УДК 551.463.5

© *О. Б. Кудинов*[1]*, *О. В. Мартынов*[1], *Р. И. Ли*[2]

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

ЗОНДИРУЮЩИЙ СПЕКТРАЛЬНЫЙ ИЗМЕРИТЕЛЬ ФЛЮОРЕСЦЕНЦИИ И РАССЕЯНИЯ, ИСПЫТАНИЯ В ЛАБОРАТОРНЫХ И ПОЛЕВЫХ УСЛОВИЯХ

Статья поступила в редакцию 30.01.2020, после доработки 12.03.2020

С целью проведения экологического мониторинга и получения оперативных данных о состоянии исследуемой акватории был создан зондирующий спектральный измеритель флюоресценции и рассеяния света. Он позволяет одновременно проводить измерения флюоресценции фитопланктона, растворенного органического вещества и рассеяния света в морской воде, в спектральном диапазоне, охватывающем область от ближнего ультрафиолета до красной границы видимого спектра. Получение спектров указанных параметров производятся в одном и том же измерительном объеме, что избавляет от необходимости согласовывать полученные сигналы по объёму флюоресцирующего вещества. Результаты проверки функционирования прибора в лабораторных условиях на образцах монокультур фитопланктона, обитающего в водах Чёрного моря, показали хорошее совпадение измерений с литературными данными. В ходе испытаний прибора во время 106-го рейса на НИС «Профессор Водяницкий» весной 2019 г. были произведены измерения на 77 гидрологических станциях следующих параметров: профилей вертикального распределения флюоресценции фитопланктона; растворённого органического вещества и упругого рассеяния света. В результате были получены сведения о вертикальном распределении измеряемых параметров на исследованных акваториях Чёрного моря. Результаты проведённых испытаний представленного измерителя позволили апробировать техническое исполнение и методологию работы с данным измерителем.

Ключевые слова: спектральный флюориметр, спектр возбуждения флюоресценции микроводорослей, флюоресценция хлорофилла, флюоресценция растворенного органического вещества, вертикальные профили флюоресценции, Чёрное море.

© *O. B. Kudinov*[1]*, *O. V. Martynov*[1], *R. I. Lee*[2]

[1]Marine Hydrophysical Institute RAS, 299011, Kapitanskaya Str., 2, Sevastopol, Russia
[2]A.O. Kovalevsky Institute of Biology of the Southern Seas RAS, 299011, Nakhimova Prospekt, 2, Sevastopol, Russia
*e-mail: kudinov_ob@mhi-ras.ru

SOUNDING SPECTRAL METER OF FLUORESCENCE AND LIGHT SCATTERING: LABORATORY AND FIELD TESTING

Received 30.01.2020, in final form 12.03.2020

In order to conduct environmental monitoring and obtain operational data on the state of the investigated water area, a sounding spectral fluorescence and scattering meter was created. It allows simultaneous measurements of the fluorescence of phytoplankton, dissolved organic matter and light scattering in the sea water, in the spectral range covering the region from the near ultraviolet to the red boundary of the visible spectrum in the same measuring volume.

The results of instrument testing in laboratory conditions on samples of phytoplankton monocultures existing in the Black Sea waters showed good agreement between the measurements and literature data. During the expedition of the R/V "Professor Vodyanitsky", at 77 hydrological stations the following parameters were measured: vertical profiles of the phytoplankton fluorescence; DOM; the elastic light scattering. As a result, information was obtained on the vertical distribution of the measured parameters in the studied waters of the Black Sea. The results of tests of the presented meter allow to approve the technical design and works methodology of this meter.

Key words: spectral fluorometer, microalgae fluorescence excitation spectrum, chlorophyll, DOM, vertical fluorescence profiles, Black Sea.

Ссылка для цитирования: *Кудинов О.Б.*, *Мартынов О.В.*, *Ли Р.И.* Зондирующий спектральный измеритель флюоресценции и рассеяния, испытания в лабораторных и полевых условиях // Фундаментальная и прикладная гидрофизика. 2020. Т. 13, № 2. С. 82–87. doi: 10.7868/S2073667320020100

For citation: *Kudinov O.B.*, *Martynov O.V.*, *Lee R.I.* Sounding spectral meter of fluorescence and light scattering: laboratory and field testing. *Fundamentalnaya i Prikladnaya Gidrofizika*. 2020, 13, 2, 82–87. doi: 10.7868/S2073667320020100

1. Introduction

Determination of water bio-optical properties, assessment of biomass and primary production of phytoplankton are modern areas of environmental monitoring [1, 2]. Biological organisms are highly sensitive to the pollutants of the environment, as well as to changes in climate, light conditions, etc. [3]. Phytoplankton as the primary link of the ecosystem trophic chain is the sensitive to the above-mentioned factors [2]. Phytoplankton distribution in the water column, its concentration and phases of growth have the highest importance for the environmental assessment. Today, phytoplankton concentrations can be measured from airplanes or satellites [4, 5]. However, modern methods of bio-optical observations, despite their fast development, are mainly concerned with the surface layer of seawater, while the vertical distribution or seasonal and interannual variability of fluorescence could be not as thoroughly studied.

Most of the contact methods for assessing the biomass and primary production of phytoplankton in water bodies require a long time to analyze water samples, which is a significant disadvantage, especially when studying large water areas. The fluorescence method is much simpler in application than the methods that require sampling, which makes it possible to carry out an express analysis of the state of aquatic ecosystems and obtain arrays of environmental data.

The estimation of phytoplankton biomass from fluorometric measurements has certain limitations, since the ratio of in situ fluorescence signal and chlorophyll concentration obtained by the extraction method is not constant over the entire measurement range [6, 7]. A significant source of variability in fluorescence intensity is the phytoplankton species composition. Fluorescence spectra are different for pigments of different taxonomic groups of phytoplankton. Variation in species composition leads to a change in the fluorescence signal by the order of magnitude [8].

Studying the possibility of using spectral fluorescence as a tool for assessing the composition of phytoplankton pigments, in the late seventies, Yentsch et al. [9, 10] conducted the first experiments, which were further developed and expanded [11–14]. It was found that different classes of algae have different fluorescence excitation spectra, but measuring the whole spectrum during large-scale field studies was too difficult. Kolbowski and Schreiber [15] solved this problem and showed that measurements at just four or five wavelengths are sufficient to distinguish between four groups of microalgae that are most common in the World Ocean [16]. Since algae from the same taxonomic group contain a similar amount and a same type of photosynthetic pigments, the fluorescence excitation spectra are similar for each group. Therefore, algae groups can be differentiated by the fluorescence excitation spectrum.

It should be noted that measurements of the vertical distribution of chlorophyll-*a* concentration require sounding fluorimeters. This makes in situ spectral fluorimeters an important 21st century oceanography tool.

2. Materials and methods

To solve the task of determining the species composition of phytoplankton a spectral meter was developed in the Department of Marine Optics and Biophysics of Marine Hydrophysical Institute of RAS. It allows to measure simultaneously the fluorescence of phytoplankton and dissolved organic matter and light scattering in seawater. The spectral range is from the near ultraviolet (360 nm) to the red border (690 nm) of the visible spectrum for the same measuring volume [1, 17, 18]. The optical diagram of the device is shown in fig. 1.

The source of phytoplankton fluorescence excitation is a 4-color LED (RGBA LED) with spectral bands 450–460, 525–535, 565–575 and 620–630 nm (with half-width 5 nm), which correspond to maxima of the excitation spectra of four microalgae groups. To excite the fluorescence of dissolved organic matter (DOM), a source of ultraviolet radiation (UV LED — 365 nm), perpendicular to the source of chlorophyll excitation, was added. LEDs are installed in the focus of the lenses, which convert the light emitted by the LEDs into a parallel beam and direct it into the measuring volume. The radiation scattered by the measuring volume at a 90° angle, as well as the excited fluorescence radiation, pass through a lens that collects the radiation and directs it to the photocathode of a highly sensitive photomultiplier tube (PMT). In front of the PMT window, a disk with interchangeable color filters is placed. The fluorescence and scattering are measured at the wavelengths of 360, 460, 530, 570, 625 and 685 nm.

The device was tested under laboratory conditions. For experiments, the samples of monocultures of phytoplankton common for the Black Sea from the collection of A. O. Kovalevsky Institute of Biology of the Southern Seas RAS were used. During the experiments, fluorescence excitation spectra for various concentrations of green microalgae (*Tetraselmis viridis* (Rouchijajnen) R. E. Norris, Hori et Chihara 1980), diatoms (*Phaeodactylum tricornutum* Bohlin 1898), dinophytic (*Prorocentrum caspicum* (Kiselev) Krachmalny 1993), and chrysophytes (*Isochrysis galbana* Parke 1949) were measured. The measurements were carried out in filtered seawater with increasing concentrations of phytoplankton. The resulting signal was normalized by the elastic scattering intensity in the corresponding spectral ranges.

The obtained fluorescence excitation spectra (at the emission 680 nm) of microalgae of various cultures, normalized by the maximum values, are presented in fig. 2. In the same figure, the obtained spectra are compared with data from published sources [19].

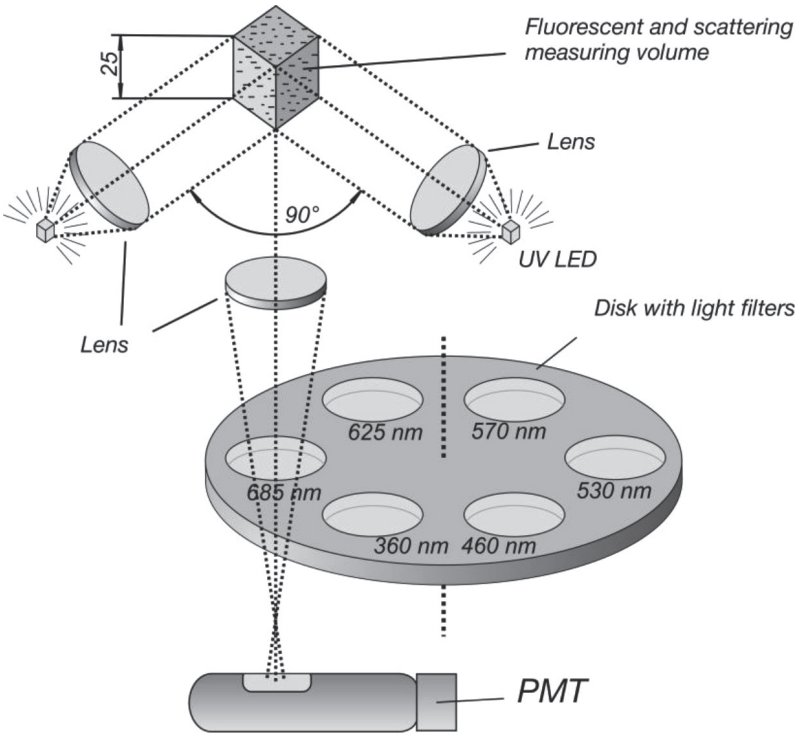

Fig. 1. Optical diagram of the instrument for measuring the fluorescence and light scattering.

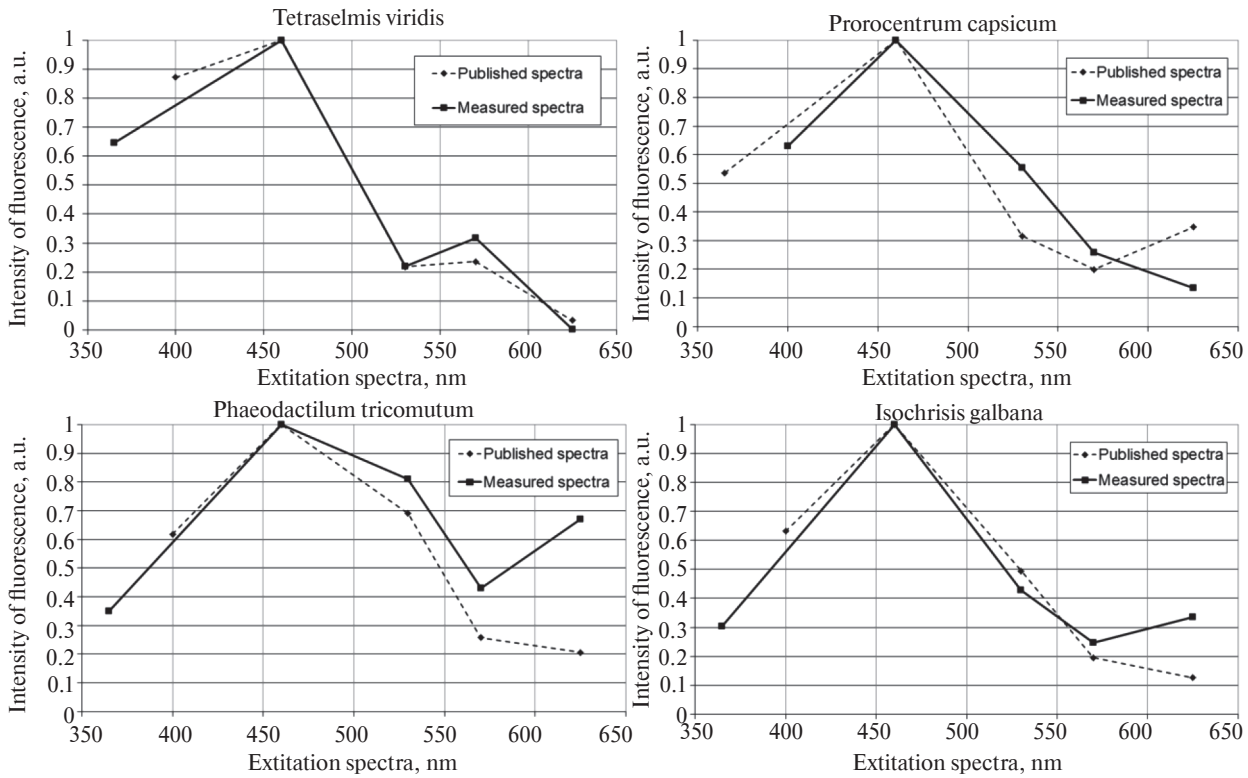

Fig. 2. Normalized fluorescence excitation spectra of various monocultures of phytoplankton common for the Black Sea. Comparison of measured and published data [19]. Dotted line — fluorescence excitation spectra of published data; solid line — data obtained from our meter.

The compared spectra are similar in shape, which indicates that the applied methodological approach and the design of the device are correct.

Also, the spectrograms were obtained for the following cases: a) tap water filtered with a household filter and b) with a reverse osmosis filter, c) sea water, d) seawater filtered with a 1 μm filter, e) own spectrogram of the device and f) spectrograms of various phytoplankton cultures. Fig. 3, *a* (see Insert) shows the own spectrogram of the device, it is formed by the intersection of the passbands of the filters and the emission of LEDs and lies on the diagonal of 350,350–650,650 nm.

The spectrogram is based on the reference spectra of the optical filters transmission and LED emission. The excitation spectrum of the LEDs is plotted along the horizontal axis, and the registration spectrum (transmission of light filters) is along the vertical axis. Thus, on a diagonal the radiation of the elastic light scattering is shown, fluorescence is above the diagonal, and anti-Stokes luminescence is below the diagonal.

Fig. 3, *b* (see Insert) shows the spectrogram of filtered seawater. Filtration was made using a 1 μm cell filter. Three main spots can be seen on the spectrogram. Two of them are associated with the elastic scattering, because they fall on the diagonal of the own spectrogram of the device in the range of excitation wavelengths 500–650 nm. And the spot at 350–400 nm lies above the diagonal and appears to be the fluorescence of the dissolved organic matter, which cannot be eliminated using filter (only by using sorbents).

Spectrograms for various phytoplankton cultures were also plotted. Fig. 4 (see Insert) shows the spectrogram of the fluorescence intensity of *Isochrysis galbana* where the fluorescence of pure water and own spectrum of the device were subtracted to remove the contribution of scattering.

As a result, a spectrogram with three areas of fluorescence was obtained: 350–400 nm, 400–500 nm and 600–650 nm bands of the excitation spectrum.

The fluorescence intensity spectrum of *Isochrysis galbana* shown in fig. 4 and in fig. 2 were obtained in different experiments on samples of the same culture taken in different seasons, nevertheless, the spectra are the same. Also, the spectrogram shows that the fraction of dissolved organic matter is present in the studied sample. The spectrograms of other monocultures used in the experiment are presented in fig. 5 (see Insert).

Using the results of laboratory tests, the device was modified in accordance with the requirements for sounding instruments, equipped with an autonomous data storage module "LAKUNA" [20], and subjected to immersion testing.

During the 106th cruise of the R/V "Professor Vodyanitsky" (April 18—May 13, 2019), test soundings were made, as well as measurements of the following parameters at 77 hydrological stations: vertical distribution of phytoplankton fluorescence, DOM, the elastic light scattering. Intercalibration with the Indronaut-fluorometer probe showed a significant degree of agreement between the measured parameters. Fig. 6 (see Insert) shows vertical temperature profiles in arbitrary units, as well as fluorescence excitation spectra at a wavelength of 680 nm, normalized by the elastic scattering, presumably these spectra belong to different groups of phytoplankton monocultures, such as chlorophytes (green microalgae), cyanobacteria (blue-green), and cryptophytic. The fluorescence signal increases below the layer of maximum gradient of temperature. The maximum values of fluorescence and, correspondingly, maximum chlorophyll-*a* concentration are observed at the bottom of the photic zone, where the supply of nutrients and the illumination level form optimal conditions for the development of phytoplankton.

The vertical distributions of DOM fluorescence and elastic light scattering in seawater at the station 130 are shown in fig. 7. The fluorescence of DOM was excited at 365 nm and registered at 460 nm. Usually the DOM content in the Black Sea water generally increases with depth and has a step at depths more than 80 m. These depths in the Black Sea correspond to a transition zone between the oxygen and hydrogen sulfide waters (redox zone).

3. Conclusion

Tests of a spectral device for simultaneous *in situ* measurement of fluorescence and light scattering in seawater in the spectral range 360–685 nm in the same measuring volume confirmed the use of correct methodological approach used and design of the device. The data obtained are in good agreement with data from literary sources and are of interest for further detailed analysis.

4. Financing

This work was carried out as part of a state assignment No. 0827–2018–0002 (code "Operational Oceanology") and No. 0827–2018–0006.01 (code "Biooptics") and on the topic "Functional, metabolic and toxicological aspects of the existence of aquatic organisms and their populations in biotopes with different physicochemical conditions"; State registration number AAAA-A18–118021490093–4.

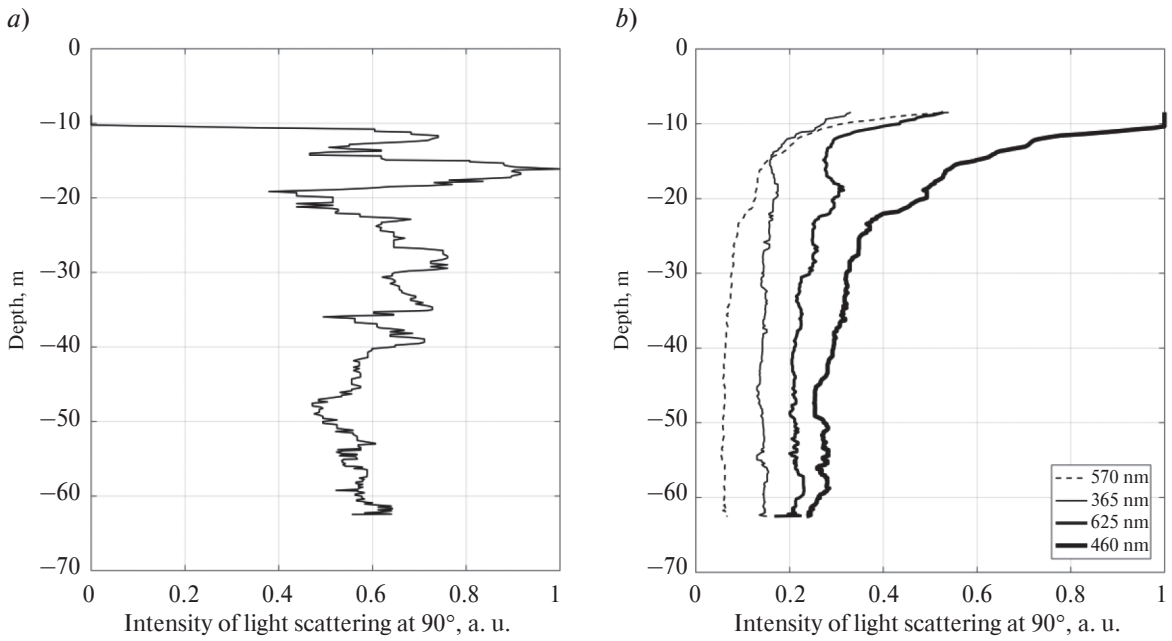

Fig. 7. The vertical profiles of the DOM (*a*) and light scattering (*b*) fluorescence in sea, measured at the station 130.

Литература

1. *Ли М.Е.* Определение биооптических свойств вод по измерениям спектральных характеристик флюоресценции и рассеяния света в морской среде / М.Е Ли., Р. И. Ли, О. В. Мартынов // Системы контроля окружающей среды / Под. ред. В. А. Гайского. Севастополь: НПЦ «ЭКОСИ-Гидрофизика», 2014. С. 74–83.
2. *Винберг Г.Г.* Продуктивность и охрана морских и пресных водоёмов. М: Наука, 1989. 135 с.
3. *Попик А.Ю.* Динамика спектров лазерной индуцированной флюоресценции хлорофилла-а фитопланктона в условиях меняющихся параметров внешней среды: дис. канд. физ-мат. наук. Институт автоматики и процессов управления. Владивосток, 2015. 145 с.
4. *Суетин В.С.*, *Кучерявый А.А.*, *Суслин В.В.*, *Королев С.Н.* Концентрация пигментов фитопланктона в северо-западной части Черного моря по данным измерений спутниковым цветовым сканером CZCS // Морской гидрофизический журнал. 2000. № 2. С. 74–82.
5. *Fedorov S.V.* Comparison of the secondary products obtained by the optical sensor installed at the satellite «Resurs-P» with the products of the analogous optical sensors // Physical Oceanography. 2018. V. 34, № 1. 2018. P. 29–39.
6. *Marra J.*, *Langdon C.* An evaluation of an in situ fluorometer for the estimation of Chlorophyll a. Tech. Rep. LDEO-93–1. Lamont-Doherty Earth Observatory, Palisades. NY. 1993. 41 p.
7. *Marra J.* Analysis of diel variability in Chlorophyll fluorescence // J. Mar. Res. 1997. 55. P. 767–784. doi: 10.1357/0022240973224274
8. *Proctor C.W.*, *Roesler C.S.* New insights on obtaining phytoplankton concentration and composition from in situ multispectral Chlorophyll fluorescence // Limnol. Oceanogr.: Methods. 2010. 8. P. 695–708.
9. *Yentsch C.S.*, *Yentsch C.M.* Fluorescence spectral signatures — characterization of phytoplankton populations by the use of excitation and emission-spectra // J. Mar. Res. 1979. 37. P. 471–483.
10. *Yentsch C.S., Phinney D.A.* Spectral fluorescence — an ataxonomic tool for studying the structure of phytoplankton populations // J. Plankt. Res. 1985. 7. P. 617–632.
11. *Karabashev G.S.*, *Khanaev S.A.* Underwater multichannel fluorimeter for marine ecological research // J. Appl. Spectrosc. 1988. 49. P. 979–981. http://dx.doi.org/10.1007/BF00662796
12. *Poryvkina L.*, *Babichenko S.*, *Kaitala S.*, *Kuosa H.*, *Shalapjonok A.* Spectral fluorescence signatures in the characterization of phytoplankton community composition // J. Plankton Res. 1994. 16. P. 1315–1327.
13. *Babichenko S.*, *Leeben A.*, *Poryvkina L.*, *van der Wegt R.*, *de Vos F.* Fluorescent screening of phytoplankton and organic compounds in sea water // J. Env. Monit. 2000. 2. P. 378–383.
14. *Beutler M.*, *Wiltshire K.H.*, *Meyer B.*, *Moldaenke C.*, *Lüring C.*, *Meyerhöfer M.*, *Hansen U-P.*, *Dau H.* A fluorometric method for the differentiation of algal populations in vivo and in situ // Photosynth. Res. 2002. 72. P. 39–53.
15. *Kolbowski J.*, *Schreiber U.* Computer-controlled phytoplankton analyzer based on 4-wavelengths PAM chlorophyll fluorometer // Photosynthesis: from light to biosphere / Ed. Mathis P. 1995. V. V. Kluwer, Netherlands. P. 825–828.

16. *Van den Hoek C.*, *Mann D. & Jahns H.* Algae: An introduction to phycology. Cambridge University Press, Cambridge; New York. 1996. 640 p.
17. *Кудинов О.Б.*, *Мартынов О.В.*, *Ли М.Е.* Спектральный измеритель флюоресценции и рассеяния // Тр. IX Всероссийской конференции «Современные проблемы оптики естественных вод». СПб., 2017. С. 217–222.
18. *Кудинов О.Б.* Автономный измеритель спектра флюоресценции фитопланктона // Комплексные исследования Мирового океана: Материалы IV Всероссийской научной конференции молодых ученых. 2019. С. 361–362.
19. *Poryvkina L.*, *Babichenko S.*, *Kaitala S.*, *Kuosa H.*, *Shalapjonok A.* Spectral fluorescence signatures in the characterization of phytoplankton community composition // J. Plankton Res. 1994. 16. P. 1315–1327.
20. *Кудинов О.Б.*, *Латушкин А.А.* Универсальный автономный накопитель для повышения оперативности гидрооптических исследований in situ // Процессы в геосредах. 2018. № 3 (17). С. 74–75.

References

1. *Lee M.E.*, *Lee R.I.*, *Martynov O.V.* Determination of bio-optical properties of waters from measurements of spectral characteristics of fluorescence and light scattering in a marine environment. *Monitoring System of Environment*. 2014, 74–83 (in Russian).
2. *Winberg G.G.* Productivity and protection of marine and fresh water basins. *Moscow*, *Nauka*, 1989, 135 p. (in Russian).
3. *Popik A.Yu.* The spectra dynamics of laser-induced fluorescence of phytoplankton chlorophyll-a under conditions of changing environmental parameters. *Dr. Sci. thesis. Vladivostok*, 2015. 145 p. (in Russian).
4. *Suetin V.S.*, *Kucheryavyy A. A.*, *Suslin V. V.*, *Korolev S. N.* Phytoplankton pigment concentration in the northwestern Black Sea as measured by the satellite color scanner CZCS. *Morskoy Gidrofizicheckiy Zhurnal*. 2002, 2, 74–82 (in Russian).
5. *Fedorov S.V.* Comparison of the secondary products obtained by the optical sensor installed at the satellite «Resurs-P» with the products of the analogous optical sensors. *Physical Oceanography*. 2018, 34, 1, 29–39.
6. *Marra J. and C. Langdon.* An evaluation of an in situ fluorometer for the estimation of Chlorophyll a. *Tech. Rep. LDEO-93–1. Lamont-Doherty Earth Observatory*, *Palisades*, *NY*, 1993. 41 p.
7. *Marra J.* Analysis of diel variability in Chlorophyll fluorescence. *J. Mar. Res*. 1997, 55, 767–784. doi: 10.1357/0022240973224274
8. *Proctor C.W. and C.S. Roesler.* New insights on obtaining phytoplankton concentration and composition from in situ multispectral Chlorophyll fluorescence. *Limnol. Oceanogr.*: *Methods*, 2010, 8, 695–708.
9. *Yentsch C.S., Yentsch C.M.* Fluorescence spectral signatures — characterization of phytoplankton populations by the use of excitation and emission-spectra. *J. Mar. Res*. 1979, 37, 471–483.
10. *Yentsch C.S.*, *Phinney D.A.* Spectral fluorescence — an ataxonomic tool for studying the structure of phytoplankton populations. *J. Plankt. Res*. 1985, 7, 617–632.
11. *Karabashev G.S.*, *Khanaev S.A.* Underwater multichannel fluorimeter for marine ecological research. *J. Appl. Spectrosc*. 1988, 49, 979–981. http://dx.doi.org/10.1007/BF00662796
12. *Poryvkina L.*, *Babichenko S.*, *Kaitala S.*, *Kuosa H.*, *Shalapjonok A.* Spectral fluorescence signatures in the characterization of phytoplankton community composition. *J. Plankton Res*. 1994, 16, 1315–1327.
13. *Babichenko S.*, *Leeben A.*, *Poryvkina L.*, *van der Wegt R.*, *de Vos F.* Fluorescent screening of phytoplankton and organic compounds in sea water. *J. Env. Monit*. 2000, 2, 378–383.
14. *Beutler M.*, *Wiltshire K.H.*, *Meyer B.*, *Moldaenke C.*, *Lüring C.*, *Meyerhöfer M.*, *Hansen U-P.*, *Dau H.* A fluorometric method for the differentiation of algal populations in vivo and in situ. *Photosynth. Res*. 2002, 72, 39–53.
15. *Kolbowski J.*, *Schreiber U.* Computer-controlled phytoplankton analyzer based on 4-wavelengths PAM chlorophyll fluorometer. *Photosynthesis*: *from light to biosphere* / Mathis P. (ed.). V. V, *Kluwer*, *Netherlands*, 1995, 825–828.
16. *Van den Hoek C.*, *Mann D. & Jahns H.* Algae: An introduction to phycology. *Cambridge University Press*, *Cambridge*, *New York*. 1996. 640 p.
17. *Kudinov O.B.*, *Martynov O.V.*, *Lee M.E.* Spectral measurer of fluorescence and scattering. *Proc. of IX All-Russian Conf.* "*Current problems in optics of natural waters* (*ONW'2017*)", *St. Petersburg*, 2017, 217–222 (in Russian).
18. *Kudinov O.B.* Autonomous phytoplankton fluorescence spectrum meter. *Proc.: Integrated World Ocean Researches*, *Materials of the IV All-Russian Scientific Conference of Young Scientists*, *KIMO-2019*. 2019, 361–362 (in Russian).
19. *Poryvkina L.*, *Babichenko S.*, *Kaitala S.*, *Kuosa H.*, *Shalapjonok A.* Spectral fluorescence signatures in the characterization of phytoplankton community composition. *J. Plankton Res*. 1994, 16, 1315–1327.
20. *Kudinov O.B.*, *Latushkin A.A.* Universal standalone storage device to increase the efficiency of *in situ* hydrooptical studies. *Processy v Geosredakh*. 2018, 3 (17), 74–75 (in Russian).

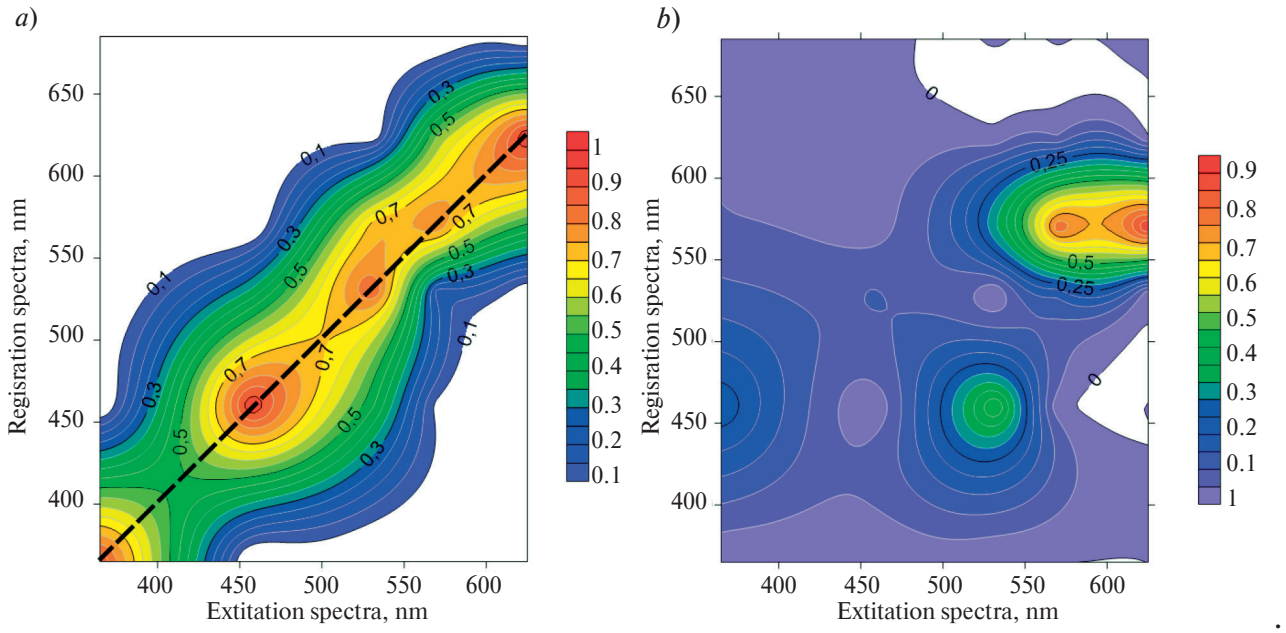

Fig. 3. Spectrograms: *a* — own spectrogram of the device; *b* — filtered seawater.

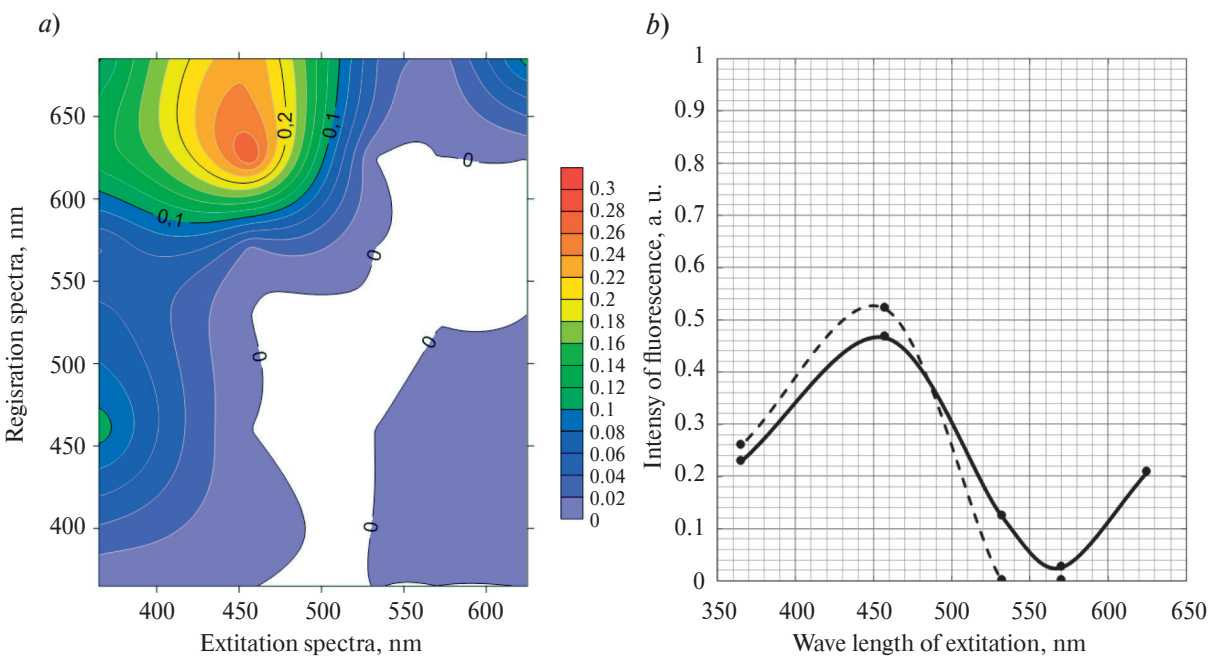

Fig. 4. Spectrogram (*a*) and fluorescence intensity spectrum (*b*) of *Isochrysis galbana*. The solid line is the registration at a wavelength of 680 nm, the dotted one is at 625 nm.

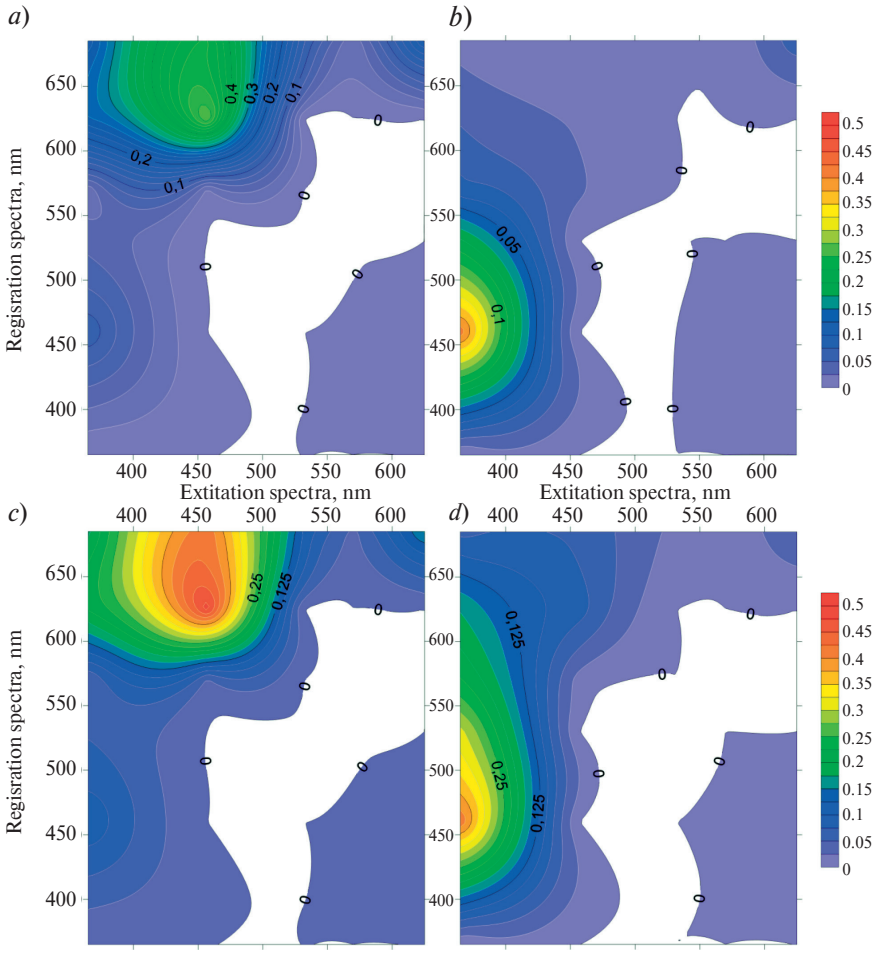

Fig. 5. Spectrograms of various monocultures (*a* — diatoms *Phaeodactylum Tricornutum*; *b* — dimatoms *Chaetoceros Affinis*; *c* — dinophytic *Prorocentrum cordatum*; *d* — dinophytic *Scrippsiella Trochoidea*).

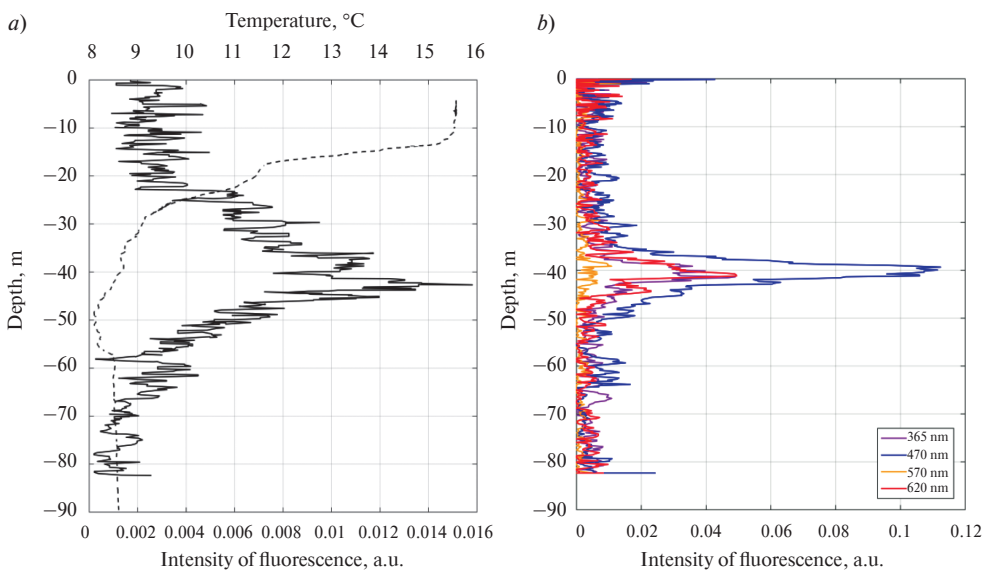

Fig. 6. The vertical profiles of fluorescence at the emission 680 nm and temperature, measured at the station 138 (*a*); the vertical profiles of normalized spectra fluorescence at the emission 680 nm of phytoplankton (colors correspond to local maxima of the excitation spectrum), measured at the station 97 (*b*).