Evaluation of primary production of phytoplankton based on chlorophyll delayed fluorescence in sea water

OCEANOLOGIA, 28, 1990
PL ISSN 0078-3234

Primary production
Delayed fluorescence
Chlorophyll
DCMU influence

V. I. ZVALINSKY
Pacific Ocean Oceanological Institute
of the Far East Scientific Centre,
Academy of Sciences of the USSR,
Vladivostok

O. D. BEKASOVA
A. N. Bach Institute of Biochemistry,
Academy of Sciences of the USSR,
Moscow

S. KACZMAREK, A. KOSAKOWSKA
Institute of Oceanology,
Polish Academy of Sciences,
Sopot

Manuscript received June 24, 1988, in final form July 25, 1989.

Abstract

The intensity of chlorophyll delayed fluorescence in the presence and absence of *diuron* — a photosynthesis inhibitor, phytoplankton primary production and chlorophyll concentration were measured simultaneously in the Gulf of Gdańsk during the International Ecological Experiment "Sopot-87" (1st-8th May, 1987). A correlation coefficient between delayed fluorescence and chlorophyll concentration was equal to $r = 0.7$, while that between delayed fluorescence in the presence of *diuron* and chlorophyll concentration — to $r = 0.9$. A correlation coefficient between the primary production and delayed fluorescence (calculated using a previously proposed formula taking into account an intensity of the incident light at the studied depth) was equal to $r = 0.95$. The obtained results imply that the method of delayed fluorescence in sea water is promising for the determination of both chlorophyll concentration and primary production of phytoplankton.

1. Introduction

Since the first report of the delayed fluorescence (DF) of chlorophyll (Chl) in plant cells (Strehler, Arnold, 1951), it has been demonstrated in numerous studies that this fluorescence is a result of reversible photochemical reactions taking place during photosynthesis (Litvin *et al*, 1960; Krasnovsky, 1982; Lavorel, Dennery, 1982). Delayed fluorescence reflects the production processes more adequately than prompt fluorescence, since it is closely related to light energy

storage processes (Karabashev, 1987). This property of DF is widely used for evaluation of the production characteristics and physiological state of both higher plants (Veselovsky, Veselova, 1983; Havaux, Lannoye, 1985; Matorin *et al*, 1985; Grigorev *et al*, 1986) and algae (Latsko *et al*, 1980; Gerhardt, Krauze, 1984). Delayed fluorescence has been recently used for the evaluation of primary production (PP) in sea (Krauze *et al*, 1984; Krauze, Gerhardt, 1984; Zakharkov *et al*, 1985; Matorin *et al*, 1986). In these studies an intensity of the delayed fluorescence of chlorophyll was shown to be strongly dependent on the value of primary production (correlation coefficient $r = 0.9$) within a wide range of phytoplankton concentrations ($10^{-2} - 10^{3}$ mg $Chlm^{-3}$).

The paper presents the results of investigations on the nature of a relationship between the DF intensity, primary production value and Chl concentration, aiming at developing applications of the DF method in oceanological studies, *eg* for testing the anthropogenic impact on the ecosystem of the Baltic Sea.

2. Methods

The delayed fluorescence was measured on board of a ship. A cylindrical phosphoroscope in a millisecond fluorescence decay range (time between the end of excitation and beginning of measurement being equal to *ca* 1.5 ms) was used. The signal from a FEU-84 photomultiplier, measuring the fluorescence intensity, was amplified by a millivoltameter and recorded by a strip-chart recorder.

Samples of sea water from each depth were drawn 4 times a day (at 0.00, 6.00, 12.00 and 18.00) and placed in a special 70 ml perspex vial. Samples were excited with red light (a 100 W lamp equipped with a KS-15 red light filter, light intensity 20 $W \cdot m^{-2}$) in order to reduce the fluorescence of the vial and the impurities contained in sea water. Samples were drawn from 10 depths, *viz* 0, 1, 2, 3, 5, 7, 10, 15, 20, and 30 m. Determination of the delayed fluorescence of all samples took 1 hour.

In some cases, the determinations of the delayed fluorescence intensity (L) were carried out after additions of *diuron* — a photosynthesis inhibitor (DCMU) at a concentration of $5 \cdot 10^{-8}$ M. The ratio of fluorescence intensity with the inhibitor added to the intensity without the inhibitor ($E = L^{DCMU}/L$) was used as a measure of the increase of delayed fluorescence intensity (enhancement coefficient). The applied concentration of the inhibitor was chosen experimentally as a concentration at which the DF enhancement was maximum.

The primary production was measured by a radiocarbon method (Strickland, Parsons, 1986), using 100 ml vials and a 6 hour exposure (6.00 to 12.00 and 12.00 to 18.00).

A pigment content in 210 samples was determined using a standard spectrophotometric method (SCOR-UNESCO, 1966).

All the measurements were carried out in the Gulf of Gdańsk at G-2 and Z stations, on the board of r/v "Oceania", belonging to the Polish Academy of Sciences, between 1st and 7th May, 1987.

3. Results

The delayed fluorescence of chlorophyll was detectable in samples of sea water from all depths at both stations. The DF intensity at the deepest level (75 m) was more than an order of magnitude lower than that at the layer of maximum intensity (Fig. 1).

Vertical profiles obtained at station G-2 are characterized by a gradual decrease of the DF intensity beginning at a depth of 10 m (Fig. 1A,B). A decrease

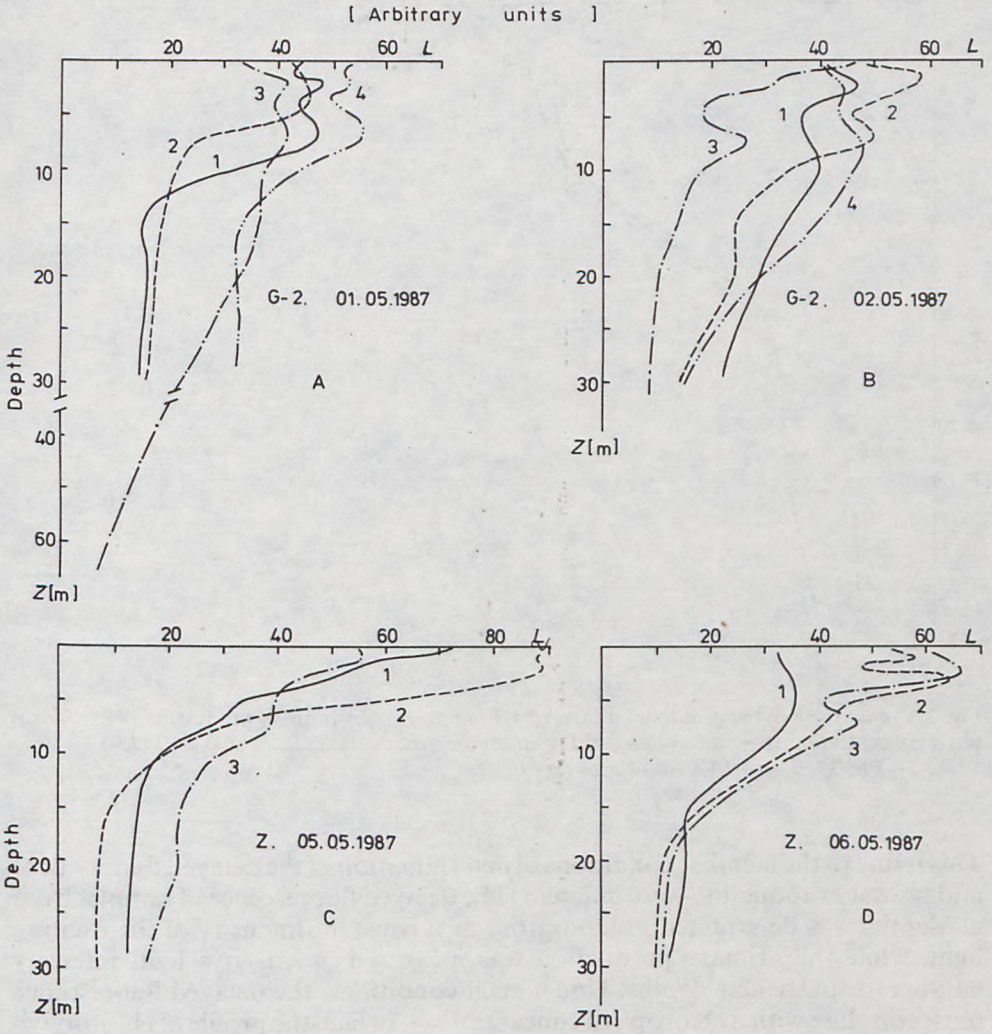

Fig. 1. Profiles of delayed fluorescence (DF) intensities at stations G-2 (A–B) and Z (C–D) Time of the day: 1–6.00, 2–12.00, 3–18.00, 4–24.00 hours

of the DF intensity at station Z was more rapid and started at a depth of 5–6 m. This was due to lower transparency of water at this station, resulting in a more rapid decrease of light intensity with depth.

A comparison of the shapes of profiles determined at different time of the day revealed that during the day phytoplankton was located close to the surface, while at night it was distributed more uniformly within the euphotic layer.

The DF and PP profiles do not coincide (Fig. 2, curves 1 and 2, respectively).

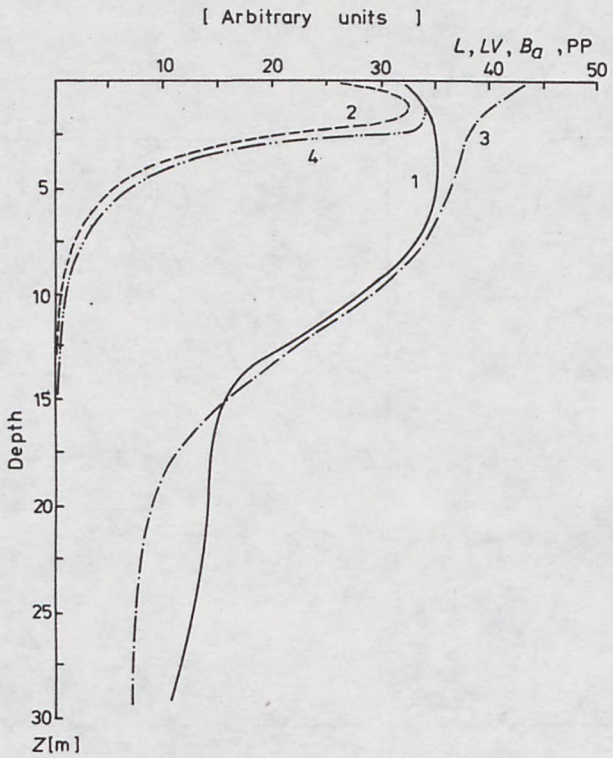

Fig. 2. Comparison of the profiles of measured DF intensities (L), primary production (PP), chlorophyll concentration (B_a), and profiles of DF intensities, recalculated from eqs. 2–3 (LV)
1–L, 2–PP, 3–B_a, 4–LV. All in arbitrary units

This is due to the fact that conditions of determination of the delayed fluorescence and primary production were different. The delayed fluorescence of samples from all depths was determined in laboratory at a constant intensity of the exciting light, while the primary production was measured *in situ* at a light intensity varying for particular depths. Under such conditions, the delayed fluorescence must correlate with a chlorophyll concentration. In fact, the profile of chlorophyll "a" concentration (B_a) better corresponds to that of DF (Fig. 2, curves 3 and 1), though the coincidence is not full. This is due to the fact that curve B_a reflects the total Chl concentration, while that of L corresponds only to the variations of photosynthetically active chlorophyll (see below).

In order to determine an extent of correlation between the DF intensity and the PP value, it is necessary to know the intensity of photosynthetically active radiation (PAR) at the studied depths and the light dependence of of photosynthesis. The intensity of PAR was determined by D. Bogucki, W. Czyszek and A. Rozwadowska, by means of instrumentation and methods used in IOPAS (Woźniak, 1977). The obtained data were used in further calculations.

The results of mathematical modelling of the photosynthesis light dependence carried out by Dr. Zvalinsky, as well as the results of experimental examination of the light saturation curves determined for various species of sea algae, demonstrated that these curves can be described by an equation of an non-rectangular hyperbola (Zvalinsky, Litvin, 1983):

$$I/I_k = \frac{P}{P^{max}} \cdot \frac{1-\gamma P/P^{max}}{1-P/P^{max}}, \tag{1}$$

where:

P – the rate of photosynthesis,

P^{max} – the maximum value of the photosynthesis rate (at saturating intensity of light),

I – the intensity of incident light,

I_k – the light intensity at the point of intersection of an extension of a linear part of the light curve with the plateau (proposed by Talling, 1957),

γ – a parameter of an non-rectangular hyperbola.

At $\gamma \to 0$ eq. (1) describes an rectangular hyperbola (Fig. 3, curve 1). At $\gamma \to 1$ the

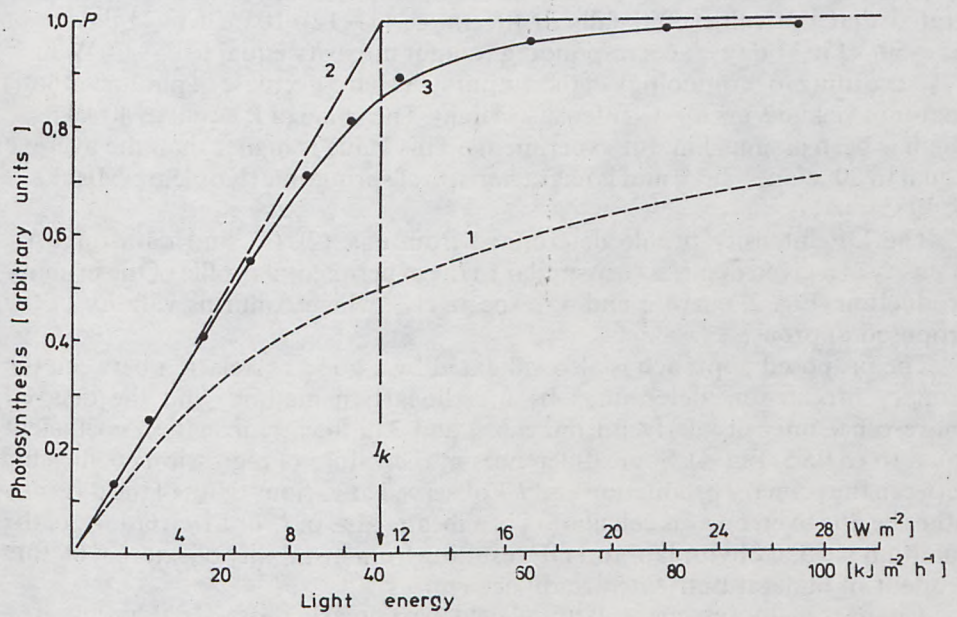

Fig. 3. Calculated and experimental light curves of photosynthesis
1 – rectangular hyperbola (eq. 1, $\gamma = 0$), 2 – Blackman broken line (eq. 1, $\gamma \approx 1.0$), 3 – black circles designate the experimental points for a unicellular red alga *Porphyridium cruentum*, solid line is an non-rectangular hyperbola calculated by eq. 1 ($\gamma = 0.96$). The value of $I_k = 11.2$ W·m^{-2}

plot approaches the Blackman broken line (Fig. 3, curve 2). Actual experimental light curves for a majority of the examined algae (Zvalinsky, Litvin, 1983) can be described by eq. (1) with γ equal to *ca* 0.95 (Fig. 3, curve 3). The value of γ can therefore be assumed to be constant and equal to 0.95 with an accuracy sufficient for the description of light curves of phytoplankton photosynthesis.

If the light intensity and photosynthesis rate are expresed in arbitrary units, *viz* $i = I/I_k$ and $V = P/P^{max}$, then all the light curves can be described by the same equation:

$$i = V(1-\gamma \cdot V)/(1-V). \tag{2}$$

The value of relative photosynthesis rate (V) can be readily derived from eq. (2) on the basis of data on relative fluorescence intensity at the investigated depths. Since the delayed fluorescence intensity determined by the above method is proportional to the saturation photosynthesis $L \cong K \cdot P^{max}$, the DF intensity (L_i) and the production value (P_i) at the investigated depth can be determined:

$$L_i = V \cdot L,\ P_i = V \cdot P^{max} = V \cdot L/K. \tag{3}$$

If follows from eqs. (2) and (3) that the production value reaches a maximum at high values of light intensity ($i > 1.0$, $P \approx P^{max}$, and is independent on it. At low intensities ($i < 1.0$, $P \approx i \cdot P^{max}$), P_i is proportional to light intesity, and at moderate light intensities ($i \approx 1.0$) it reaches 80–90% of its maximum value.

To calculate the relative light intensity, it is necessary to know the value of I_k apart from the light intensity at a given depth (I). Numerous experimental determinations of phytoplankton photosynthesis light dependence, performed by Koblentz-Mishke in the Baltic Sea (Koblentz-Mishke *et al*, 1985), demonstrated that the value of I_k falls in the range $(4-12) \cdot 10^4$ cal m^{-2} day^{-1} or (17–50) kJ m^{-2} day^{-1}, corresponding to light intensity equal to 5–14 W m^{-2} (η_{VK} according to terminology of the authors). A sharp decrease of photosynthesis quantum yield occurs at this intensity of light. The value of I_k equal to 40 kJ m^{-2} h^{-1} has been assumed in our experiments. This value is higher than the average (equal to 30 kJ m^{-2} h^{-1}) and is characteristic of springtime (Koblentz-Mishke *et al*, 1985).

The DF intensity profile determined from eqs. (2), (3), and data on PAR intensity at a given depth is very similar to the experimental profile of the primary production (Fig. 2, curve 2 and 4, respectively), which confirms validity of the proposed approach.

The proposed approach is also validated by a good correlation between the primary production, determined by a radiocarbon method, and the delayed fluorescence intensity derived from eqs. 2 and 3 (a linear correlation coefficient equal to *ca* 0.95, Fig. 4). Some differences in the values of regression coefficients between the primary production and LV observed at various times of the day may either be due to erroneous calculation of a mean value of L, or to variations of the quantum yield of phytoplankton DF resulting from instability of external factors (content of mineral nutrients, irradiance *etc*).

Changes in fluorescence quantum yield, particularly those of delayed fluorescence of chlorophyll, resulting from variations in external factors, have been known for a long time and used for evaluation of functional states of plant organisms (Veselovsky, Veselova, 1983; Andreyenko *et al*, 1985; Grigorev *et al*,

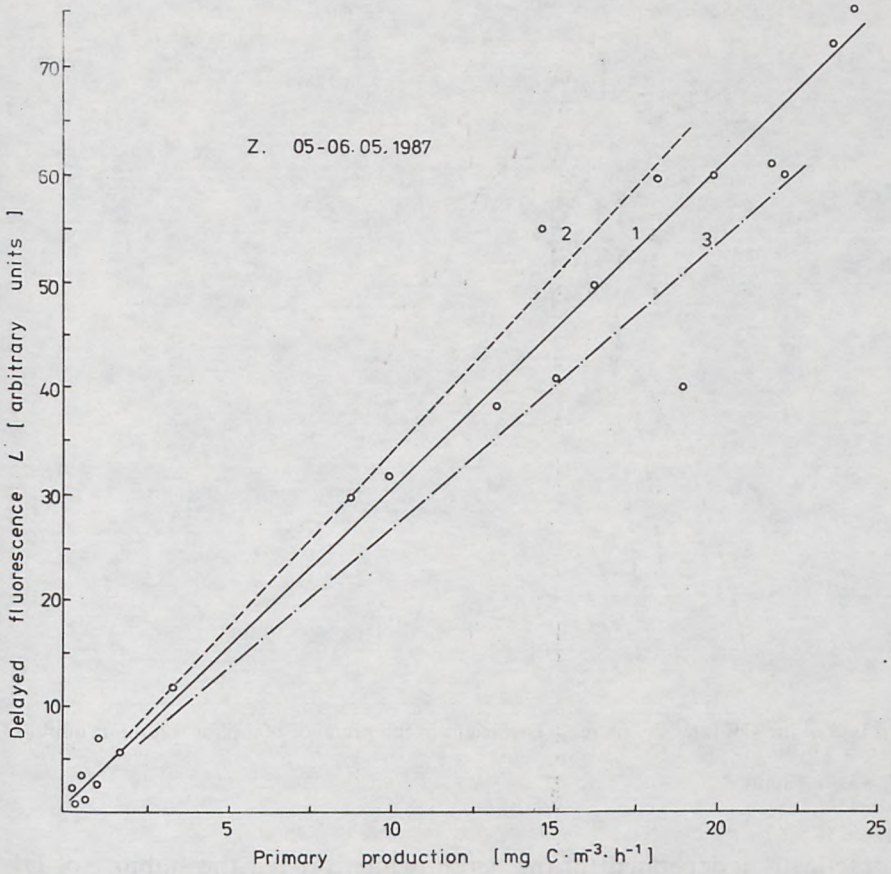

Fig. 4. Correlation between DF intensity (arbitrary units) calculated from eqs. 2 – 3 (*LV*) and primary production (PP) value measured by the radiocarbon method
1, 2, 3 – mean for profiles obtained at 6.00 to 12.00 to 18.00, respectively ($r = 0.98$)

1986). Changes in quantum yield of phytoplankton photosynthesis due to variable irradiance in the Baltic Sea were reported by Koblentz-Mishke *et al* (1985). Since the photosynthesis processes and the delayed fluorescence generation are competitive, the changes in the yield of photosynthesis are accompanied by changes in the DF yield.

Changes in quantum yield of the delayed fluorescence were confirmed by experiments on the influence of DCMU on the DF intensity. DCMU is known to interrupt the electron transport between photosystem 2 and photosystem 1, which leads to photosynthesis inhibition. This results in an increase of the rate of reverse photochemical reactions and of the yield (enhancement) of prompt and delayed fluorescence, reaching the values close to maximum. The higher the enhancement of the DF intensity in the presence of DCMU, the lower the DF intensity (and higher the yield of photosynthesis) in the absence of the inhibitor.

The profiles of the DF enhancement coefficient varied significantly during the day (Fig. 5). At night and in the morning (at 0.00 and 6.00 am) the enhancement

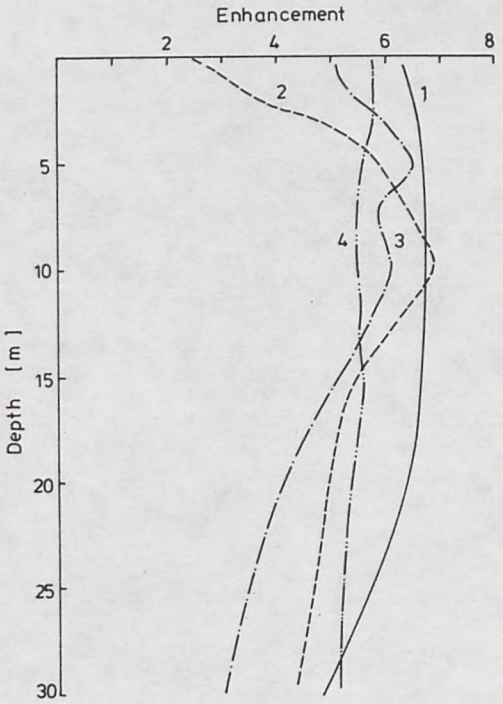

Fig. 5. Profiles of the DF intensity increase coefficient in the presence of a photosynthesis inhibitor DCMU ($E = L^{DCMU}/L$)
1, 2, 3, 4, – as in Figure 1

E was practically independent of the depth, which proved the stability of DF yield. At noon and in the evening (at 12.00 and 18.00) quite considerable changes of E were observed with depth (Fig. 5). A rapid decrease of E at the daytime (at maximum irradiance), observed at small depths, testifies the increase of DF yield, the phenomenon being probably related to a decrease of the photosynthesis yield due to inhibitory action of light of high intensity (Koblentz-Mishke *et al*, 1985). The same may also account for higher values of the regression coefficients between the delayed fluorescence and primary production during the day (Fig. 4).

As already mentioned, the DF intensity correlates with Chl concentration of phytoplankton. Statistic analysis of data on Chl concentration and DF intensity revealed a linear correlation between these parameters ($r \approx 0.7$, Fig. 6A). A relatively low value of the correlation coefficient between L and B_a may be due to the variations in quantum yield of the delayed fluorescence mentioned above. In such a case, an addition of DCMU to the samples should result in suppression of these variations. In fact, the correlation coefficient between L^{DCMU} and B_a was found to be higher and equal to *ca* 0.9 (Fig. 6B). This allows to use the intensity of the delayed fluorescence in the presence of DCMU (L^{DCMU}) as a measure of the chlorophyll content in sea water, the accuracy of such an estimation being sufficiently good. It is remarkable that the yield of the delayed fluorescence increases by a factor of 2 to 6 in the presence of DCMU; therefore, the sensitivity of the method also increases.

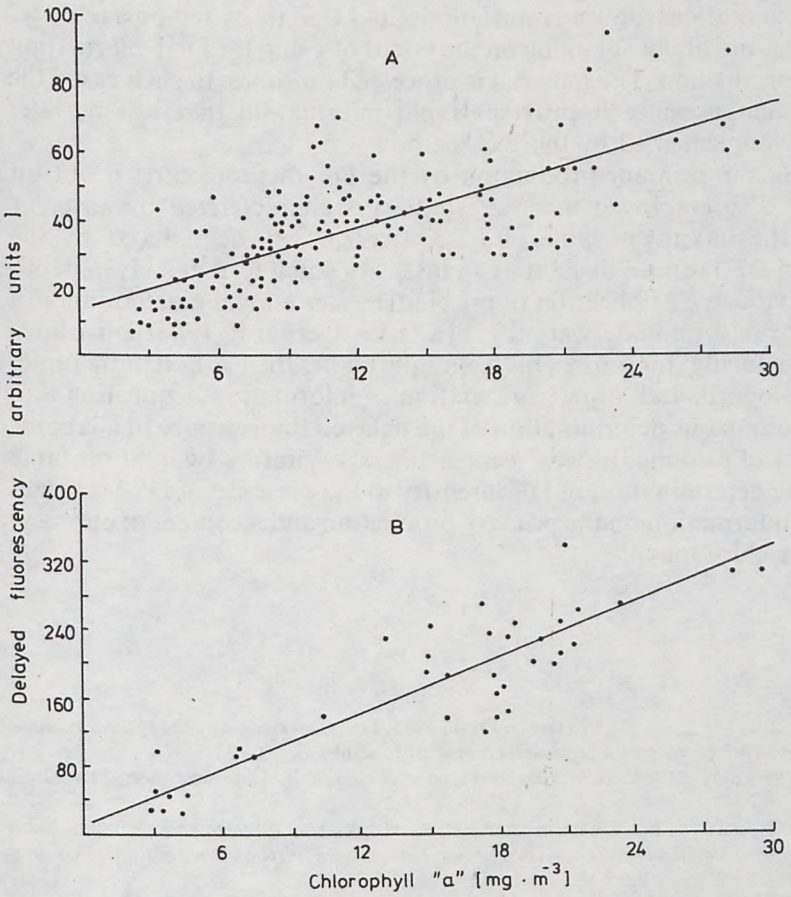

Fig. 6. Correlation between DF intensity of chlorophyll in the absence (L) and presence of DCMU (L^{DCMU}) and chlorophyll concentration (B_a) in sea water
A – in the absence of DCMU ($r = 0.7$), B – in the presence of DCMU ($r = 0.9$)

4. Discussion

The presented results confirmed the previous report on a good correlation between the DF intensity and PP value (Krauze, Gerhardt, 1984; Zakharkov *et al*, 1985). A large value of the correlation coefficient between the LV value obtained from eqs. (2) and (3), and PP determined by the ^{14}C method indicates that the proposed approach to estimation of the primary production by the delayed fluorescence intensity is promising. Hence, the DF intensity may be used for the determination of primary production of phytoplankton and concentration of photosynthetically active chlorophyll directly in sea water.

Taking into account a rapidity of the DF method, it is worth utilizing even despite greater errors observed in our experiments or reported in the literature, since it can provide rapid and detailed information on large sea areas, while the

possibilities of the radiocarbon method are limited due to its tediousness. ^{14}C method often does not allow obtaining on the board of a ship the final information on the primary production. The material is processed on shore. In such cases, the DF method is unique, since it provides rapid information that can be later confirmed and complemented by the ^{14}C method.

Estimation of the primary production by the DF method, carried out on board (which is also employed in other studies) is characterized by an error resulting from the unknown value of I_k. This error can be reduced by the determination of DF induced by light of an intensity equal to that a given depth (determined previously). Application of probing devices allowing determination of DF induced by natural underwater light (Krauze, Gerhardt, 1984) constitutes another way of reducing this error. This technique makes the method more rapid, accurate, physiological, and allows automation of information acquisition and processing. Simultaneous determination of the delayed fluorescence in a laboratory or by means of probing devices, using artificial excitation by light of stable intensity, and the determination of DF intensity in the presence of DCMU yield complementary information on the primary production and a content of photosynthetically active chlorophyll.

References

Andreyenko T. I., Bystrykh E. E., Matorin D. E., 1985, *Izucheniye zamedlennoy fluorestsentsii listev yarovoy pshenitsy v svyazi s produktivnostyu*, Bill. Nauki, 3, 30–35.

Gerhardt V., Krauze H., 1984, *Delayed fluorescence of algae*, J. Luminescence, **31–32**, 2, 895–898.

Grigorev Y. S., Morgun V. N., Gekhman A. V., Gold V. M., 1986, *Svyazi millisekundnoy zamedlennoy fluorestsentsii s pervichnymi protsessami fotosinteza: vliyanie temperatury*, Fiziolog. Rast, **33**, 1, 15–22.

Havaux M., Lannoye R., 1985, *In vivo chlorophyll fluorescence and delayed light emission as rapid screening techniques for stress tolerance in corp plants*, Z. Pflanzenzucht., **95**, 1, 1–13.

Karabashev G. S., 1987, *Fluorestsentsiya v more*, Gidrometeoizdat, Leningrad.

Koblentz-Mishke O. I., 1985, *Roli svetovogo faktora v pervichnoy produktsii morya.* [In:] *Usvoyeniye solnechnoy energii v protsesse fotosinteza chernomorskogo i baltiyskogo fitoplanktona*, M. Izdat. Inst. Okeanology, 10–58.

Koblentz-Mishke O. I., Konovalov B. V., Woźniak B., Bogucki D., 1985, *Effektivnost ispolzovaniya solnechnoy energii v protsesse fotosinteza fitoplanktona.* [In:] *Usvoeniye solnechnoy energii v protsesse fotosinteza chernomorskogo i baltiyskogo fitoplanktona*, 152–171.

Krasnovsky A. A., 1982, *Delayed fluorescence and phosphorescence of plant pigments*, Photochem. Photobiol., 36, 6, 733–741.

Krauze H., Gerhardt V., 1984, *Application of delayed fluorescence of phytoplankton in limnology and oceanography*, J. Luminescence, **31–32**, 2, 888–891.

Krauze H., Kretsch G., Gerhardt V., 1984, *Differential equations for delayed fluorescence kinetics in living plants*, J. Luminescence, **31–32**, 2, 885–887.

Latsko G., Maroti Z., Ringler A., Shalay L., 1980, *Zamedlennaya fluorestsentsiya khlorofilla v millisekundnom diapazone*, Biofizika, **25**, 3, 531–536.

Lavorel J., Dennery J. M., 1982, *The slow component of photosystem II luminescence*, Biochem. Biophys. Acta, **680**, 3, 281–289.

Litvin F. F., Vladimirov G. A., Krasnovsky A. A., 1960, *Khemiluminestsentsiya khlorofilla pri fotokhimicheskikh reaktsiyakh*, Uspekhi Fiz. Nauk, **71**, 5, 149–156.

Matorin D. N., Venediktov P. S., Rubin A. B., 1985, *Zamedlennaya fluorestsentsiya, es ispoljzovania dlya otsenki sostoyaniya rastitelnogo organizma*, Izd. Akad. Nauk SSSR, Biol. ser, 4, 508–520.

Matorin D. N., Vasilev I. R., Venediktov P. S., Zakharkov S. P., 1986, *Metod neprerývnogo zondirovaniya aktivnogo fitoplanktona v vodoemakh putem zamedlennoy fluorestentsii*, Gidrobiol. Zhurn., **22**, 2, 87–89.

SCOR-UNESCO, 1966, *Determination of photosynthetic pigments in sea water*, Monographs on oceanography methodology, Paris, 1, 69.

Strehler B. L., Arnold W. A., 1951, *Light production by green plant*, J. Chem. Phys., **34**, 6, 809–820.

Strickland J. D. H., Parsons T. R., 1968, *A practical handbook of seawater analysis*, Bull. Fish. Res. Biol. Canada.

Talling J. F., 1957, *The phytoplankton population as a compound photosynthetic system*, New Phytol., 56, 133–149.

Veselovsky V. A., Veselova T. V., 1983, *Recombinatsionnaya luminestsentsiya organismov, eyo prakticheskoye ispolzovanye*, Bioluminestsentsiya, Moscow, 241–258.

Woźniak B., 1977, *Wpływ składników wody morskiej na podwodne pole światła w morzu*, Ph. D. dissertation, IO PAN, Sopot, Poland.

Zakharkov S. P., Matorin D. N., Vasilyev I. R., Starodubtsev E. G., Venediktov P. S., 1985, *Izucheniye raspredeleniya fitoplanktona v okeane s pomoshchyu metoda izmereniya zamedlennoy fluorestsentsii*, Biol. Nauki, 3, 100–105.

Zvalinsky V. I., Litvin F. F., 1983, *Svetovye krivye fotosinteza dlya razlichnoy organizatsii fotosinteticheskikh edinitz*, Fiziolog. Rast., **30**, 5, 881–892.