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Influence of solar radiation on chlorophyll *a* concentration assessment using fluorescence measured by the submersible sensor in Lake Baikal

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ABSTRACT. Assessment of chlorophyll *a* concentration based on fluorescence intensity is actively used at present. In natural waters, profile fluorescence is measured using submersible sensors. These sensors are equipped with no special chamber for phytoplankton dark adaptation before measurement. Effect of irradiance in the upper layer leads to a decrease in chlorophyll *a* fluorescence due to closing some reaction centers of photosystem 2. The conducted research on Lake Baikal has revealed the relationship between the share of open reaction center in photosystem 2 and photosynthetically available radiation in the lake. The relationship between these parameters was described by an exponential function with a high determination coefficient ($r^2 = 0.97$). Based on the obtained relationship, an algorithm was developed to compensate for the decrease in chlorophyll *a* fluorescence intensity due to the light influence *in situ*. The algorithm enables to retrieve the "real" fluorescence profile, which is necessary for the correct retrieval of the vertical distribution of the chlorophyll *a* content.

Keywords: chlorophyll *a*, fluorescence, submersible sensor, photochemical and non-photochemical quenching, open and closed reaction centers

1. Introduction

Content of chlorophyll *a* (C_a), which is the main photosynthetically active pigment, is widely used as water trophic status and productivity indicator. At present, values of the chlorophyll *a* fluorescence measured in situ by submersible sensors are used for the analysis of temporal and spatial C_a variability as well as for validation of remote sensing data (C_a) (Odermatt et al., 2012; Xing et al., 2012; Wojtasiewicz et al., 2018).

In situ fluorescence intensity (F) depends on a number of variables: the photosynthetically available radiation (PAR, μmol/m^2/s); the C_a values (in mg/m^3) and phytoplankton functional characteristics, in particular, the chlorophyll *a* specific light absorption coefficient of phytoplankton ($a^*_{ph}(\lambda)$ m^2/mg), the quantum yield of fluorescence (φ_F, mol emitted quantum (mol absorbed quantum)$^{-1}$) and fluorescence intracellular reabsorption factor (Q^*_a, dimensionless) (Babin, 2008).

Submersible fluorescence sensors that measure in situ the chlorophyll *a* fluorescence intensity (F_{CTD}) are not equipped with a special chamber for adapting phytoplankton to the dark (so-called "dark chamber"). Due to this technical peculiarity, the sensors can measure the fluorescence of phytoplankton adapted to the environmental conditions, in particular, light intensity. In this case, some of the reaction centers (RC) of photosystem 2 are in an inactive state. These inactive RC are closed for acceptance of the electron, pathing the electron transport chain (Govindjee et al., 1990). It results in decreased F values (Pogosyan and Matorin, 2005) due to the effect of light only but not C_a (Falkowski and Raven, 2007).

Correct assessment of chlorophyll *a* concentration via fluorescence requires additional processing of the fluorescence signal to compensate the decreasing effect of PAR on the fluorescence intensity, which is especially obvious in the upper part of the euphotic layer, i.e. in the upper mixed layer (UML).

Quasi-synchronous in situ measurements of temperature, fluorescence and PAR profiles as well as pulse amplitude modulation (PAM) fluorescence parameters were carried out during the scientific cruise on Lake Baikal in September 2019. The obtained results represent the required scientific basis, allowing an attempt for a correction of the PAR influence on the fluorescence intensity measured by the submersible sensor.

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Therefore the aim of this research is to investigate in situ the effect of PAR on the fluorescence intensity and PAM fluorescence parameters; to develop a general algorithm for restoring the fluorescence affected by PAR and test the algorithm for Lake Baikal.

2. Methods

The research was carried out in different areas of Lake Baikal (Fig. 1) during the scientific cruise onboard the RV "Titov" on September 3-11, 2019. The measurements were fulfilled at 21 stations during daylight time (from 7:00 AM to 7:00 PM).

The vertical profiles of temperature, salinity, density, fluorescence (chlorophyll *a* concentration), and photosynthetically available radiation (PAR) were measured by a JFE Rinko AAQ-177 water quality probe (Japan) (Table 1).

The measured parameters (downcast data) were displayed in real time on the screen of a laptop.

The submersible F sensor provided the fluorescence measurements of the sample (phytoplankton) adapted to the environment, i.e. the light intensity at the sampling depth (z) - PAR(z).

For the laboratory measurements, water samples were collected from different depths within the UML using Niskin bottles. PAM fluorescence parameters were measured with laboratory fluorometer "Smart", which was developed in Moscow State University, Biophysical Department (Konyukhov et al., 2017). The fluorescence intensity due to colored dissolved organic matter (F_{CDOM}) was used as a background fluorescence, which was subtracted from each sample measurement (Moiseeva et al., 2018). For measurement of F_{CDOM}, the sample was filtered through a membrane filter (Sartorius), which was prerinsed with 50 ml of deionized water. Before measurements, the samples were adapted to the dark for 15-30 minutes (Gaevsky and Morgun, 1993). The laboratory fluorometer "Smart" provided the measurements of maximum (F_m) and minimum (F_0) fluorescence intensity of the sample: the F measured at 0 μmol/m²/s (dark measurement) corresponds to the parameter F_0, when all RC are open; the F measured at saturating light flash – F_m, when all RC are closed (Schreiber et al., 1994; Matorin et al., 2012). Using laboratory fluorometer, we measured the dependence of the fluorescence parameters (F_0, F_t, F_m and $F`_m$) on the light intensity, varying from 0 to 1000 μmol/m²/s (90 s light adaptation to each light intensity before measurements). The F value measured at light intensity PAR(z) (the sampling depth) was denoted as F_t (steady-state fluorescence). The difference between the maximum F value measured after continuous illumination ($F`_m$) and $F_t$ is proportional to the number of open RC (Schreiber et al., 1994; Matorin et al., 2012). The relative amount of open RC in phytoplankton that exists at a depth –z with radiance – PAR(z) can be assessed based on parameters $F_0$, $F_t$, $F_m$ and $F`_m$ according to (Falkowski and Kiefer, 1985):

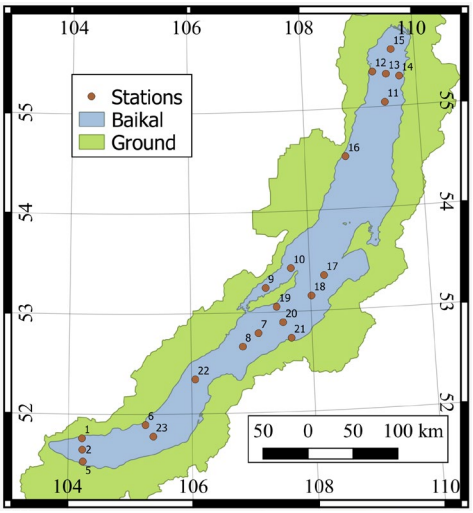

Fig.1. Map of the stations (●) that were investigated during the scientific cruise onboard the RV "Titov" on Lake Baikal on September 3-11, 2019

$$d_{open} = \frac{F'_m - F_t}{F_m - F_0} \qquad (1)$$

3. Results and discussion

Due to different cloudiness and different daytime of sampling, the solar radiance incidence on the lake surface varied from ~200 to 1950 μmol/m²/s (Table 2). The UML depth changed from 3 m to 19 m between almost all stations, except for three stations with more shallow UML (< 3 m) and one station with deepest UML (21m) (Table 2).

Table 1. Characteristics of the JFE Rinko AAQ-177 water quality probe (Japan) and sensors equipped

Measured parameter	Measurement range	Resolution	Accuracy	Response time
Depth	0 - 100 m	0.002 m	±0.3% of full scale	0.2 s
Water temperature	-3 - 45 °C	0.001 °C	±0.01 °C (0 - 35 °C)	0.2 s
Fluorometer	0 to 400 ppb (Uranin reference)	0.01 ppb	±1% of full scale	0.2 s
Photosynthetically available radiation in water	0 – 5000 μmol/m²/s	0.1 μmol/m²/s	±4%	0.2 s

For the UML, we have revealed a relationship between the d_{open} and the intensity PAR_z (Fig. 2) and described it by the exponential function:

$$d_{open} = A \cdot e^{-0.0025 \times PAR}, \; n = 42, \; r^2 = 0.97 \quad (2)$$

where the coefficient A = 1 since all RC are open (d_{open} = 1) in the dark (PAR = 0).

The fraction of closed RC, which do not contribute to the fluorescence intensity recorded by the CTD probe, can be calculated using equation 6:

$$d_{closed} = 1 - d_{open} \quad (3)$$

Consequently, the F value of closed RC (potential fluorescence, F_{closed}), which is not detected by a submersible sensor (without a dark chamber), can be calculated as follows:

$$F_{closed}(z) = F_{CTD}(z) \times d_{closed} \quad (4)$$

To compensate for the decreasing effect of PAR and restore the fluorescence profile, it is necessary to take into account the value of $F_{closed}(z)$:

$$F_{real} = F_{CTD} + F_{closed}, \quad (5)$$

where F_{real} is the fluorescence intensity provided that all RC are in the open state.

Based on equations 4 and 5, we get equation 6:

$$F_{real}(z) = F_{CTD}(z) + \left(F_{CTD}(z) \times (1 - e^{-0.0025 \times PAR_z})\right) \quad (6)$$

which is transformed into equation 7:

$$F_{real}(z) = F_{CTD}(z) \times (2 - e^{-0.0025 \times PAR_z}) \quad (7)$$

The fluorescence profiles were restored at all stations based on the obtained relationship (equation 7). Figure 3 shows the restored F profiles, which were measured in different day-time. We have revealed that F_{real} exceeds F_{CTD} within the UML at all stations. The relative differences between F_{real} and F_{CTD} (ΔF) reflect a decrease in chlorophyll *a* fluorescence intensity in the upper layer due to light effect. We have correlated the ΔF values with the PAR_0 (Table 2).

Early in the morning, when PAR_0 was 11 μmol/m²/s, there was almost no effect of light on F. At noon, under a cloudless sky, PAR_0 reached 1950 μmol/m²/s. In this case, the F values in the surface layer decreased ~ 2 times (Table 2).

Correct assessment of C_a based on the fluorescence measured by the submersible sensor requires the compensation of the PAR effects of on the chlorophyll *a* fluorescence. This problem remains relevant to date (Barbieux et al., 2019). To solve this problem, it was proposed to use chlorophyll *a* fluorescence profiles measured at night only when there is no PAR effect (Wojtasiewicz et al., 2018). However, this approach

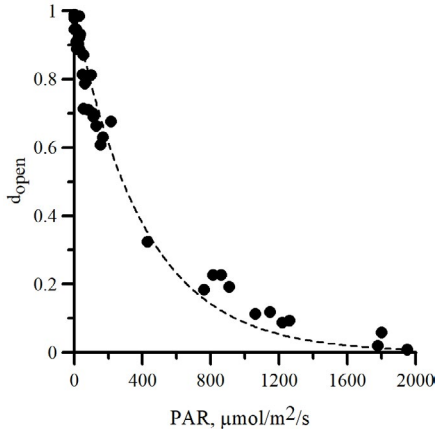

Fig.2. Dependence of the fraction open reaction centers (dopen) on light intensity (PAR) in the UML layer of Lake Baikal on September 3-11, 2019

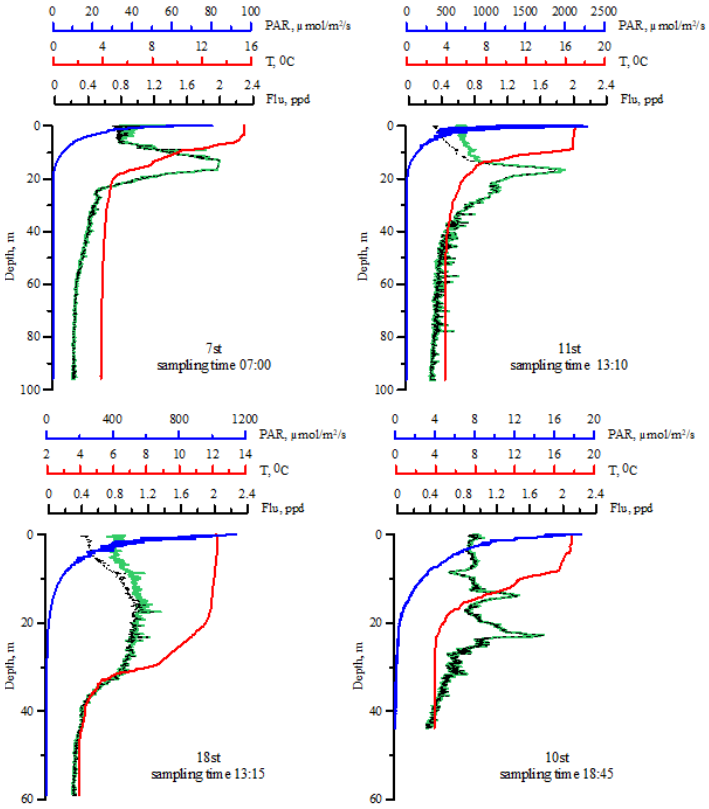

Fig.3. Vertical profiles: photosynthetically available radiation (PAR, blue line), temperature (T, red line), fluorescence intensities (F) measured by the submersible sensor (black line) and F, which was reconstructed, taking into account the light intensity (green line) in Lake Baikal on September 3-11, 2019

significantly reduces the data set for analyzing the spatial and temporal variability of C_a and limits the use of this data (nightly) for validation of satellite data (daily). In this study, the observed two-fold decrease in F under the influence of solar insolation evokes one doubt about the validation of satellite data, according to BioArgo floats, without this correction of the light effect on fluorescence (Kubryakov et al., 2017).

Table 2. The effect of the developed algorithm for compensation of chlorophyll *a* fluorescence intensity (F) decreased under the surface photosynthetically available radiation (PAR_0): increased F in the surface layer ($\Delta F = (F_{real} - F_{CTD})/F_{CTD}$) and averaged within the upper mixed layer (UML) (ΔF_{UML}) at the stations on Lake Baikal in September 2019.

Station no.	date	sampling time	UML, m	PAR_0, µmol/ m^2/s	$\Delta F = (F_{real} - F_{CTD})/F_{CTD}$, %	ΔF_{UML}, %
1	03.09.2019	16:45	-	1260	72	-
2	04.09.2019	07:05	3	30	7	5
5	04.09.2019	10:35	5	810	83	74
6	04.09.2019	18:35	-	110	18	-
7	05.09.2019	07:00	3	80	14	12
8	05.09.2019	09:35	5	910	75	69
9	05.09.2019	15:30	-	1780	82	-
10	05.09.2019	18:45	4	20	4	3
11	06.09.2019	13:10	8	1800	96	78
12	06.09.2019	16:10	4	1060	65	61
13	06.09.2019	17:40	4	170	23	17
14	07.09.2019	08:05	19	760	69	20
15	07.09.2019	13:35	8	170	30	19
16	08.09.2019	15:40	6	860	72	56
17	09.09.2019	11:05	6	430	55	49
18	09.09.2019	13:15	18	1150	87	33
19	09.09.2019	16:15	10	1220	87	44
20	09.09.2019	18:10	2	60	10	9
21	10.09.2019	07:00	28	110	15	3
22	11.09.2019	07:00	12	10	2	1
23	11.09.2019	12:10	16	1950	74	45

Conclusions

In this study, we have developed an algorithm that compensates a decrease in fluorescence intensity due to the light influence in situ. The algorithm enables to retrieve the "real" fluorescence profile, which is required for the correct retrieval of the vertical distribution of the chlorophyll *a* content as the main photosynthetically active pigment. The upper layer of water, being most illuminated, makes the main contribution to the primary productivity of the water column. Consequently, the correction of the C_a estimation affects the accuracy of the primary productivity assessment.

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References

Babin M. 2008. Phytoplankton fluorescence: theory, current literature and in situ measurement. In: Babin M., Roesler C., Cullen J. (Eds.), Real-time coastal observing systems for marine ecosystem dynamics and harmful algal blooms. Paris: UNESCO Publishing, pp. 237-281.

Barbieux M., Uitz J., Gentili B. et al. 2019. Bio-optical characterization of subsurface chlorophyll maxima in the Mediterranean Sea from a Biogeochemical-Argo float database. Biogeosciences 16: 1321-1342. DOI: 10.5194/ bg-16-1321-2019

Falkowski P., Kiefer D.A. 1985. Chlorophyll *a* fluorescence in phytoplankton: relationship to photosynthesis and biomass. Journal of Plankton Research 7: 715-731. DOI: 10.1093/plankt/7.5.715

Falkowski P.G., Raven J.A. 2007. Aquatic photosynthesis. Princeton and Oxford: Princeton University Press.

Gaevsky N.A., Morgun V.N. 1993. Using variable and slow chlorophyll fluorescence to study plant photosynthesis. Fiziologija Rastenij [Plant physiology] 40: 119-127. (in Russian)

Govindjee M., Van de Ven C., Preston C. et al. 1990. Chlorophyll *a* fluorescence lifetime distributions in open and closed photosystem II reaction center preparations.

Biochimica et Biophysica Acta 1015: 173-179. DOI: 10.1016/0005-2728(90)90017-X

Kirk J.T.O. 2011. Light and photosynthesis in aquatic ecosystems. Third edition. Cambridge: Cambridge University Press.

Konyukhov I.V., Vorobyova O.V., Pogosyan S.I. et al. 2017. Fluorometer with pulse modulation of exciting light for estimating the functional state of natural phytoplankton by chlorophyll fluorescence parameters. In: II Vserossijskaja nauchnaya konferenciya molodyh uchenyh "Kompleksnye Issledovanija Mirovogo Okeana", pp. 345-347. (in Russian)

Kubryakov A.A., Stanichny S.V., Kubryakova E.A. 2017. Variability of the bio-optical characteristics of the Black Sea from measurements of Bio-Argo buoys and satellite data. In: II Vserossijskaja nauchnaya konferenciya molodyh uchenyh "Kompleksnye Issledovanija Mirovogo Okeana", pp. 130-131. (in Russian)

Matorin D.N., Osipov V.A., Rubin A.B. 2012. Metodika izmereniy obiliya i indikatsii izmeneniya sostoyaniya fitoplanktona v prirodnykh vodakh fluorestsentnym metodom. Teoreticheskiye i prakticheskiye aspekty. Moskow: Al'teks. (in Russian)

Moiseeva N., Churilova T., Efimova T. et al. 2018. Dependence of fluorescence intensity on chlorophyll *a* concentration and light absorption coefficients by phytoplankton in the Black Sea (October 2017). Proceedings of the SPIE 10833. DOI: 10.1117/12.2505415

Odermatt D., Pomati F., Pitarch J. et al. 2012. MERIS observations of phytoplankton blooms in a stratified eutrophic lake. Remote Sensing of Environment 126: 232-239. DOI: 10.1016/j.rse.2012.08.031

Pogosyan S.I., Matorin D.N. 2005. Variability in the condition of the photosynthetic system of the Black Sea phytoplankton. Oceanology 45: 139-148.

Schreiber U., Bilger W., Neubauer C. 1995. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In: Schulze E.D., Caldwell M.M. (Eds.), Ecophysiology of photosynthesis. Berlin, Heidelberg, pp. 49-70. DOI: 10.1007/978-3-642-79354-7_3

Suggett D.J., Borowitzka M.A., Prášil O. 2010. Chlorophyll *a* fluorescence in aquatic sciences: Methods and applications. Dordrecht: Springer. DOI: 10.1007/978-90-481-9268-7

Wojtasiewicz B., Hardman-Mountford N.J., Antoine D. et al. 2018. Use of bio-optical profiling float data in validation of ocean colour satellite products in a remote ocean region. Remote Sensing of Environment 209: 275-290. DOI: 10.1016/j.rse.2018.02.057

Xing X., Morel A., Claustre H. et al. 2012. Combined processing and mutual interpretation of radiometry and fluorometry from autonomous profiling Bio-Argo floats: 2. Colored dissolved organic matter absorption retrieval. Journal of Geophysical Research: Oceans 117. DOI: 10.1029/2011jc007632