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Evaluation of photosynthetic capacity in phytoplankton by flow cytometric analysis of DCMU-enhanced chlorophyll fluorescence

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ABSTRACT: A new method is presented to evaluate the photosynthetic capacity of individual phytoplankton cells using flow cytometry. The method is based on the well-known phenomenon in which chlorophyll *a* fluorescence is enhanced in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), an inhibitor of photosynthetic electron transport. Using a mercury-cadmium arc lamp-based flow cytometer, we found significant correlations between fluorescence enhancement and photosynthetic capacity. The correlation was dependent on species and growth irradiance. The regression coefficient ranged more than 5 times according to species, and cells under light-saturated growth were enhanced less than light-limited cells, although the former had higher photosynthetic capacity than the latter. The ability to measure DCMU-enhanced fluorescence in flow cytometry depends on a weak excitation beam and a long residence time in the sensing zone to ensure maximum variable fluorescence yield. We were unable to detect enhancement using laser-based flow cytometers. The variability in fluorescence enhancement is discussed in relation to its practical use in the field. The method was applied to natural assemblages in Bedford Basin (Canada): temporal variations of fluorescence enhancement were clearly evident in dominant diatoms and cryptophytes.

INTRODUCTION

Phytoplankton communities in nature undergo continuous changes in species composition and abundance. The alterations are brought about by succession, sequential changes or their combinations (Gran & Braarud 1935). Since species succession is dependent on the outcome of differential growth activity among species, measurements of growth and photosynthetic activity at the species level are most relevant to an understanding of succession.

Several approaches have been proposed to investigate species-specific photosynthetic activity, including grain density autoradiography (Brock & Brock 1966, Watt 1971) and the labelling of single cells with radioisotope (Rivkin & Seliger 1981, Rivkin 1986). These methods require seawater samples to be incubated and therefore may suffer from artifactual effects owing to confinement of natural assemblages, and from a possible contamination by toxic substances during sample-handling procedures (Venrick et al. 1977, Carpenter & Lively 1980, Fitzwater et al. 1982). Furthermore, autoradiography and micromanipulation of isotope-labelled cells are tedious and demand considerable technical skills. Recently, other methods have been developed to probe photosynthetic activity of single cells using flow cytometry: an immunofluorescence method for ribulose bisphosphate carboxylase (Orellana et al. 1988) and the pump-and-probe fluorescence method (R. Olson & E. Zettler pers. comm.).

In this paper we describe a new method to evaluate the photosynthetic capacity of individual phytoplankton cells using flow cytometry, which enables quick assessment without the need for lengthy incubations or complicated procedures. The photosynthetic capacity was indexed by enhancement of *in vivo* fluorescence in the presence of the photosynthetic inhibitor

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3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). Light energy absorbed in photosystem II (PS II) is either re-emitted, dissipated as heat or utilized through photosynthetic electron transport. At physiological temperature, the *in vivo* fluorescence emanates mainly from the chlorophyll *a* (chl *a*) of the PS II. The fluorescence yield, that is, the intensity of fluorescence per unit amount of chl *a*, reflects the redox state of the primary electron acceptor (Q) of the PS II (Duysens & Sweers 1963): the yield is high when Q is reduced and low when Q is oxidized. In the dark, Q becomes oxidized by electron flow into PS I, and on illumination photoreduction of Q occurs by PS II. DCMU blocks further electron transport from Q and inhibits reoxidation of the reduced Q. As a consequence, the *in vivo* fluorescence is maximized in the presence of DCMU. The enhancement in fluorescence due to DCMU treatment is attributed to blockage of photosynthetic electron transport, and has been extensively used as an indicator of photosynthetic capacity of bulk assemblages (Samuelsson & Öquist 1977). Distinct enhancements are generally detected for the DCMU-induced fluorescence with standard fluorometers. On the contrary, to date the addition of DCMU has resulted in little effect on chl *a* fluorescence as measured by flow cytometry (Perry & Porter 1989). Therefore, the effect of DCMU on chlorophyll fluorescence has not been exploited in flow cytometry for evaluating photosynthetic capacity. In the work reported here, we found that DCMU-enhanced fluorescence could be detected by flow cytometry when excitation intensities were weak. We investigated the suitability of this method to index photosynthetic capacity of cultured algae and then applied this method to a study of natural phytoplankton assemblages.

MATERIALS AND METHODS

Clone cultures were used for laboratory tests. Batch cultures of *Prorocentrum minimum* strain 1PM (Dinophyceae), *Thalassiosira weissflogii* strain Tw, *Phaeodactylum tricornutum* strain Ph (both Bacillariophyceae), *Dunaliella tertiolecta* strain Dun (Chlorophyceae) and *Chroomonas salina* strain 3C (Cryptophyceae) were grown in f/2 media (Guillard & Ryther 1962) at 20 °C on a constant photon flux of 210 $\mu E\ m^{-2}\ s^{-1}$ supplied by cool-white fluorescent lamps. Preliminary experiments indicated that this irradiance was optimum for the growth of all the above species. *Pycnococcus provasolii* strain Ω48-23 (Micromonadophyceae) grown in f/2 media at 18 °C was kept in 12 h L:12 h D cycle.

Seawater was collected from discrete depths down to 20 m at a station (44° 31.3' N, 63° 38.3' W) over the deepest part (75 m) of Bedford Basin on the Atlantic coast of Nova Scotia, Canada, an enriched fjord-like inlet with a surface area of 17 km^2. The basin is characterized by high biomass and production rates, especially in spring and summer (Irwin et al. 1983, 1988a, b). Seventeen collections were made at 1 to 3 d intervals during a spring bloom from 2 to 30 March 1988 between 08:30 and 09:00 h using a Niskin bottle. A further field observation was conducted from 22 October to 11 December 1991. The samples were immediately transferred into darkened carboys and carried to shore, then gently reverse-filtered through a 50 µm mesh netting to remove larger particles to avoid blocking the orifice of the flow cytometer. Flow cytometric analysis was performed within 2 h after the sampling. In the spring bloom study, aliquots were fixed with glutaraldehyde for microscopic examination (Tsuji & Yanagita 1981).

A FACS Analyzer (Becton Dickinson, Mountain View, CA, USA) was used to measure Coulter volume, side scattering and fluorescence of both chl *a* and phycoerythrin. The particles were passed through either a 50 or a 75 µm orifice and excited by a mercury-cadmium arc lamp emitting 485 nm. *In vivo* autofluorescence from phytoplankton was reflected by a 505 nm dichroic filter, and then split into long- and short-wavelength components by a 590 nm dichroic filter. The 2 components were further isolated respectively with a 660 nm long pass absorbance filter and a 575 ± 13 nm bandpass filter, each corresponding to chl *a* and phycoerythrin fluorescence. List mode data were acquired on signals accepted on the basis that they exceeded a lower threshold on >660 nm fluorescence. Signals were recorded in relative units on 3 decade logarithmic scales. Flow rate of samples through the orifice was controlled by the pressure difference between sample and sheath water

Laser-based flow cytometers (FCS, Jasco, Tokyo, Japan; EPICS, Coulter, Hialeah, FL, USA) were also used for laboratory tests of the cultures. Particles were ejected through a 70 or 75 µm jet nozzle and excited by an Ar-ion laser emitting 400 to 500 mW at 488 nm (Innova 90-4, Coherent, Palo Alto, CA, USA). Fluorescence emissions from chl *a* and phycoerythrin were processed in the same way as with the FACS Analyzer.

Signal intensity and flow rate were maintained at constant levels by frequent analyses of standard latex beads of known particle densities. Coulter volume and side scattering were calibrated against beads of 3 different sizes (Duke Scientific, Palo Alto, CA, USA). Fluorescence intensity was calibrated using fluorescence beads (Flow Cytometry Standards Corporation, Research Triangle Park, NC, USA).

Algae were kept in the dark for 10 to 20 min before analyses. DCMU in 95 % ethanol (10^{-2} M) was then added into the remainder of the samples to a final concentration of 10^{-5} M; after a further incubation in darkness for 5 to 15 min, the analysis was repeated. The effect of the ethanol was examined by adding the same portion of DCMU-free 95 % ethanol into the cultures as above: no substantial alteration in chlorophyll fluorescence was noted. Throughout the whole procedure, phytoplankton were kept dark at culture or *in situ* temperature using a water-jacket.

The enhancement of red fluorescence was quantified using the fluorescence response index (*FRI*) of Cullen & Renger (1979):

$$FRI = \frac{(F_d - F_n)}{F_d} = 1 - \frac{F_n}{F_d}$$

where F_n and F_d are fluorescence intensities averaged for a population before and after poisoning with DCMU, respectively.

Light-saturated photosynthesis was determined by placing quadruplicate 5.0 ml aliquots from the cultures into borosilicate vials with ^{14}C-bicarbonate (ICN Biomedicals, CA, USA) at final concentrations of 9.25 kBq ml^{-1}. The algae were incubated at 20 °C for 30 min. The incubation was then terminated by acidification with 50 µl 6N HCl, and the vials were shaken overnight to eliminate inorganic ^{14}C. For *Pycnococcus provasolii*, the incubation was conducted for 60 min, and terminated by acidification with 1 ml 5 % glacial acetic acid in methanol (Li & Goldman 1981). Blanks were prepared in triplicate as time zero controls. Triplicate dark controls yielded no significant difference from the time zero controls. Activity was assayed by liquid scintillation counting (Beckman LS7800). The counts were corrected against efficiency determined by the channels ratio method.

Photosynthetic rates were measured as a function of irradiance for cultures preconditioned to 2 photon fluxes, 367 and 28 µE m^{-2} s^{-1}, which are saturating and limiting intensities for photosynthesis, respectively. Aliquots (1 ml) of cultures containing 37 kBq ml^{-1} (final concentration) of ^{14}C-bicarbonate were dispensed into glass scintillation vials and incubated at 42 different irradiances for 45 min in a light gradient box (Lewis & Smith 1983). The incubations were terminated by adding HCl and activity was measured as outlined above.

Chl *a* was measured fluorometrically (Turner Designs 10R) for replicate 90 % acetone extracts of cells collected on Whatman GF/F filters (Holm Hansen et al. 1965). The extraction was made for 24 h at 0 °C in the dark.

RESULTS

Laboratory test

Red fluorescence measured by the FACS Analyzer was enhanced by adding DCMU in all the species tested. The DCMU treatment had no significant effect on either Coulter volume or side scattering. The magnitude of the *FRI* depended on excitation intensity, being low at high excitation intensity (Fig. 1a). Weaker

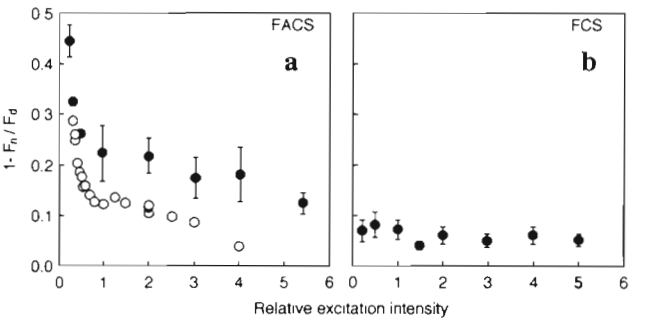

Fig. 1 *Thalassiosira weissflogii, Pycnococcus provasolii.* Variations in the enhancement of red-fluorescence (*FRI*; expressed as $1 - F_n/F_d$) as a function of excitation intensity for (a) FACS Analyzer and (b) FCS in *T. weissflogii* (●) and *P. provasolii* (○) at their exponential growth phases under saturating (210 µE m^{-2} s^{-1}) and limiting photon flux (70 µE m^{-2} s^{-1}), respectively. Excitation intensity is given in arbitrary units normalized by maximum intensity where the highest gain was obtained for standard fluorescence beads. The maximum intensity was different between the 2 instruments

excitation, produced either by combinations of neutral density filters or by mis-aligning the arc lamp in the lamp holder, gave rise to higher *FRI*. In particular, high *FRI* was induced with low excitation below one-third of the maximum excitation. Since absolute intensity of the excitation was not measured in the present study, the excitation was monitored by frequent calibration using the standard fluorescence beads. In contrast to the FACS Analyzer, distinct *FRI* was not observed with the FCS, a laser-based flow cytometer (Fig. 1b). Reduction of the excitation intensity did not raise the *FRI*. Similarly, insignificant *FRI* was obtained with the EPICS. On the basis of these results, all the flow cytometric analyses hereafter were made using the FACS Analyzer.

The *FRI* varied during growth in batch culture (Figs. 2 & 3). The enhancement increased rapidly during early exponential phase and was rather stable in midexponential phase. It decreased sharply when growth declined and became minimum at stationary phase. This fluctuation paralleled that of assimilation number, i.e. maximum photosynthetic

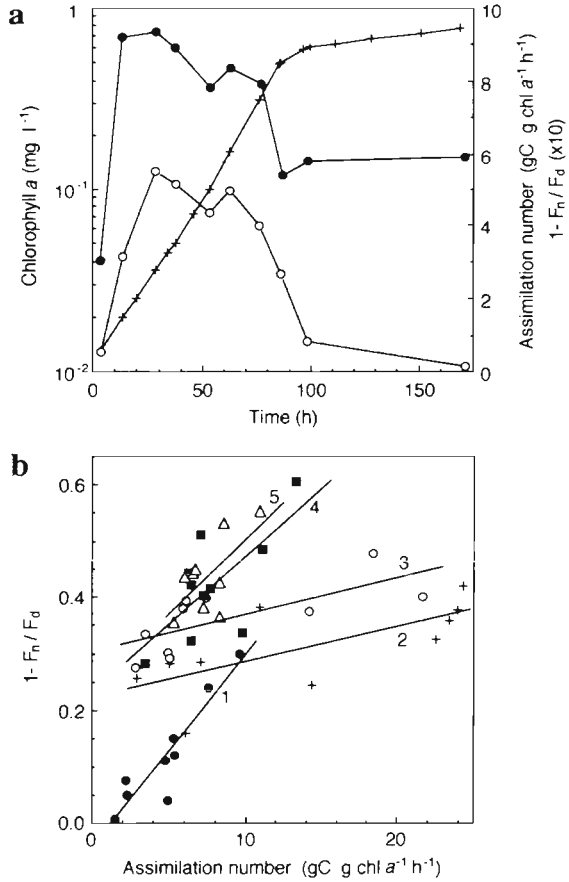

Fig. 2. (a) Time courses of the *FRI* (○) and assimilation number (●) during growth in terms of chl *a* (+) in *Thalassiosira weissflogii* in light-saturated growth (210 μE m^{-2} s^{-1}). Enhancement is multiplied by 10. (b) Relationships between assimilation number and the *FRI* in *Prorocentrum minimum* (●), *Phaeodactylum tricornutum* (+), *Chroomonas salina* (○), *T. weissflogii* (■), and *Dunaliella tertiolecta* (△), each corresponding to regression line 1, 2, 3, 4 and 5, respectively. 1: $y = 0.0340x - 0.043$ (r = 0.924, n = 9); 2: $y = 0.0062x + 0.223$ (r = 0.704, n = 11); 3: $y = 0.0067x + 0.303$ (r = 0.715, n = 10); 4: $y = 0.0240x + 0.234$ (r = 0.703, n = 10); 5: $y = 0.0266x + 0.237$ (r = 0.657, n = 9). The cultures were in light-saturated growth (210 μE m^{-2} s^{-1})

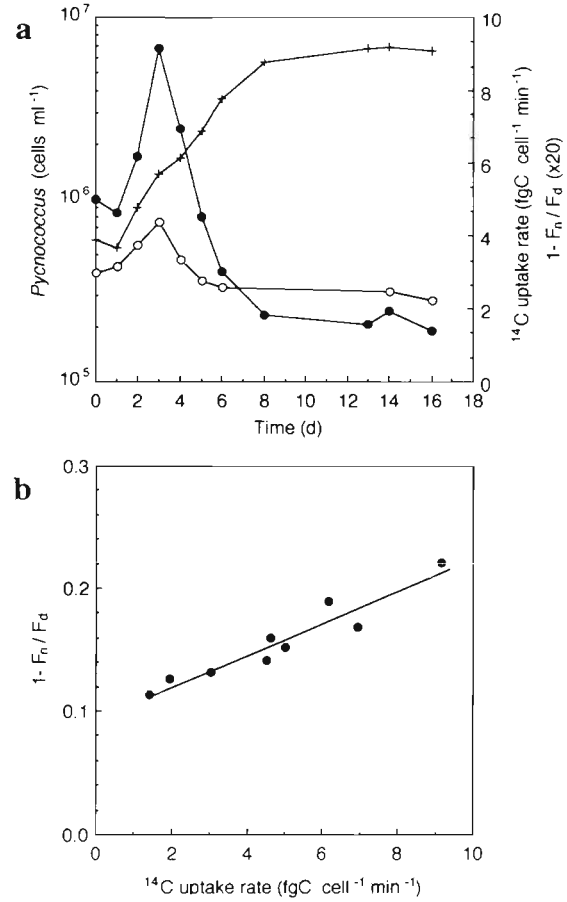

Fig. 3. *Pycnococcus provasolii*. (a) Time courses of the *FRI* (○) and light-saturated rate of ^{14}C uptake $cell^{-1}$ (●) during growth in terms of cell number (+) in *P. provasolii* in light-saturated growth (105 μE m^{-2} s^{-1}). Enhancement is multiplied by 20. (b) Relationship between P_{max} per cell and the *FRI* in *P. provasolii* in light-saturated growth (105 μE m^{-2} s^{-1})

rate P_{max} per unit amount of chl *a* (Fig. 2a). The *FRI* showed a significant relationship ($p < 0.05$) with assimilation number (Fig. 2b). Slopes of the regression varied considerably among the cultures, ranging from 0.0062 to 0.340, indicating the correlation was species specific (Fig. 2b). Interspecific variation of the *FRI* was greater than intraspecific fluctuation, as exemplified by *Thalassiosira weissflogii* and *Prorocentrum minimum*. The parallelism was also found between the *FRI* and P_{max} per cell during growth of *Pycnococcus provasolii* and resulted in a strong correlation (Fig. 3).

The magnitude of the *FRI* was dependent on growth irradiance (Fig. 4). Cells under light-limited growth showed higher *FRI* than those under light-saturated

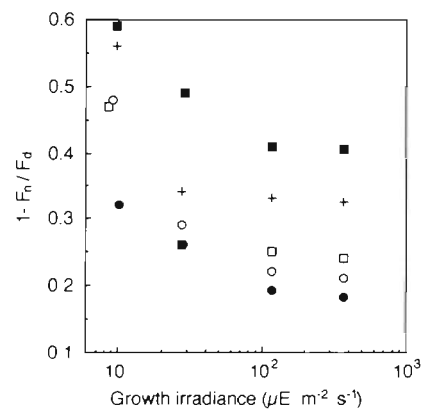

Fig. 4. Variations in the *FRI* of cultures at mid-exponential phase as a function of growth irradiance in *Prorocentrum minimum* (●), *Phaeodactylum tricornutum* (+), *Chroomonas salina* (○), *Thalassiosira weissflogii* (■), and *Dunaliella tertiolecta* (□)

growth, although the former had a lower assimilation number than the latter (Fig. 5), indicating that the correlation between the *FRI* and photosynthetic capacity varied with growth irradiance.

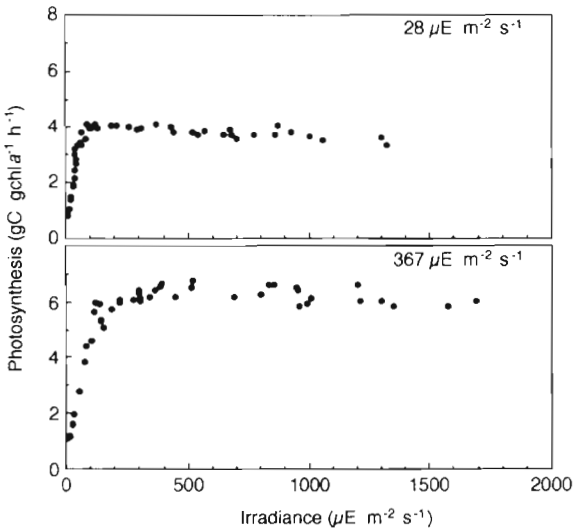

Fig. 5. *Thalassiosira weissflogii*. Photosynthesis vs light curve of cultures under light-limited (upper) and light-saturated growth (lower). Numbers in the diagrams denote growth irradiance

Field application

A spring bloom composed mainly of centric diatoms occurred in Bedford Basin from late February to March 1988, during which surface chl *a* fluctuated between 14 and 38 mg m^{-3} (Brian Irwin pers. comm.). Some species formed clear clusters in cytograms (Fig. 6). Identification to species was made for the clusters by comparing with microscopic observation of cell volume and density. The hatched cluster in Fig. 6 was identified as *Thalassiosira nordenskiöldii*. The progress of this species was followed during the bloom by monitoring the hatched cluster over time. This flow cytometric extraction of *T. nordenskiöldii* was cross-checked by microscopic determination of cell volume and numerical abundance at each observation. The cluster consistently represented this species during the period of 3 to 14 March.

The *FRI* of *Thalassiosira nordenskiöldii* fluctuated considerably during the bloom (Fig. 7). The *FRI* was high on 3 March and then decreased sharply until 9 March. In this period, the numerical abundance of *T. nordenskiöldii* increased exponentially, reached a maximum, and then decreased. A rise of the *FRI* was observed at another exponential increase of cell number from 9 to 11 March. The elevation of the *FRI* was

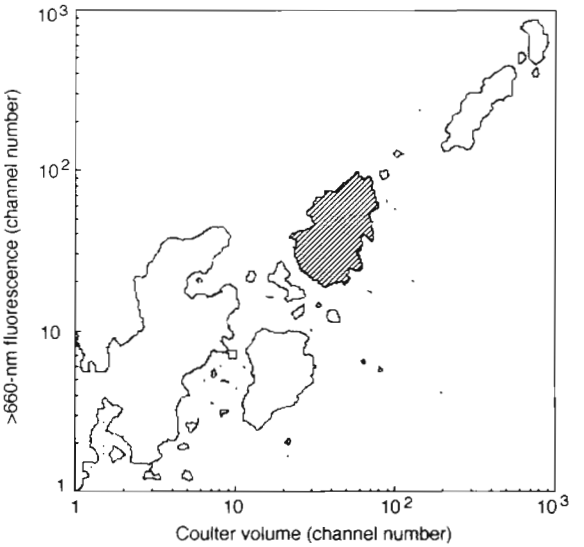

Fig. 6. Chlorophyll fluorescence vs Coulter volume of a field sample from 1 m depth of Bedford Basin on 10 March 1988. Counter plot is made for channels of 2 or more counts, where trigger source was chlorophyll fluorescence. The hatched cluster was composed of *Thalassiosira nordenskiöldii*

short-lived and had reduced sharply when cell number reached its maximum, as was observed at the previous maximum on 7 March. A phase shift between the maximum of the *FRI* and cell abundance was 1 to 4 d. The *FRI* of the near surface population tended to be lower than those of subsurface populations. The *FRI* of *T. nordenskiöldii* at 10 m depth was 1.2 to 1.8 times higher

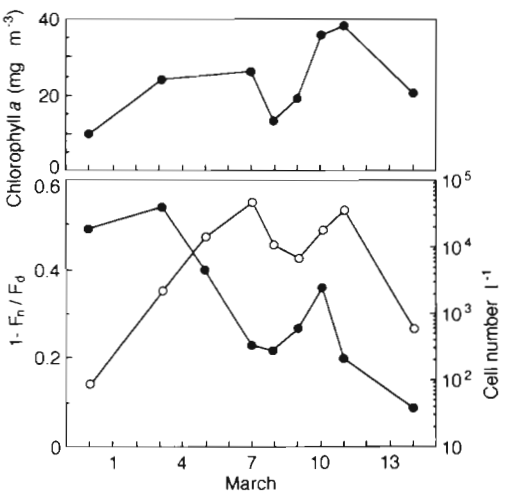

Fig. 7 *Thalassiosira nordenskiöldii*. Temporal variations in surface chl *a* averaged for 0–5 m (upper), and numerical abundance (○) and the *FRI* (●) of *T. nordenskiöldii* (lower) in the period 29 February to 14 March 1988 at 1 m depth. On 29 February cell number was counted by microscopy instead of flow cytometry, and the *FRI* was measured for only 7 cells owing to low abundance

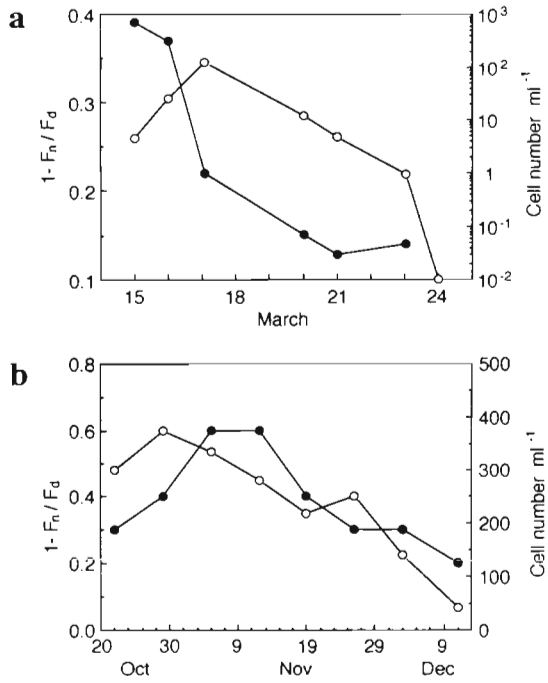

Fig. 8. *Cryptomonas* sp. (a) Temporal variations in numerical abundance (○) and the *FRI* (●) of *Cryptomonas* sp. in the period 29 February to 14 March 1988 at 1 m depth. On 24 March cell number was counted by microscopy instead of flow cytometry owing to low abundance. (b) Symbols as in (a) but cryptomonads collected from 22 October to 11 December 1991 at 1 m depth

than that at 1 m depth during the period of cell-number increase, and varied from 0.7 to 1.2 times in the decreasing phases.

Cryptomonas sp. occurred on 15 March 1988 with a high *FRI* (Fig. 8a). The temporal variation of *FRI* was similar to that of *Thalassiosira nordenskiöldii*: that is, the high *FRI* was observed during an exponential increase of cell number, and then decreased sharply at and after a cell-number maximum. In direct contrast, observations of cryptomonad abundance and *FRI* in autumn 1991 revealed a different relationship between the 2 variables. In this second field study, a peak in *FRI* (6 to 13 November) was not followed by an obvious peak in cryptomonad abundance (Fig. 8b). In fact, there was an apparently linear decrease in abundance from 30 October to 11 December. The very slight increase in abundance noted on 27 November did not, in our opinion, constitute convincing evidence indicating a response to the earlier peak in *FRI*.

DISCUSSION

Fluorescence increase due to DCMU addition was clearly detected with a FACS Analyzer, and the method presented here provides a rapid assessment of photosynthetic capacity of phytoplankton populations. Temporal variations of the *FRI* were clearly evident in natural assemblages; high *FRI* was associated with the early phase of the 1988 bloom and *FRI* tended to decrease as the bloom progressed toward its peak (Figs. 7 & 8a). In a similar manner but using the incubation method of single-cell labelling with ^{14}C, Han et al. (1992) also showed high values of species-specific photosynthetic rates (SSP) in the initial phase of a *Skeletonema costatum* bloom; thereafter, SSP decreased gradually and reached a minimum at the peak of the bloom. On the other hand, we also noted, in the 1991 field study, that a peak in *FRI* did not indicate that a peak in cell abundance must necessarily follow (Fig. 8b). It is important to remember that although *FRI* is related to photosynthetic capacity, there is not necessarily any relationship between photosynthetic capacity and photosynthetic performance *in situ*; nor between any of these variables and net population growth which includes all the loss processes, physiological and trophic.

The large variability among species in the correlation between the *FRI* and photosynthetic capacity (Fig. 2b) demonstrates an advantage of flow cytometry over the standard fluorometry. In a natural assemblage in which each species shows different correlations, the DCMU effect could be obscured in measurements of bulk fluorescence by standard methods. This interspecific diversity may partly explain the variable degree of success of previous studies (as reviewed by Bates & Platt 1984) which attempt to correlate DCMU-enhancement and photosynthesis for bulk phytoplankton composed of diverse species of various physiological status.

In our hands, *FRI* was measurable only in the FACS Analyzer but not the laser-based flow cytometers (Fig. 1). A previous study also reported negligible effect of DCMU using a laser-based flow cytometer (Perry & Porter 1989). There are at least 2 possible explanations for the lack of measurable enhancement. One is based on a consideration of fluorescence induction which is completed within milliseconds to several tens of milliseconds after onset of excitation (Bates & Platt 1984, Ishimaru et al. 1985). Since the residence time of cells in the sensing zone of laser-based flow cytometers is on the order of microseconds, fluorescence is detected at the early rising phase of induction (Ashcroft et al. 1986, Cullen et al. 1988, Neale et al. 1989). A consequence of the poorly-induced fluorescence is the similarity in measured response with and without DCMU. Neale et al. (1989) concluded that in an EPICS flow cytometer operated at 250 mW excitation at 488 nm, the effective fluorescence lies between minimum and maximum fluorescence yields. The

other explanation ascribes the lack of DCMU enhancement to a high irradiance from the laser beam which leads to rapid saturation of the reaction center in PS II. Although we present only relative light intensity in this paper, measurements of irradiance around the sensor position with a quanta meter (Biospherical QMS-100, San Diego, CA, USA) indicated a much higher intensity in laser-based flow cytometers than the FACS Analyzer. This was noted by Cullen et al. (1988) in their compilation of manufacturers' specifications. At high irradiance, photon absorption exceeds turnover of reaction center of PS II; consequently, all of the absorbed energy is re-emitted as fluorescence, or dissipated as heat even without DCMU (Murata et al. 1966). Under this condition, little of the absorbed photon energy is utilized in photosynthesis, and DCMU would have minimal effect on chlorophyll fluorescence. As a further complication at high excitation intensities, the induction patterns of fluorescence are altered through shifts in the pigment state for the energy distribution between the PS II and PS I (Murata 1969, Shimura & Fujita 1973).

Our experiments indicate the both explanations are possible. The residence time of cells in the sensor of the FACS Analyzer is almost 2 orders of magnitude longer than that in the laser-based flow cytometers used in the present study and by Perry & Porter (1989) (Cullen et al. 1988). The long residence time allows fluorescence induction to approach to its steady level such that the the effect of DCMU may be detected. In our experiments with the cultures, the *FRI* varied as flow rates changed. Moreover, the coefficient of variation for chlorophyll fluorescence with added DCMU was low when flow rate was kept constant, but tended to be high when flow became unstable due to fluctuating differential pressure (partial blockages of the orifice, low sheath water flow). These observations support the above inference, and suggest that maintenance of a constant flow rate is requisite in our method.

A suitable excitation intensity is another important consideration in this method. The minimal effect of DCMU (Fig. 1) may be in part attributable to the high irradiance supplied by the Ar-ion laser. In using flow cytometry to examine DCMU enhancement, it is therefore important to reduce excitation intensity as much as possible. However, this imposes a limitation in the kind of cell that can be studied by this method. The limitation arises because small phytoplankton cells, with their low chlorophyll content, fluoresce very weakly on an individual basis. The reduction of excitation intensity, which is necessary to achieve DCMU enhancement, effectively places the weakly fluorescent cells below the level of instrument detection. In the present study, we have successfully applied our method to *Pycnococcus provasolii* which is only 1.5 to 4.0 µm in diameter (Guillard et al. 1991). It is conceivable that our method may be improved by using photomultipliers of higher sensitivity and by lengthening the residence time of cells in the sensing zone.

The dependence of the *FRI* on growth irradiance (Fig. 4) imposes a constraint in applying our method to natural assemblages. Since light-limited cells with low assimilation number have higher *FRI* than light-saturated cells (Figs. 4 & 5), direct comparison of the *FRI* between shallow and deep populations of stratified waters is not warranted for evaluation of photosynthetic capacity. Similarly, the *FRI* of a population with unknown irradiance history may not be interpreted properly. Hence, we confined time-series analyses of natural phytoplankton to surface populations. The Bedford Basin bloom initiated at the surface and during the phase of cell-number increase of *Thalassiosira nordenskiöldii*, supplies of macronutrients seemed to be replete (Brian Irwin pers. comm.); accordingly, the photosynthetic capacity of the surface population was most likely higher than that at 10 m depth. Thus, the higher *FRI* observed in subsurface *T. nordenskiöldii* compared to the surface population appears to be a manifestation of the same phenomena observed in the laboratory test. Although it is not possible to infer from our data why cells grown at low irradiance give the higher *FRI*, photoadaptation in the light-harvesting pigments seems to be an important factor. Various phytoplankton are known to increase the amounts of light-harvesting pigments per photosystem in response to low growth irradiance (e.g. Halldal 1970). At the same time, the transfer efficiency of excitation energy from accessory pigments to chl *a* may increase (Shimura & Fujita 1975). As a consequence, under the same excitation intensity, Q is expected to be photoreduced more rapidly: that is, the time scale for the fluorescence induction to reach its steady level would be shorter in cells grown at low than at high irradiance. This may result in the higher *FRI* of dark-adapted cells.

The key of our method lies in distinction and identification of individual species in flow cytometry before and after adding DCMU. Combinations of Coulter volume, side scattering, chlorophyll and phycoerythrin fluorescence enabled us to separate particular species from others. For cryptophytes and cyanobacteria, phycoerythrin fluorescence was used as the primary criterion; and on the whole, Coulter volume was the most useful among the 4 signals. Cell sizing by electrical impedance on the FACS Analyzer was a significant advantage for our method since microscopic-based cell size of both cultures and natural assemblages corresponded better to Coulter volume than to forward angle light scatter (FALS). Measurements of FALS tended to give higher coefficients of variation than Coulter volume. The presence of strongly absorbing

pigments in variable amounts affects the amplitude of FALS at 488 nm, and may enhance magnitude of the variation (Shapiro 1988, Phinney & Cucci 1989). Furthermore, the lowering of excitation intensity required to obtain fluorescence enhancement has no effect on Coulter volume but severely constrains the measurement of FALS.

In conclusion, the FACS Analyzer is a useful tool for evaluating photosynthetic capacity of single cells in natural assemblages. However, field application of the flow cytometric DCMU method needs caution, especially in examinations of populations having different irradiance histories. A better understanding of the nature of chlorophyll fluorescence in flow cytometry (Neale et al. 1989) is needed for a more robust interpretation of measurements.

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