

# Fluorescence of Chlorophyll *a* during Seasonal Water Stratification in the Black Sea

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**Purpose.** At present the express method of estimating the chlorophyll *a* concentration by its fluorescence is widely spread. The applied in calculations coupling coefficient between these two values is assumed to be constant (in time and space); but it does not correspond to reality. Therefore, the basic purpose of the paper consists in studying dependence of fluorescence on the chlorophyll *a* concentration in the deep part of the Black Sea in a warm period when seasonal water stratification is observed.

**Methods and Results.** Relation between the light absorption by the phytoplankton pigments, and the chlorophyll *a* concentration and fluorescence is investigated. It is shown that the ratio between the chlorophyll *a* concentration and fluorescence was different in the quasi-isolated layers during the seasonal water stratification. Revealed is the vertical heterogeneity of fluorescence normalized for the chlorophyll *a* concentration in the euphotic layer. It is conditioned by change of the phytoplankton ability to absorb light quanta at the wavelength ~ 455 nm (fluorescence excitation) calculated for a unit of the chlorophyll *a* content. Comparison of the specific values of fluorescence and light absorption index on the wavelength ~ 455 nm showed their strong mutual correlation that testifies to insignificant variation of the values of the fluorescence quantum yield within the photosynthesis zone.

**Conclusions.** The obtained dependences between the chlorophyll *a* concentration and fluorescence can be used to specify the algorithms applied at present for reconstructing the chlorophyll *a* concentration profiles based on the data of either the fluorescence submerged probes or the drifting floats of the international project Biogeochemical Argo.

**Keywords:** chlorophyll *a*, fluorescence, light absorption, quantum yield, phytoplankton, the Black Sea.

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## Introduction

The use of chlorophyll *a* (*F*) fluorescence measurements in hydrobiological studies counts an almost century-old history [1, 2]. The *Biogeochemical-Argo* (*BGC-Argo*) drift float research program has been launched in the Black Sea since 2008 (<http://biogeochemical-argo.org/>). Currently, *BGC-Argo* floats measure only one biological parameter – chlorophyll *a* fluorescence. Recalculation of

the fluorescence  $F$  values recorded applying BGC-Argo floats in the Black Sea into the concentration values of the main photosynthetically active pigment – chlorophyll  $a$  ( $C_a$ ). It is carried out using the algorithm [3], created for Case 1 waters according to the optical classification. However, the Black Sea belongs to the Case 2 waters [4]. The algorithm suggested in [5, p. 8] assumes a constant coefficient of proportionality in relationship between  $C_a$  and  $F$ .

It is known that the chlorophyll  $a$  fluorescence intensity  $F$  ( $\mu\text{E}\cdot\text{m}^{-3}\cdot\text{s}^{-1}$ ) under the natural conditions of the existence of phytoplankton depends on several parameters [6, p. 242]:

$$F = PAR \cdot C_a \cdot \bar{a}_{ph}^* \cdot Q_a^* \cdot \phi_F, \quad (1)$$

where  $PAR$  is photosynthetically active radiation (PAR) in the range from 400 to 700 nm,  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ;  $C_a$  is the chlorophyll  $a$  concentration,  $\text{mg}\cdot\text{m}^{-3}$ ;  $\bar{a}_{ph}^*$  is the average spectrum value of the specific (normalized to  $C_a$ ) coefficient of light absorption by phytoplankton pigments ( $a_{ph}^*(\lambda)$ ),  $\text{m}^2\cdot\text{mg}^{-1}$ ;  $Q_a^*$  is fluorescence intracellular reabsorption factor;  $\phi_F$  - quantum yield of fluorescence *in vivo*.

Value of the coefficient  $a_{ph}^*(\lambda)$ , and consequently the value of  $\bar{a}_{ph}^*$  vary by almost an order of magnitude, according to theoretical [7] and *in situ* [8] studies in the oceans. The quantum yield of fluorescence depends on the light intensity, nutrient availability and species composition of the phytoplankton community [9, 10]. As can be seen from equation (1), the relationship between the parameters  $F$  and  $C_a$  is far from straightforward and is due to the influence of the environmental factors on the phytoplankton functional characteristics:  $a_{ph}^*(\lambda)$  and  $\phi_F$  [9]. In accordance with the results obtained during long-term studies in the Black Sea [11], values of  $a_{ph}^*(440)$  change 2–3 times between seasons (winter – summer). The influence of environmental factors on the values of  $a_{ph}^*(\lambda)$  and  $\phi_F$  causes the variability of the relationship between the fluorescence  $F$  and the chlorophyll  $a$  concentration  $C_a$  within the same water area during the year, when seasonal stratification divides waters into quasi-isolated layers.

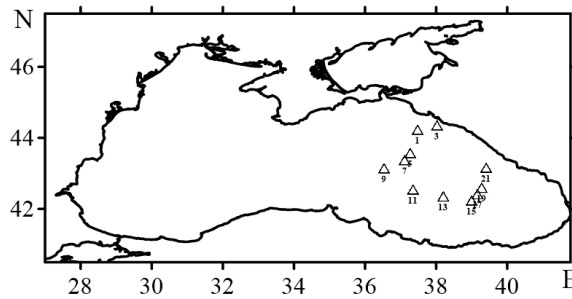
In this regard, the present paper is aimed to study the dependence of fluorescence intensity  $F$  on the chlorophyll  $a$  concentration  $C_a$  in the Black Sea in the warm season, when seasonal stratification of waters is observed.

### Methods

The research was carried out in the deep-water part of the Black Sea (Fig. 1) during the 97<sup>th</sup> scientific cruise of the R/V “Professor Vodyanitsky”, October 24–28, 2017. Water samples were taken from different depths of the euphotic layer using a cassette of Niskin bottles attached to the SBE-911plus probe (*Sea-Bird Electronics*). The depths were chosen based on the temperature, the chlorophyll  $a$  fluorescence ( $F$ ) profiles, and the water transparency, assessed based on

the Secchi disk ( $Z_s$ ) visibility depth. For the research aim implementation the sampling was executed at the depths within the following four layers:

- 1) the upper mixed layer (UML),
- 2) the thermocline (TC),
- 3) the layer between the thermocline and the depth of 1% of the PAR incident on the sea surface,
- 4) the layer between the depth of 1% and 0.1% of the PAR incident on the sea surface.



**Fig. 1.** Map of the stations ( $\Delta$ ) in the 97<sup>th</sup> cruise of RV “Professor Vodyanitsky”, October 24–28, 2017

The bottom of the  $Z_{eu}$  photosynthesis zone was assumed to be at the depth, where photosynthetic available radiation (PAR) is 1% of its surface value. The  $Z_{eu}$  values were assessed using the dependence of the diffuse light attenuation coefficient (for downwelling irradiance) on  $Z_s$  [12].

#### *Chlorophyll a and phaeopigment concentration*

Seawater samples (1.5 L) were filtered through glass fiber filters (GF/F, Whatman) under low vacuum (<0.2 atm). Then the filters were folded twice (by the particles collected inward), wrapped in foil and stored in liquid nitrogen in a Dewar ( $t = -196$  °C) until further measurement in the laboratory. Optical measurements were carried out with a double-beam spectrophotometer Lambda 35 (PerkinElmer).

Concentration of chlorophyll *a* ( $C_a$ ) and phaeopigments ( $C_{phae}$ ) was estimated by spectrophotometric method using the equations [13]:

$$C_a = [2,7(D_o(665) - D_a(665))V_E] / V_F l, \quad (2)$$

$$C_{phae} = [2,7(1,7D_o(665) - D_a(665))V_E] / V_F l, \quad (3)$$

where  $D_o(665)$  and  $D_a(665)$  are the optical density values at 665 nm wavelength before and after acidification of the pigment extract in acetone solution (90%), respectively;  $V_E$  is the extract volume,  $cm^3$ ;  $V_F$  is the water sample volume,  $dm^3$ ;  $l$  is the cuvette optical path length, cm. In the present research  $V_E = 5$   $cm^3$ ;  $V_F = 1.5$   $dm^3$  and  $l = 1$  cm.

### *Light absorption by particles, phytoplankton pigments*

The light absorption by a particles, phytoplankton pigments was estimated in accordance with the NASA protocol [14] using the filter pad technique (“wet filter technique”) [15]. Optical density of the samples was measured at the wavelength from 350 to 750 nm using a dual-beam spectrophotometer Lambda 35 (*PerkinElmer*) equipped with an integrating sphere. The particulate light absorption coefficient was calculated by the following equation:

$$a_p(\lambda) = [2,3 \cdot OD_p(\lambda) \cdot S] \cdot \beta(\lambda) / V, \quad (4)$$

where 2.3 is the conversion factor of lg to ln;  $S$  is the filter “working” area,  $m^2$ ;  $V$  is the water sample volume,  $m^3$ ;  $\beta$  – correction of a difference in an optical path between the particles collected on the filter and particles suspended in water (Mitchell, 1995);  $OD_p(\lambda)$  is the optical density of the particles (collected on the filter) [16].

To separate the  $a_p(\lambda)$  coefficients into its components – light absorption by phytoplankton pigments ( $a_{ph}(\lambda)$ ) and non-algal particles ( $a_{NAP}(\lambda)$ ) – the sample was bleached by a solution of sodium hypochlorite (NaClO) [17]. The  $a_{ph}(\lambda)$  value was calculated by the following equation:

$$a_{ph}(\lambda) = a_p(\lambda) - a_{NAP}(\lambda). \quad (5)$$

At all the stations, in addition to the fluorescence profile (the SBE-911plus probe data), fluorescence intensity was measured using a laboratory MEGA-25 fluorimeter with exciting radiation at the  $\sim 455$  nm<sup>1</sup> wavelength. Before the measurements, seawater samples were adapted to darkness for 10–30 min [18] depending on the light intensity in the environment (light “history” of the phytoplankton existence). The following fluorescence parameters were measured:  $F_0$  – the chlorophyll *a* fluorescence intensity, measured when reaction centers (RC) of photosystem 2 are open, and  $F_m$  – the chlorophyll *a* fluorescence intensity, when RCs are closed.  $F_0$  and  $F_m$  are corresponding to constant and maximum fluorescence intensity<sup>1</sup>. These chlorophyll *a* fluorescence parameters were calculated relative to the background fluorescence caused by a colored dissolved organic substance (CDOM). To estimate CDOM fluorescence the seawater samples were filtered through a nucleopore filter (*Sartorius*) with a pore size of 0.2  $\mu m$  under vacuum  $< 0.2$  atm. The filters were rinsed preliminary with  $\sim 50$  ml deionized waters. The assessment of the chlorophyll *a* concentration ( $C_a$ ) based on fluorescence intensity  $F$  suggests<sup>1</sup> a linear relationship between these parameters:

$$F = A \cdot C_a + b, \quad (6)$$

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<sup>1</sup> Matorin, D. N., Osipov, V. A. and Rubin, A. B., 2012. Metodika Izmerenij Obiliya i Indikacii Izmeneniya Sostoyaniya Fitoplanktona v Prirodnikh Vodakh Fluorescentnym Metodom. Teoreticheskie i Prakticheskie Aspekty [*Methodology for Measuring Abundance and Indicating Changes in the State of Phytoplankton in Natural Waters Using the Fluorescence Method. Theoretical and Practical Aspects*]. Moscow: Alteks, 138 p.

where  $A$  - the coefficient of proportionality between the  $F$  and the  $C_a$ ;  $b$  - the coefficient corresponding to the background fluorescence, mainly caused by CDOM. This type of dependence between the  $F$  and the  $C_a$  (equation 6) corresponds to the case of CDOM constant concentration. Based on the CDOM light absorption data, it can be concluded that the content of this optically active component in the Black Sea significantly changes during the year. Moreover, for a particular season, the variability of this parameter is characteristic with a change in both depth and distance from the coast [19–21]. Therefore, in our studies, the CDOM fluorescence  $F$  was measured in all samples, which was subtracted from the initial measurement to obtain fluorescence associated only with phytoplankton. As a result, the dependence of fluorescence on the chlorophyll  $a$  concentration (6) takes the following form

$$F = A \cdot C_a, \quad (7)$$

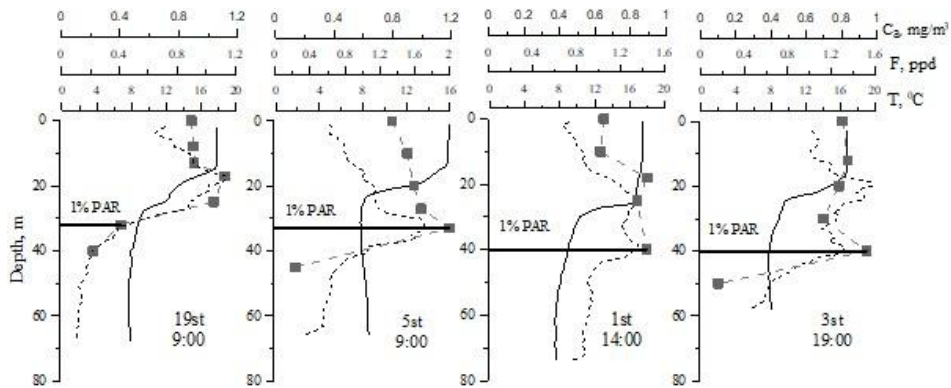
where  $A$  is the coefficient equal to specific (normalized to  $C_a$ ) fluorescence  $F^*$ .

## Results

### *Chlorophyll a concentration*

In the research area (Fig. 1) at the end of October 2017, the surface water temperature was 16–18 °C (Fig. 2). The UML varied between stations from 12 to 20 m. The TC was recorded in the 12–25 m layer at almost all stations, with the exception of the last two ones in the cruise (stations 21 and 19), which were worked at after the storm. The  $Z_{eu}$  (1% PAR) varied between stations within 30–38 m. Values of 0.1% PAR was observed at 50–57 m depths. For fluorescence vertical distribution a maximum located near the bottom of the euphotic zone (1% PAR) was typical for almost all the stations.

The relative content of phaeopigments (relative to from value) was in a range 0–18%. It should be noted that relative concentration of the phaeopigment exceeded 10 % at, and only at the lower horizons depths, where PAR was less than 1% of the sea surface PAR value. Due to the low content of phaeopigments, the chlorophyll  $a$  concentration  $C_a$  was used in the present research. Typical profiles of the vertical distribution of chlorophyll  $a$  concentration  $C_a$  are presented in Fig. 2. Distribution of  $C_a$  was almost uniform in the UML (Fig. 2). However, between stations the value  $C_a$  in the UML varied almost twice – from 0.56 to 0.96  $\text{mg} \cdot \text{m}^{-3}$ , being equal to  $0.78 \pm 0.11 \text{ mg} \cdot \text{m}^{-3}$  on average for the sea area under study. At many stations in the TC layer, an increase in the chlorophyll  $a$  concentration from 0.7 to 1.01  $\text{mg} \cdot \text{m}^{-3}$  (an average of  $0.88 \pm 0.1 \text{ mg} \cdot \text{m}^{-3}$ ) was noted. Maximum in the profile  $C_a$  was observed at depth of 1% of the sea surface PAR. In the maximum the  $C_a$  values were in the range from 0.89 to 1.2  $\text{mg} \cdot \text{m}^{-3}$  and was equal to  $1.0 \pm 0.12 \text{ mg} \cdot \text{m}^{-3}$  on average.



**Fig. 2.** Profile of temperature vertical distribution ( $T$ ) (solid line), fluorescence intensity ( $F$ ) (dotted line) and chlorophyll  $a$  concentration ( $C_a$ ) (grey squares) at separate stations in various periods of a light day, October, 2017

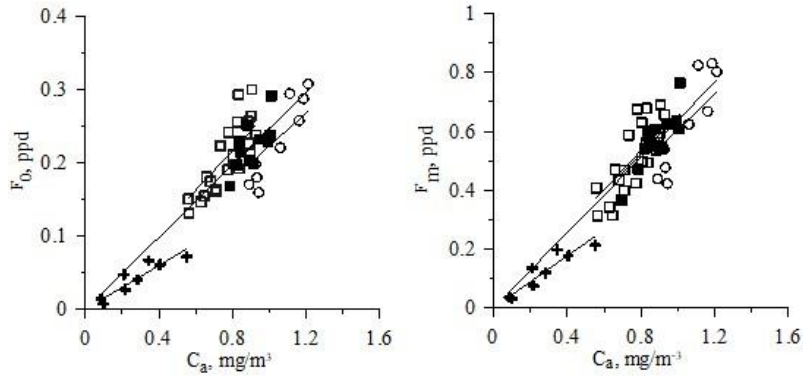
The type of vertical distribution of the chlorophyll  $a$  concentration, temperature and fluorescence  $F$  allowed designating four layers: the first is the UML, the second is TC, the third is between TC and depth of 1% of the sea surface PAR and the fourth is between depths of 1 and 0.1% of the surface PAR. The dependence of fluorescence on the chlorophyll  $a$  concentration was estimated for both all data set and particular layers (Fig. 3). It was found out that for all data set (for whole water layer investigated), this dependence was generally described by the following equations:

$$F_0 = 0,24 C_a, \quad r^2 = 0,97, \quad (8)$$

$$F_m = 0,64 C_a, \quad r^2 = 0,98. \quad (9)$$

From the graphs in Fig. 3 it is seen that the obtained dependencies of  $F$  on  $C_a$  (8) – (9) overestimate the values in the 3<sup>rd</sup> and 4<sup>th</sup> layers located deeper than the TC and underestimate – in the 1<sup>st</sup> layer (UML). Therefore, for each of the layers selected, the values of coefficient  $A$  were calculated from equation (7), given in Tab.1.

The coefficient  $A$  decreases with depth within the water column (Tab. 1). At that, the value of the coefficient  $A$  decreases by 10–20% in the upper three layers and it decreases most sharply in the 4<sup>th</sup> layer. In fact, the change in the coefficient  $A$  reflects a decrease in the chlorophyll  $a$  specific fluorescence intensity ( $F^* = F/C_a$ ) with depth. It should be noted that the correlation ( $r^2$ ) between  $F$  and  $C_a$  was high (Tab. 1). The value  $r^2$  was the largest for the dependence  $F_m$ , which is explained by higher values of  $F_m$  and, as a consequence, a smaller error introduced.



**Fig. 3.** Comparison of the measured values of the chlorophyll *a* concentration ( $C_a$ ) with intensity of chlorophyll *a* fluorescence ( $F_0$  and  $F_m$ ) for the water layers designated in October, 2017: the upper mixed layer ( $\square$ ), the temperature stratification layer ( $\blacksquare$ ), the layers between the temperature stratification layer and the depth of 1% of the sea surface photosynthetic available radiation (PAR) ( $\circ$ ) and layer between the depth of 1 and 0.1 % of the surface PAR (+)

Table 1

**Coefficient *A* of dependence of the constant ( $F_0$ ) and maximum ( $F_m$ ) fluorescence intensity on the chlorophyll *a* concentration and the correlation coefficient ( $r^2$ ) in the water layers**

Layer	Coefficient <i>A</i>		$r^2$		Number of data pairs, <i>n</i>
	$F_m$	$F_0$	$F_m$	$F_0$	
1. Upper mixed layer	0.67	0.27	0.98	0.98	21
2. Thermocline	0.66	0.25	0.99	0.99	11
3. Layer between the thermocline and the depth of 1% of the sea surface PAR	0.61	0.22	0.98	0.98	9
4. Layer between the depths of the 1 and 0.1% of the sea surface PAR	0.45	0.16	0.95	0.91	8

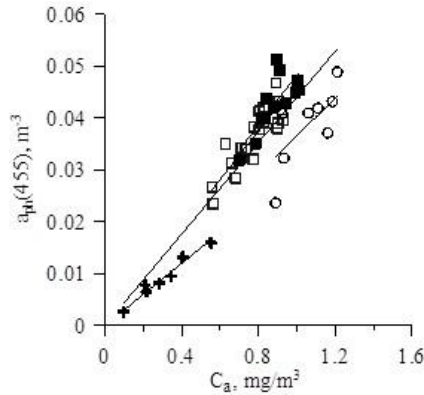
*Light absorption by phytoplankton pigments*

The value of the  $a_{ph}(\lambda)$  coefficient at 455 nm ( $a_{ph}(455)$ ), corresponding to the wavelength of fluorescence excitation in the MEGA-25 fluorimeter [22], varies depending on the chlorophyll *a* concentration (Fig. 4). The relationship between these parameters is described by a power function [8]

$$a_{ph}(455) = A(455) \cdot C_a^{B(455)}. \quad (10)$$

For the whole water column under the study (for the four selected layers generally), the equation (10) has the form as follows:

$$a_{ph}(455) = 0,045 C_a^{1,2}. \quad (11)$$



**Fig. 4.** Dependence of the phytoplankton pigments light absorption coefficient at the wavelength  $\sim 455$  nm ( $a_{\text{ph}}(455)$ ) on the chlorophyll  $a$  concentration ( $C_a$ ) for the layers distinguished in October, 2017 (symbols are the same as in Fig. 3)

The coefficient  $A(455)$  was obtained as  $0.045 \text{ m}^2 \cdot \text{mg}^{-1}$ . In fact, this coefficient corresponds to the chlorophyll  $a$  specific (normalized to  $C_a$ ) coefficient of light absorption by phytoplankton pigments at 455 nm wavelength ( $a_{\text{ph}}^*(455)$ ) in the case when  $C_a$  is equal to  $1 \text{ mg} \cdot \text{m}^{-3}$ . The coefficient  $a_{\text{ph}}^*(455)$  will increase with  $C_a$  growth if the power-law coefficient  $B(455)$  in equation (10) is higher than 1, or decrease if  $B(455)$  is less than 1.

The relationship between coefficients  $a_{\text{ph}}(455)$  and  $C_a$  was investigated for each designated layer. The coefficients  $A(455)$  and  $B(455)$  of the dependence described by equation (10) obtained for each layer are presented in Tab. 2.

**Table 2**

**Coefficients  $A(455)$  and  $B(455)$  in the dependence equation (10) and the correlation coefficient ( $r^2$ ) in the water layers**

Layer	$A(455)$	$B(455)$	$r^2$	Number of data pairs, $n$
1. Upper mixed layer	0.047	0.99	0.78	21
2. Thermocline	0.048	0.93	0.61	11
3. Layer between the thermocline and the depth of 1% of the sea surface PAR	0.036	1.25	0.66	9
4. Layer between the depths of the 1 and 0.1% of the sea surface PAR	0.031	1.01	0.97	8



The value of coefficient  $A(455)$  decreases with depth from the 1<sup>st</sup> to the 4<sup>th</sup> layer by ~ 35% (Tab. 2). The value of the power-law coefficient  $B(455)$  is close to 1 in almost all layers, with the exception of the 3<sup>rd</sup> layer. Taking into account the type of relationship between  $(a_{ph}(455))$  and  $C_a$  (equation 10), it was found out that for the range of variability of chlorophyll  $a$  concentration  $C_a$  noted in each layer, the  $a_{ph}^*(455)$  value remained almost unchanged and equal to coefficient  $A(455)$ . The  $a_{ph}^*(455)$  values had a significant difference (~ 35%) between the layers due to a decrease in  $A(455)$  coefficient from the 1<sup>st</sup> to the 4<sup>th</sup> layer (Tab. 2).

*Relationship between fluorescence intensity and phytoplankton pigments light absorption coefficient*

The values of the phytoplankton light absorption coefficient normalized to the chlorophyll  $a$  concentration  $a_{ph}^*(455)$  and fluorescence  $F_m^*, F_0^*$  decreased with depth from 0.047 to 0.031  $m^2 mg^{-1}$  and from 0.67 to 0.45  $mg^{-1}$ , from 0.27 to 0.16  $mg^{-1}$ , respectively (Tab. 3). A comparison of the depth-dependent variability in these parameter revealed the coincidence in their vertical distribution type.

Table 3

**Coefficient  $A(455)$  in equation (10) corresponding to the chlorophyll  $a$  specific phytoplankton pigments light absorption coefficient at the wavelength 455 nm ( $a_{ph}^*(455)$ ), and coefficient  $A$  corresponding to the specific fluorescence ( $F_m^*$  and  $F_0^*$ ) in the water layers**

Layer	$A(455),$ $m^2 mg^{-1}$	$A,$ $mg^{-1}$	
	$a_{ph}^*$	$F_m^*$	$F_0^*$
1. Upper mixed layer	0.047	0.67	0.27
2. Thermocline	0.048	0.66	0.25
3. Layer between the thermocline and the 1% of the sea surface PAR	0.036	0.61	0.22
4. Layer between the depths of the 1 and 0.1%-PAR penetration	0.031	0.45	0.16

The fluorescence intensity  $F_m$  and  $F_0$  were measured on a laboratory fluorimeter with a constant exciting light intensity. As result, the PAR value in equation (1) is taken constant. It should be noted that there are no closed RCs remaining after dark adaptation of phytoplankton carried out before measuring fluorescence  $F_0$ . The reabsorption coefficient  $Q_a^*$  can be neglected. Taking into account three statements mentioned above, the equation (1) will have the following form for  $F_0$ :

$$F_0 = C_a \cdot a_{ph}^*(455) \cdot \varphi_F. \quad (12)$$

The equation (12) is transformed by dividing by  $C_a$  into:

$$F_0^* = a_{ph}^*(455) \cdot \varphi_F. \quad (13)$$

Using the values of  $a_{ph}^*(455)$  and  $F_0^*$  (Tab. 3), obtained on average for each layer, the values of  $\varphi_F$  were calculated. In the first layer  $\varphi_F$  was 5.7; in the second – 5.1; in the third – 6.1 and in the fourth – 5.1.

### Discussion

The obtained relationships between the chlorophyll  $a$  concentration  $C_a$  and the phytoplankton pigments light absorption coefficient  $a_{ph}(\lambda)$  is consistent with the previously noted type and degree of change with depth, obtained on the basis of long-term biooptical investigations in the Black Sea [11, 23]. Along with this, the fluorescence intensity  $F$  is directly dependent on  $a_{ph}^*(\lambda)$  (equation (1)). These results (relationships and regularities) justify the depth-dependent variability in the coefficient ( $A$ ) of link between  $F$  and  $C_a$  within euphotic layer of the sea.

In the period of seasonal stratification of waters, provided that the thermocline is located within the euphotic zone, the ratio between fluorescence and chlorophyll  $a$  concentration varies with depth. Due to the acclimation of the phytoplankton community to the main environmental factors (temperature, nutrient availability and light intensity), the intracellular concentration of pigments and the ratio between them change, which leads to change of  $a_{ph}^*(\lambda)$  [7, 24, 25]. At the same time, with the adaptation of the phytoplankton community to changing conditions, the species composition of the community changes and, as a result, the pigment complex and the size structure of the phytoplankton community change. This is manifested in a change in the spectrum shape and the values of the  $a_{ph}^*(\lambda)$  coefficients [8, 23].

The obtained results showed that the use of a constant coefficient of the link between  $C_a$  and  $F$  (for the upper euphotic layer of the sea) in the Black Sea will lead to at least a 40% error in estimating the chlorophyll  $a$  concentration ( $C_a$ ) based on the fluorescence data ( $F$ ). The error is mainly ( $\sim 88\%$ ) due to the variability of the phytoplankton ability to absorb light quanta per unit of chlorophyll  $a$  ( $a_{ph}^*(\lambda)$ ). It should be noted that during the period of the research (October) the values of the  $PAR$  incident on the sea surface were less than its summer values [26, 11], and the UML was deeper than in summer period. The UML thickness and light conditions in this layer vary from the moment of formation of seasonal stratification of waters (late spring) to the UML deepening period (late autumn), which is associated with the annual solar insolation cycle of the sea [26] and the ratio between the UML and  $Z_{eu}$  thickness. Such changes lead to the fact that the intracellular concentration in the phytoplankton existing in the UML increases the proportion of accessory pigments decreases with a decrease in the average photosynthetic available radiation within the UML [24]. The noted changes in the pigment content in microalgae cells are accompanied by a decrease in  $a_{ph}^*(\lambda)$  values [7, 25]. Earlier, in [11], these parameters were compared in

the UML of deep-water part of the Black Sea during the winter and summer periods, which showed an almost 3-fold difference in the values in the blue part of the spectrum (at  $\sim 440$  nm).

Taking into account the influence of the UML thickness and the solar insolation of the sea surface on the value  $a_{ph}^*(\lambda)$  in the UML, the degree of depth-dependent variability of  $a_{ph}^*(\lambda)$  will change during the period of seasonal water stratification. It should be noted that the vertical variability  $a_{ph}^*(\lambda)$  will be maximum at the beginning of summer, when the maximum solar activity and the minimum thickness of the UML coincide in time, and the PAR value on average for the UML reaches  $1000 \mu E \cdot m^{-2} \cdot s^{-1}$  [27]. As a result, both the coefficient  $A$  in equation (7), which describes the relationship of  $F$  with  $C_a$  in the UML, and its change with depth in the euphotic layer will be maximum at the beginning of the summer period.

### Conclusions

The vertical variability of the relationship between the chlorophyll  $a$  concentration and its fluorescence during the period of seasonal waters stratification is shown. Four different water layers were identified within euphotic layer based on vertical distribution of the fluorescence intensity, temperature and photosynthetic available radiation. It was shown that using a constant coefficient of link between  $C_a$  and  $F$  (for the entire upper productive layer) in the Black Sea will lead to at least 40%-error in the  $C_a$  estimation based on  $F$ .

Due to the noted inconsistency of the relationship between the chlorophyll  $a$  concentration and its fluorescence, as well as the significant influence of environmental conditions on the relationship between the chlorophyll  $a$  concentration and the ability of phytoplankton to absorb solar quanta, it is planned to carry out further studies to estimate the chlorophyll  $a$  concentration based on the fluorescence data measured using BioArgo floats.

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**Olga V. Krivenko** – preparation of the article text, consultations on the article topic

**Dmitriy N. Matorin** – preparation of the article text, consultations on the topic of the article

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