PROCEEDINGS OF THE YEREVAN STATE UNIVERSITY
УЧЕНЫЕ ЗАПИСКИ ЕРЕВАНСКОГО ГОСУДАРСТВЕННОГО УНИВЕРСИТЕТА
Geology and Geography **60**(2), 2026 ***Геология и география***

Geography

УДК 581.1:551.468.3

PARAMETERS OF CHLOROPHYLL FLUORESCENCE INDUCTION CURVES TO STUDY WHITE SEA PHYTOPLANKTON AND ITS SENSITIVITY TO H_2S

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This paper briefly discusses the potential for using high-temporal-resolution chlorophyll fluorescence induction curves to assess the state of natural phytoplankton in meromictic waters of the White Sea and its resistance to H_2S.

https://doi.org/10.46991/PYSUC.2026.60.2.316

Keywords**:** White Sea, phytoplankton, hydrogen sulfide, chlorophyll fluorescence, photosynthesis, ecology.

Introduction. The activity of natural phytoplankton in the presence of adverse factors is actively studied using chlorophyll fluorescence. This method is characterized by its noninvasiveness and high sensitivity [1–6]. It is based on the fact that chlorophyll, located in the thylakoid membranes of microalgae, is capable of emitting fluorescence quanta and serves as a natural sensor of algal cell state [7–10]. Previously, we designed a submersible probe-fluorometer to study natural phytoplankton, which was used in various regions of the World Ocean [1, 2, 6].

This article briefly reviews the results of applying the high-temporal-resolution fluorescence induction curve method to assess the state of natural phytoplankton in meromictic reservoirs of the White Sea in the autumn of 2024–2025. The sensitivity of the light-induced photosynthesis processes of natural cryptophyte algae *Rhodomonas sp.* to the effects of H_2S at different concentrations was studied.

Materials and Methods. The study objects were coastal meromictic reservoirs of the White Sea, isolated from the sea to varying degrees [4, 5, 7]. In the autumn of 2024, pronounced water stratification was observed in these reservoirs, with a surface aerobic layer subject to wave and convective mixing (mixolimnion)

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and a lower anaerobic layer, where mixing does not occur (monimolimnion). Between these layers, a chemocline zone (10–20 *cm*) with sharp physicochemical gradients was formed [4, 7]. Colored layers caused by the massive development of bright red or green phototrophic microorganisms were detected in the chemocline zone. The methods for sampling and determining the physicochemical parameters of water have been described previously [7].

Fluorescence induction curves (OJIP curves) were recorded using an Aquapen-C-100 fluorometer ("Photon System Instruments", Brno, Czech Republic) with excitation by light with a wavelength of 455 *nm* and an intensity of 3000 μmol photons $m^2 \cdot s^{-1}$ for 2 *s* [1]. Fluorescence intensities at different times were used to calculate fluorescence parameters from the induction curves using the JIP test, according to [9, 10]. The sensitivity of the method allowed for direct analysis of natural phytoplankton populations without preliminary enrichment.

Results and Discussion. Recently, methods for measuring fluorescence induction curves with high temporal resolution (from 20 μs) under excitation by intense light using PEA-type instruments have been developed to evaluate the performance of the photosynthetic apparatus of algal cultures. The fluorescence intensity was determined at different sections of the induction curves and the photosynthetic parameters were calculated using the standard JIP test [10]. This test uses the following characteristics of the induction curve: fluorescence intensities at 50 μs (E_O), 2 *ms* (F_J), 30 *ms* (F_I), 6 *s* (F_{6s}), and F_M (maximum fluorescence intensity). These values are used to analyze the characteristics of the primary processes of photosynthesis: F_V/F_M is the maximum quantum yield of the primary photochemical reaction in photosystem II (PSII); V_J is the number of closed reaction centers (RC) in relation to the total number of RC; φE_O is the quantum yield of electron transport beyond Q_A; ABS/RC is the energy flux absorbed by one active reaction center; PI_{ABS} is the productivity index and DI_0/RC is the total amount of energy dissipated by one RC as heat. A more detailed description of the JIP test parameters is given in [1, 9, 10]. Based on the results of chlorophyll fluorescence measurements, the Department of Biophysics of the Biological Faculty of Moscow State University developed the "Methodology for measuring the abundance and indicating changes in the state of phytoplankton in natural waters using the fluorescence method" (FR.1.39.2011.11246, PND F 14.2.268-2012) [1]. The method is approved for the purposes of state environmental control under the section "quantitative chemical analysis of waters". The initial fluorescence intensity E_O is recommended for assessing the biomass and growth rate of algae, and the ratio of variable to maximum fluorescence (F_V/F_M) is recommended for assessing the quantum yield of photosynthesis, which is a measure of the physiological activity of algae.

Fluorescence methods were used to study natural phytoplankton in 2024 in the stratified Lake Kislo-Sladkoe and the meromictic lagoon at Cape Verde in the White Sea. Phytoplankton abundance was assessed using F_O [6], and the functional state of the algal photosynthetic apparatus was assessed using JIP test parameters [10]. It was shown that in 2024, a red layer of water with chlorophyll a concentrations of 4.2 $\mu g/L$ and 18 $\mu g/L$, respectively, was observed in the chemocline (at the boundary between the aerobic and anaerobic zones) of Lake Kislo-Sladkoe at a depth of 2.3 *m* and the lagoon at Cape Verde at a depth of 4.8 *m*. Cryptophyte algae (*Rhodomonas* sp.) dominated this water layer, displaying high activity of light-induced photosynthetic reactions, similar to those observed in algal cultures maintained under optimal conditions and well-supplied with nutrients. Maximum quantum yields ranged from 0.7 to 0.73.

The prevalence of cryptophyte algae (*Rhodomonas* sp.) was determined using fluorescence induction parameters. High functional activity of PSII (PI_{ABS}) was recorded, expressed through high values of the quantum yield of PSII photochemistry F_V/F_M, the proportion of active reaction centers (ABS/RC), and the quantum yield of electron transport in PSII (φE_O). Such high functional activity of the photosynthetic apparatus in the presence of hydrogen sulfide may indicate the cryptophyte algae's resistance to the toxic effects of H_2S. Phytoplankton exhibited lower photosynthetic activity in the surface water layer.

The effect of H_2S at different concentrations on the light-induced photosynthesis of natural cryptophyte microalgae *Rhodomonas* sp. was studied using chlorophyll fluorescence induction curves. It was shown that exposure to H_2S (8 *mg*/*L*) decreased the relative maximum electron transport rate (ETR_{max}) and the maximum light energy utilization coefficient (α), while non-photochemical fluorescence quenching (NPQ), associated with a relative increase in the dissipation of excess energy, increased. Analysis of fluorescence induction curves revealed that incubation of microalgae for 10 *min* with H_2S at concentrations above 2.3 *mg*/*L* significantly reduced the functional activity of PSII (PI_{ABS}), expressed as high values of the quantum yield of PSII photochemistry (F_V/F_M), the proportion of active reaction centers (ABS/RC), and the quantum yield of electron transport in PSII (φE_O). A decrease in electron transport is accompanied by an increase in the dissipation of unused light energy (DI_0/RC). H_2S at concentrations above 2.3 *mg*/*L* reduced the amplitude of all phases, primarily shortening the I-P phase, indicating disruption of electron transport in the plastoquinone-cytochrome B_6 complex between photosystems.

Thus, natural *Rhodomonas* sp. microalgae experience stress when exposed to 2.3 *mg*/*L* H_2S, which is significantly higher than the concentrations typical for the chemocline and anaerobic zones of water bodies, where *Rhodomonas* sp. are found.

The use of fluorescence methods is proposed for studying the state of phytoplankton in coastal meromictic water bodies with varying H_2S concentrations.

Поступила 01.06.2026
Получена с рецензии 18.06.2026
Утверждена 26.08.2026

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ՔԼՈՐՈՖԻԼԻ ՖԼՈՒՈՐԵՍՑԵՆՑԻԱՅԻ ԻՆԴՈՒԿՑԻԱՅԻ ԿՈՐԵՐԻ ՊԱՐԱՄԵՏՐԵՐԸ ՍՊԻՏԱԿ ԾՈՎԻ ՖԻՏՈՊԼԱՆԿՏՈՆԻ ԵՎ ԴՐԱ H_2S-Ի ՆԿԱՏՄԱՄԲ ԶԳԱՅՈՒՆՈՒԹՅԱՆ ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅԱՆ ՀԱՄԱՐ

Ամփոփում

Այս հոդվածում համառոտ քննարկվում է բարձր ժամանակային լուծաչափով քլորոֆիլի ֆլուորեսցենցիայի ինդուկցիայի կորերի օգտագործման ներուժը՝ Սպիտակ ծովի մերոմիկտիկ ջրերում բնական ֆիտոպլանկտոնի վիճակը և դրա H_2S-ի նկատմամբ դիմադրողականությունը գնահատելու համար:

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ПАРАМЕТРЫ КРИВЫХ ИНДУКЦИИ ФЛУОРЕСЦЕНЦИИ ХЛОРОФИЛЛА ДЛЯ ИЗУЧЕНИЯ ФИТОПЛАНКТОНА БЕЛОГО МОРЯ И ЕГО ЧУВСТВИТЕЛЬНОСТИ К H_2S

Резюме

В данной статье кратко обсуждается потенциал использования кривых индукции флуоресценции хлорофилла с высоким временным разрешением для оценки состояния природного фитопланктона в меромиктических водоемах Белого моря и его устойчивости к H_2S.