# Calibration of the method of laser fluorometry for measuring the chlorophyll A concentration

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Calibration coefficients are given for measuring the absolute values of the chlorophyll A concentration by the method of laser fluorometry. The coefficients obtained in different regions of the Pacific Ocean, as well as in the Sea of Okhotsk and Sea of Japan are compared. It is shown that they coincide within the measurement error. Exclusions are only the situations that the measurements were conducted in the zones of intense temperature fronts.

The problem of finding the chlorophyll A concentration from the laser-induced fluorescence spectra of sea water is very important, because the fluorescent response of pigments to laser excitation can depend on many factors. In the first turn, it depends on specific composition of phytoplankton, its state, stage of development, as well as hydrological parameters of the sea water, etc.[1–3] When the laser fluorescent method is used, the chlorophyll A concentration is determined as a ratio of fluorescence signals to Raman scattering of water.[4] However, now there are only a few papers in the literature devoted to determination of calibrating coefficients from conversion from the relative intensity of fluorescence line to the absolute concentration of the chlorophyll A for various types of the sea water. Only Ref. 1 gives the conversion coefficients for some oceanic regions. This is caused by the fact that standard measurements of the chlorophyll A concentration are very labor- and time-consuming, and such a calibration requires a large number of measurements.

In spite of the fact that for solution of most problems by the laser fluorometry method it is sufficient to study relative changes in fluorescence signals, the need in such a calibration is high, because it far extends the domain of applicability of this method.[5,6] For example, the present time is characterized by intense exploitation of biological resources, the efficient control over which requires real-time measurements of the absolute chlorophyll A concentration, and one of the methods of such measurements is just the method of laser fluorometry.

In this paper, we present the experimental data obtained during several research expeditions, in which the concentration of the chlorophyll A was measured by standard methods along with measurements of the intensity of the chlorophyll A fluorescence line. The data were obtained in the expeditions of 1992 and 1993 aboard Research Vessel *Akademik Lavrent'ev* in the Sea of Okhotsk, aboard Sailing Training Vessel *Nadezhda* in 1997–1998 in the open waters of the Pacific Ocean and in August 2000 in the shelf waters of the Sea of Okhotsk and the Sea of Japan. A total of about 30 calibrations were made. The data of the last mentioned expedition are of most interest from the viewpoint of analysis of possible dependence of the calibration coefficient on various factors. The shelf waters of the Sea of Okhotsk are characterized by a wide diversity of the specific composition of phytoplankton and wide variety of hydrological parameters and phytoplankton concentration on different spatial scales.

In all these cases, we used a flow laser fluorometer, which allowed fluorescence spectra of the sea water to be recorded simultaneously with temperature and salinity measurements. A description of the fluorometer in more detail can be found, for example, in Ref. 7.

In the case of linear dependence between the phytoplankton concentration and the intensity of the chlorophyll A fluorescence under exposure to laser radiation, the relation between them can be expressed simply as[8]

$$C_{\text{chl}} = K\,\Phi,$$

where $C_{\text{chl}}$ is the chlorophyll A concentration; $\Phi$ is the normalized intensity of the fluorescence signal of the chlorophyll A (in our case, the signal was normalized to the intensity of the Raman line of water); $K$ is the calibration constant, which is determined from calibration to standard methods of measurement of the chlorophyll A concentration.

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Figure 1 shows the route of the run of the Vessel *Nadezhda* in 2000. Circles in this figure mark the points of sea water sampling for standard measurements of the chlorophyll A concentration.

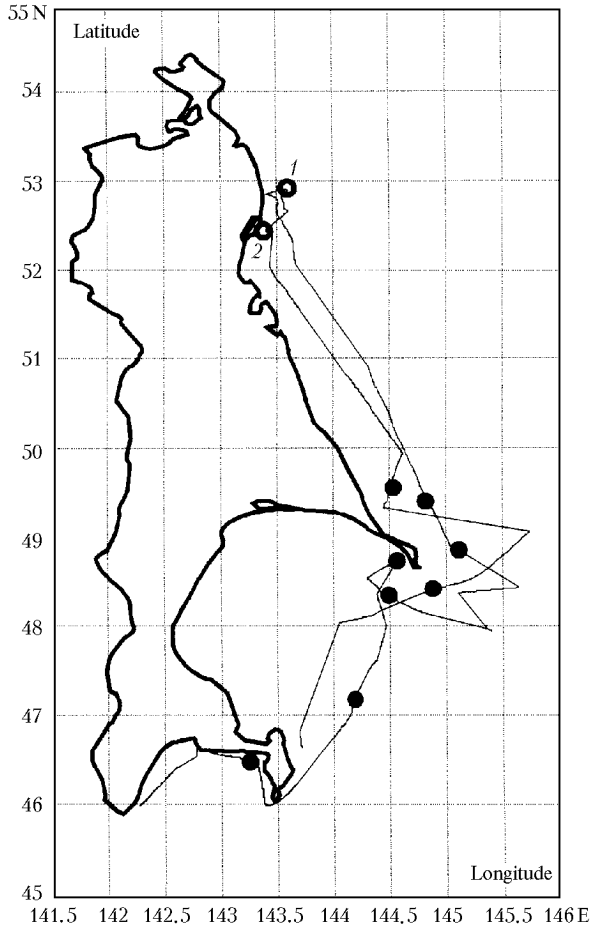


**Fig. 1.** Route of Vessel *Nadezhda* in 2000.

Contact measurements and study of the absorption spectra of phytoplankton samples were conducted with the use of the standard technique. The samples were obtained by filtering water from the flow-through cell of the laser fluorometer. The error in the chlorophyll A concentration measured by this technique varies from 10 to 50% depending on the concentration in a sample.[9]

Figure 2 shows the results of calibrations made during the trip along the Okhotsk shelf of Sakhalin in 2000. The values of the chlorophyll A concentration obtained by standard measurements are plotted along the vertical axis in Fig. 2, and the normalized intensity of the fluorescence signal is plotted along the horizontal axis. The vertical and horizontal bars denote absolute measurement errors. The solid line is the regression straight line obtained by the least-square method using the "moving control" procedure. This procedure allows a selection of points most distant from the regression line, as well as estimation of the regression error and its parameters in the set of subsamples generated from the initial data by excluding one, two, and more points.[10] The need to use the "moving control" procedure is connected with the smallness of the initial data sample. It gives stable estimates under the conditions that the distribution law of initial data is unknown. Once the points *1* and *2* (see Fig. 2) most distant from the regression line were excluded, we obtained the value of the regression coefficient $K$ (the coefficient of conversion of the relative intensity of fluorescence to absolute values of the chlorophyll A concentration) equal to $(2.3 \pm 0.3)$ μg/l. Peculiarities of hydrological situations, in which deviations similar to points *1* and *2* were observed, are considered below.

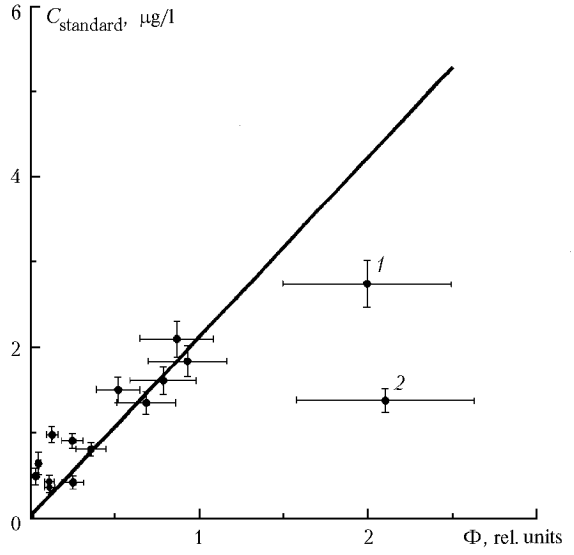


**Fig. 2.** Calibration of the relative fluorescence signal to standard measurement methods.

The amount of data obviously does not allow us to obtain statistically significant estimates of the second moments. Nevertheless, we present some of them for interpretation of the obtained results. The correlation coefficient between the data shown in Fig. 2 is equal to 0.8. It is rather high value for indirect determination of the chlorophyll A concentration by optical methods.

During the calibration measurements of 2000 the temperature varied from 9 to 16.6°C. The correlation coefficient between the temperatures, at which the calibration measurements were conducted, and the calibration coefficient $K$ obtained in every measurement is equal to 0.46. This indicates that the coefficient $K$ is temperature independent (within our errors and temperature range) or, in other words, the quantum yield of the fluorescence depends on the temperature in the given range. The same conclusion follows from the analysis of $K$ values conducted by means of the data sample separation into two subsamples of the same length for the temperatures above and below 12°C. In the both samples, the values of the conversion coefficients are equal within the measurement error.

Let us consider in greater detail the situations, in which calibrations at the points *1* and *2* (Fig. 2) were carried out (the corresponding positions of the stations are shown by open circles in Fig. 1). The values of the coefficient $K$ at these points differed significantly from the above values (exceeded the measurement error). The both measurements were obtained in the zone of intense temperature fronts. Figure 3 shows the examples of these realizations. The relative intensity of the chlorophyll A fluorescence signal was measured simultaneously with the temperature.

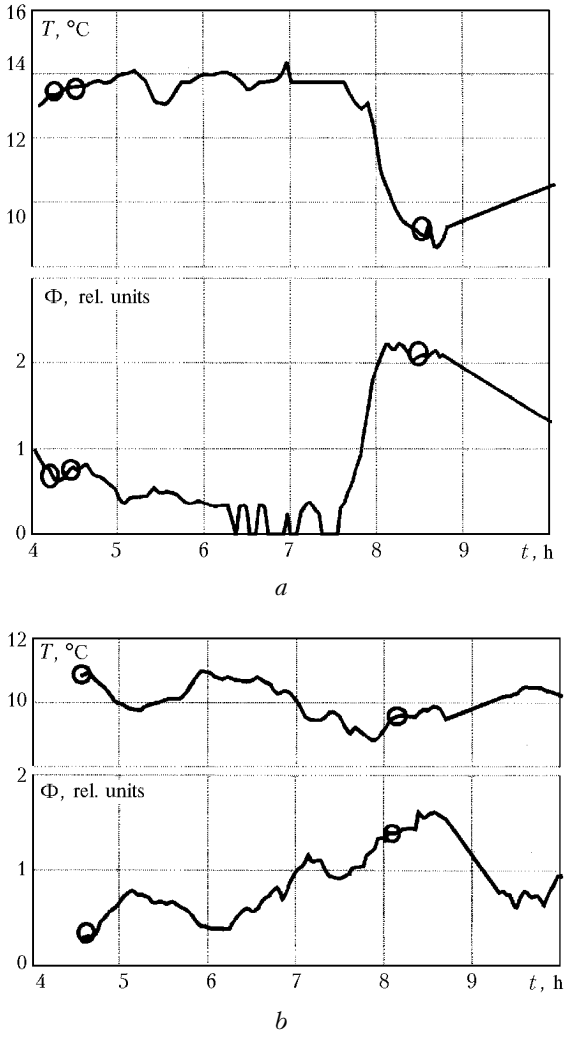


**Fig. 3.** Changes of fluorescence signals in frontal zones.

The open circles in Fig. 3 show the time, at which sea water was sampled for standard measurements of the chlorophyll A concentration. Two measurements (at 04:26 and 04:55 of local time, Fig. 3*a*) were conducted before the cold front and one measurement was conducted at 08:26 L.T. after the front. The temperature change at the path about 5 mile long was 4.5 °C. It was the most significant temperature front, during which we succeeded to carry out calibration to the standard method of chlorophyll A measurement. The coefficient $K$ for the case shown in Fig. 3*a* is equal to $(0.6 \pm 0.2)$ μg/l, and for the case shown in Fig. 3*b* it is $(1.4 \pm 0.4)$ μg/l. It is seen from Fig. 3 that the coefficient $K$ can depend in a complex way on a number of difficult-to-monitor parameters, the main among which, in our opinion, are the specific composition and the state of the plankton community. The specific composition of plankton can hardly be determined in such experiments, because this is a very labor-consuming process, which requires sufficient statistics to be collected. As to the state of the plankton community, the methods for its monitoring are now only at the beginning of their development. In this case, the cold front is connected with local upwelling and lifting of cold water rich in detritus and dissolved oxygen to the surface. This creates rather favorable conditions for development of phytoplankton and can serve as a cause of the change in the pigment reaction to laser excitation as compared to the plankton studied in other regions.

It is interesting to note that the obtained value of $K$ coincides, within the error, with the value of the conversion coefficient, $(2.6 \pm 0.3)$ μg/l, reported in Ref. 1. The conversion coefficient obtained from the data of the research expeditions of 1992 and 1993 in the southern part of the Sea of Okhotsk is equal to $(1.9 \pm 0.4)$ μg/l. In the open waters of the Pacific Ocean, $K = (2.9 \pm 0.6)$ μg/l as follows from the data of the research expeditions of 1997–1998. This value also coincides with the above value within the error.

The coefficient of relation between the chlorophyll concentration and the relative fluorescence signal is not of universe character. It depends on such parameters, as instrumental constants and quantum efficiency of the fluorescence signal. It can be different depending on the type of the used fluorometers, as well as the region and season of measurements. Unfortunately, the description of the experiment in Ref. 1 does not allow us to compare the used fluorometers for we could state reliably that the coincidence of our results with the coefficient from Ref. 1 was not accidental. The presented estimates give only the range (scale) of the coefficients for fluorometers similar to that used by us.[7] In our opinion, it is more important that the calibration coefficient was almost unchanged in different ocean regions, where we succeeded to carry out the calibrations. This allows us to assert that all variations connected with different specific composition and dependence of the quantum yield of fluorescence on hydrological parameters lie within the measurement error for those specific hydrophysical situations that took place during the expeditions.

However, it should be kept in mind that the statistics of measurements is obviously insufficient (in the number of measurements and, especially, in the range of hydrological parameters of sea water, as well as species diversity of plankton, etc.) to generalize the above result. The situation observed in two intense

temperature fronts, in which the coefficients varied significantly, is also indicative of this fact.

## Acknowledgments

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