Fluorescence induction as a measure of photosynthetic capacity in marine phytoplankton: response of *Thalassiosira pseudonana* (Bacillariophyceae) and *Dunaliella tertiolecta* (Chlorophyceae)

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ABSTRACT: The possibility was investigated of using *in vivo* chlorophyll fluorescence induction (timescale ms) as an index of photosynthetic capacity in marine phytoplankton. Batch cultures of the diatom *Thalassiosira pseudonana* Hustedt (3H) and of the chlorophyte *Dunaliella tertiolecta* Butcher were preconditioned for 24 h at 8 different light intensities (2 to 60 W m\(^{-2}\)). Photosynthetic rates at light saturation (*P_s*) and the area above the fluorescence induction curve (*A_{DCMU}*), were then determined; *A_{DCMU}* was obtained using dark-adapted cells in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), with a fluorometer having a shutter opening speed of about 0.3 ms. After 24 h, values of *P_s* and of *A_{DCMU}* were greater in the low than in the high irradiance cultures. In a second type of experiment, *P_s* and *A_{DCMU}* were measured throughout a 24 h incubation period after *T. pseudonana* was shifted from 60 to 9 W m\(^{-2}\) of irradiance. Both types of experiments showed a strong positive correlation between *P_s* and *A_{DCMU*}. The method offers the possibility of indexing the photosynthetic capacity of phytoplankton without lengthy incubation.

INTRODUCTION

The accurate and precise estimation of the rate of primary production by natural populations of phytoplankton is one of the major goals of aquatic ecologists. To this end, the \(^{14}\)C-technique (Steemann Nielsen, 1952) has been most widely adopted because it is sensitive and easy to use. However, the validity of this technique as used in the field has recently been challenged on methodological and on theoretical grounds (Gieskes et al., 1979; Carpenter and Lively, 1980; Peterson, 1980; Dring and Jewson, 1982; Smith, 1982; Jackson, 1983). Alternatively, it is now possible to measure photosynthetic rates of open ocean phytoplankton using changes in oxygen concentration (Williams and Jenkinson, 1982). This method may prove reliable provided sufficient ancillary data are available to estimate a reliable photosynthetic quotient.

However, both the \(^{14}\)C and oxygen methods suffer from the drawback that the sample must first be incubated in bottles for a given period before the photosynthetic rate can be determined. Significant changes in taxonomic composition (Venrick et al., 1977), and possible toxicity due to contamination from incubation bottles and/or the \(^{14}\)C-tracer stock solution may occur during the incubation period (Carpenter and Lively, 1980; Fitzwater et al., 1982).

Problems associated with long incubations and with the \(^{14}\)C-technique may be avoided by exploiting the variability in the fluorescence intensity of *in vivo* chlorophyll a. Upon addition of the photosynthetic inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), the fluorescence intensity rises to a maximal value (Papageorgiou, 1975; Prézelin, 1981). In some cases, good correlations have been found between DCMU-enhanced fluorescence and photosynthetic capacity (Samuelsson and Öquist, 1977; Samuelsson et al., 1978; Roy and Legendre, 1979; Kulandaivelu and Daniell, 1980; Öquist et al., 1982). In other cases, the correlation was not present or was obscured by other factors (Prézelin and Sweeney, 1977; Cullen and Renger, 1979; Harris, 1980; Roy and Legendre, 1980;
Parker and Tranter, 1981). The variable success in correlating fluorescence indices and photosynthesis in these studies is due in part to differences in fluorometers used, as well as to differences in the excitation irradiance, dark-adaptation time, and time-interval over which the fluorescence change was measured. The latter point is especially important, as the change in fluorescence intensity in the absence of DCMU is variable with time (Kautsky effect), given the proper irradiance (Papageorgiou, 1975). Different fluorescence intensities would, therefore, be obtained depending on the time interval over which the fluorescence signal was measured. For example, some researchers (Samuelsson and Oquist, 1977; Cullen and Renger, 1979; Roy and Legendre, 1979; 1980) time-integrated the first 5 or more seconds of fluorescence after the onset of illumination. Others (Harris, 1980; Kulandaivelu and Danieli, 1980) waited for a reasonably stable time-course in fluorescence intensity (30 s to 1 min) prior to starting the measurement. A long exposure to the fluorometer's excitation source alters the configuration of the chloroplast membrane in relation to that in situ and further changes the fluorescence signal (Papageorgiou, 1975), obscuring the correlation between fluorescence and photosynthesis.

To avoid these problems, one could measure fluorescence events occurring immediately (within ms) after the onset of illumination, and relate these events directly to the organization of the photosynthetic apparatus of photosynthesis. This approach was taken by Malkin et al. (1981) and Malkin and Fork (1981) who demonstrated a strong correlation between the photosynthetic capacity \( P_{\text{m}} \) (photosynthetic rate at light saturation) and the concentration of reaction centers (RC) in photosystem II (PSII) in 4 desert plant species representing different photosynthetic capacities. The RC concentration is defined as the amount of primary electron acceptor \( Q \) per unit chlorophyll \( a \) in the photosynthetic unit (PSU) of PSII. The quantity of \( Q \) is obtained as the area above the fluorescence signal (fluorescence induction curve) in the presence of DCMU (see 'Theoretical Considerations'). In this paper we examine the possibility of indexing, without lengthy incubation, the photosynthetic capacity of marine phytoplankton by measuring the area above the fluorescence induction curve. To obtain cells of differing photosynthetic capacities, cultures of 2 species of phytoplankton were studied during and subsequent to a shift to low irradiances.

### THEORETICAL CONSIDERATIONS

When a phytoplankton cell is placed in the dark, electrons drain from \( Q \) into photosystem I. In this oxidized state, \( Q \) acts as a quencher of fluorescence (Duysens and Sweers, 1963), and fluorescence is at its lowest intensity \( F_0 \). In the presence of light, \( Q \) becomes photoreduced and if the electrons cannot be drained rapidly enough from \( Q \) by the non-cyclic electron transport system, then the radiant energy absorbed cannot be used in photosynthesis but is emitted instead as fluorescence. The presence of DCMU blocks further electron transport from \( Q \), and fluorescence achieves its maximum level \( F_m \) (fluorescence induction).

The time required to accomplish the photoreduction of \( Q \) in the presence of DCMU is known as the fluorescence induction time, and is proportional to the number of RC containing \( Q \) (Dubertret and Joliot, 1974; Malkin et al., 1981). Geometrically (Fig. 1), the induction time is equal to the area above the fluorescence induction curve \( \Delta(DCMU) \), bounded by a vertical axis situated at the point where the optical shutter is completely open (0.3 ms after the initial rise in fluorescence), and a horizontal line situated at \( F_m \) (Malkin and Kok, 1966). Absolute estimates of the concentration of \( Q \) may be made using certain quantitative assumptions (Malkin et al., 1981), but in this paper we seek to determine only if \( A_{DCMU} \) is related in any systematic way to photosynthetic capacity.

### MATERIALS AND METHODS

Organisms used were *Thalassiosira pseudonana* (Hustedt) Hasle and Heimdal, Bacillariophyceae, Clone 3H (kindly supplied by R. Geider from the culture collection of R. R. Guillard), and *Dunaliella tertiolecta* Butcher, Chlorophyceae (kindly supplied by Dr. Subba Rao of the Marine Ecology Laboratory). Stock cultures were maintained axenically in f/10 medium (Guillard and Ryther, 1962) at 20°C under 60 W m\(^{-2}\) of constant irradiance provided by a bank of Cool White fluorescent bulbs. Experiments were carried out at 20°C and in f/10 medium using organisms taken from the mid-to late-exponential growth phase of batch culture.

In a first type of experiment, 100 ml of phytoplankton culture, grown at 60 W m\(^{-2}\) of constant irradiance, were each preconditioned for 24 h at 60.0, 42.0, 27.6, 19.2, 11.4, 9.2, 4.8, and 2.1 W m\(^{-2}\) of constant irradiance in single 150 ml borosilicate incubation bottles stirred by bubbling with sterile air. The different irradiance levels were achieved by surrounding the bottles with neutral-density filters made from nickel screens (Perforated Products, Inc.). The relatively short (24 h) preconditioning period at the eight irradiances was chosen to minimize changes in the photosynthetic capacity due solely to growth in batch culture (Bear-
At the end of the 24 h incubation period, the photosynthetic rate at 60 W m\(^{-2}\) (the highest light intensity available), the area above the fluorescence induction curve (A\(_{DCMU}\)), chlorophyll \(a\) concentration, and cell number were obtained, in triplicate, as described below.

In a second type of experiment, 250 ml of *Thalassiosira pseudonana* culture, grown at 60 W m\(^{-2}\), were each exposed to 60 and 9 W m\(^{-2}\) of constant irradiance in single 500 ml Erlenmeyer flasks stirred with a magnetic stirrer and sterile air. Triplicate measurements were made of the photosynthetic rate at 60 W m\(^{-2}\), A\(_{DCMU}\), chlorophyll \(a\) concentration, and cell number throughout the 24 h incubation period.

Fluorescence measurements to obtain A\(_{DCMU}\) were made with an induction fluorometer designed and built at the Bedford Institute of Oceanography (B.I.O.). Actinic light provided by a 100 W tungsten lamp (Model 6129, Oriel Corp., Stamford, CT) was passed through a heat absorbing filter (Melles Griot SQ), and was isolated by filters (Melles Griot 03 FIB 004; Corning 1–75) passing a band centered at 458 nm (40 nm half-band). A sharp cut long pass red filter (Schott RG 665) was used to isolate the fluorescence which was detected by a silicon photodiode (Model 125 DP, United Detector Technology, Inc., Santa Monica, CA; responsivity = 0.19 amps W\(^{-1}\) at 560 nm). An electronic shutter (model 884, Newport Research Corp., Fountain Valley, CA), using a shutter drive designed and built at B.I.O., introduced the actinic light onto the sample; a shutter opening time of about 0.3 ms was possible with this system. The fluorescence induction signal was displayed on a storage oscilloscope (Model 1703A, Hewlett-Packard) and was photographed with an oscilloscope camera (Model 123A, Hewlett-Packard). The sample was held in a fluorescence cuvette (101-OX, 10 mm light path, Hellma Canada, Ltd., Toronto). Mirrors were placed at a 90° angle to each other, parallel to the vertical sides of the cuvette facing the actinic light beam and the photodiode. In this way the actinic and fluorescent light could be reflected through the sample to increase the sensitivity of the fluorometer. The irradiance of the actinic light at the sample surface was about 150 W m\(^{-2}\).

A fluorescence induction curve was obtained by placing a cuvette containing the sample (3.0 ml of phytoplankton culture) into the darkened sample chamber of the fluorometer at room temperature. A magnetic stirrer and cell spinbar (F37150, Bel Art Products, Pequannock, NJ) kept the culture in suspension during a 5 min dark-adaptation period. After dark-adaptation, the fluorometer was triggered and the fluorescence induction signal was displayed and photographed for later analysis. A second aliquot, containing DCMU (10\(^{-5}\) M final concentration, added from a 10\(^{-2}\) M ethanolic solution) was then dark-adapted for 5 min prior to obtaining the fluorescence signal. A blank reading (actinic light signal) was obtained with an empty cuvette in the sample holder. A\(_{DCMU}\) was then determined, in triplicate, by tracing the perimeter of the area above the fluorescence curve (Fig. 1) 10 times with a compensating planimeter, giving a precision of about ± 2%.

Photosynthetic rates at 60 W m\(^{-2}\) were measured by placing triplicate 5.0 ml aliquots from each of the preconditioning irradiances into glass scintillation vials, and adding 14C-bicarbonate (0.2 \(\mu\)Ci ml\(^{-1}\) final activity, New England Nuclear Corp.). After a 15 min incubation period (20°C), the samples were acidified (50 \(\mu\)l of concentrated hydrochloric acid), and the scintillation vials were placed on a shaker table overnight to remove inorganic 14C. Aquasol (10 ml, New England Nuclear Corp.) was then added, and the radioactivity was measured using a liquid scintillation counter (Model LS 7800, Beckman). The time-zero controls were treated identically, except that they were acidified at the beginning of the incubation.

Photosynthetic rates as a function of irradiance were determined on another occasion using cells preconditioned, as above, to each of the eight light intensities. Aliquots (1.0 ml) containing 14C-bicarbonate (0.8 \(\mu\)Ci ml\(^{-1}\) final activity) were dispensed into glass scintillation vials and were incubated (20°C) at 36 irradiances for 20 min in a light gradient box (Lewis and Smith, 1983). The incubations were then terminated and the samples were processed as outlined above. Data were fitted to the light-saturation curves according to Platt and Gallegos (1980).

Irradiance was measured with a 4 \(\pi\) collector (Bio-spherical Instruments Inc., San Diego, CA) placed at the position of the cuvette in the fluorometer, or inside the incubation vessel in the case of the photosynthesis experiments. Chlorophyll \(a\) was determined using a...
flourometer (Model 10, Turner Designs) calibrated with pure chlorophyll a (Sigma Chemical Co.). Cells were counted with a bright line hemacytometer (American Optical). Excitation and emission spectra of *Thalassiosira pseudonana* and of *Dunaliella tertiolecta* were obtained at room temperature with a Zeiss spectrofluorimeter (Model LX501 xenon lamp, M4QII excitation unit, MB3 emission unit, PMQII read-out unit).

### RESULTS

*Thalassiosira pseudonana* exhibited fluorescence excitation and emission peaks at 461 nm and 677 to 678 nm, respectively; those for *Dunaliella tertiolecta* were at 475 nm and 683 to 684 nm, respectively. The sharp cut red filter used in our induction fluorometer isolated a wavelength of greater than about 670 nm.

A concentration higher than about 7 µM DCMU was required to achieve the smallest ΔADCₐwr relative to a control without added DCMU (Table 1). The volume of ethanol added to the sample had no significant influence on ΔADCₐwr. The initial fluorescence intensity (F₀) increased by about 16 % with an increase in the concentration of added DCMU (Table 1). A standard concentration of 10 µM DCMU was used in all subsequent experiments.

The effect of dark-adaptation time on the fluorescence induction signal when DCMU was added prior to or after a given dark-adaptation period is shown in Table 2. A dark-adaptation time longer than 30 s was required to obtain the smallest value of ΔADCₐwr. For a given set of conditions, a larger value of ΔADCₐwr would indicate that a portion of the reaction centers was still in the reduced form (see 'Discussion'). No consistent difference was seen in the value of ΔADCₐwr when the DCMU was added before or after a given period of dark-adaptation.

The replicatability of the fluorescence induction signal was studied using 12 aliquots of *Thalassiosira pseudonana* withdrawn successively from a batch cul-
Photosynthetic rates and values of $A_{DCMU}$ Chl $a^{-1}$ for both the high and the low irradiance cultures, showed apparent maxima after about 9 h into the incubation period. In accordance with the previous experiments (Fig. 3 and 4), values of $P_{m}$ and of $A_{DCMU}$ Chl $a^{-1}$ were consistently greater for the culture shifted to the lower irradiance compared to the control (Fig. 7).

Values of $A_{DCMU}$, whether normalized to chlorophyll a (Fig. 8a) or to culture volume (Fig. 8b), correlated well with the photosynthetic capacity; values for the cultures adapted to both 60 W m$^{-2}$ and to 9 W m$^{-2}$ fell along a common straight line. In this experiment, the linear regression showed a y-intercept which was not significantly different from zero when the values were normalized to culture volume (Fig. 8b).

**DISCUSSION**

Alternative approaches for measuring primary productivity of marine phytoplankton are desirable, especially in light of the controversy concerning the magnitude of primary production in oligotrophic waters (Eppley, 1980). The preliminary laboratory results presented here are promising, and show the potential for using in vivo chlorophyll fluorescence induction to index the photosynthetic capacity of phytoplankton, without potentially-deleterious lengthy incubations. The approach is based on about 20 yr of experience by plant physiologists in the area of chlorophyll fluorescence.

We have attempted to optimize the conditions necessary for obtaining reliable and reproducible fluorescence induction curves. Most of the research directed at chlorophyll fluorescence has been carried out using higher plants or freshwater green algae (Papageorgiou, 1975; Vincent, 1983). Few fluorescence excitation and emission spectra are available for marine phytoplankton (Yentsch and Yentsch, 1979). In the case of diatoms, *Phaeodactylum tricornutum* showed emission peaks at 680 and 705 nm at room temperature, while the chlorophyte *Dunaliella tertiolecta* emitted at 683 to 684 nm. This compares with a maximum emission of 685 nm for green plants (Papageorgiou, 1973). In our study, the diatom *Thalassiosira pseudonana* gave an emission band at 677 to 678 nm, while the chlorophyte *Dunaliella tertiolecta* emitted at 683 to 684 nm. This compares with a maximum emission of 685 nm for green plants (Papageorgiou, 1975). Fluorescence peaks may actually vary slightly depending on the ambient growth conditions (Shimura and Fujita, 1973). In our study, the diatom *Thalassiosira pseudonana* gave an emission band at 677 to 678 nm, while the chlorophyte *Dunaliella tertiolecta* emitted at 683 to 684 nm. This compares with a maximum emission of 685 nm for green plants (Papageorgiou, 1975). Fluorescence peaks may actually vary slightly depending on the ambient growth conditions (Shimura and Fujita, 1973). The filters used here to excite and to isolate the fluorescence peaks were broad-banded, and encompassed the observed excitation and emission peaks.

The concentration of DCMU used in this study (10 μm) is similar to that used in other studies.
Fig. 3a. Photosynthesis vs. irradiance curves for *Dunaliella tertiolecta*, measured after a 24 h incubation period at each of the preconditioning irradiances indicated.

(Samuelsson et al., 1978; Harris, 1980; Kulandaivelu and Daniell, 1980). A concentration lower than about 7 μM resulted in an increase in $A_{DCMU}$ (Table 1). This suggests that in the presence of an insufficient concentration of DCMU, a portion of the intermediary chain (e.g. plastoquinone) between photosystem I (PSI) and photosystem II (PSII) is measured in addition to $Q$. (Duysens and Sweers, 1963; Malkin and Kok, 1966; Forbush and Kok, 1968).

Likewise, the increase in $A_{DCMU}$ at dark-adaptation times of less than 1 min (Table 2) suggests that the measurement is not restricted to the concentration of $Q$, but also includes a portion of the photosystem intermediates. In the case of *Thalassiosira pseudonana*, a dark-adaptation time of 5 min proved adequate for the measurement of $A_{DCMU}$ (Table 2); dark-adaptation times in other studies were: zero (Cullen and Renger, 1979; Roy and Legendre, 1979); 10 min (Samuelsson and Oquist, 1977); 30 min (Prézelin and Sweeney, 1977); 2 h (Vincent, 1981). The utility of the fluores-
Fig. 3b. Photosynthesis vs. irradiance for *Thalassiosira pseudonana*, measured after a 24 h incubation period at each of the preconditioning irradiances indicated.

cence induction method, which aims to eliminate sample incubation, is lost at these longer dark-adaptation times. A standard dark-adaptation time may not always be reliable due to variabilities introduced by
Fig. 4. Saturated rate of chlorophyll a-normalized photosynthesis, $P_m$, (a) and chlorophyll a-normalized area above the fluorescence induction curve, $A_{DCMU} \text{ Chl } a^{-1}$ (c) for *Dunaliella tertiolecta* (a) and for *Thalassiosira pseudonana* (b), measured after a 24 h incubation period at several irradiances as indicated by the data points.

Differences in species composition and the physiological state of the cell (Prézelin and Ley, 1980). These difficulties may be resolved in future work by (1) vacuum infiltrating the cells with DCMU to ensure complete penetration of the inhibitor (Kulandaivelu and Daniell, 1980; Malkin et al., 1981); (2) avoiding a long period of dark-adaptation by irradiating the sample for less than 1 min with far-red light to accelerate the reoxidation of Q (Malkin and Kok, 1966), or by providing a reductant.

Dark-adaptation allows the re-oxidation of Q by the pool of intersystem oxidants, ensuring that all of the reaction center electron 'traps' are open, and that the initial intensity of fluorescence ($F_o$) is at a minimum (Malkin and Kok, 1966; Forbush and Kok, 1968; Papageorgiou, 1975). Apparently, Q becomes at least partially reoxidized, even in the presence of DCMU, as no consistent difference was seen in the value of $A_{DCMU}$ when the DCMU was added before or after the dark-adaptation period (Table 2); it is not known where the electrons drain to in the presence of DCMU (D. Fork, pers. comm.). In this study, the DCMU was added prior to dark-adaptation as a matter of convenience, to avoid exposure to even dim light if the DCMU were to be added to the sample after dark-adaptation.

Fig. 5. Relation between photosynthetic rate at light saturation ($P_m$) and area above fluorescence induction curve ($A_{DCMU}$) when normalized to chlorophyll a (a, c) and to culture volume (b, d) for *Dunaliella tertiolecta* (a, b) and for *Thalassiosira pseudonana* (c, d), measured after a 24 h incubation period at several irradiances as indicated by data points.
Fig. 6. Cellular chlorophyll a concentration measured over 24 h for *Thalassiosira pseudonana* incubated at 60 W m\(^{-2}\) of irradiance (O), or transferred to 9 W m\(^{-2}\) of irradiance (●). Preconditioning irradiance = 60 W m\(^{-2}\).

Fig. 7. Saturated rate of chlorophyll a-normalized photosynthesis, \(P^\text{N}\) (a), and chlorophyll a-normalized area over fluorescence induction curve, \(A_{\text{DCMU}} \text{ Chl a}^{-1}\) (b), measured over a 24 h incubation period for *Thalassiosira pseudonana*. Symbols as in Fig. 6.

The goal of this paper was to examine the possibilities of indexing the photosynthetic capacity of marine phytoplankton by measuring \(A_{\text{DCMU}}\). In this respect, the strong positive correlations obtained between \(A_{\text{DCMU}}\) and photosynthetic capacity (Fig. 5a,b,d; 8a,b), and the way in which the pattern of \(A_{\text{DCMU}} \text{ Chl a}^{-1}\) parallels that of \(P^\text{N}\) at 2 irradiances over a 24 h period (Fig. 7) are encouraging. Such a technique offers the possibility of measuring photosynthetic capacity without lengthy incubation. However, several drawbacks of the present system deserve examination.

As presently used, the method needs calibration against assimilation numbers measured in the conventional way on cultures grown under a wide range of environmental conditions. However, the ultimate goal is a direct (rather than correlative) estimate of photosynthetic rate; to achieve this we need to interpret the geometry of the induction curve in terms of the flow of electrons through Q.

A measure of photosynthetic capacity rather than of the rate of photosynthesis at *in situ* irradiances is obtained. However, knowledge of the variability of photosynthetic capacity and its relation to environmental factors is important in mathematical models used to predict phytoplankton primary productivity (e.g. Platt and Gallegos, 1980).
The sensitivity of the fluorometer was not great enough to allow measurement of induction curves of natural populations of phytoplankton. Chlorophyll a concentrations of the order of 200 to 300 μg l⁻¹ were generally used in these experiments; the lowest chlorophyll a concentration providing a measurable AD_{DCMU} was only 20 to 30 μg l⁻¹. The presently-used more convenient silicon photodiode will be replaced by a more sensitive photomultiplier tube to detect the lower intensity fluorescent light. Alternatively, the fluorescence induction signal of an 'artificial leaf' may be observed by concentrating cells onto a glass fiber or membrane filter.

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