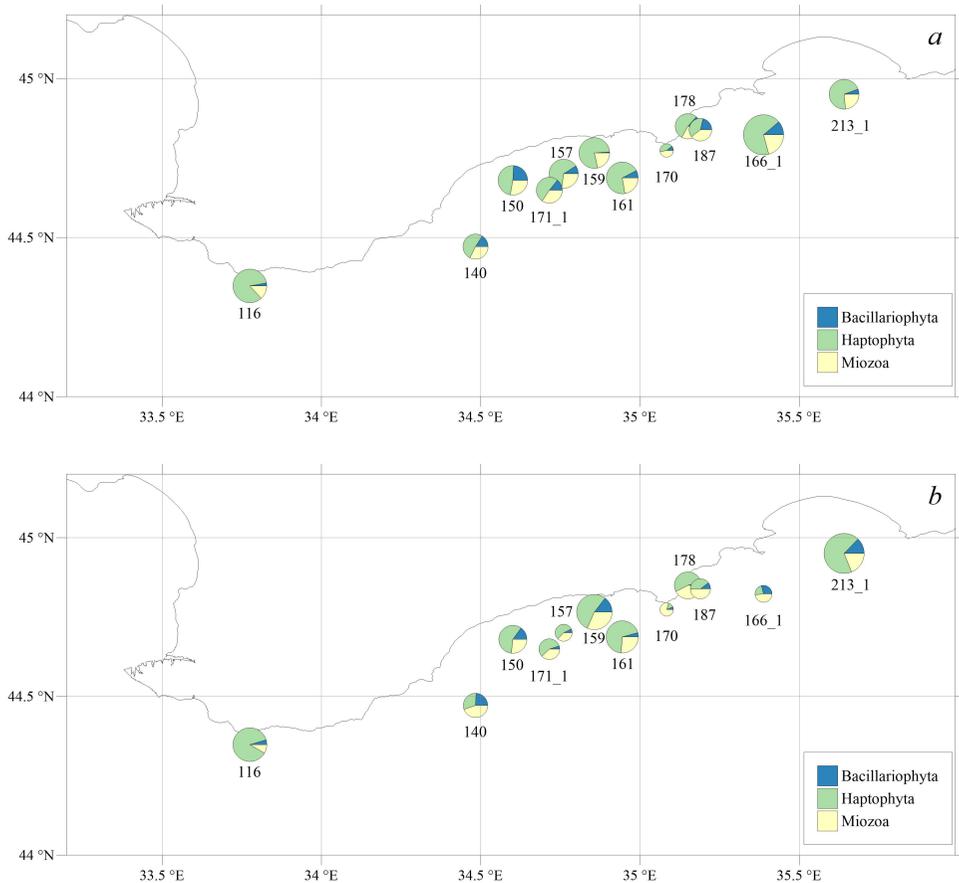


Fig. 4. Proportion of the abundance of three main phytoplankton divisions at 0 m (a) and 10 m (b) horizons

The maximum contributions to biomass were made by representatives of dinoflagellates: *Diplopsalis lenticula*, *Gymnodinium fuscum*, *Lingulodinium polyedra* (F. Stein) M.J. Head, K.N. Mertens & R.A. Fensome 2024, *Phalacroma rotundatum*, *Prorocentrum balticum*, *Prorocentrum compressum*, *Prorocentrum micans* Ehrenberg 1834, *Protopteridinium divergens*, *Scrippsiella acuminata*, *Triadinium orientale* (Lindemann) J.D. Dodge 1981, *Tripos muelleri* and diatom algae *Proboscia alata* (Brightwell) Sundström 1986, the contribution of each species to biomass was 30–199.1 mg/m³ (3–28%). The abundance was contributed the most by the haptophyte alga *Gephyrocapsa huxleyi*; its values ranged from 33 to 471 million cells/m³ (Fig. 5). At this horizon, the average

abundance was 418 ± 145 million cells/m³, biomass 812.6 ± 150.16 mg/m³. The main contribution to biomass was made by dinoflagellates (691.6 ± 149.2 mg/m³, 85%), diatoms (71.78 ± 44.07 mg/m³, 8%) and the haptophyte alga *Gephyrocapsa huxleyi* (46.78 ± 25.08 mg/m³, 5%).

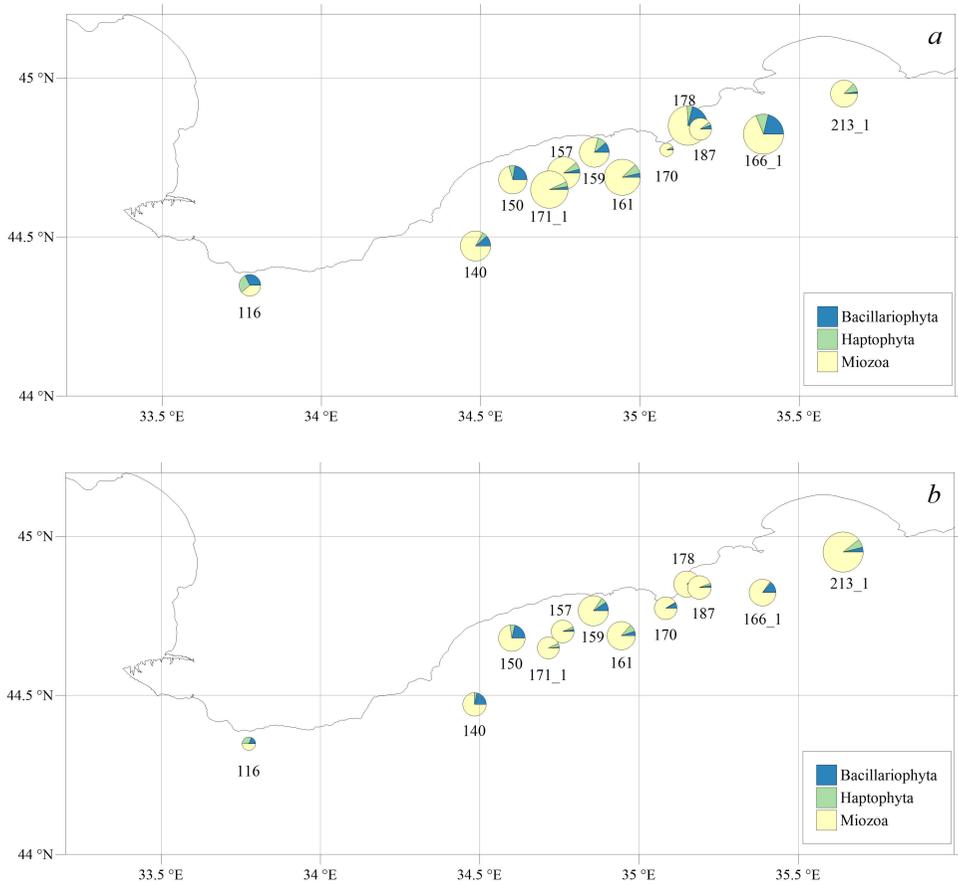


Fig. 5. Proportion of the biomass of three main phytoplankton divisions at 0 m (a) and 10 m (b) horizons

It should be noted that at station 116, where the maximum proportion of haptophyte algae abundance was recorded (Fig. 4), the highest RC values were obtained – the maximum spectrum given in Fig. 2. At the same time, at station 170, where the total phytoplankton abundance was minimal, the RC spectrum was close to the average for all measurements. The cause may be that the cell abundance of *Gephyrocapsa huxleyi* has only an indirect effect on the seawater optical properties, while the fine mineral suspension it produces plays a greater role. The Czekanowski–Sørensen species similarity index between the 0 and 10 m horizons was 0.83, indicating high species similarity [22].

The chlorophyll a concentration, backscattering coefficient, and numerical concentration of suspended matter were calculated. Average chlorophyll a concentration was 0.50 ± 0.16 mg/m³. The backscattering coefficient at

a wavelength of 550 nm averaged 0.01 ± 0.002 1/m with typical values of 0.003–0.009 m^{-1} [23]. Assuming the dominance of coccolithophores and the suspension they produce among the total suspended matter, the numerical concentration of suspension can be estimated using the empirical formula by V.I. Mankovsky [24] $b_{b_cocc}(546) = 1.1 \cdot 10^{-13} N_c$, if changes in scattering within 5 nm are neglected. It amounts to $(1.10 \pm 0.27)10^{11}$ $1/\text{m}^{-3}$, which in the case of coccolith suspension corresponds to a developed “bloom” [2]. However, the *in situ* determined abundance of coccolithophores is approximately two times lower than the boundary defined as a “bloom”, indicating a significant contribution of terrigenous suspension to backscattering and upwelling radiance. The correlation of total phytoplankton abundance, as well as the abundance of *Gephyrocapsa huxleyi* separately, with the backscattering coefficient is very weak. This also indicates that *Gephyrocapsa huxleyi* cells, as optically large particles, make a minimal contribution to backscattering.

As can be seen from the temperature and chlorophyll a concentration profiles from the fluorescence sensor (Fig. 6), the mixed layer during the studies was ~ 10 m. The thermocline layer, as a rule, coincides with the layer of increased turbidity; indirect confirmation of this is also that the Secchi disk visibility depth at the studied stations did not exceed 13 m and averaged 10 m. Despite the fact that at depths of ~ 30 m there is a maximum of chlorophyll a concentration, it practically does not affect the integral optical characteristics of the water column, such as upwelling radiance.

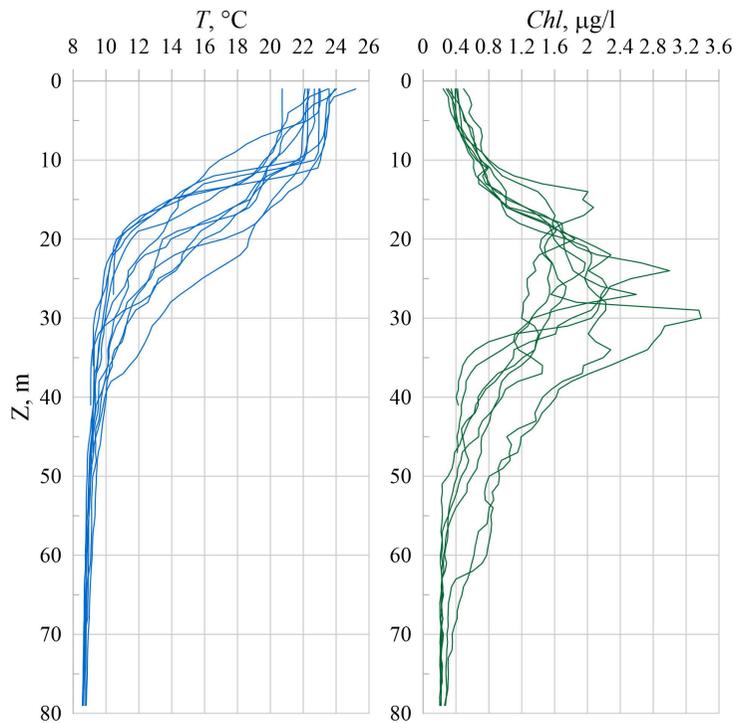


Fig. 6. Temperature and chlorophyll a concentration profiles at the sampled stations derived from Idronaut probe data

The chlorophyll *a* concentration calculated using the semi-analytical algorithm correlates quite well with the phytoplankton biomass (Fig. 7). This correlation is on the border between moderate and strong, but is significant with $p < 0.01$. It should be noted, however, that dinoflagellates dominate the phytoplankton community in terms of biomass, and heterotrophic species (e.g., from the genera *Protoperidinium* and *Diplopsalis* [25]), whose cells do not contain chlorophyll *a*, constitute from 3 to 36% of the total biomass (average 17%). Their presence may reduce the correlation with the calculated pigment concentration.

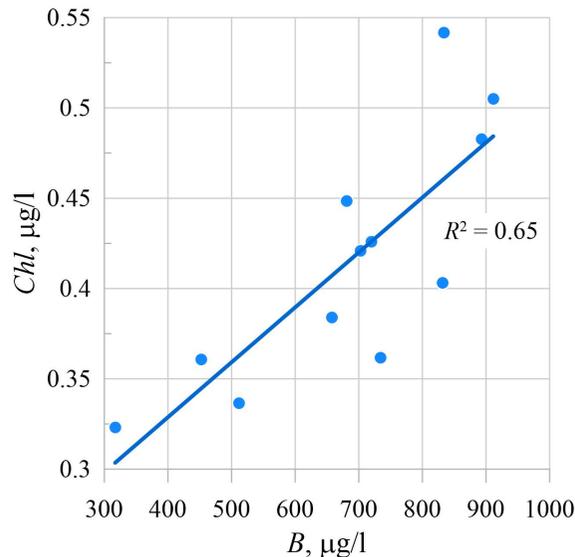


Fig. 7. Comparison of the effective phytoplankton biomass with the chlorophyll *a* concentration for the 0–10 m layer

Furthermore, it can be assumed that there is some proportionality in the development of heterotrophic and mixotrophic species, due to which the total biomass will be proportional to the biomass of mixotrophic species and will correlate with the chlorophyll *a* concentration.

However, the correlation between chlorophyll *a* concentration and phytoplankton abundance is low (Fig. 8) and not significant ($p = 0.18$). This can be explained by the presence of a large number of coccolithophore cells covered with a mineral shell, which limits the influence of chlorophyll *a* contained within them on the integral optical properties of the water. When subtracting the abundance of coccolithophores, the correlation increases slightly, but it is not meaningful to assert the significance of a change in such a weak correlation.

In conclusion, it can be said that during the mass development of coccolithophores, remote optical methods can fairly reliably estimate phytoplankton biomass through chlorophyll *a* concentration, as well as the abundance of fine suspended matter (detached coccoliths) through the backscattering coefficient. Finding relationships between optical parameters and cell abundance is difficult due to the small contribution that cells make to the integral light-scattering properties of seawater.

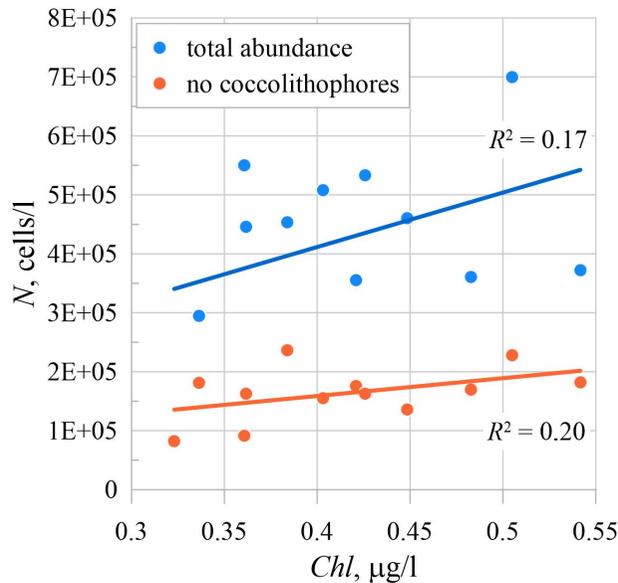


Fig. 8. Comparison of the phytoplankton cell abundance with the chlorophyll a concentration for the 0–10 m layer

Conclusions

As a result of the phytoplankton study in the Black Sea coastal zone from Foros to the Feodosia Gulf, 123 species of microalgae were identified in the samples (of which 14 were identified only to the genus level), belonging to six divisions: Miozoa (dinoflagellates), Bacillariophyta, Haptophyta, Chlorophyta, Ochrophyta, Euglenophyta. Dinoflagellates (88 species), diatoms (20), and haptophytes (9) dominated in the phytoplankton community; from the remaining divisions, one or two species were present. The Czekanowski–Sørensen species similarity index between the 0 and 10 m horizons was 0.83, indicating high species similarity.

The average abundance was 422.2 ± 109.3 million cells/m³, and the average biomass was 742.95 ± 128.36 mg/m³. The main contribution to the total raw biomass was made by mixotrophic and heterotrophic forms of dinoflagellates (612.77 ± 148.49 mg/m³), autotrophic forms of diatoms (76.15 ± 52.0 mg/m³), and the haptophyte alga *Gephyrocapsa huxleyi* (51.42 ± 24.88 mg/m³). The quantitative development of phytoplankton was characterized by high abundance values, primarily due to *Gephyrocapsa huxleyi*; its contribution to the total abundance at the stations ranged from 30 to 70%. Thus, *Gephyrocapsa huxleyi* makes a significant contribution to phytoplankton abundance but a small one to biomass, which determines its effect on the optical properties of seawater: a significant increase in scattering and a minor one in absorption.

A significant correlation was obtained between the calculated pigment concentration and the phytoplankton biomass determined in the samples, while the correlation with cell abundance was not significant. Despite the fact that no separation into heterotrophic and mixotrophic plankton species took place in the biomass, the correlation with biomass indicates that heterotrophic species constitute a fairly stable fraction of the phytoplankton community. This relationship

is statistical and does not account for the chlorophyll a content in the cells of specific species; therefore, it can be used assuming that the contribution of heterotrophic species remains constant.

The mass development of coccolithophores complicates the description of the marine environment by optical methods. When studying such optically different objects as phytoplankton cells covered and not covered with an “opaque” shell, as well as detached coccoliths, a more correct approach would be to use complex optical measurements, such as simultaneous measurements of the light attenuation coefficient and the reflectance coefficient. Estimating both total scattering and the backscattering fraction, as shown previously, provides a more complete understanding of the nature of suspended particles and their size composition.

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PHYSICAL OCEANOGRAPHY VOL. 33 ISS. 1 (2026)

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