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[1]Marine Hydrophysical Institute, Russian Academy of Sciences, 299011, Kapitanskaya Str., 2, Sevastopol, Russia
[2]A.O. Kovalevsky Institute of Biology of the Southern Seas of RAS, 299011, Pr. Nakhimova, 2, Sevastopol, Russia
*kudinov_ob@mhi-ras.ru

# FEATURES OF FLUORESCENCE PROFILES AND SPECIES COMPOSITION OF PHYTOPLANKTON IN THE BLACK SEA AND THE SEA OF AZOV IN EARLY AUTUMN 2020

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**Abstract**

The aim of this work was to study the properties of vertical fluorescence intensity (FI) profiles of phytoplankton pigments (chlorophyll-*a*, phycocyanin, phycoerythrin, and beta-carotene) using data on the phytoplankton species composition obtained during the cruise 114 of the R/V 'Professor Vodyanitsky'. The analysis of covariance matrices of phytoplankton pigment FI profiles in the upper 50-meter layer of the Black Sea indicates that the pigment composition of phytoplankton changes with depth, which may be associated with changes in its species composition. At the same time, 80% variability of phytoplankton pigment FI profiles in the upper 20-meter layer is described by the first eigenvector. It agrees well with direct observations of the phytoplankton species composition, indicating the dominance of one phytoplankton division in the 20-meter layer. In addition, there are regional peculiarities: for example, the average FI values of the phytoplankton pigments in the Sea of Azov are significantly higher than those in the Black Sea, which is associated with a higher concentration of phytoplankton in the Sea of Azov.

**Keywords:** phytoplankton pigment fluorescence, phytoplankton species composition, photosynthetic zone, the Black Sea, the Sea of Azov

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[1]Морской гидрофизический институт РАН, 299011, Севастополь, ул. Капитанская, д. 2
[2]Институт биологии южных морей имени А.О. Ковалевского РАН, 299011, Севастополь, пр. Нахимова, д. 2
*kudinov_ob@mhi-ras.ru

# ОСОБЕННОСТИ ПРОФИЛЕЙ ФЛУОРЕСЦЕНЦИИ И ВИДОВОГО СОСТАВА ФИТОПЛАНКТОНА В ЧЁРНОМ И АЗОВСКОМ МОРЯХ В НАЧАЛЕ ОСЕНИ 2020 ГОДА

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**Аннотация**

Цель работы состояла в изучении свойств вертикальных профилей интенсивности флуоресценции пигментов фитопланктона (хлорофилла-*a*, фикоцианина, фикоэритрина и бета-каротина) с привлечением данных о видовом составе фитопланктона, полученных в 114 рейсе НИС «Профессор Водяницкий». Проведённый анализ ковариационных матриц профилей ИФ пигментов фитопланктона в верхнем 50-метровом слое Чёрного моря указывает, что с глубиной происходит изменение пигментного состава фитопланктона, что может быть связано со сменой его видового состава. В то же время, изменчивость профилей ИФ пигментов фитопланктона на уровне 80% в верхнем 20-метровом слое описывается первым собственным вектором, что хорошо согласуется с прямыми наблюдениями видового состава фитопланктона, свидетельствующие о доминировании одного отдела фитопланктона в 20-метровом слое. Кроме того, имеют место региональные особенности, в частности, средние значения ИФ пигментов фитопланктона в Азовском море существенно выше, чем в Чёрном море, что связано с более высокой концентрацией фитопланктона в Азовском море.

**Ключевые слова:** флуоресценция пигментов фитопланктона, видовой состав фитопланктона, зона фотосинтеза, Чёрное море, Азовское море

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## 1. Introduction

Phytoplankton is a living, constantly changing substance that has various set of pigments, depending not only on the phytoplankton species composition, but also on the external conditions: the ambient temperature, the concentration of minerals in the sea water, the spectral composition of light and its intensity. The ability to determine the vertical profiles of the concentration of various phytoplankton divisions using simultaneous measurements of the phytoplankton fluorescence intensity (FI) in various spectral channels, as well as using the spectrum of the sea brightness emerging from the water column (the sea brightness coefficient), is an urgent and long-term task for several science areas, such as phytoplankton physiology, optics of the sea, remote sensing in the visible range of the spectrum, ecology of marine ecosystems, etc. Sea brightness coefficient data are received via optical scanners installed on satellites for remote sensing of the Earth. These data are used in algorithms for restoring the species and size composition of phytoplankton [1, 2], being actively developed today. The works [1, 3–13] attempt to identify the species composition of phytoplankton based on the relation between the spectral characteristics of the primary hydrooptical characteristics obtained from satellite data and various phytoplankton divisions. However, the sea brightness coefficient is only formed by the upper sea layer, which approximately corresponds to the first optical depth, and, as a result, the remote sensing data characterize only this layer. Therefore, the simultaneous analysis of vertical profiles of the phytoplankton FI spectra and species composition, as well as (in prospect) of the satellite data on the sea brightness coefficient is a long-term task, and after its achievement we will be able to restore the three-dimensional structure of the phytoplankton species composition in the entire photosynthetic layer. The approach to restoring the primary hydrooptical characteristics of the Black Sea was tested in the work [14].

The aim of the work is (i) to identify regional features of the vertical FI profiles of phytoplankton pigments — *f*-parameters: chlorophyll-*a* excited in the blue *f*-Chl(blue) and red *f*-Chl(red) areas of the spectrum, phycocyanin *f*-PC, phycoerythrin *f*-PE and beta-carotene *f*-β-carotene; and (ii) to analyze the correlation of these data with one another and with data from direct measurements of phytoplankton species composition. To achieve this goal the following tasks were set and completed.

**Task 1.** Perform an analysis of the vertical profiles of all *f*-parameters and cell concentrations of various phytoplankton divisions, obtained from three horizons (0, 10 and 20 m) of the Black Sea.

**Task 2.** Perform a statistical and comparative analysis of the vertical profile variability of all *f*-parameters for the Black Sea and the Sea of Azov separately in 0–50 m and 0–10 m layers, respectively.

**Task 3.** Perform a simultaneous analysis of all *f*-parameter vertical profiles in pairs at different horizons of the Black Sea using the vertical profile *f*-Chl(blue) as a reference.

## 2. Materials and methods

The work is based on optical and biological measurements obtained during the cruise 114 of the R/V 'Professor Vodyanitsky', which took place from September 15 to October 8, 2020, in the Black Sea and the Sea of Azov within the territorial water of the Russian Federation exclusive economic zone. During the cruise simultaneous measurements of the vertical profiles of five *f*-parameters were performed. They were recorded using a multichannel probing fluorescence meter (FR-1) developed by Marine Optics and Biophysics Department of RAS Marine Hydrophysical Institute [15]. The *f*-parameters were measured at 64 stations in the probing mode to a depth of 100 m in the Black Sea and 10 m in the Sea of Azov, or to the bottom where the station depth was less than 100 m or 10 m, respectively.

The operating principle of FR-1 fluorimeter is based on a patented method [16]. FI of various phytoplankton pigments (*f*-parameters) is registered near-simultaneously to using one photomultiplier tube (PMT). The registered fluorescence radiation excited in different parts of the spectrum enters PMT through rotating disk with light filters placed in front of entrance window from one measuring volume, where there is the same composition of suspended matter at each specific moment. The digitized FI values are recalculated into relative units proportional to the value of the PMT cathode current. The recalculation takes into account the background illumination signal (without the use of exciting radiation), the PMT spectral sensitivity, and the dependence of the PMT gain on the supply voltage. Thereby, the obtained data are comparable. Data logging frequency: 2 Hz at a probing speed of 0.2–0.25 m/s. The measuring volume is protected from external radiation by the light-protect housing. The logging parameters are shown in Table 1.

*Table 1*

**Parameters for logging *f*-parameters**

| Pigment | Abbreviation | Excitation spectrum half-width, nm | Fluorescence signal detection range, nm |
|---|---|---|---|
| Chlorophyll-*a* (blue) | *f*-Chl(blue) | 450–470 | 672–800 |
| Phycocyanin | *f*-PC | 580–600 | 672–800 |
| Chlorophyll-*a* (red) | *f*-Chl(red) | 610–630 | 672–800 |
| Phycoerythrin | *f*-PE | 525–550 | 600–800 |
| β-carotene | *f*-β-carotene | 450–470 | 490–560 |

This method differs from the flow cytometry in that during the probing the integral fluorescence signals of phytoplankton cells composition in their habitat are recorded instead of the fluorescence intensity signals from single elements of the dispersed phase. This is called 'integral cytometry'. Thus, vertical profiles of five *f*-parameters were obtained simultaneously for each station.

Water samples for subsequent qualitative and quantitative analysis of phytoplankton were taken using bathometers of the OCEAN SEVEN320 PlusM, Idronaut probing complex. There were fixed horizons for taking water samples: at 0, 10 and 20 m. To determine the species and quantitative composition of phytoplankton the samples up to 1.5 l were concentrated on a reverse filtration funnel, equipped with 1-μm mesh diameter filter, to a volume of 60 ml and fixed with 2.5 ml of neutralized 40 % formalin [17, 18].

Cell counting and determination of the phytoplankton size and species composition were performed using LOMO Mikmed-2 light microscope with 40x-1500x magnification. Cell volume and biomass were estimated according to standard methods [19].

The obtained data were used to complete Task 2 and included information on the phytoplankton species and size composition, as well as the value of calculated biomass for all species on three horizons of the Black Sea (0, 10 and 20 m). The task was performed using statistical methods, which include the analysis of average fluorescence profiles, the calculation of eigenvectors and eigenvalues (both for a single parameter and for two parameters using joint analysis at a fixed depth), etc. A separate method was used to complete each of the three tasks above. These methods are described below.

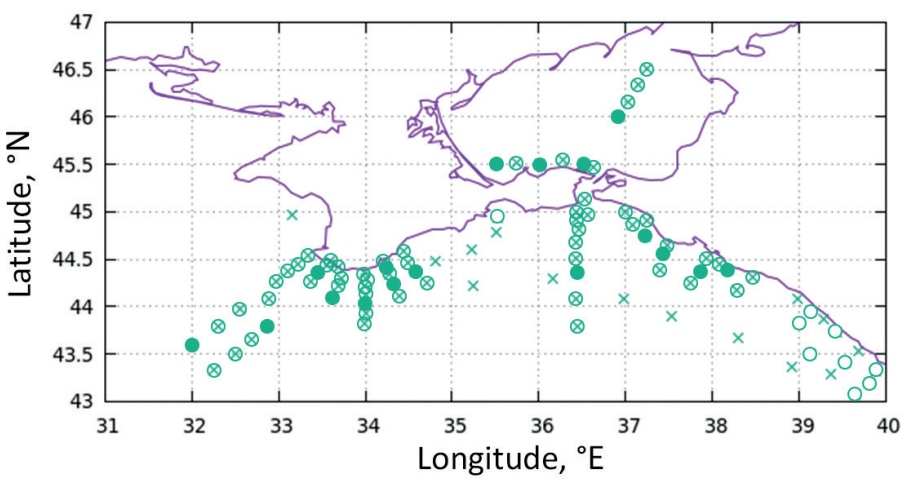


**Fig. 1.** Station positions for cruise 114 of the R/V "Professor Vodyanitsky":
⊗ stations where vertical profiles of all *f*-parameters were measured;
× stations where samples were taken to determine phytoplankton species composition;
● joint stations where the vertical profiles of all *f*-parameters were measured
and samples were taken to determine phytoplankton species composition

To complete **Task 1** we selected stations in the Black Sea where simultaneous observations of the phytoplankton species composition and *in situ* measurements of five *f*-parameters were performed. The phytoplankton species composition was analyzed at 0 m, 10 m and 20 m horizons. In situ measurements of *f*-parameters were taken in the vicinity of ±1 m for each sampling horizon and averaged. This approach resulted in 3–30 values of each *f*-parameter for each horizon. For each i-station, a value vector of the corresponding *f*-parameter $\overrightarrow{x_i}$ was formed. It consisted of three components (each of them corresponded to the average value of the *f*-parameter at the corresponding horizon), considering the vicinity:

$$\overrightarrow{x_i} = \begin{pmatrix} d_{1,i} \\ d_{2,i} \\ d_{3,i} \end{pmatrix}. \quad (1)$$

There were seventeen stations of this kind in total, and we managed to form a matrix *M* for each *f*-parameter:

$$M = \overrightarrow{x_1},..,\overrightarrow{x_k}, \quad (2)$$

where *k* is a station. number.

We calculated the average value over stations for each of the three horizons:

$$s_j = \frac{1}{k} \cdot \sum_{i=1}^{k} d_{j,i} \quad (3)$$

or

$$\vec{s} = \begin{pmatrix} s_1 \\ s_2 \\ s_3 \end{pmatrix}. \quad (4)$$

Next, we got the mean deviation matrix:

$$A = \overrightarrow{x_1} - \vec{s},..,\overrightarrow{x_k} - \vec{s} \quad (5)$$

and as a consequence, 3 × 3 covariance matrix *K*: $K = A \cdot A^T$.

Our aim is to analyze the eigenvalues $\lambda_i$ and eigenvectors $\vec{v}_i$ of the covariance matrix *K*: $K \cdot \overrightarrow{v_i} = \lambda_i \cdot \overrightarrow{v_i}$.

The step-by-step procedure for data processing during **Task 2** consisted of the following steps. For the Black Sea, a vertical profile was formed from the measurement data for the selected *f*-parameter over 10 horizons, starting from 0 m with a step of 5 m. For each horizon, the average value of *f*-parameter within ± 0.5 m was calculated ( $\vec{x} = x_i$ ). It was followed by the calculation of the mean profile $\vec{x}$ and covariance matrix $K = \vec{x} - \vec{x}$, where *i* is a horizon number. At the final stage, the eigenvalues $\lambda_i$ and eigenvectors $\vec{v}_i$ of the covariance matrix *K* were found. A similar procedure was followed for the measurements in the Sea of Azov. The difference was in selected horizons and their vicinity. There are eight horizons in total: 3, 4, 5, 6, 7, 8, 9 and 10 m. The vicinity is ± 0.1 m.

In order to complete **Task 3**, the vertical profiles of *f*-parameters for the Black Sea only were used. They consisted of pairs ($x_i$, $y_i$): *f*-Chl(blue) vs *f*-PE, *f*-Chl(blue) vs *f*-PC, *f*-Chl(blue) vs *f*-Chl(red), and *f*-Chl(blue) vs *f*-β-carotene for a fixed horizon. *f*-Chl(blue) profile was used as a reference. There were 10 horizons in total, starting from 0 m with a step of 5 m. For each horizon, the average value of the *f*-parameter = <x>, <y> within ±1 m was calculated and eigenvalues of the 2×2 covariance matrix *K* ( $K \cdot \overrightarrow{v_i} = \lambda_i \cdot \overrightarrow{v_i}$ ) were analyzed at a separate horizon (the matrix was obtained from pairs ($x_i$ –<x>, $y_i$ –<y>) for all profiles). To determine the difference between daytime and nighttime measurements, the corresponding samples were formed without morning and evening measurements.

### 3. Results and discussion

**Task 1.** In total for all stations, the laboratory analysis of water samples showed the presence of 113 phytoplankton species and intraspecific taxa belonging to 7 divisions: Miozoa (Dinophyta), Bacillariophyta, Haptophyta, Ochrophyta, Euglenozoa, Cercozoa, Eukaryota unasigned phylum. The studies of

*Table 2*

**The results of eigenvalues ($\alpha_i$, $i = 1-3$) analysis for covariance matrices compiled for five f-parameters at three fixed horizons of 0, 10 and 20 m in the vicinity of ±1 m in the Black Sea**

| Calculation parameters* | *f*-parameters | | | | |
|---|---|---|---|---|---|
| | Chl (blue) | Chl (red) | PC | PE | β-carotene |
| $\lambda_1$ | 0.106 | 0.373 | 0.00305 | 0.0798 | 2.34 |
| $\lambda_2$ | 0.014 | 0.015 | 0.00087 | 0.0144 | 0.12 |
| $\lambda_3$ | 0 | 0.002 | 0.00002 | 0.0004 | 0.01 |
| ε,% | 88 | 96 | 77 | 84 | 95 |

*N = 17, N is a number of stations.

the phytoplankton species and size composition with the calculation of biomass for the Black Sea stations are presented in Fig. 2. As can be seen from the figure, in the period under consideration the Dinophyta microalgae division dominated at all three horizons with a small contribution of Bacillanophyta and Haptophyta divisions.

The analysis of the first eigenvector contribution to the overall variability of five *f*-parameters at three horizons at the Black Sea stations is presented in Table 2.

The calculations were performed according to the following formula:

$$\varepsilon = \frac{\lambda_1}{\lambda_1 + \lambda_2 + \lambda_3}. \quad (6)$$

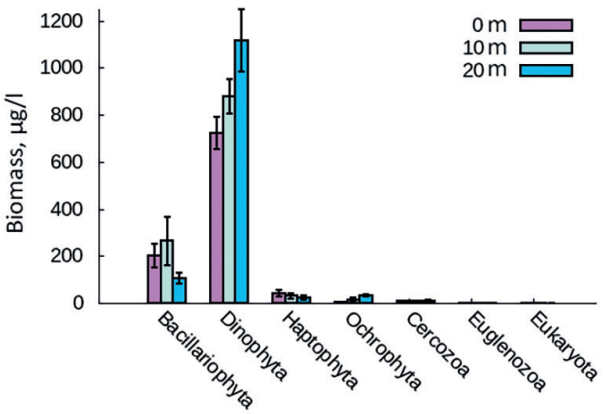


**Fig. 2.** The average value of biomass and the standard deviation (µg/l) at three horizons for the entire array of stations in the Black Sea

The analysis result showed that ≥80 % of the variability is described by the first eigenvector. ε calculation results are in good agreement with the measurement data of the contribution to the biomass of the three dominant phytoplankton divisions (see Fig. 2 and Tab. 2). Given that the microalgae mentioned above are similar in pigment composition [20], ε values were expected. Thus, in early autumn 2020 in the northern part of the Black Sea central and eastern regions 80 % of the vertical variability of five *f*-parameters at three fixed horizons is explained by the first eigenvalue, which is in good agreement with the dominance of Dinophyta microalgae division and its variability.

**Task 2.** Fig. 3 and Fig. 4 show the average profiles of five *f*-parameters for the Black Sea and the Sea of Azov, respectively. Fig. 3 includes profiles for two samples: optical stations (A-sample) where FR-1 fluorimeter probing was performed, and stations where simultaneous optical and biological studies were performed (KL-sample). As you can see, the average profiles taking into account the standard deviation are almost identical. Thus, to the first approximation, the conclusions obtained only for the KL-sample (which covers a smaller count of stations) can be applied to all Black Sea stations. The average profiles of five *f*-parameters in the Sea of Azov taking into account the standard deviation can be considered homogeneous.

As can be seen from Fig. 3, the average value of the fluorescence signal for four of the five *f*-parameters is approximately the same. Phycocyanin fluorescence value is an order of magnitude smaller than the other values. In general, the profile is homogeneous. The standard deviation is 10–20 % of the average value for all depths. An exception is observed for *f*-β-carotene standard deviation, which decreases significantly with

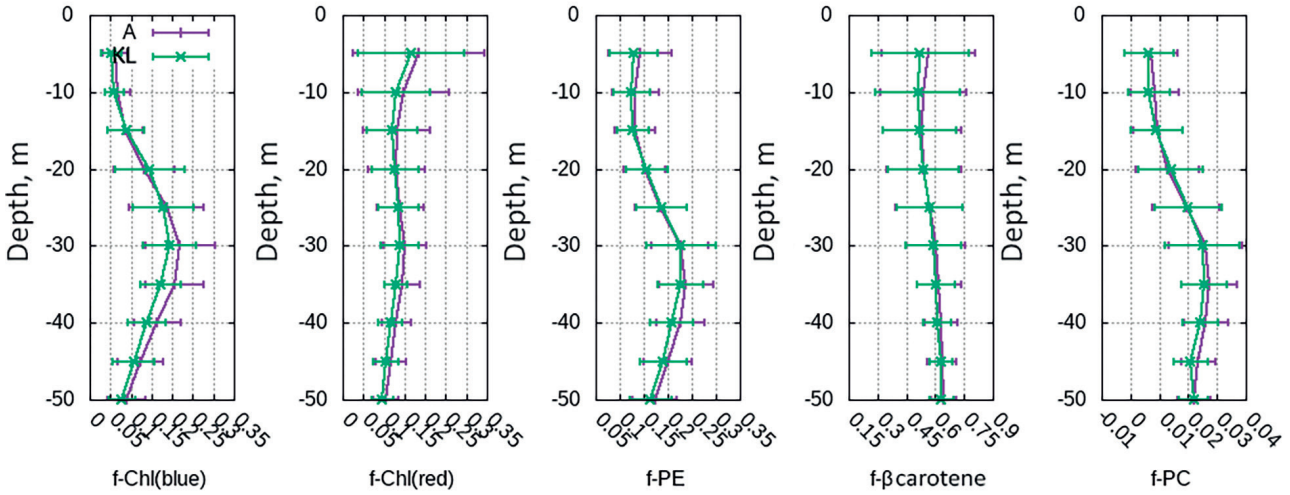


**Fig. 3.** Average profiles for five *f*-parameters (*f*-Chl(blue), *f*-Chl(red), *f*-PC, *f*-PE, *f*-β-carotene, from left to right) of two samples: A — stations where optical measurements were performed; KL — stations where simultaneous optical and biological measurements were performed. The legend shown in the left figure is valid for all *f*-parameters

depth. For the Black Sea, the standard deviation for at least three *f*-parameters (*f*-Chl(blue), *f*-PC, *f*-PE) appears as an absolute maximum located at depths of 25–35 m. In the Black Sea and the Sea of Azov, the standard deviation for the *f*-β-carotene profile decreases with depth.

The results of covariance matrices analysis for each of the five *f*-parameters are shown in Figures 5–7. Fig. 5 shows the calculations of the first eigenvector and first two eigenvectors contribution, respectively, to the description of the total variability of each *f*-parameter as a function of depth for the Black Sea and the Sea of Azov. The calculations are performed according to the following formulas:

$$\varepsilon_1 = \frac{\lambda_1}{\lambda_1 + \lambda_2 + .. + \lambda_k} \text{ and } \varepsilon_2 = \frac{\lambda_1 + \lambda_2}{\lambda_1 + \lambda_2 + .. + \lambda_k},$$

where $k$ — is a number of horizons.

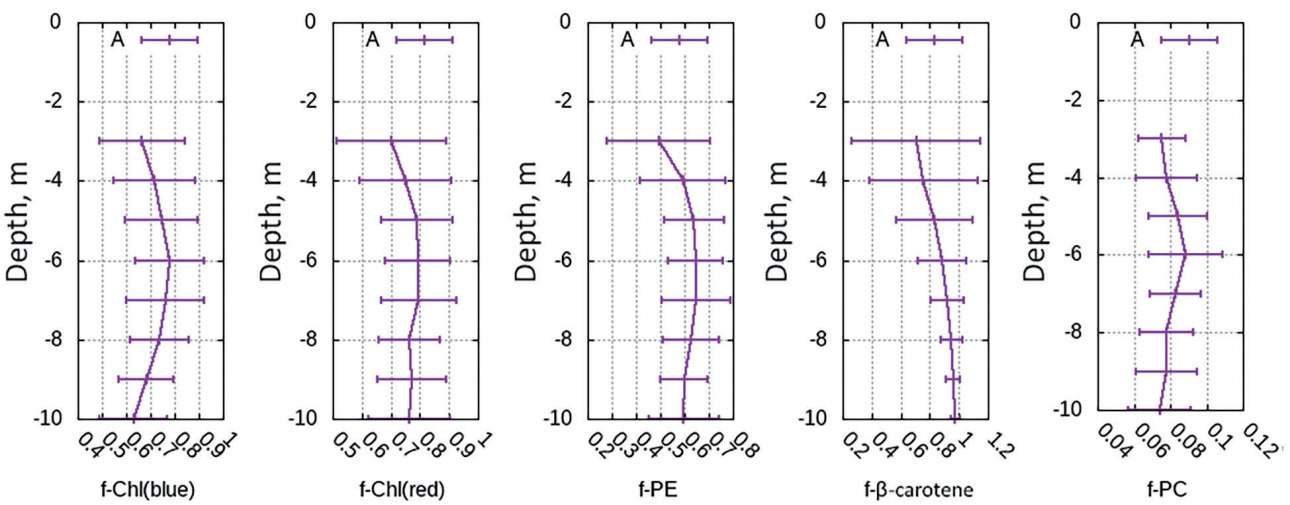


**Fig. 4.** Average profiles for five *f*-parameters in the Sea of Azov for all stations for the A-sample where optical measurements were performed

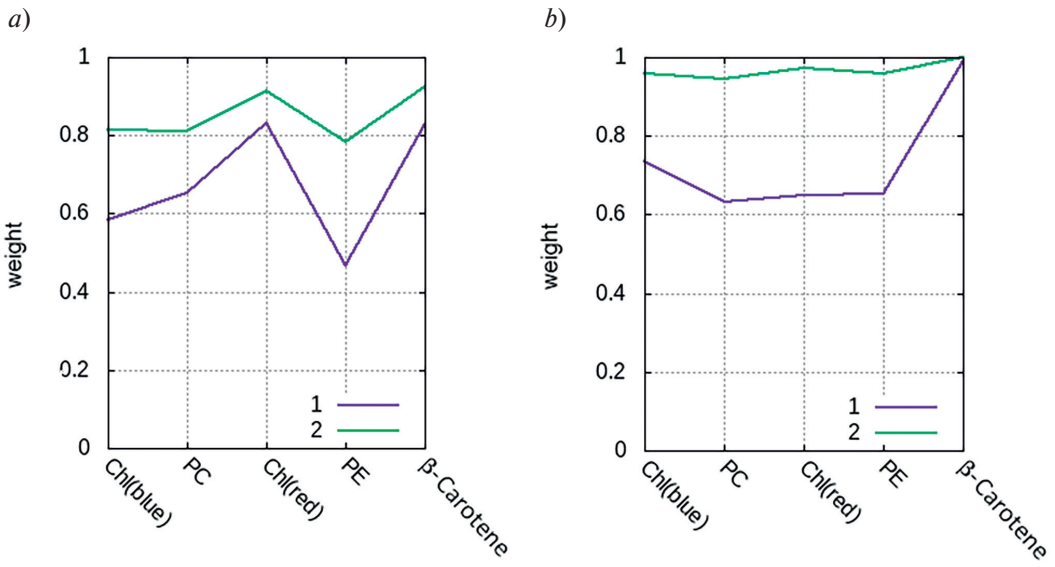


**Fig. 5.** The percentage of profile variability description for each of the five *f*-parameters (1, $\varepsilon_1$) by the first eigenvector and (2, $\varepsilon_2$) by first two eigenvectors in (*a*) the Black Sea and (*b*) the Sea of Azov

The results of covariance matrix analysis for each of the five *f*-parameters confirm that the first two eigenvectors for *f*-Chl(blue), *f*-PC, *f*-PE in the Black Sea and *f*-Chl(red), *f*-PC, *f*-PE in the Sea of Azov should be used to describe the depth variability at the level of 75 %. And only *f*-β-carotene variability is well described by the first eigenvector. The minimum variations from the considered range of depths (0–50 m) in the Black Sea for all five *f*-parameters are observed at 50 m depth. In the Sea of Azov, the first eigenvector for all five *f*-parameters does not depend on the depth (except for *f*-β-carotene).

Summarizing the results obtained for the Black Sea, we can state the following:

– for *f*-Chl(blue), *f*-PC and *f*-PE vertical profiles, the description of 80 % variability is provided by at least two first eigenvectors;

– for *f*-Chl(red) and *f*-β-carotene vertical profiles, the first eigenvector is sufficient to provide a description at the same level of variability.

Based on the above, considering the results of **Task 1**, it can be assumed that the variability of the pigment content in microalgae at a depth below 20 m is more complex. Therefore, the analysis of phytoplankton species composition in the season under consideration must be performed to the depths of the photosynthetic layer lower boundary.

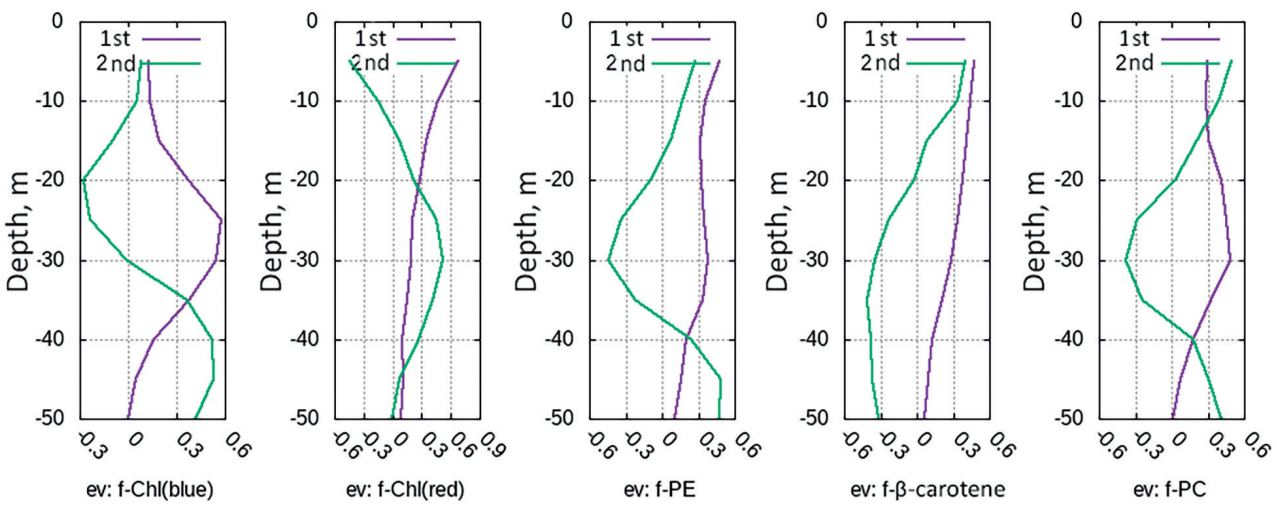


**Fig. 6.** The first two eigenvectors for five *f*-parameter profiles in the Black Sea

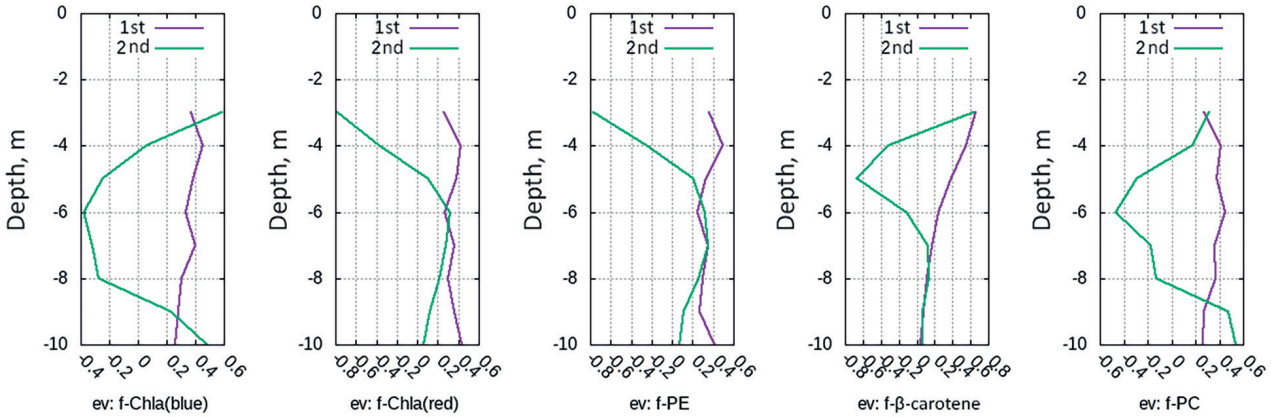


**Fig. 7.** The first two eigenvectors for *f*-parameter profiles in the Sea of Azov

For the Sea of Azov:

– the first harmonic is homogeneous in depth, except for *f*-β-carotene, for the latter it decreases significantly from the surface to the bottom;

– at a depth of 5–6 m, a maximum variability of the second harmonic is observed for all five *f*-parameters.

The comparison of phytoplankton pigment FI profiles in the Black Sea (0–50 m) and the Sea of Azov (0–10 m) shows that the average values of all Black Sea *f*-parameters are significantly lower than the ones of the Sea of Azov (Figures 3–4). This difference is explained by the higher trophicity of the Sea of Azov water [21, 22]. In addition, 80 % profile variability for three f-parameters in the Black Sea and four f-parameters in the Sea of Azov are described by the first two eigenvectors of the covariance matrix, which, apparently, is associated with a change in the phytoplankton species composition with depth.

**Task 3.** Fig. 8 shows two samples of the Black Sea profiles of five *f*-parameters: the upper part presents profiles obtained in the daytime; the bottom one — profiles obtained at night. The twilight period was excluded. For ease of comparison, the profile of each fixed *f*-parameter is presented on the same scales for both daytime and nighttime samples. At first glance, there are no other significant differences between the nighttime and daytime profiles, apart from increase in phytoplankton pigment FI at night.

In order to identify the features between the behavior of *f*-parameter profiles for daytime and nighttime samples (if any), the following procedure was followed. A covariance matrix consisting of two *f*-parameters was formed at each horizon. For calculation of such a matrix, *f*-Chl(blue) was used as a reference along with another changing parameter. Obviously, such 2 × 2 matrices have two eigenvectors. Fig. 9 shows the analysis of the angle between the first eigenvector and the direction associated with *f*-Chl(blue) positive variability in a particular sample as a function of depth and a pair of *f*-parameters. The physical meaning of this parameter is that the angle characterizes the correlation coefficient between two *f*-parameters: if the angle tends to zero, then the correlation coefficient tends to one, which means the connection is strong, and if it is close to 90°, then the correlation coefficient tends to zero, which means there is no connection.

Analysis of the angle as a function of depth between the first eigenvector of the covariance matrix for two *f*-parameters and the direction associated with the positive variability of *f*-Chl(blue) in a particular sample showed the following:

– there is no difference between daytime and nighttime samples (except for *f*-Chl(blue) and *f*-β-carotene pair);

– *f*-Chl(blue) contribution to the first eigenvector with depth was increased with all *f*-parameters.

## 4. Conclusion

The summary of the results for all three tasks is provided below:

– The vertical structure of phytoplankton pigment FI profiles has its own characteristics and different behavior in the Sea of Azov and the Black Sea.

– The analysis of the covariance matrix of phytoplankton pigment FI profiles in the upper 50-meter layer of the Black Sea indicates that the pigment composition of phytoplankton changes with depth, which may be associated with changes in its species composition.

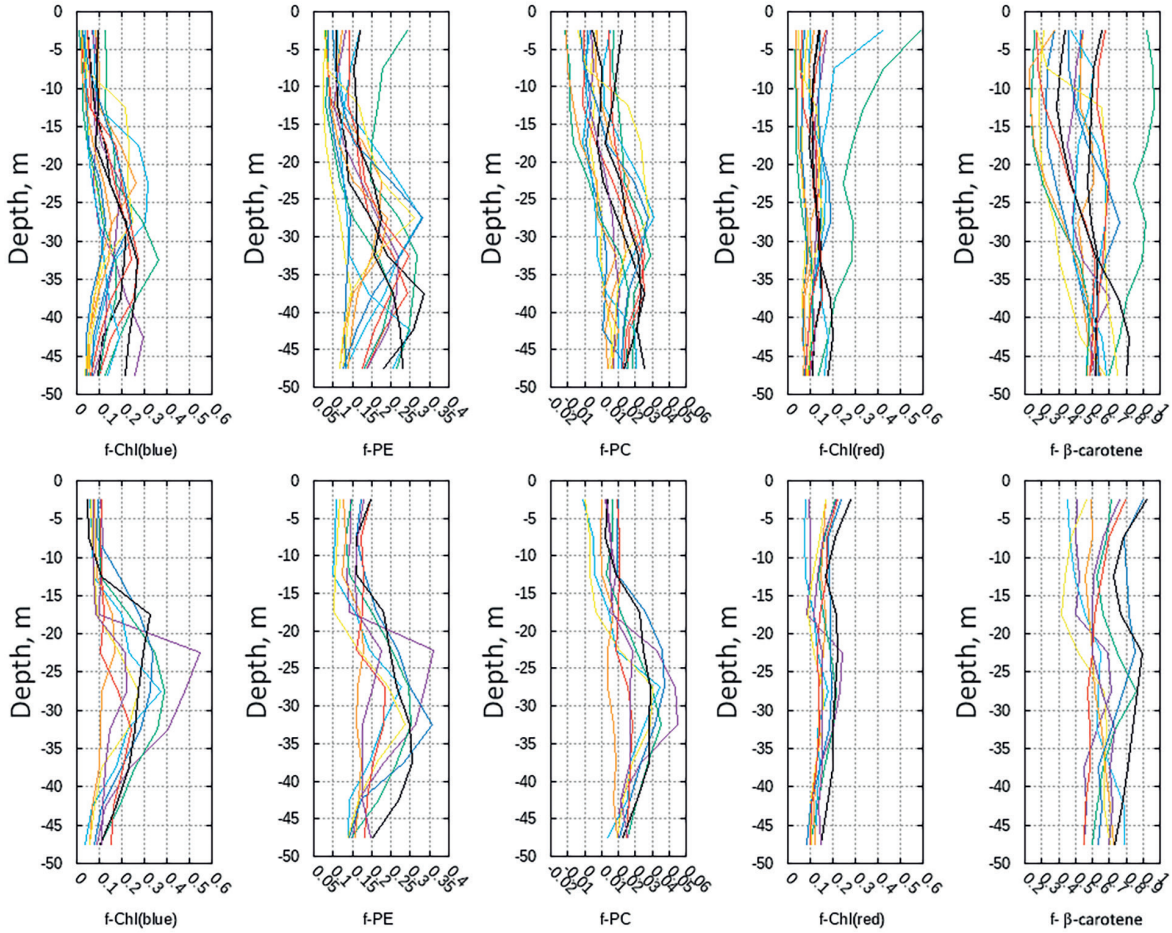


**Fig. 8.** Profiles of five *f*-parameters for daytime (top row) and nighttime (bottom row) studies in the Black Sea

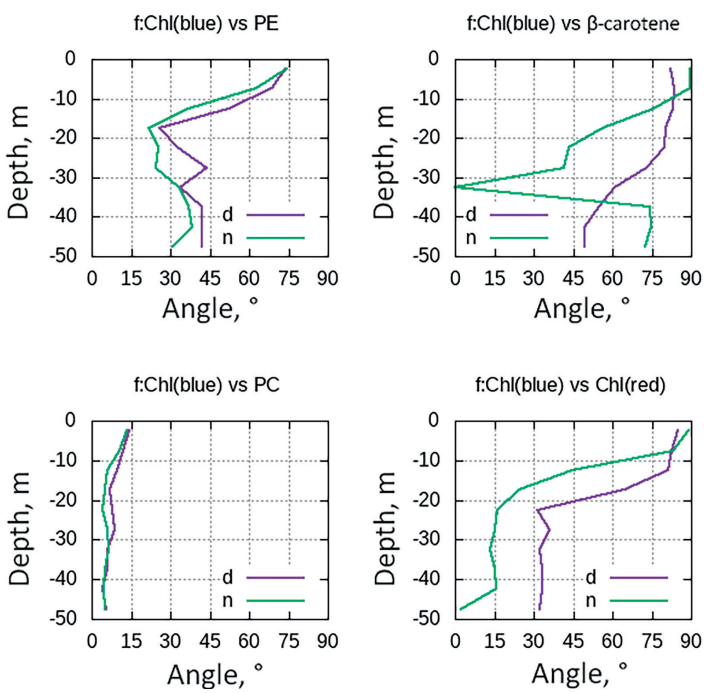


**Fig. 9.** The change in the angle between the first eigenvector of the covariance matrix for two *f*-parameters and the direction of *f*-Chl(blue) variability as a function of depth in the Black Sea for daytime (*d*) and nighttime (*n*) profiles

– In early autumn 2020, in the northern part of the Black Sea central and eastern regions the variability of the vertical profiles *f*-Chl(blue), *f*-PC, *f*-PE at the level of 80 % is described by at least the first two eigenvectors, and for the vertical profiles *f*-Chl(red) and *f*-β-carotene the first eigenvector is sufficient, which is in good agreement with direct observations of the phytoplankton species composition, indicating the dominance of one phytoplankton division in the 20 m layer.

– The description of the profile variability in the upper 50 m layer of the Black Sea and in the 10 m layer of the Sea of Azov at the same level requires the use of the first two eigenvectors, which can be explained by the change in the phytoplankton species composition with depth.

– It is still difficult to explain several discovered properties of f-parameter profiles due to the lack of direct measurements of the phytoplankton species composition. Therefore, while conducting joint biooptical measurements, given the complexity of biological measurements, the phytoplankton species composition should be calculated up to the lower boundary of the photosynthetic layer with a step of 10 m in the Black Sea and with a step of 2 m in the Sea of Azov, especially in the warm season — during the period of the formed thermocline.

– The obtained results showed that the autonomous probing multichannel fluorescence meter FR-1 developed at Marine Hydrophysical Institute is an informative and future proven measuring device for studying the species composition of phytoplankton *in situ*.

The development of this study is seen in a series of laboratory measurements of fluorescence intensity signals, considered in the work of *f*-parameters, for individual phytoplankton monocultures typical of the Black Sea, grown under controlled fixed conditions of lighting, nutrition, and temperature.

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**About the authors**

SUSLIN, Vyacheslav V., Cand.Sc. (Phys.-Math.), РИНЦ AuthorID: 825673, ORCID: 0000-0002-8627-7603, Scopus AuthorID: 6602501867, WoS ResearcherID: B-4994-2017, slava.suslin@mhi-ras.ru

KUDINOV, Oleg B., РИНЦ AuthorID: 861503, Scopus AuthorID: 57201072321, kudinov_ob@mhi-ras.ru

RYABOKON, Daniil A., РИНЦ AuthorID: 1144660, akronis_white@mail.ru

LEE, Raisa I., РИНЦ AuthorID: 937587, ORCID ID: 0000-0002-2482-0105, raisa-lee@yandex.ru