Peculiarities of the laser-induced fluorescence spectra of seawater during algae blooming in different regions of the World Ocean

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We present some results of investigations into the laser-induced fluorescence (LIF) of phytoplankton pigments and dissolved organic matter (DOM). The LIF spectra were studied in three regions of the World Ocean, characterized by high bioproductivity, namely, the North Sea and the northeastern part of the Atlantic Ocean, the southwestern part of the Atlantic Ocean, and Pacific coast of Chile, as well as the Sea of Okhotsk. The measurements were carried out in 2002 and 2003 during the time of algae blooming. It is shown that the concentration of the primary phytoplankton pigment, chlorophyll-a, is linearly related to the parameter characterizing the fluorescent part of DOM in the periods of algae blooming. In the water areas, where this relation is observed, it is possible to introduce parameters characterizing the rate of the DOM production by phytoplankton community.

The study of laser-induced fluorescence (LIF) of seawater is interesting from different points of view. First of all, the parameters of LIF spectra characterize the stage of development and the state of a phytoplankton community. The investigation of, for example, laser-induced fluorescence allows us to evaluate the state of photosynthesizing phytoplankton cells from the relation between the intensities of fluorescence lines of different pigments contained in the cells. In addition, dynamic parameters of the chlorophyll-a fluorescence spectra can be used for determination of the electron transport rates in photosynthesis.[1,2] The method of LIF spectroscopy provides for large arrays of continuous measurements of the chlorophyll-a concentration and the concentration of the fluorescing part of organic matter in the surface ocean layer. In combination with hydrophysical and hydrochemical parameters, this enables one to study the influence of different processes (including pollution of marine waters) on the development of plankton communities.[3,4]

Laser-induced fluorescence spectra bear information about both the living phytoplankton cells and the organic matter, which is reproduced by the phytoplankton community and is present in seawater in dissolved and suspended states. Thus, the LIF method can be used to study the carbon cycles of organic matter on our planet. Various problems of ecological monitoring of phytoplankton communities and investigation into the transformation processes and sources of dissolved organic matter (DOM) necessitate the development of methods for a continuous monitoring of vast water areas. The data of such monitoring will permit us to understand the main features in the transformation and cycles of organic matter in the ocean on large scales. Discrete measurements at stations are obviously insufficient for studying different-scale spatiotemporal variability of biological and biochemical parameters of seawater, especially, in shelf zones and for determining the relations between parameters characterizing the plankton community and DOM.

The study of carbon cycles of organic matter is one of the basic problems in oceanology and geochemistry of the biosphere, because the processes of reproduction of organic matter and its transformation by living organisms determine the functioning of bioproductivity chains in the ocean.[5] The processes of the organic carbon transformations and degradation of living cells into the DOM part resistant to biochemical transformations, mineralization of carbon in deep water or its further participation in the bacterial development and creation of fractions, which are transformed into organic forms in the upper ocean layer, are now investigated quite intensively.[6] The dissolved organic matter amounts to about 90–95% of the total organic matter, while the rest 5–10% of the organic matter present in the water in the form of suspensions.[7,8] It is just the DOM and phytoplankton that play the main role in the formation of spectra of radiation upwelling from the sea depth, that is, in the formation of the sea surface color or its biooptical parameters.[9] From this viewpoint, the DOM concentration, as well as the concentration of phytoplankton pigments, can be considered important biooptical parameters.

Phytoplankton communities form the main source of organic matter in the ocean.[7,8] The dissolved organic

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matter is continuously transformed, and, according to data from Refs. 6–10, its quickly degrading (labile) part amounts to 75%, while the rest 25% form humus resistant to degradation, and carbon itself makes up to 50% of the total DOM.

The main contribution to the intensity of the line of DOM fluorescence induced by laser radiation is due to just the degrading part of DOM or the chromophoric (colored) DOM – an important fraction of the DOM pool. This fraction serves a moderator in photochemical reactions in the seawater, determines the quantity and quality of the sunlight reaching the photosynthesizing phytoplankton cells, and thus forms the color of the ocean, which is recorded by space scanners and serves the basis for remote methods of evaluation of the phytoplankton characteristics. This DOM fraction is most easily measurable by optical methods.[11]

In recent years, fluorimetric methods have been widely applied to studying the organic matter (including petroleum hydrocarbons) in seawaters (see, for example, Refs. 6, 12, and 13). Despite some papers indicate the low correlation between the intensity of the fluorescence signal and the total DOM concentration (see, for example, Ref. 14), more and more authors use the technique of laser-induced fluorescence to study the dynamics of the concentration of fluorescing DOM in seawater,[1,4,11,15] especially, in the regions with high concentration of chlorophyll-a, in which the concentration of the labile DOM fraction approaches the total amount of DOM.

1. Description of the regions under study

This paper analyzes the parameters of LIF spectra of organic matter and phytoplankton pigments, as well as the relations between the intensities of LIF spectral lines of the primary and additional pigments of phytoplankton cells and the concentration of the fluorescing fraction of DOM (expressed in relative units of the Raman line intensity of water) as reconstructed from the LIF spectra. The analysis is performed in the algae bloom and neighboring periods for three most bioproductive regions of the World Ocean: the North Sea and the northeastern part of the Atlantic Ocean near the islands of Ireland and Great Britain (denoted as Region I), the southwestern part of the Atlantic Ocean in the region of the Falkland Islands and the Pacific coast of Chile (Region II), and the Sea of Okhotsk (Region III). The study involved a total of about 12 thousands of LIF spectra recorded from on board *Nadezhda* sailing vessel during the cruise in the Sea of Okhotsk in 2002 and the round-the-world cruise in 2003.

The routes of the vessel are shown in Figs. 1*a–c*. The parts of the route, at which the LIF spectra and hydrological parameters were recorded continuously with a flow-through fluorimeter, are shown by bold lines.[16] Figures 1*d–f* depict the results of processing the SeaWiFS (Sea-viewing Wide Field of view Sensor) data (SeaWiFS is a scanner of the sea color deployed onboard the SeaStar spacecraft) for 2002, from which we can determine the periods of the algae blooming in the regions under study. Months are plotted as a horizontal, and the concentrations of chlorophyll-a 8-day average over the region studied (second-level SeaWiFS data were used)[17] are plotted as a vertical. The regions, corresponding to the route of the vessel, were separated from the SeaWiFS data.

In the North Sea and the adjacent region of the Atlantic, in the Atlantic Ocean (in the water area adjacent to the Falkland Islands), and near the Pacific Coast of Chile, the measurements were conducted just within the period of algae blooming, namely, in April in the North Sea and in November near the Falkland Islands (spring in the Southern Hemisphere). In the Sea of Okhotsk, the measurements were conducted in July, which is about 1.5 months after the maximum of the concentration observed by SeaWiFS. The large number of cloudy days in the Sea of Okhotsk in the period from May through August did not allow the period of algae blooming to be determined from the SeaWiFS data with sufficient accuracy. Nevertheless, at some parts of the route, the shipborne measurements evidence very high concentration of chlorophyll-a, which is characteristic of the blooming period.

For geographic reference to the route and for analysis and display of the data, the specially developed "Far East Seas" GIS[18] along with the "Ocean data view" software[19] were used.

2. Characteristic features of the LIF spectra

The characteristic LIF spectrum corresponding to the high chlorophyll-a concentration, equal to 7.2 μg/l (after correction for the spectral transmission function of the filter used to suppress Raman scattering and for the spectral sensitivity of the photocathode of the detecting PMT), is shown in Fig. 2. The initial spectrum recorded by the flow-through laser fluorimeter[16,20] without averaging and accumulation of signal corresponds to curve *1*.

For further processing, the spectrum was smoothed and split into the following biooptical components (BCs): phycoerythrin fluorescent line (Ph), whose shape can be described by the Gaussian function centered at the wavelength of 580 nm; line with the center at 610 nm (*Y*); Raman scattering of water (RS, line with the center at 648 nm); fluorescence of chlorophyll-a (Ch-A, line with the center at 680 nm); fluorescence of chlorophyll-b (Ch-B, line centered at 710 nm); DOM fluorescence, described by the descending exponent (solid line below the LIF spectrum in Fig. 2, in the wavelength range from 540 to 750 nm). The parameters of all BC functions were determined by the nonlinear least-squares method.[21] The spectrum composed of BCs calculated in this way is presented by curve *3*.

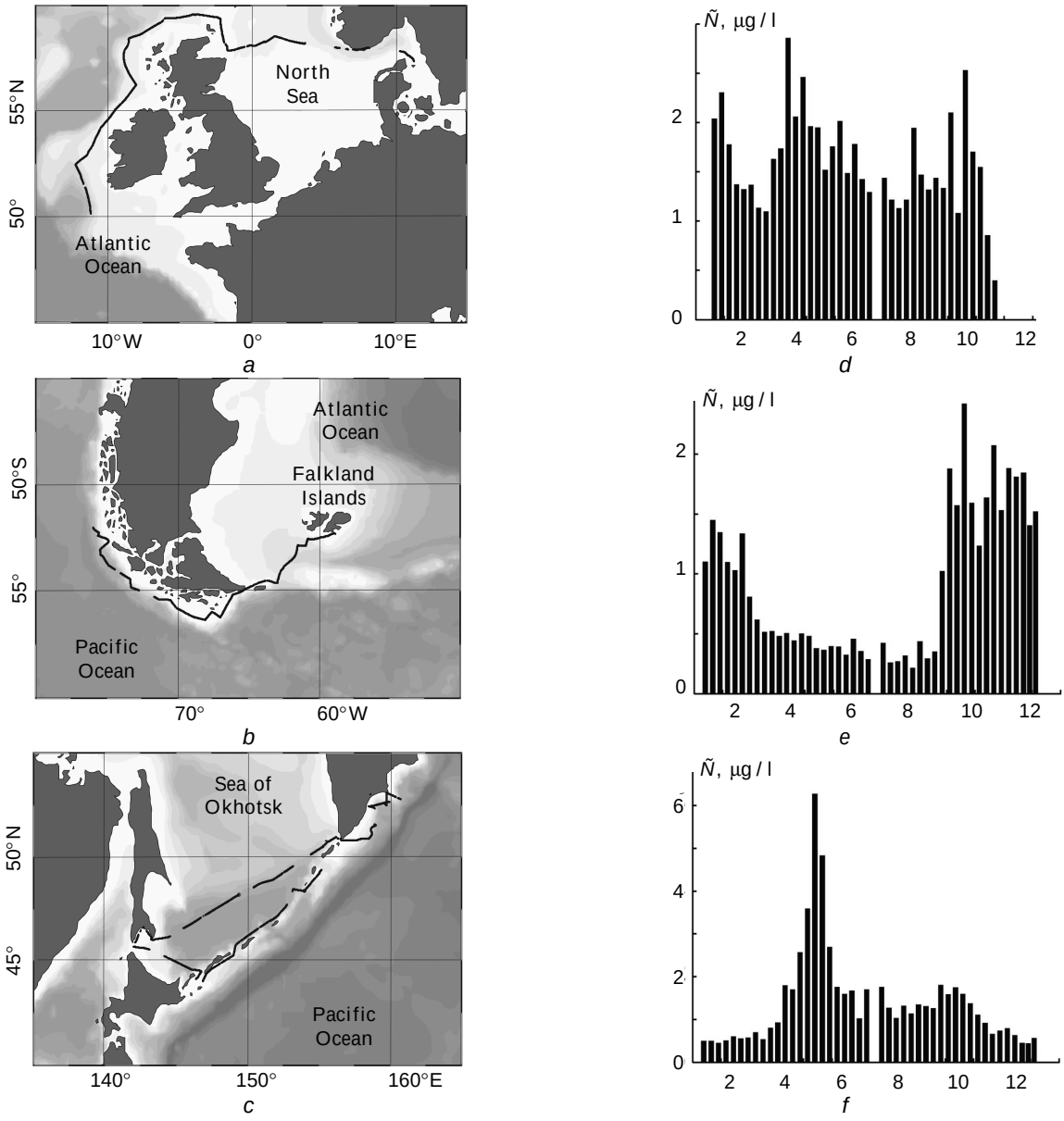

Fig. 1. Regions of LIF measurements (*a*–*c*) and annual profile of the regionally mean chlorophyll concentrations (*d*–*f*) reconstructed from the SeaWiFS data.

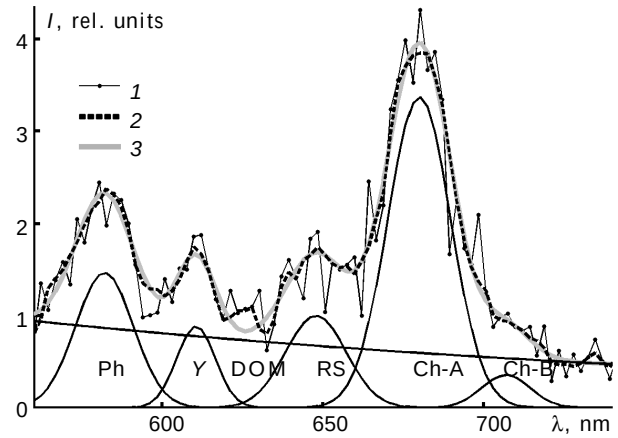

Fig. 2. The LIF spectrum of seawater normalized by the intensity of water Raman band: recorded spectrum (curve *1*); smoothed spectrum (*2*); sum of the biooptical components (*3*).

The fluorescence line peaking at the wavelength of 580 nm was assigned to fluorescence of one of the additional pigments in the phytoplankton cell, namely, phycoerythrin, according to Refs. 1, 22, and 23. This additional pigment absorbs the radiation in the green spectral region, where the absorption of chlorophyll-a is low; its content is especially high in cyanobacteria and *Oscillatoria erythraea* (Refs. 9 and 23).

According to data of our laboratory experiments, which agree with the results from Ref. 23, the broad DOM fluorescence band had a peak at the wavelength of 560 nm, when excited by the laser radiation with the wavelength of 532 nm. A close-to-exponential fall off was observed starting from 560 nm and into the long-wave region. The results of the experiments conducted with filtered seawater, bidistillate, and tap water are shown in Fig. 3 (sea water was filtered using filters with the pore diameter of 2.5 μm).

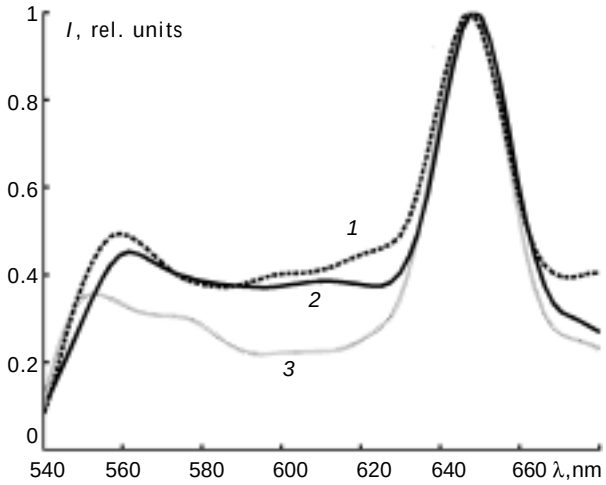

Fig. 3. The LIF spectrum (normalized to the Raman line intensity) of filtered seawater (*2*), bidistilled water (*3*), and tap water (*1*).

Consider the LIF spectral band of 580 to 645 nm, that is, the band lying between the phycoerythrin fluorescence line and the line of Raman scattering from water, in a more detail. The line peaking at 610 nm is observed quite stably in the regions under study, as shown in Fig. 2 (this line is denoted by *Y*). The laboratory experiments showed that this line is absent in the fluorescence spectrum of bidistilled water, tap water, and filtered seawater, when excited by laser radiation with the wavelength of 532 nm (see the spectra shown in Fig. 3).

We have analyzed the correlation between the fluorescence intensities of the biooptical component *Y* and other components. The intensity of this component was found to correlate best of all with the intensity of the chlorophyll-a and b lines (maximum of 0.75 in the Sea of Okhotsk and minimum of 0.4 in the North Sea), while the worst correlation was observed with DOM. The biooptical component *Y* was observed in all regions, but its fluorescence had different intensity. The intensity histograms of the component *Y* (normalized to the intensity of water Raman band) were used to determine the most probable intensity values, which turned out to be 0.3, 0.2, and 0.4 for regions I (Fig. 1*a*), II (Fig. 1*b*), and III (Fig. 1*c*), respectively. For a comparison, the modes in the phycoerythrin histograms are 0.4, 0.3, and 0.25 for these regions.

The high correlation of the intensity of *Y* component with the intensity of chlorophyll suggests that this spectral component is likely caused by pigments entering into the phytoplankton cells. It should be noted that the component *Y* can also appear in the LIF spectra of algae and some bacteria.[23]

In all the three regions at the sites with the high concentration of chlorophyll-a, the band nearby 710 nm was observed, which was assigned to chlorophyll-b fluorescence.[24] The correlation coefficients determined from the scatter diagrams of the relative fluorescence intensity of chlorophyll-a and chlorophyll-b had high values of 0.9, 0.82, and 0.9 for regions I, II, and III, respectively. On the average, the relative intensity of chlorophyll-a exceeded that of chlorophyll-b by 5.2, 4.2, and 3 in the regions studied. This allows us to estimate the intensity ratio of these BC lines for each of these regions.

3. Peculiarities of the LIF spectra of the dissolved organic matter

The variation of the intensity of DOM fluorescence in the wavelength range from the green (from 560 nm) to the red can be approximated rather well by the exponential function:

$$I_{\mathrm{DOM}}(\lambda) = a\exp(-b\lambda). \qquad (1)$$

The parameters of the exponent are mostly determined by DOM should represent the specific conditions of DOM formation and the stage of degradation of the organic compounds being the components of DOM. This specificity consists, first of all, in the rate of DOM production by a phytoplankton community, which depends on the season, the types of algae, and the local conditions of phytoplankton development (hydrological, hydrochemical, hydrophysical, and other features of regions). In addition, the parameters of the exponent should, likely, depend on the rate of degradation and transformation of the organic matter produced by the phytoplankton cells. The degradation processes, in their turn, depend on the ambient conditions.[7] Therefore, the constant in the exponent in Eq. (1) reflects, most probably, the "quality" of the DOM studied. To relate the parameters of the LIF spectrum of seawater to the concentration of the fluorescing part of DOM, we introduce the integral parameter *Q*:

$$Q = \int_{560}^{740} \frac{I_{\mathrm{DOM}}(\lambda)}{I_{\mathrm{RS}}} \mathrm{d}\lambda. \qquad (2)$$

From here on, we shall consider this parameter as some conditional concentration of the fluorescing part, because we are interested in the peculiarities of statistical relations between the chlorophyll-a concentration and the parameter characterizing not only the quantity of DOM, but also its quality (that is, the composition and the stage of degradation). In Refs. 20, 25, and 26, we compared the chlorophyll-a concentration (*C*) and the parameter *S*, which, in contrast to *Q*, includes fluorescence bands of additional pigments. This fact complicated the interpretation of the results obtained from the viewpoint of determining the relations between DOM and phytoplankton. If the correlation between *C* and *Q* is low, this means that there is a large amount of fluorescing DOM, not attributed to the phytoplankton community functioning in the considered period.

Earlier we defined such waters as waters of the second type.[25] In the cases of the linear relation between $\tilde{N}$ and *Q* (first-type water), the relationship between these parameters can be written as follows:

$$Q(C) = Q_0 + \nu C, \quad (3)$$

where $Q_0 = Q(0)$ is the background DOM value; ν is the specific reproduction of DOM by the phytoplankton community functioning in the analyzed water area during the measurements. In fact, Q_0 corresponds to the conditional concentration of the fluorescing organic matter, which would be observed in the studied region at the zero chlorophyll-a concentration, that is, in the absence of phytoplankton. The value of νC corresponds to that labile part of the DOM, which is produced by the phytoplankton community in this region for unit time. Then the parameter ν can be considered as a parameter characterizing the production of fluorescing DOM by the number of phytoplankton cells containing 1 μg/l of the chlorophyll-a. This parameter must depend on the ambient conditions, under which the cell develops, as well as on the species composition and the period of development of the phytoplankton cells.

Figure 4 shows the scatter diagrams of Q and C for regions I–III, histograms of the chlorophyll-a concentration, the parameter Q, and the constant b.

The linear relation between the chlorophyll-a concentration and the parameter Q is most pronounced in regions I and II, just where the measurements were conducted exactly in the period of algae blooming. In region III (see Fig. 4*c*), the linear relation between Q and C is not so pronounced. Analyzing individual small samples in the scatter diagrams of Q and C, we can separate clusters with the high coefficient of correlation R between C and Q. However, these clusters differ rather strongly, and their superposition showed no common linear dependence. This is either due to different hydrological characteristics in different subregions of the Sea of Okhotsk or because of some specific features of the period of algae development. Nevertheless, the major part of small-scale subregions (with the size of 10 to 30 km) show rather high correlation, which indicates the prevalence of the linear relation between the biooptical components C and Q in region III as well, but on smaller scales. In region I (Fig. 4*a*), the correlation coefficient is $R_1 = 0.62$ for all measured values (3 500 LIF spectra). It is also possible to separate out smaller clusters. Nevertheless, the superposition of all clusters keeps the linearity of the relation between Q and C on the large scales (the length of the sample along the vessel route was about 2 500 km).

In region II (Fig. 4*b*), the correlation coefficient was also high: $R_2 = 0.7$ (3 300 LIF spectra) at the route leg of 1700 km in length. Here it is possible to separate out, at least, two smaller clusters, though they fit rather well in one large-scale cluster.

In the regions with R higher than 0.4, we conducted the regression analysis by Eq. (3). For regions I and II, the following values of the background level and the DOM reproduction rate were obtained: $Q_{01} = 77$, $Q_{02} = 31$, $\nu_1 = 5.2$, $\nu_2 = 6$. It is also worth noting the very close values of the parameter ν, that is, the specific reproduction of DOM by the phytoplankton community in regions I and II in the period of blooming.

From Figs. 4*d–f* we can see that the chlorophyll-a concentration has a bimodal distribution with peaks at 4 and 16, 3 and 8, 2 and 6 μg/l, respectively, for regions I, II, and III (see Fig. 4*d*). These peaks can be also observed in the scatter diagrams, in which the small-scale clusters with the characteristic DOM parameters are grouped near these values of the chlorophyll-a concentration. Since the waters of the analyzed regions are mostly the waters, in which the linear relation between Q and C is pronounced, the high values of the chlorophyll-a concentration must lead to the high DOM concentrations. This is seen from Fig. 4*e*, in which the peak of the Q distribution equal to 100 is the highest in the region I.

Although in the Sea of Okhotsk we failed to find the linear relation between Q and C on the large scale, it is still possible to perform some comparison using the histograms shown in Figs. 4*d* and *e*. Since the shape of the $\tilde{N}$ distribution in regions II and III is roughly the same (Fig. 4*d*), the comparison of the peaks in the distribution of Q (40 and 70 in Fig. 4*e*) suggests that in the region III the background DOM values were high or the specific reproduction of DOM was higher. To find the true reason, we have to perform small-scale analysis. From the comparison of regions III and I, we can only conclude that the higher values of the chlorophyll-a concentration lead to higher values of Q. It should be noted that, unlike the chlorophyll-a distribution, the distribution of the parameter Q is unimodal in all the three regions.

It is seen from Fig. 4*f* that similar unimodal distribution of the constant b with the mode at about 0.005 and 0.006 are observed in the regions II and III, respectively. The distribution of the constant b in region I is also unimodal with the peak at 0.004, but here we can see a wide "tail" into the region of high values. These high values correspond to the region of the Norwegian Hollow and the Skagerrak Strait.

The lowest value of the constant b (more slightly descending DOM fluorescence) was observed in the North Sea, where the phytoplankton blooming was more intense. This can be connected with the fact that DOM included relatively more components fluorescing in the red spectral region, which, in turn, can be attributed to higher concentration of suspended matter at strong wind-induced and tidal mixing in shallow waters of the North Sea (turbid water was observed visually during measurements). All the three regions are well seen in the histograms of the constant b (see Fig. 4*f*).

The results presented demonstrate the possibility of describing the reproduction of the fluorescing part of the dissolved organic matter by the plankton community with the use of biooptical components of LIF spectra of organic matter in seawaters.

In the period of algae blooming, at least in two most productive regions of the World Ocean, the linear relation is observed between the chlorophyll-a concentration and the conditional concentration of the fluorescing part of DOM.

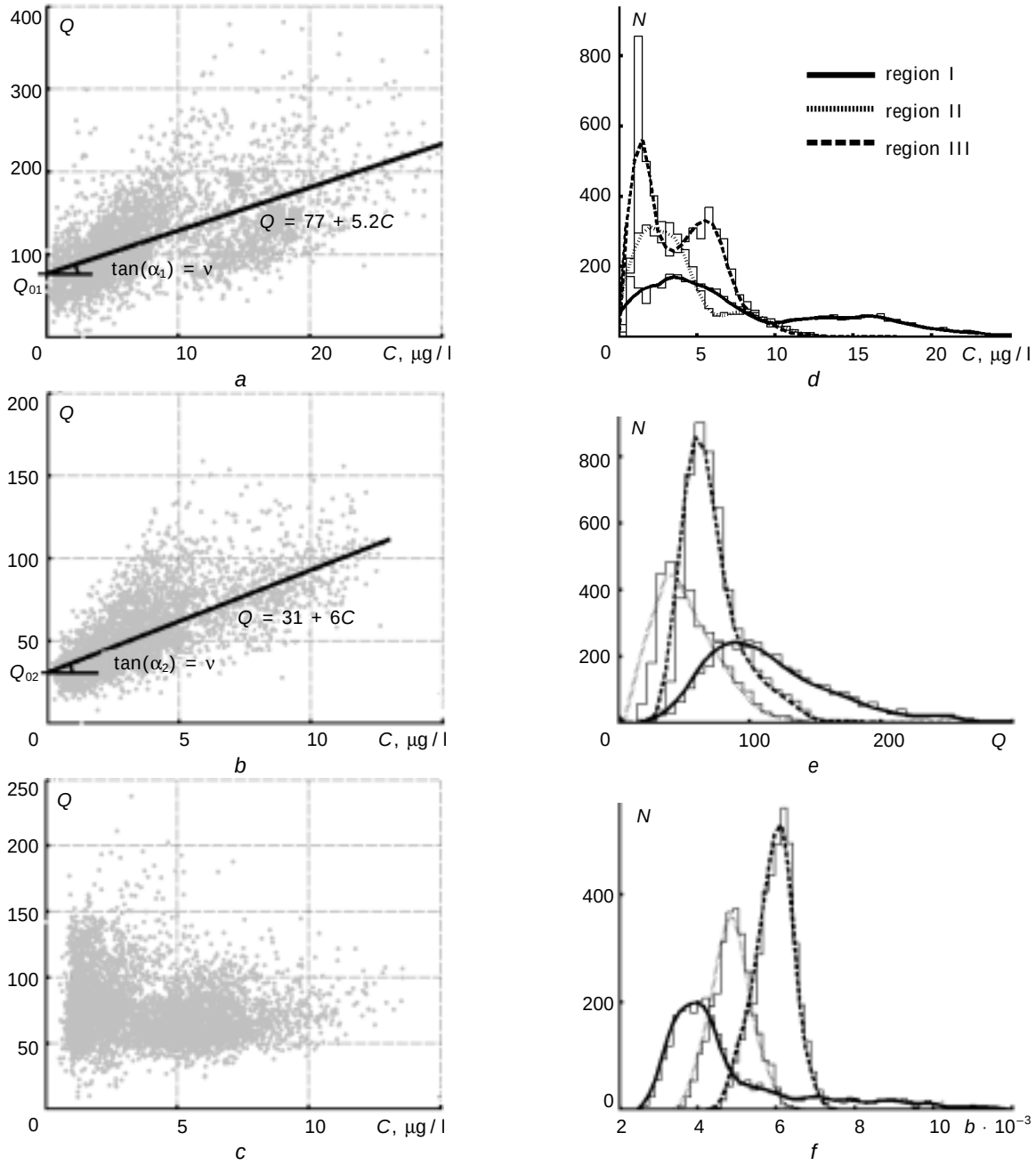

Fig. 4. Scatter diagrams of the conditional concentration Q of the fluorescing part of DOM and the chlorophyll-a concentration C for regions I (*a*), II (*b*), and III (*c*); histograms of $\tilde{N}$ (*d*), Q (*e*), the constant b of the exponent, characterizing the DOM fluorescence (*f*); N is the number of points.

In the case of such a linear relation, it is possible to introduce a parameter characterizing the background values of the fluorescing DOM, in which the reaction of photosynthesis occurs, and a parameter determining the specific reproduction of DOM due to vital activity of the phytoplankton community in the period under study.

The scales, at which the parameters $\tilde{N}$ and Q are linearly related, are rather large in the period of algae blooming and amount to about 2000 km in regions I and II. Beyond the period of algae blooming, as in region III, these relations likely keep true only on small scales, but break on the larger scales.

References

1. R. Barbini, F. Colao, R. Fantoni, A. Palucci, and S. Ribezzo, Int. J. Remote Sens. **22**, Nos. 2/3, 369–384 (2001).
2. Z.S. Kolber and P.G. Falkowski, Limnol. and Oceanogr. **38**, No. 8, 1646–1665 (1993).
3. O.A. Bukin, M.S. Permyakov, A.N. Pavlov, A.Yu. Maior, A.V. Maleenok, T.I. Tarkhova, G.V. Skorokhod, and D.A. Akmaikin, Atmos. Oceanic Opt. **13**, No. 9, 787–790 (2000).
4. V.N. Pelevin, O.I. Abramov, G.G. Karlsen, V.V. Pelevin, A.M. Strogov, and D.V. Khlebnikov, Atmos. Oceanic Opt. **14**, No. 8, 646–650 (2001).
5. V.V. Klimov, Soros. Obraz. Zh., No. 8, 6–13 (1996).

6. L. Guo, P.H. Santchi, and K.W. Warnken, Limnol. and Oceanogr. **40**, No. 8, 1392–1403 (1995).
7. B.A. Skopintsev, Okeanologiya **21**, No. 5, 821–830 (1981).
8. O.K. Leont'ev, ed., *Atlantic Ocean* (Mysl', Moscow, 1977), 296 pp.
9. R.W. Spinrad, K.L. Carden, and M.J. Parry, *Ocean Optics* (Clarendon Press, Oxford; Oxford University Press, New York, 1994), 283 pp.
10. B. Norrman and U.L. Zweifel, Limnol. and Oceanogr. **40**, No. 5, 898–907 (1995).
11. R.F. Chen, Org. Geochem. **30**, 397–409 (1999).
12. S.M. Glushkov, V.V. Fadeev, and V.V. Chubarov, Atmos. Oceanic Opt. **7**, No. 4, 246–250 (1994).
13. S.M. Glushkov, V.V. Fadeev, E.M. Filippova, and V.V. Chubarov, Atmos. Oceanic Opt. **7**, No. 4, 231–238 (1994).
14. G.S. Karabashev and A.I. Agatova, Okeanologiya **24**, No. 6, 906–909 (1984).
15. V. Drozdowska, S. Babichenko, and A. Lisin, Oceanologia **44**, No. 3, 339–354 (2002).
16. A.Yu. Maior, O.A. Bukin, A.N. Pavlov, and V.D. Kiselev, Prib. Tekh. Eksp., No. 4, 151–154 (2001).
17. http://daac.gsfc.nasa.gov
18. E.L. Podoprigora, T.S. Chistyakov, V.A. Khovanets, and M.S. Permyakov, Issledovano v Rossii, No. 128, 1517–1526 (2003); http://zhurnal.ape.relarn.ru/articles/2003/128.pdf
19. R. Schlitzer, *Ocean Data View*; http://www.awi-bremerhaven.de/GEO/ODV, 2003.
20. O.A. Bukin, M.S. Permyakov, A.Yu. Maior, S.G. Sagalaev, E.A. Lipilina, and V.A. Khovanets, Atmos. Oceanic Opt. **14**, No. 3, 203–206 (2001).
21. D. Marquardt, SIAM J. Appl. Math. **11**, 431–441 (1963).
22. F.E. Hoge and R.N. Swift, Appl. Opt. **20**, No. 18, 3197–3205 (1981).
23. R.J. Exton, W.M. Houghton, W. Esais, R.C. Harriss, F.H. Farmer, and H.H. White, Appl. Opt. **22**, No. 1, 54–64 (1983).
24. F.V. Bunkin and A.F. Bunkin, Atmos. Oceanic Opt. **13**, No. 1, 54–60 (2000).
25. O.A. Bukin, M.S. Permyakov, A.Yu. Maior, A.N. Pavlov, G.V. Skorokhod, V.V. Chekunkova, O.S. Tsareva, and T.I. Tarkhova, Atmos. Oceanic Opt. **13**, No. 11, 936–939 (2000).
26. O.À. Bukin, A.N. Pavlov, M.S. Permyakov, A.Yu. Major, O.G. Konstantinov, A.V. Maleenok, and S.A. Ogay, Int. J. Remote Sens. **22**, Nos. 2/3, 415–428 (2001).