Studies of organic matter reproduction in phytoplankton cells by laser-induced fluorescence method

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The functional relationships between the bio-optical components of the seawater characterizing the content of chlorophyll-*a* and the part of dissolved organic matter capable of fluorescing have been studied in different regions of the World Ocean. Phytoplankton communities (clusters) have been revealed, for which these relations are linear. In the case of clusters with high correlation between the above-mentioned components regression parameters have been calculated. These parameters characterize the rates of reproduction of the dissolved organic matter and the background amounts of the fluorescing part of the dissolved organic matter. It is shown that these parameters can be used in studying the reproduction of organic matter by phytoplankton cells and to classify the phytoplankton communities (in relation to the organic matter reproduction).

Introduction

Today, the problem of monitoring the phytoplankton communities assumes a special importance because of so much obvious climatic changes on the Earth and growing anthropogenic impact onto the aquatic ecosystems. However, the monitoring of phytoplankton communities implies measurement of the parameters characterizing separate cells and the functioning of photosynthetic system at a molecular level, but applied to large spatiotemporal scales.[1,2]

For a more comprehensive study of the states of phytoplankton communities and effects of different processes (including anthropogenic ones) on the functioning of phytoplankton cells, we need data on the whole set of parameters, such as concentrations of chlorophyll-*a* and other cellular pigments, the electron transportation rates in photosynthesis of organic matter, concentrations of primary intracellular elements, etc. All this must be measured efficiently and over wide offshore zones. The laser spectroscopy methods allow us to carry out such measurements.[3–5]

Application of the laser-induced fluorescence (LIF) in studies of reproduction of organic matter by phytoplankton cells is of great interest not only because it allows us to control the state of aquatic ecosystems, but also because it helps to estimate the role of phytoplankton in the global cycle of reproduction of organics on our planet. The amount of dissolved organic carbon in the ocean is comparable with that of CO_2 in the atmosphere and makes ~ 20% of the total content of organic matter on the Earth (except for kerogens and coal).[6]

The phytoplankton is the main source of dissolved organic carbon in the ocean. According to the estimates by Romankevich,[7] phytoplankton annually produces about 20 billion tons of dissolved organic carbon, while the land and primary phytobenthos production yield as little as 5% of the incoming organic matter. The phytoplankton biomass expressed in the units of organic carbon is relatively small (just 80 million tons). Thus, the mass of its yearly products exceeds its biomass by 250 times. This points to an important role of photosynthesis in the process of reproduction of organics on our planet and determines the interest in the cycles of reproduction of organic matter by phytoplankton cells.[8]

Most organic carbon (90–98% by different estimates) in the sea exists in dissolved state[7,9] and thus it is a component of dissolved organic matter (DOM). Dissolved organic matter consists of very complex compounds. Only 10 to 20% of these compounds can be described as separate components. The rest, 80–90%, comprise complex organic mixtures, which cannot be split into components and presented analytically.[10,11] Due to a very complex structure, measurement of DOM is a complicated task. Chemical methods are extremely laborious and do not always allow us to separately measure the concentrations of each DOM component. Despite the fact that optical methods allow detection of just chromophores and fluorophores (i.e., the compounds absorbing the light or fluorescing under the influence of incident light), they still are most suitable for monitoring the organic matter reproduction processes in the seawater for they allow us to perform efficient measurements over large offshore zones.

Earlier,[4] we have demonstrated that it is possible to apply the LIF spectroscopy of seawater to investigation of the cycles of fluorescing DOM reproduction by phytoplankton. At large spatial scales, during algae bloom we established a linear relationship between the bio-optical components of LIF spectra, which are determined by the chlorophyll-*a* concentration, and the relative intensity of DOM fluorescence.

In this paper, we consider the scales, at which there is a uniform linear ratio between the bio-optical

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parameters of LIF spectrum for those regions of the World Ocean, where we have measured LIF spectra, and the ratio between the bio-optical components at different periods of alga growth (not only during their bloom). We also analyze the parameters of linear relations between the bio-optical components of LIF spectra that characterize reproduction of organics by phytoplankton and allow us to classify phytoplankton communities from this viewpoint.

Measurement techniques

The measurement technique and the instrumentation were described in detail in Refs. 12 and 13. Here we describe them just briefly. Fluorescence spectra of seawater were excited by the second harmonic of an Nd : YAG laser at 532 nm wavelength. Fluorescence intensity was measured in the range from 560 to 740 nm. The spectra of LIF were corrected for the spectral transmission function of the filter, which served for suppressing the elastic scattering, and for the spectral sensitivity of the PMT's photocathode used. The fluorimeter was installed onboard a ship and was operated in a continuous-flow mode; spatial resolution in measurements of one spectrum did not exceed 500 m and depended on the speed of the vessel.

In the study of reproduction of organics by phytoplankton, it is essential to find the amount of DOM reproduced by the cells, or specific reproduction (the amount of DOM reproduced by such an amount of phytoplankton, which contains 1 μg/l of the chlorophyll-*a*). Here, is important the possibility of measuring only "young" DOM, i.e., select that part of DOM, which is reproduced by intravital plankton excretions.[15] This part can be described by one of the bio-optical parameters (namely, the parameter Q) of LIF spectrum.[4]

The chlorophyll-*a* concentration (in the equation it is designated as C) and the parameter Q, which represents the area normalized under the envelope of the DOM fluorescence spectrum (reduced to a spectral interval of 1 nm), are determined from LIF spectra as follows:

$$C = kI_{\text{Ch-}a}/I_{\text{RS}}; \quad (1)$$

$$Q^* = qQ = q\int_{560}^{740}\frac{I_{\text{DOM}}(\lambda)}{I_{\text{RS}}}d\lambda, \quad I_{\text{DOM}}(\lambda) = a\exp(-b\lambda), \quad (2)$$

where I_{RS} is the maximum of intensity of the Raman scattering of seawater; $I_{\text{Ch-}a}$ is the maximum of intensity of the chlorophyll-*a* fluorescence line; $I_{\text{DOM}}(\lambda)$ is the DOM fluorescence intensity; k is the calibration constant determined from comparison with the standard measurements of the chlorophyll-*a* concentration[12]; q is the coefficient that converts the dimension of the normalized area under the DOM fluorescence line in the considered spectral region into the dimension of DOM concentration in μg/l, Q^*; a and b are the coefficients of regression exponent that describes the envelope of DOM fluorescence spectrum. The values of intensities were determined by the least squares method in splitting the spectrum into separate bio-optical components, where the pigment fluorescence peaks were represented by Gaussian curves, and DOM fluorescence spectrum in the considered region was described by the exponent.[4]

We used the Monte Carlo method to determine the random and systematic errors in measurements of C and Q. With the allowance made for the calibration error introduced by the least squares method and for the noise in LIF spectra, the total relative error (δC) of the chlorophyll-*a* concentration equal to 0.1 μg/l (at a 30% noise) made 105%. It is quite comparable with the sensitivity of other methods.[14] With the increase in concentration, the relative error decreased abruptly. For example, at $C = 0.25$ μg/l, $\delta C = 58\%$, and in the range of concentrations from 1 to 8 μg/l, $\delta C = 17$–19%. With the further increase in concentration, relative error grew continuously, reaching 35% at 30 μg/l. The above evaluations were obtained in simulations of 100 LIF spectra with preset parameters.

The question on calibration of DOM fluorescence intensity (determination of the coefficient q) is quite complicated. This is because common techniques often do not allow obtaining total DOM concentration, but only the composition of its separate components. In Ref. 15 its author has comprehensively studied the relation between the DOM fluorescence intensity and the concentration of organic carbon. There are cases, when it is impossible to establish a relation between them. One of possible explanations of this is the fact that the majority of carbon atoms can be arranged in the groups that either fluoresce weakly or do not fluoresce at all, depending on to which DOM degradation stage a particular complex belongs to. However, we have demonstrated[4] that in highly productive regions of the World Ocean, where the concentrations of the chlorophyll-*a* are high, and also in the period of algae bloom, when the percent of "young" DOM is high, there is a linear relation between the DOM fluorescence intensity and the concentration of the chlorophyll-*a*, i.e., in this case, we can establish a direct relationship between the DOM fluorescence intensity and the concentration of the chlorophyll-*a* in the cells that produce this DOM.

Regions covered in measurements

We used data on 100 thousand LIF spectra, which we obtained during our expeditions from 2002 to 2004 to the open ocean and marginal seas of the Pacific, Indian, and Atlantic Oceans. The section, along which we measured LIF spectra, temperature, and salinity at a depth of four meters, is shown in Fig. 1. We have now data on virtually all types of waters from different climatic zones and at different algae bloom periods.

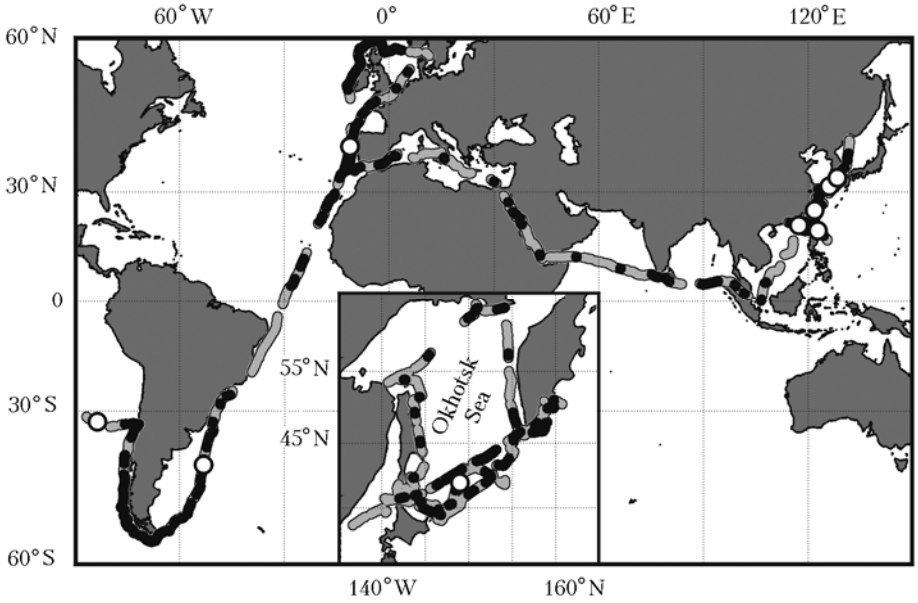

Fig. 1. Regions of measuring the LIF spectra.

In the aquatic system of Okhotsk Sea, we have carried out measurements in the period from 2000 to 2004, each year from July to September. The peak of algae bloom falls on May.[4] But in some areas of the Okhotsk Sea, high concentrations of the chlorophyll-*a* were also recorded in the summer–fall period because of the local algae bloom. For example, in August 2002, along the Kuril Straits we recorded the chlorophyll-*a* concentrations about 20 μg/l as high.

In the period from January 2003 to April 2004, in our overseas expedition, we conducted measurements in the East China Sea and in the South China Sea twice: in February 2003, when there was no bloom, and in March 2004 at early algae bloom. Then we measured in the Indian Ocean and in the Red Sea in February–March 2003, in the Mediterranean sea and in the Atlantic Ocean round the Portuguese coast in April. In all the above-mentioned regions, the chlorophyll-*a* concentrations did not exceed 3 μg/l. High concentrations of the chlorophyll-*a* (up to 20 μg/l) were recorded in the North Sea in April 2003 and in the southern Atlantics and along the Chilian coast of the Pacific Ocean in November–December 2003. In these regions, we have carried out our measurements during the algae bloom.

Data analysis

We analyzed the relationship between the DOM fluorescence intensity excited by radiation at 532 nm wavelength, which we have numerically expressed by the parameter Q, and the concentration of the chlorophyll-*a* (retrieved from LIF spectra) expressed as C. To do this, we have built the Q–C scatter diagrams (Fig. 2). In some regions, a linear dependence between Q and C could be observed. In this case, if the coefficient of correlation between Q and C (denoted as R) is statistically significant, we introduce a linear regression for description of the Q–C relationship. In the regression, the free term is denoted as Q_0, and the slope ratio as v:

$$Q(C) = Q_0 + C_{\text{Ch-a}}v. \qquad (3)$$

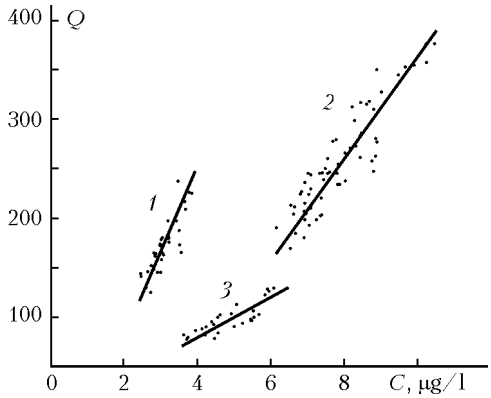

Fig. 2. An example of linear scatter diagrams for different regions of the World Ocean: the Okhotsk Sea, August 3, 2002 (*1*); the North Sea, April 28, 2003 (*2*); the south-western part of the Atlantic Ocean, December 10, 2003 (*3*).

From the general considerations, Q_0 can be interpreted as some background value of the fluorescing DOM (i.e., either the DOM that has not been produced by the considered phytoplankton community in the considered time period or the DOM that has come from the sources not related to phytoplankton community at all). The coefficient v can be regarded as production of the fluorescing DOM by one unit of the chlorophyll-*a* during the considered time period.[4]

Then, the question arises on how to properly specify the boundaries of the area, within which the relationship between C and Q would be linear and

uniform. In other words, how to choose a linear data cluster, where the process of organics reproduction could be described by the same parameters? Visual separation using the Q–C scatter diagrams is difficult and sometimes impossible to perform, for often, in one diagram, there are many different clusters with similar parameters. To clusterize, we have chosen the following parameters: the coefficient of correlation R between C and Q, the slope of linear regression v, and its free term Q_0 (see. Eq. (3)), i.e., the clusters are determined only by the parameters of linear dependence between C and Q and do not depend directly on the quantities C and Q.

The clusterization itself consisted in the following: within a window of a specified width we calculated the correlation coefficient R, the coefficients of linear regression, and their corresponding statistical errors. The size of the window was chosen to be 60 points (about 30 km). The reduction of window size resulted in the growth of errors in the correlation coefficient and regression parameters and in the loss of fine scales. Then, the selection went on over the whole array of points with a one-point increment. To avoid pooling together the points from different regions, we skipped the data samples with time span between the neighbor points more than 20 min. The calculated parameters were assigned a sample-average time. Thus, we obtained three new time series R, v, and Q_0 with the corresponding errors (Figs. 3*a*–*c*).

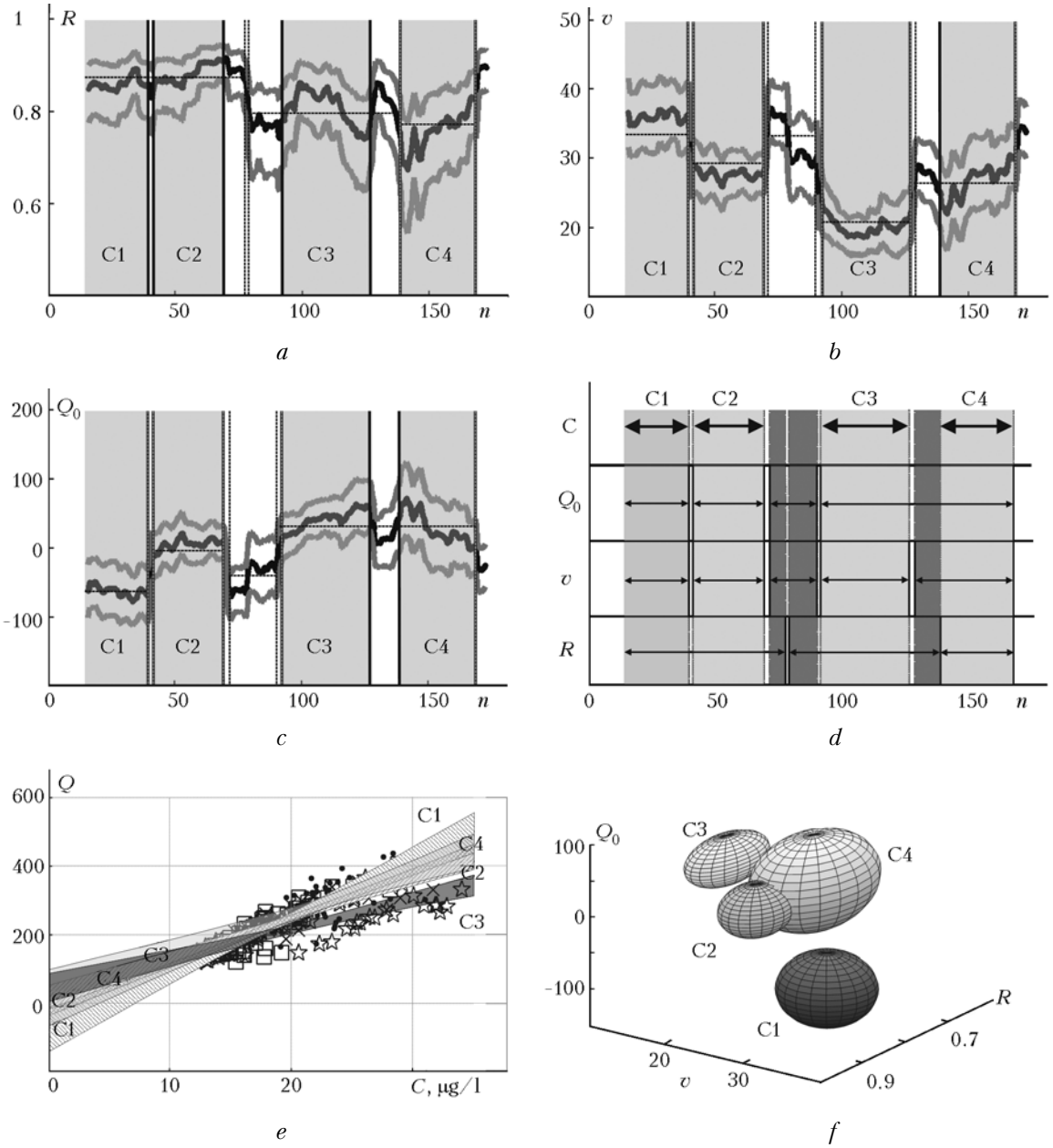

Fig. 3. An example of determining the stationary segments in the time series R, v, and Q_0. The clusters C1–C4 are emphasized.

We believe that equal values (within the error limits) of the resulting parameters describe a separate cluster or a separate phytoplankton community (from the viewpoint of reproducing DOM). It features the same relationship between the amount of phytoplankton and that of the resulting DOM, taking into account that DOM is reproduced with equal rates at a certain DOM background level. From the time series of R, v, and Q_0 formed we determined the boundaries of the area, where all the three parameters would be equal within the error limits (Fig. 3*d*). The corresponding scatter diagrams are shown in Fig. 3*e*, and Fig. 3*f* shows the examples of 3D diagrams of the cluster parameters, which describe homogeneous phytoplankton communities. Such diagrams are built to provide a pictorial view of different clusters. Here, separate clusters are much better to discern than in a 2D Q–C scatter diagram (Fig. 3*e*).

Using this procedure, in our data array we distinguished 319 clusters with the statistically significant correlation coefficient R. Of most interest for us are those phytoplankton communities, which exhibit a strong linear relationship between the chlorophyll-*a* concentration and the phytoplankton-generated DOM. Note that if the concentration of chlorophyll-*a* exceeds 5 μg/l, the correlation coefficient R does not drop below 0.4, and at $C > 10$ μg/l $R > 0.6$ (Fig. 4*a*). That is, we may state that at a sufficiently high concentration of the chlorophyll-*a* (at least, when it is above 5 μg/l), the fluorescence signal is indicative of the presence of DOM produced by the phytoplankton community in the considered area in the considered time period ("young" DOM).

The clusters with the correlation coefficient above 0.5 were examined mostly during algae bloom: for example, in the north-eastern part of the Atlantic Ocean and in the water area of the North Sea in the end of April, 2003, and on the way from Falkland Islands to the Pacific side of the Strait of Magellan early in December, 2003. But there were also occasional linear clusters in the Red and the Mediterranean Seas and the whole series of them in the Okhotsk Sea in 2001 and 2002 beyond the blooming period. We associated these clusters with local algae bloom.

During our voyage, we often recorded the phytoplankton communities with $R < 0$. Many negative values of R could be neglected, for they did not differ much from zero, but there were also such communities, where R was significantly below zero. For example, we recorded 32 communities with $R < -0.5$ (while $R > 0.5$ was recorded for 120 communities). The black segments in Fig. 1 are those in our route, where the values of R are statistically significant (by the Student criterion), and the white circles indicate the regions with statistically significant negative R. Almost all the negative R were observed in the period before algae bloom. Thus, we suppose that at the early stage of phytoplankton development, its cells actively consume DOM.

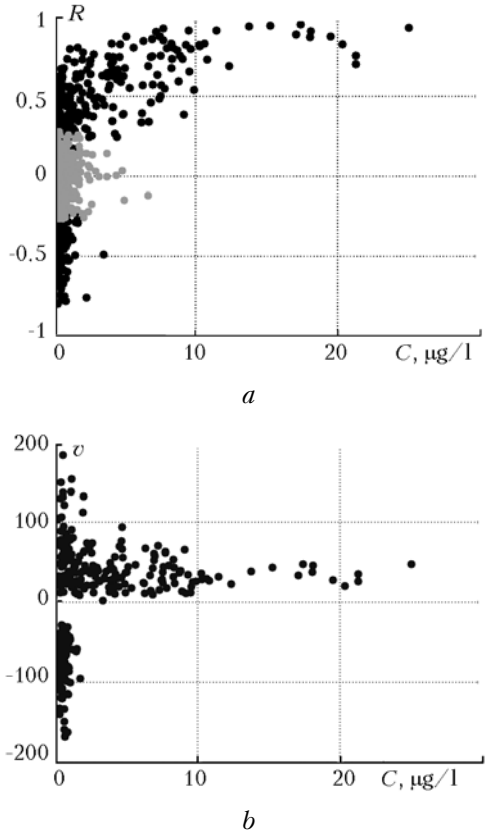

Fig. 4. Dependence of the correlation coefficient R on the chlorophyll-*a* concentration (*a*), statistically insignificant values of R (according to the Student criterion, with the significance level of 5%) are shown in grey; dependence of the parameter v on the chlorophyll-*a* concentration (*b*).

As seen from the v–C scatter diagram (Fig. 4*b*), with the increase in the chlorophyll-*a* concentration we observe a decrease in the organics reproduction rate v. Most likely, with the increase in the chlorophyll-*a* concentration to a certain value, DOM reproduction rate decreases. Here, the parameter v is approximately the same (about 30) for the clusters with the chlorophyll-*a* concentration above 8 μg/l.

The possible negative influence of high population density on the photosynthetic phytoplankton activity has been considered in Ref. 14. We also note that for the linearly negative clusters, the value of v does not exceed 30, and the chlorophyll-*a* concentration in these linearly negative clusters does not exceed 1.5 μg/l. This confirms again the supposition that in the beginning of development of the phytoplankton community, DOM is actively used by phytoplankton cells for them to increase in concentration. Figure 5 shows the histograms of the parameter v for the regions of the World Ocean with most intense biological production.

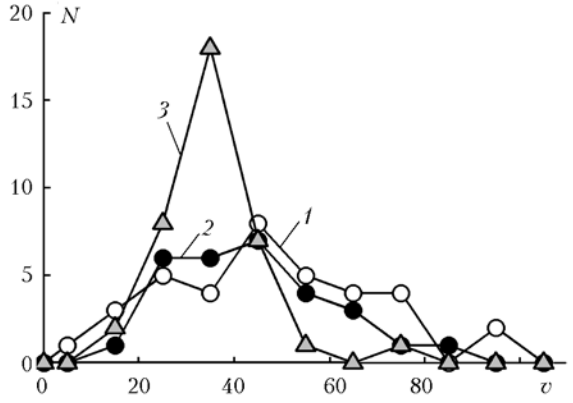

Fig. 5. Histograms of v for most biologically productive regions of the World Ocean: the Okhotsk Sea, July 20 – August 10, 2002 (*1*); the North Sea, April 20 – May 5, 2003 (*2*); the south-west part of the Atlantic Ocean and the Chilean Pacific coast, November 25 – December 10, 2003 (*3*).

In all the three regions, we recorded a single-mode distribution. Convergence (by the order of magnitude) of most probable values of v for different geographical and climatic zones of the World Ocean during the algae bloom time or near-blooming periods (35 for the Southern Atlantics and the Pacific Ocean; ~45 for the North and Okhotsk Seas) is indicative of the universal character of the photosynthesis process from the viewpoint of organics reproduction by phytoplankton cells. This is most obvious for the histograms of two regions of the northern hemisphere (the North and Okhotsk Seas), where the histograms almost coincide. But the differences in the histograms of the examined regions of the northern and southern hemispheres can be associated with the specific features in functioning of phytoplankton communities in different climatic and hydrophysical conditions.

A question arises on what part of DOM starts fluorescing at excitation by a 532 nm wavelength. A number of works (e.g., Refs. 16 and 17) are devoted to the studies of 3D LIF spectra, where the intensity of seawater fluorescence is presented as a function of two variables, namely, the fluorescence excitation wavelength and the fluorescence emission wavelength. On the basis of *in situ* or laboratory measurements, intensity peaks in 3D LIF spectra are attributed by authors to a certain type of DOM. We thus distinguish three main types of fluorescence: protein-like, humus-like, and tyrosine-like. Unfortunately, in the literature that we have found, the fluorescence is excited only by ultraviolet radiation. In the works, where the authors use green radiation to excite the fluorescence (e.g., Ref. 18), they do not analyze what part of DOM yields this fluorescence. Therefore, we have compared the results from Refs. 15, 19, and 20, where the authors study the relation between water salinity and DOM fluorescence intensity at excitation by UV radiation, and our measurements.

In the cited works, almost everywhere we observe an inverse relationship between seawater salinity and UV-excited DOM fluorescence intensity. This allows us to assume that at excitation by the UV radiation, the greatest contribution to the total fluorescence comes from "old" DOM and the terrigenous DOM. From the measurements that we have conducted in water areas with sharp salinity changes (estuary of the Amur in the Okhotsk Sea and passage from the North Sea to the Baltic Sea) we have not revealed any dependence between the 532 DOM fluorescence intensity and seawater salinity. The latter can serve as one argument more in favor of the statement that the major contribution to the total DOM fluorescence intensity at excitation by a 532 nm wavelength comes from young DOM (our first argument is a strong correlation between Q and C at high chlorophyll-*a* concentrations).

Conclusion

The analysis of correlations between the bio-optical parameters of seawater LIF spectra allows us to classify phytoplankton communities in different geographical and climatic regions of the World Ocean by their rates of reproduction of dissolved organic matter. In the main biologically productive regions of the World Ocean, the process of DOM reproduction is generally the same and has similar statistical characteristics. But in the northern and southern hemispheres, phytoplankton communities have somewhat different parameters.

The regions with high chlorophyll-*a* concentrations (the North Sea, Okhotsk Sea, southern parts of the Atlantic and Pacific Oceans) feature approximately the same most probable values of the parameter v (about 40). Measurements in these regions were performed in the period of algae bloom or a local bloom (the Okhotsk Sea). The histograms of distribution of v for the biologically productive regions of the northern hemisphere are almost identical, while in the southern parts of the Atlantic and Pacific Oceans, the phytoplankton communities are more uniform in the organic matter reproduction rates (a narrower histogram).

During our measurements in the Indian Ocean and the equatorial part of the Atlantic Ocean, only in few cases we established some linear relationship between Q and C within the spatial scales analyzed. This can be explained by low mean values of the chlorophyll-*a* concentration in these regions, which results in a minor contribution of "young" DOM to the total signal of DOM fluorescence.

This approach to the description of functioning of phytoplankton communities supported by the methods of analysis of chemical composition of seawater and phytoplankton cells,[21] the ratio in the cell pigment composition, and determination of the electronic transportation rates in the photosynthesis reaction[3] will allow us to make a more thorough description and a more detailed classification of the phytoplankton communities.

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