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Investigation on the Chlorophyll-*a* Content of Phytoplankton in the Sea of Azov and the Don River by the Fluorescence Method

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Abstract

Results of in situ fluorescence investigations on chlorophyll-*a* (chl-*a*) of phytoplankton in the Sea of Azov (May to June 2018) and in the Don River estuary (September to November 2019) are presented. Continuous data series of chl-*a* fluorescence were obtained with flow-through fluorometers. Discrete reference measurements of chl-*a* concentration and phytoplankton biomass were performed by standard methods for the sake of comparison and eventual corrections. The fluorescence intensity values measured in a lateral surface salinity gradient of the Sea of Azov were found correlating with the obtained data on the chl-*a* concentration ($R^2 = 0.88$, $n = 27$) and phytoplankton biomass ($R^2 = 0.90$, $n = 11$). Instead, there was a weak correlation ($R^2 = 0.40$, $n = 33$) between the fluorescence and phytoplankton biomass found in the estuary. This disparity in correlations was explained by the difference in conditions during measurements, which affected the fluorescence. There were no significant changes in both temperature and coenotic composition of phytoplankton in the course of the marine expedition. The measurements on the river covered a period characterized by the seasonal variations in the phytoplankton composition and by noticeable temperature fluctuations.

Keywords: chlorophyll-*a*, intravital fluorescence, chlorophyll-*a* concentration, biomass and coenotic composition of phytoplankton, the sea of Azov, the Don River

1. Introduction

Fluorescence methods for chlorophyll-*a* (chl-*a*) estimation are widely used in modern natural sciences, including hydrobiology. Despite the rapid development of satellite methods for the assessment of phytoplankton concentration in natural water, including case II waters (i.e., turbid and productive) in the Sea of Azov [1], fluorescence methods are used for the correction of satellite imagery [2] and as substantive assessment methods for phytoplankton distribution [3, 4].

Standard methods for determining chl-*a* concentration in water are based on light absorption [5, 6]; they are time-consuming and require samples in large quantities. Initially, fluorescence was only used to determine the chl-*a* concentration in extracts [7]. Later on, the technology advancement has made modern fluorometers became not inferior to spectrophotometers in the accuracy of the pigment content determination [8–10]. Nowadays, the high speed and ease of measurements, good sensitivity, and a variety of instrumentations are the undoubted advantages of fluorescence methods [8, 11].

Recently, scientists have been expressing a growing interest in the in situ fluorescence studies of chl-*a*, which is the main plant pigment. In contrast to solutions, the fluorescence intensity of living chlorophylls depends not only on their concentration in microalgae but also on the state of the photosynthesis system [12]. The specific yield of fluorescence depends on the state of the photosynthesis electron transport chain and can be significantly reduced by spending a fraction of the absorbed energy on photochemical reactions. In living phytoplankton, not all absorbed light energy goes into optical emission. In all situations, living organisms containing chlorophyll strive to maximize the use of absorbed light energy for synthesis and return only the excess of it in the form of radiation. Photosynthetic activity depends on the condition of living cells, which is determined by the parameters of the environment at a given moment and by the previous state [13]. The higher is the photosynthetic capacity of microalgae, the lower is the specific yield of fluorescence.

The fluorescence of living chlorophylls depends on temperature [14] and illumination [15]. Both have to be controlled for eventual corrections of the measurements considering the inertia the photosynthetic system demonstrates at rearrangement. The dissolved oxygen, acidity [16], dissolved salts [16, 17], and other abiotic factors of the aquatic environment all have an impact on fluorescence [16]. Ultimately, researchers are mostly interested in the quantity of biomass rather than in the intensity of fluorescence [18, 19]. To get reliable estimations of biomass, it is necessary to consider not only the environmental parameters mentioned above but also changes in microalgae species composition [19].

Despite the mentioned difficulties in quantitative analysis of phytoplankton distribution, fluorescence methods are widely used for its assessment in either fresh or salt waters. Among monitoring methods a special place is occupied by vessel surveys using a continuous vessel-based horizontal and vertical profiling [20]. Vessels allow to collect large datasets with a precise time and geo-referencing. While the volume of measured data is comparable to that provided by satellites, it significantly exceeds in accuracy and detail [20]. Fluorometers have a clear advantage over satellites allowing for continuous measurements of chl-*a* concentration during day-and-night cycles. Measurements can be automated, and data can be transmitted in real time, enabling quick adjustments of the research program according to findings. A regular sampling on-board for subsequent measurements in an on-ground laboratory is a common practice during vessel expeditions [2, 20, 21]. Collected samples allow for further in-depth analysis with identification of the composition of phytoplankton species [19].

A number of studies carried out in the most developed areas of the World Ocean by using continuous series of fluorescence data enabled a deeper inquiry into the seasonal and spatial dynamics of phytoplankton in aquatic ecosystems. The findings were used for the prediction of the productivity of waters and their conditions under climate change and anthropogenic pressure [22]. Monitoring of phytoplankton is essential for planning actions against the growth of harmful algal blooms. It is possible to determine the bio-productivity of water based on chl-*a* concentration [23]. In addition, fluorescence-based monitoring of water quality in rivers permits a

rapid response to bio-induced deviations of its parameters at water-treatment sites for tap-water distribution networks [24].

In the Taganrog Bay of the Sea of Azov, the phytoplankton concentration was estimated from the in situ chl-*a* fluorescence measurements for the first time in June 2008 [25]. The studies continued then in 2014–2016, thanks to the grant (RFMEFI60714X0059) from the Russian Ministry of Science and Education. In July 2016, continuous measurements of chl-*a* concentration and of dissolved oxygen in the surface layer of the Sea of Azov were performed on the research vessel (R/V) “Deneb” using a custom designed fluorometer [26] in order to justify the new method for calculation of gross primary production based on satellite data [27]. Simultaneously, the flow-through fluorometer, which later has been employed for the present study, was developed and installed into the vessel’s seawater system for the tests [28]. They concluded successfully and the device is still in operation. The experience gained about the development and operation of flow-through fluorometers in case II waters with complex optical properties determined the goals and objectives of this work.

In this contribution we report on the complex studies of spatial distribution and temporal dynamics of phytoplankton in the Sea of Azov and the Don River, where significant variations have been noted previously. The main task was to establish the relationship between the parameters of the aquatic environment, the total biomass, the phytoplankton species composition, and chl-*a* fluorescence measurements. It was required to determine the range of applicability of flow-through fluorometers operating on vessels and at permanent stations. To foresee the changes in water quality in the studied waters, it is necessary to understand the main trends that affect the state of microalgae.

2. Materials and methods

The empirical data for analysis were collected during the Southern Scientific Center of the Russian Academy of Sciences (SSC RAS) expedition on R/V “Deneb” to the Sea of Azov in May to June 2018 and directly from the natural water intake point of JSC “Rostovvodokanal” within the confines of Rostov-on-Don City in September to November 2019 (**Figure 1**).

The in situ fluorescence of chl-*a* in phytoplankton was measured using flow-through fluorescence sensors developed in the SSC RAS. The sensors of a similar design [29] were installed on R/V “Deneb” and on the intake of the water supply system of JSC “Rostovvodokanal.” The installations were different in water pumping systems and in customized adaptation to the location and operation conditions. Similar to other studies, fluorescence of chl-*a*, which is the most abundant pigment in microalgae, was registered. The excitation light had a maximum intensity at a wavelength of 447.5 nm with a bandwidth of 20 nm [30]. The source was a set of powerful LEDs equipped with the SZS-20 stained-glass filters. The fluorescence emission was cut out in the 650–790 nm range by a combined filter and was detected by a silicon photodiode. The signal was amplified, digitized, and averaged over a set of pulses. Taking into account the well-known temporal behavior of fluorescence [16, 31], the excitation was performed by 10 µs pulses with a current-stabilized power.

The intake for seawater submitted to the flow system of the fluorometer on-board of R/V “Deneb” was situated at a depth of 0.5 m, while that of JSC “Rostovvodokanal” for the water supply system was located at the river bottom horizon at least 1 m from the bottom.

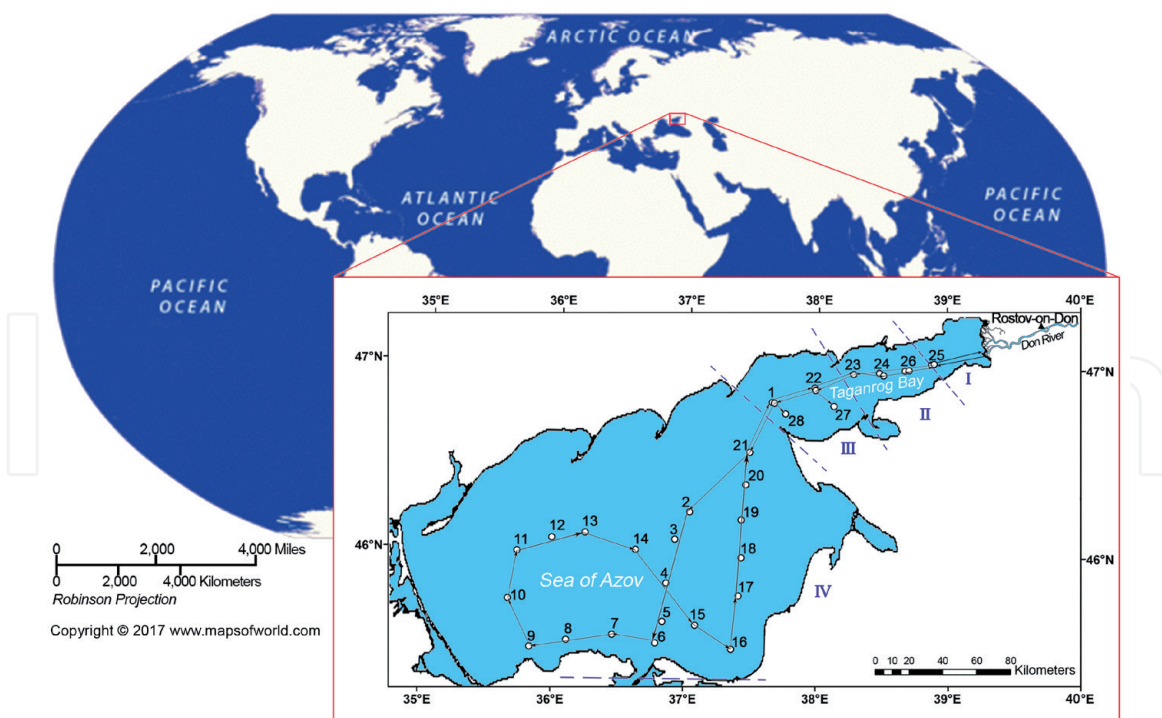


Figure 1.

Schematic map of sampling stations in May to June 2018 in the sea of Azov. Designations: 1–26, station numbers (arrows indicate the route of the R/V “Deneb”); I–IV, conditional division of the sea into four areas, eastern (I), central (II), western (III) areas of the Taganrog Bay, and central part of the sea (IV).

The continuous series of fluorescence measurements on living chl-*a* were transmitted via satellite and cellular channels to the server of SSC RAS and then presented on the website <http://meteo.ssc-ras.ru>. For the sake of calibration, a set of samples was collected at the stations, and the chl-*a* content was determined by the standard method [6] using an Evolution 3000 double-beam spectrophotometer. Another set of samples from the stations was used for the quantitative and qualitative analysis of phytoplankton composition according to the previously reported method [32]. The vertical profile of temperature and electrical conductivity of water was determined with the SBE 19 oceanographic probe and the horizontal distribution of thermohaline characteristics along the vessel’s route using SBE 21 thermosalinograph.

3. Results and discussion

3.1 Chlorophyll-*a* concentration and phytoplankton development in the Sea of Azov under a salinity gradient condition research result

The fluorescence values of phytoplankton chl-*a* measured in situ during the expedition to the Sea of Azov were confined in the range from 4.0 to 10.8 a.u. The corresponding concentrations of chl-*a* were estimated by spectrophotometry of the extracts and ranged from 1.2 to 30.8 µg/l, respectively. A continuous series of fluorescence values and spatial changes in the concentration of chl-*a* measured in situ during the expedition are presented in **Figure 2**.

Figure 2A shows that fluorescence decreases from the level of 5.0–10.8 a.u. in the Taganrog Bay to the level of 4.0–8.0 a.u. in the central part of the Sea of Azov. The values of chl-*a* concentration at complex stations have a similar distribution pattern across the water as presented in **Figure 2B**. The fluorescence intensity and

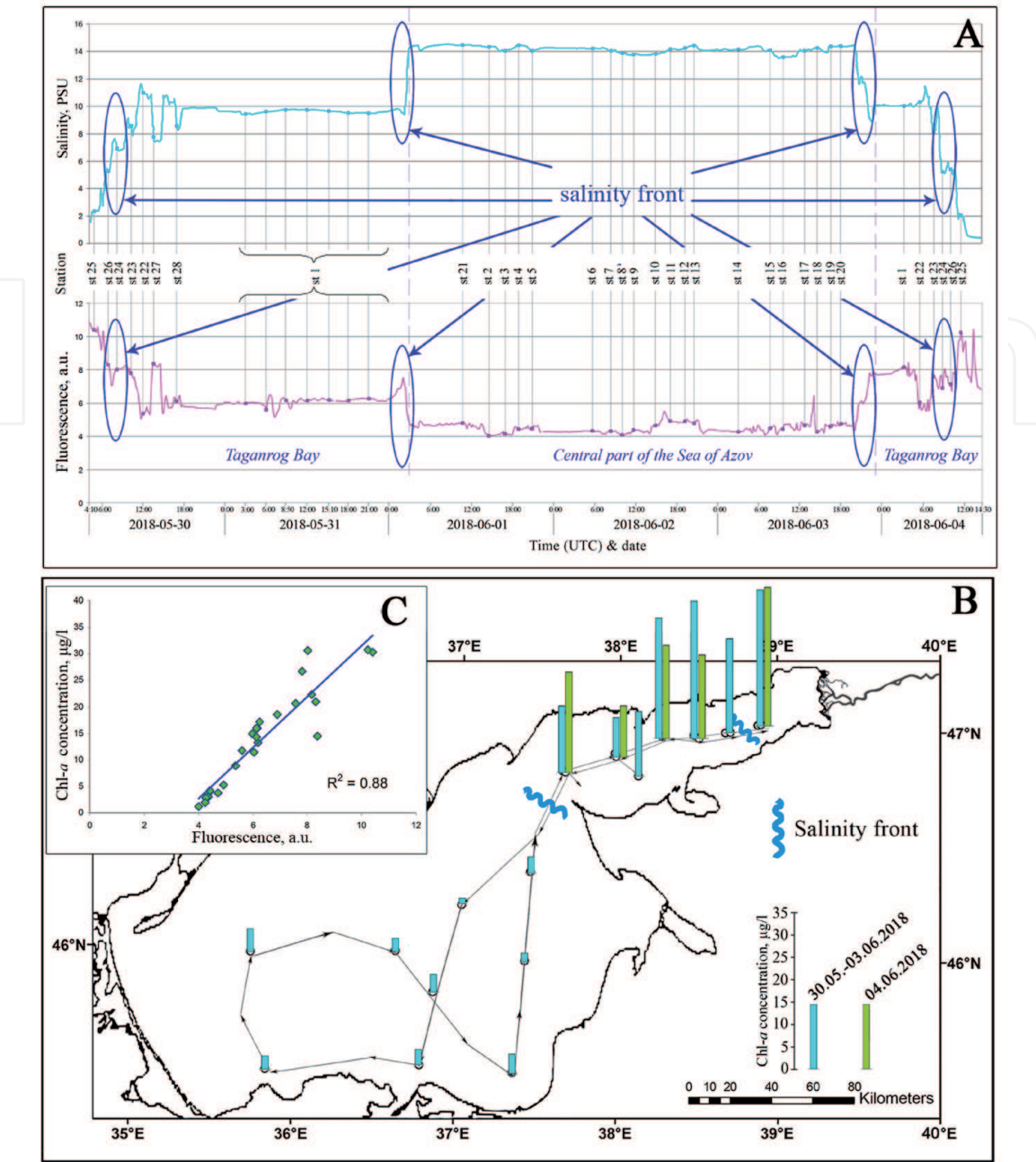


Figure 2.
(A) Fluorescence of chl-a in phytoplankton (a.u.) measured in situ during the R/V “Deneb” survey (arrows indicate the salinity fronts); (B) the spatial distribution of chl-a concentration (µg/l) across the sea of Azov; (C) the strength of relationship between these quantities.

the concentration of chl-a demonstrate a tight relationship ($R^2 = 0.88$, $n = 27$) over the entire set of examined data (**Figure 2C**).

Such a rather wide range of fluorescence and chl-a concentration values can be explained by differences in quantitative indicators as well as by a variety in the phytoplankton species composition in the areas of different seawater salinity. The measured water salinity in the Sea of Azov ranges from 1 to 14 PSU. In particular, there was a sharp increase in salinity (from 1 to 6 PSU) identified in the eastern part of the Taganrog Bay when moving West from the mouth of the Don to the Sea of Azov. Similar increments were also observed in the central part (from 7 to 11 PSU) and in the western one (from 11 to 14 PSU). The water salinity in the central part of the Sea of Azov was not less than 14 PSU [33, 34]. The phytoplankton community was characterized by the rich diversity in species, a large quantity and biomass particularly in the Taganrog Bay, where the salinity gradient is the most pronounced.

Variety is dwindling toward the central part of the Sea of Azov, where there were no sharp fluctuations in salinity detected.

In the studied waters, some specific features in the structure of phytoplankton were noted (**Figure 3**). The Taganrog Bay is rich in species compared to the central part of the Sea of Azov. There were 141 taxa of eukaryotic algae and cyanobacteria belonging to 8 divisions identified in the algal plankton from the bay. In the waters of the central part of the Sea of Azov, the number of revealed species decreased to 48 and they represent 6 divisions.

Such a distribution of phytoplankton in the studied waters is typical not only for the Sea of Azov but also for all estuary-type waters. The fact is that the Taganrog Bay is the zone where waters of various origins mix, leading to the intensification of production and destruction processes [35]. Both marine and freshwater algae can develop here, as it has been observed in the present study. There were 106 species with a rank below the genus (154), constituting 69% of the total number of identified algal taxa with known ecological characteristics. Among them, 21% of species were marine (diatoms and dinophytes), while the remaining 79% belonged to freshwater and brackish-water algae (mainly green algae, cyanobacteria, and diatoms as well).

Since 2007, the composition and spatial structure of phytoplankton in the Taganrog Bay and the central part of the Sea of Azov have been transformed, due to a decrease in feeding from rivers and the associated increase in water salinity. This transformation has led to the situation when habitats of freshwater-brackish-water species are reducing while habitats of marine species are expanding [36]. The tendency of periodic expansions of the marine phytoplankton habitat toward the delta of the Don River was noted in 2017, when the average biomass of phytoplankton had reached its highest values in the eastern and central regions of the Taganrog Bay [37]. In the recent years (comparing to 2002–2012), the maximum concentration of chl-*a* in the western part of the bay has decreased and shifted eastern [38]. In 2018, the Don River experienced the most severe high water over the past 15 years, reaching the maximum in May to June. It also had some impact on the phytoplankton species composition and spatial distribution in the Taganrog Bay.

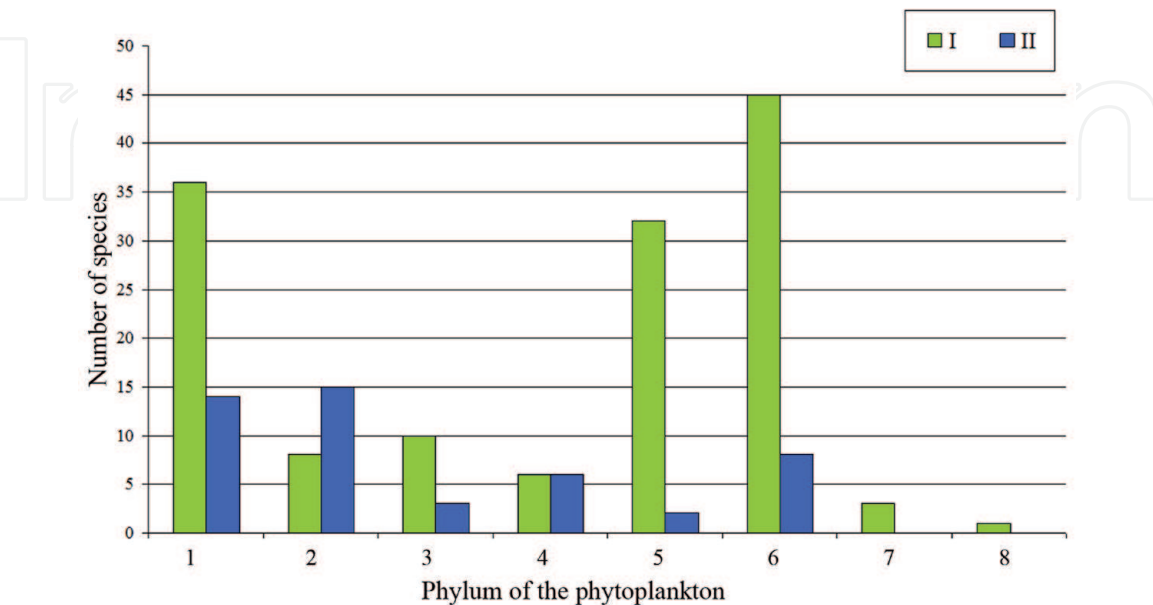


Figure 3. The number of identified species of different phyla of phytoplankton (1–8) in the Taganrog Bay (I) and in the central part of the Sea of Azov (II): 1, Bacillariophyta; 2, Miozoa; 3, Euglenozoa; 4, Cryptophyta; 5, Cyanobacteria; 6, Chlorophyta; 7, Charophyta; 8, Cercozoa.

The data obtained during the expedition concerning the fluorescence and concentration of chl-*a* as well as the abundance and biomass of microalgae demonstrate the intensive development of phytoplankton throughout the Taganrog Bay water. Also, according to the continuous data series from the flow-through fluorometer, two additional sharply defined zones with high values of chl-*a* concentration were found in the frontal salinity zones (**Figure 2A**), which were formed due to the high water in 2018. The qualitative analysis of microalgae has shown that the distribution of freshwater-brackish-water species is not limited by the eastern and central parts of the bay but extends to the western part too.

In the almost entire water area under study, which was evenly warm ($t = 19.5 \pm 0.5^\circ\text{C}$), the dominance of *Coscinodiscus granii* Gough and *Actinopterychus senarius* (Ehrenberg) Ehrenberg from the diatom plankton complex was noted. In the Taganrog Bay, they were subdominated by cyanobacteria *Woronichinia* cf. *compacta* (Lemmermann) Komárek and Hindák and green alga species of the *Oocystis* genus. This happened due to the beginning of summer succession.

A graphical representation (**Figure 4**) of the obtained microalgae biomass shows that the total biomass significantly decreases with increasing salinity and the phytoplankton biomass is significantly lower in the central part of the Sea of Azov than in the Taganrog Bay. The total phytoplankton biomass also led to high concentrations of chl-*a*, because these parameters have a tight relationship ($R^2 = 0.90$, $n = 11$). Also, a reliable relationship ($R^2 = 0.88$, $n = 27$) was obtained for the concentration of chl-*a* measured in laboratory conditions in connection to the in situ fluorescence data from the flow-through fluorometer operating continuously on a vessel. Results of the analysis prove the efficiency of the method used in the present study of the phytoplankton in the Sea of Azov under conditions of varying salinity. The principles of operation and the design of the flow-through fluorometer allow for a larger number of measurements, in order to obtain data in conditions difficult to access for sampling (e.g., when the vessel cannot be stopped, anchored, etc.).

3.2 Chlorophyll-a concentration in the Don River delta under temperature variations

In contrast to the studies in the Sea of Azov, observations in the Don River are held in September to November 2019. During that period, the mineralization of water in the river was changing in a narrow range of 0.6–0.8 g/l, the temperature of water lowered from 23.4 to 7.4°C [39], and the completion of the summer stage with transition to autumnal phytoplankton succession was observed (**Figure 5**). A seasonal change in dominant species has occurred accompanied by the reduction of the total biomass of phytoplankton. The predominant cyanobacteria in September and partly in October (*Microcystis* spp., *Aphanizomenon flosaquae* Ralfs ex Bornet & Flahault) were gradually replaced by diatoms (*Nitzschia* spp., *Gyrosigma* sp., *Ulnaria ulna* (Nitzsch) Compère). The total phytoplankton biomass decreased from 0.94 to 0.17 mg/l. The fluorescence values were decreasing from 14.95 to 4.84 c.u. over the period of studies, which corresponded to the range of chl-*a* concentrations from 35.7 to 5.36 µg/l.

The observed fluctuations in the measured fluorescence have an implicit correlation with the phytoplankton biomass variations ($R^2 = 0.40$, $n = 33$). It might be attributed to the influence of water temperature [40]. However, from the available dataset, it was not possible to derive a reliable relationship between the fluorescence and water temperature ($R^2 = 0.20$, $n = 7$), which can be explained that rather a small dataset was collected under conditions of abrupt changes in temperature. It has been clearly seen (**Figure 5**) that the values of fluorescence and of biomass demonstrate

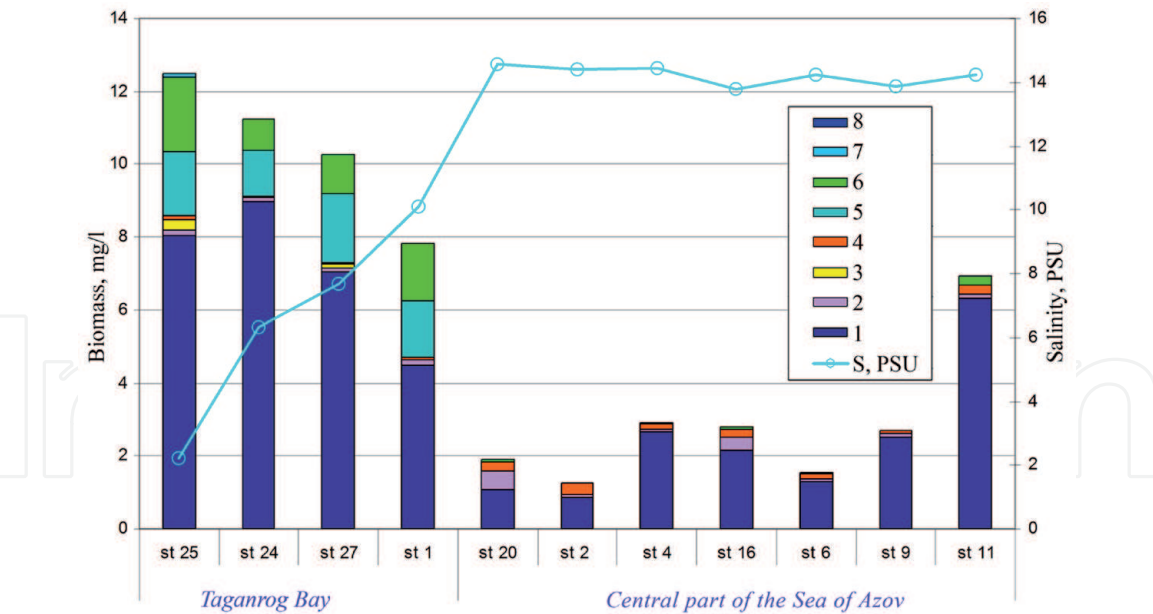


Figure 4. Variations in water salinity (S, PSU) and phytoplankton (1–8) biomass (B, mg/l) in the Taganrog Bay and in the central part of the sea of Azov: 1, Bacillariophyta; 2, Miozoa; 3, Euglenozoa; 4, Cryptophyta; 5, Cyanobacteria; 6, Chlorophyta; 7, Charophyta; 8, Cercozoa.

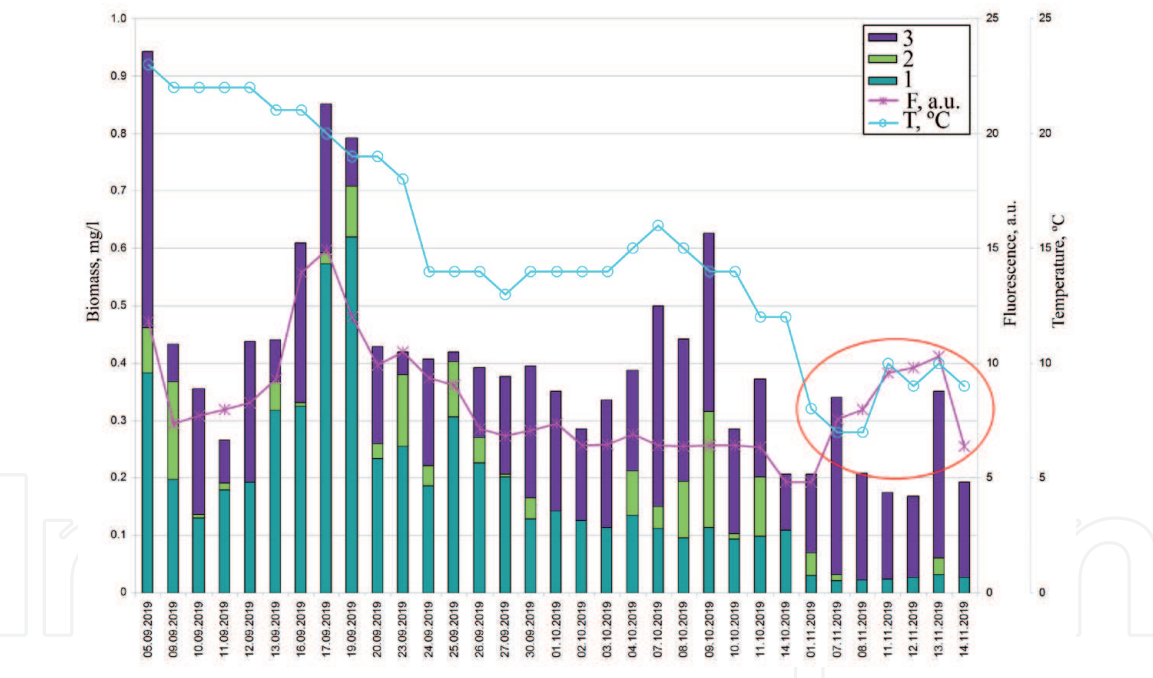


Figure 5. Variations in the biomass (B, mg/l) of the major divisions of phytoplankton (1–3), water temperature (T, °C), and chl-a fluorescence (F, a.u.) in September to November 2019: 1, Cyanobacteria; 2, Chlorophyta; and 3, Bacillariophyta. Red oval denotes a region of decrease in water temperature $\leq 10^{\circ}\text{C}$ and coenotic change of phytoplankton.

the same trend in the temperature range of 13–20°C. When the temperature decreases below 8°C, a coenotic change in phytoplankton occurs, apparently, causing a change in the chl-a fluorescence (highlighted by a red oval in **Figure 5**).

In the future, it is necessary to continue collecting empirical data, especially under the changes in the dominant phytoplankton groups and significant variations of water temperature, in order to reveal the influence of environmental parameters on the chl-a fluorescence and the correct interpretation of the results. Nevertheless, the fluorescence method of rapid assessment of water entering the water supply

network is very useful and allows for a round-the-clock remote monitoring. It is a good alternative to satellite methods, the use of which is very limited for rivers with a narrow channel and does not provide information about water at the depth of installation of water intake systems.

4. Conclusions

As a result of the studies, a reliable relationship was established between the fluorescence intensity of chl-*a* measured in the Sea of Azov and the Don River by flow-through fluorometer and the concentration of chl-*a* determined by standard methods in a wide range of environmental conditions and species compositions of phytoplankton.

Results of continuous vessel-based chl-*a* fluorescence measurements, total biomass values, and species composition of microalgae in the selected probes evidently demonstrate that salinity is one of the main factors regulating the distribution of phytoplankton in the Sea of Azov.

It was shown that the high water of 2018 is temporarily affected by the spatial distribution of freshwater and brackish-water species of phytoplankton in the waters of the Taganrog Bay. But, the general trend of marine microalgae species expansion from the Sea of Azov to the Taganrog Bay under conditions of weakening river flow and growing salinity remains unchanged for over 15 years.

Long-term observations in the lower reaches of the Don have demonstrated that the development of phytoplankton and variations in its species composition reflected in the changes of chl-*a* fluorescence are strongly influenced by temperature.

Fluorescence measurements have demonstrated to be an effective method for studying water productivity and rapid diagnostics of phytoplankton status. Despite the relative simplicity in design and operation, the developed flow-through fluorometers allowing for a round-the-clock remote monitoring of the environment have become an effective research tool, providing sufficient data for revealing spatial and temporal dynamics.

The fluorescent method of monitoring the quality of water entering the urban water supply system is promising for taking preventive measures to combat water blooming and rising population of cyanobacteria.

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Conflict of interest

The authors declare no conflict of interest. This article does not contain any research using animals as objects.

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