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Phytoplankton photoadaptation to vertical excursion as estimated by an in vivo fluorescence ratio*

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ABSTRACT: To measure the photoadaptation status of phytoplankton, Demers et al. (1985) used a fluorescence ratio which was dependent on the recent history of light exposure. A field survey of different phytoplankton populations in different oceanographic conditions was carried out to investigate the variations in this fluorescence ratio for natural populations. Results indicated that despite different stratification regimes, the vertical profiles of this fluorescence ratio could be interpreted adequately in terms of mixing and sinking. Using a simple conceptual model, which depends on the different time scales required for adaptation to bright irradiance and darkness, it is shown that this interpretation of the fluorescence ratio is consistent with a physical estimation of mixing calculated from wind data. Additional information on photoinhibition and sinking was obtained. This simple model may prove useful as a tool for identifying effects of vertical excursion on phytoplankton photoadaptation directly from vertical profiles of the proposed fluorescence ratio.

INTRODUCTION

It has long been recognized that phytoplankton grow in a rapidly changing environment, where hydrodynamic processes expose them to large fluctuations in light intensity, over a wide range of time scales (Falkowski 1980, 1984, Harris 1980, 1986, Marra & Heinemann 1982, Lewis et al. 1984a). Phytoplankton respond to these environmental changes in light by continuously adapting several of their photoadaptive characteristics (e.g. fluorescence yield; size, density, composition and distribution of the photosynthetic pigments units; chemical composition as well as level of activity of photosynthetic enzymes) to the new conditions (see reviews on photoadaptation by Harris 1978, Falkowski 1980, Richardson et al. 1983, Harding et al. 1987). In that context, it can be argued that phytoplankton photosynthesis in the upper ocean may well be mainly controlled by vertical excursions of the cells in an exponential light gradient decreasing from the surface (Lewis et al. 1984b). The extent of this vertical excursion is determined by the relative importance of turbulent mixing (Lewis et al. 1984b) and cell sinking (Bienfang 1980) processes, as influenced by the mixed layer depth (Marra 1980) and the vertical migration behaviour of some motile species (Rivkin et al. 1982, Cullen 1985).

It has been suggested that the recent light history of phytoplankton and the kinetics of photoadaptation can be used to provide information about the physical environment and, in particular, vertical mixing processes in the mixed layer (Falkowski 1983, Harris 1984, Cullen & Lewis 1988). Therefore, the possibility exists that vertical mixing rates in the upper layer may be inferred from vertical distributions of appropriate photoadaptive properties of phytoplankton which have characteristic rates of change faster than the temporal scale of vertical mixing. Fluorescence is an example of such a property (Lewis et al. 1984a, b, Cullen & Lewis 1988).

Demers et al. (1985) have suggested the measure of an instantaneous in vivo fluorescence ratio for characterizing the photoadaptation status of phytoplankton in the laboratory and in the natural environment. They found significant relationships between this ratio and the parameters of the photosynthesis-irradiance curves

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(estimated according to the model of Platt et al. 1980) which are often used to characterize physiological adaptations of phytoplankton to their light environment (e. g. Talling 1957, Yentsch & Lee 1966, Platt et al. 1980, 1982). In light of their results, Demers et al. (1985) suggested that their fluorescence ratio, which can be continuously measured in situ, could be used as a useful tool in deriving models such as the one proposed by Lewis et al. (1984a) to study the effects of vertical mixing on phytoplankton photoadaptation.

The objective of the present study is therefore to test the hypotheses that the instantaneous in vivo fluorescence ratio proposed by Demers et al. (1985) can be used (1) to describe temporal and vertical changes in the photoadaptation status of phytoplankton in the natural environment, and (2) to infer meaningful information on the effects of upper water column mixing and sinking on photoadaptation dynamics of phytoplankton.

MATERIALS AND METHODS

Measurement and significance of the in vivo fluorescence ratio of Demers et al. (1985). The experimental setup used by Demers et al. (1985) to measure the fluorescence ratio consists of 2 intercalibrated Turner Designs fluorometers separated by a length of transparent tubing exposed to bright light (2250 µEin m^{-2} s^{-1}), through which water is circulated. The length of tubing and the circulation speed determine exposure time. This allows the measurement of fluorescence before (F_b) and after (F_a) exposure to bright light for chosen differences in time between the 2 measurements.

Using this setup, Demers et al. (1985) measured the in vivo fluorescence of laboratory cultures and observed a typical relationship between the ratio F_a/F_b and the time of exposure to bright light. A plateau was reached after about 15 to 20 s of exposure, and the relative level of this plateau depended on the light intensity at which the cultures were grown (see Figs. 1 and 2 in Demers et al. 1985). By changing the time of exposure to bright light, they were able to reconstruct a curve reminiscent of a fluorescence induction curve (Papageorgiou 1975). Using aliquots of natural plankton samples, the fluorescence ratio F_a/F_b after 16 s of exposure to bright light was found to be well correlated with the value F_t/F_o (t = 16 s) of fluorescence induction curves measured by the method of Neveux (1982)

In reference to the various stages of the fluorescence time course described for in vivo chlorophyll *a* (Kautsky induction curves), 16 s would correspond to the time scale of transient changes in the thylakoid membranes caused by changes in photophosphorylation, redox potential, etc. (see review by Prézelin 1981). It is well known that when dark-adapted algae are illuminated with bright light, changes occur in their thylakoid membranes which modify the distribution of absorbed energy between photosystems (PS) I and II (Papageorgiou 1975, Butler 1978). After seconds to minutes exposure, a large proportion of energy absorbed by pigments of PS II is redirected away from the reaction centres of this photosystem to PS I (a process called spillover), which results in a decreased fluorescence yield. A number of mechanisms have been invoked for the regulation of the excitation of PS II: (1) reduction in photosystem II cross-section following phosphorylation of the light-harvesting complex II (Staehlin & Arntzen 1983); (2) dissipative cyclic electron flow around PS II (Falkowski et al. 1986); (3) resynthesis of a PS II core protein which may suffer damage as a result of electron transport (Kyle 1985); and (4) changes in the average composition and behaviour of the light-harvesting complex II which regulates the effective absorption cross-section of PS II (Sukenik et al. 1987).

Vincent (1979) was the first to present fluorometric evidence that spillover operates in the natural environment. He showed that chlorophyll fluorescence of phytoplankton samples suddenly exposed to sunlight (ca 1500 µEin m^{-2} s^{-1}) for 1 min exhibited a rapid initial increase of fluorescence in the first few seconds, followed by an almost equally rapid decrease to a minimum fluorescence threshold. The level of this threshold was dependent on the depth of collection, its higher relative fluorescence being associated with samples coming from lower depths. This minimum threshold was reached quickly (within 15 to 30 s), but recovery to the initial fluorescence level (before exposure to sunlight) took more than 15 min and was dependent on the length of the adaptation period. High near-surface light intensities would therefore favour spillover, thereby causing a large reduction in the minimum threshold of chlorophyll fluorescence. Recently, Sakshaug et al. (1987) proposed an alternative mechanism based on laboratory observations of a similar rapid decrease in chlorophyll fluorescence upon exposure to bright light. By analogy, they attributed this decrease to the relationship between fluorescence quenching and the rapid formation cycle of violaxanthin/zeaxanthin which was observed for a variety of higher plants by Demmig & Björkman (1987) and Demmig et al. (1987). The rapid nature of the spillover or fluorescence quenching adjustments is comparable to the time scales of changes in light experienced by the phytoplankton cells due to vertical mixing and to diurnal variations in the mixed layer (see Lewis et al. 1984a, Neale 1987, Cullen & Lewis 1988) Moreover, it corresponds closely to the time scale of changes used by Demers et al. (1985) for the measurement of their fluorescence ratio.

The results of Demers et al. (1985) are very similar to those of Vincent (1979). Their fluorescence ratio measures the minimum threshold of phytoplankton fluorescence after a sudden 16 s exposure to a very high irradiance of 2250 μEin m^{-2} s^{-1}. This exposure is much higher than that which phytoplankton usually experience in the natural environment at temperate latitudes (see Table 1 for typical values), and it can be expected that phytoplankton will exhibit a strong photoinhibition response that will vary according to their physiological adaptation status (see review by Neale 1987). In other words, it is expected that phytoplankton will show differences in their ability to cope with this strong light signal, depending on their most recent light history. Demers et al. (1985) documented vertical and diel variations in their fluorescence ratio characterized, respectively, by higher values (higher absolute fluorescence in this case) at depth (10 m) and during the night, and lower values (lower fluorescence) at the surface (0 to 5 m) and during the day. Therefore, one can argue that the fluorescence ratio of Demers et al. (1985) can be used as an instantaneous index for characterizing the photoadaptation status of phytoplankton.

Field measurements. Sampling was carried out at 5 anchor stations in the St. Lawrence Estuary, between 28 August and 6 September 1982 (Table 1). The 5 stations were chosen mainly for their different biological and physical characteristics (particularly in terms of

Table 1. Characteristics of the sampling stations. Range and/or (mean) values

	Stn 1	Stn 2	Stn 3	Stn 4	Stn 5
Location and bathymetry					
Coordinates	70° 04.00′ W 47° 38.50′ N	68° 65.00′ W 48° 44.30′ N	68° 30.90′ W 48° 04.50′ N	68° 05.00′ W 48° 44.30′ N	67° 17.30′ W 48° 57.30′ N
Sampling date (1982)	4–5 Sep	6–7 Sep	28–29 Aug	2–3 Sep	30 Aug–1 Sep
Depth (m)	56	68	52	85	100
Physical factors					
Surf. temp. (°C)	5.3–7.7	6.2–8.4	5.2–6.4	6.2–8.1	6.7–8.7
Surf. sal. (‰)	22.2–27.5	27.4–29.0	28.2–29.8	26.2–29.5	29.0–29.4
I_0 (μE in m^{-2} s^{-1})	(330)	(446)	(133)	(111)	(428)
Z_m (depth of mixed layer; m)	1–10 (8.4)	2–14 (8.7)	37–55 (43.0)	1.5–7.2 (5.1)	32–46 (40.0)
Z_e (depth of euphotic layer; m)	3–7 (4.5)	2–7.5 (5.1)	5–13 (10.2)	3.5–7.5 (5.9)	2–8.5 (5.0)
Type of variations in Z_m and Z_e	tidal (13 h)	tidal (13 h)	tidal (13 h)	non-periodical	non-periodical
Ri_0 (Richardson number)	0.01–1.0 stability periods of ca 1 h at 12–13 h interval	0.01–3.0 numerous stability periods of ca 1 h duration, non-periodical	0.01–0.05 12 to 13 h variability in mixed layer	0.01–3.5 numerous peaks of stability of ca 1 h duration, non-periodical	< 0.01 in first part of series; > 1.0 in 2nd half with 12–13 h periods
Biological and chemical factors					
Chl *a* (mg m^{-3})	(0.3)	(14.6)	(0.8)	(10.5)	(11.1)
10^6 cells l^{-1}	(1.1)	(3.1)	(0.8)	(2.5)	(2.8)
μflagellates/diatoms	(63.1)	(1.37)	(10.56)	(2.63)	(1.79)
Dominance	– μflagellates – few diatoms in poor shape – lot of detritus – small number of other unident. diatoms	– μflagellates – *T. gravida*[a] – *C. debilis* – many other diatoms	– μflagellates – *L. minimus* – small number of diatoms in good shape	– μflagellates – *T. gravida* – *C. debilis* – many diatoms but less than at Stn 2	– μflagellates – *T. gravida* – *C. debilis* – *L. minimus* – *N. delicatissima* – high diversity
NH_4 (μmol l^{-1})	(3.5)	(2.5)	(2.0)	(4.1)	(3.1)
NO_3 (μmol l^{-1})	(10.8)	(3.9)	(10.5)	(5.4)	(2.1)
SiO_4 (μmol l^{-1})	(11.2)	(3.9)	(11.4)	(4.9)	(3.0)
PO_4 (μmol l^{-1})	(0.80)	(0.63)	(0.91)	(0.64)	(3.5)

[a] *T.* = *Thalassiosira*; *C.* = *Chaetoceros*; *L.* = *Leptocylindrus*; *N.* = *Nitzschia*

vertical stratification conditions), based on previous data collected by Therriault & Levasseur (1985).

Using an automatic yoyo profiling system, we recorded, every 30 min during 30 consecutive h, vertical profiles of light (Biospherical Instrument, 4 π collector) temperature and salinity (Guildline CTD probe), and the fluorescence ratio F_a/F_b (determined using the setup described above). All measurements were integrated over 0.5 m intervals for vertical profiles that covered the upper 30 m of the water column. During the same period, CTD profiles encompassing the entire water column were recorded every 30 min using a second CTD probe (Guildline). Two Aanderaa current meters were also suspended from the ship at each station. One current meter was located above the pycnocline and the other 10 m below, at depths depending on the stratification characteristics of the station. The sampling frequency was 2 min. Using unfiltered data from the 2 current meters, Richardson numbers (Ri_0) were calculated as follows:

$$Ri_0 = g \frac{\Delta\rho / \Delta z}{\rho (\Delta U / \Delta z)^2} \quad (1)$$

where g = acceleration due to gravity; ρ = density; U = horizontal velocity; and z = depth of the current meters. In addition, wind data were obtained from the meterological station of the Mont-Joli airport (Québec) located near the sampling stations.

Every second hour, Niskin bottles were used to sample water at 4 depths for the measurement of chlorophyll *a* and nutrient (NO_2, NO_3, SiO_4 and PO_4) concentrations. Chlorophyll *a* was determined on acetone-extracted Whatman GF/C filters (250 ml aliquots), using the fluorometric method of Yentsch & Menzel (1963). Nutrients were analysed from frozen filtrates using a Technicon autoanalyser, according to the methods described in Strickland & Parsons (1972). Phytoplankton cell counts and identification were done using the inverted microscope method (Lund et al. 1958).

RESULTS

Characteristics of the stations

The 5 sampling stations were located in different hydrological regions of the St. Lawrence Estuary (Therriault & Levasseur 1985, Therriault et al. in press), and were all sampled within 6d. Daily mean irradiance (I_0) regimes, however, differed between stations (Table 1). As expected, the 5 stations, with depths varying between 52 and 100 m, showed different physical, chemical and biological characteristics, as summarized in Table 1

Surface temperatures were similar at the 5 stations, but Stn 1, which was located upstream, had less saline surface waters. Stns 3 and 5 had a deep mixed layer (Z_m) of about 40 m, whereas Stns 1, 2 and 4 had shallow mixed layers (5 to 8 m). Stn 3 had the deepest euphotic zone ($Z_e \approx 1\%\ I_0$) of ca 10 m, whereas the other 4 stations had similar euphotic zones (ca 5 m). Upstream Stns 1, 2 and 3 showed semi-diurnal tidal cycles in Z_m and Z_e, while variations were non-periodical at Stns 4 and 5. Ri_0 was much lower at Stns 3 and 5 (deep mixed layer) than at the other 3 stations. There was also evidence of semi-diurnal tidal variability in Ri_0 at some of the stations, as indicated in Table 1. At Stns 2, 4 and 5 phytoplankton biomass was high and was dominated by diatoms (Table 1). Microflagellates were present in about equal numbers at these 3 stations but were clearly dominant at Stns 1 and 3. Stn 5 was the one with the highest species diversity. Nutrients were always present in significant concentration and generally showed inverse relationships with biomass values.

The 5 stations were characterized by different stratification characteristics and associated vertical distributions of phytoplankton biomass and of nutrients (Fig. 1). For example, Stns 1 and 3 showed low chlorophyll *a* and high NO_3 concentrations throughout the water column. Interestingly, these 2 stations were characterized by quite different stratification regimes as illustrated by a comparison of the σ_t profiles in Fig. 1b. On the other hand, Stns 2, 4 and 5 showed much higher chlorophyll *a* values and total cell numbers, with discrete subsurface peaks. Much lower NO_3 concentrations were found in the water column at these stations, inversely related to biomass values. Again, stratification conditions were very different for these 3 stations. For all stations, in vivo fluorescence profiles adequately reflected the vertical distributions of extracted chlorophyll *a*. As expected, variations in the ratio F_a/F_b were independent from the vertical distributions of in vivo fluorescence. The data also show that the fluorescence ratio F_a/F_b was independent from chlorophyll *a*, cell numbers and nutrient concentrations.

Vertical profiles of the fluorescence ratio

Fig. 2 shows the temporal variations observed in the vertical profiles of σ_t and of the fluorescence ratio F_a/F_b at the 5 sampling stations; for the sake of simplicity and clarity, the ratio data have been averaged over 4 h periods (e.g. 16 h = 14:00 to 18:00 h) and then smoothed vertically using a 3-point moving average. Consistent and strong diurnal variations were evident in all profiles of the fluorescence ratio, but not in the σ_t profiles which were instead associated with the semi-diurnal tide. Higher values of F_a/F_b were always found

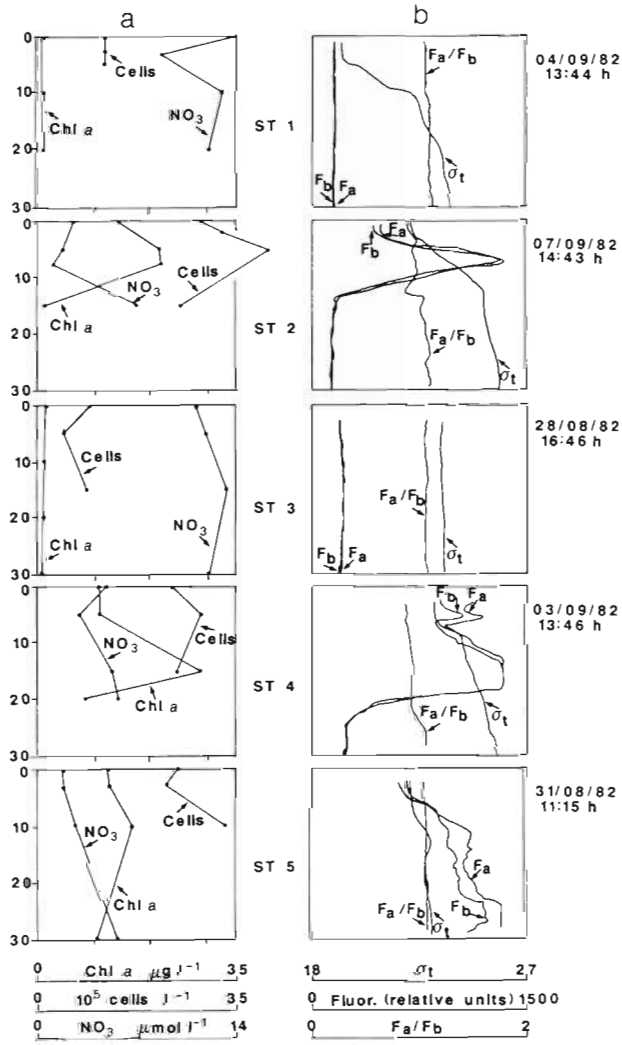

Fig. 1. Typical profiles of (a) total cell numbers, chlorophyll *a* and NO_3, and (b) σ_t, fluorescence before (F_b) and after (F_a) exposure to bright light, and the ratio $R = F_a/F_b$. Time and date of sampling are indicated on the right for each station

during evening and night at all stations (note the inverse scale of the ratio in Fig. 2), whereas minimum values were observed at around noon, usually in subsurface waters. A subsurface minimum (ca 5 to 10 m) in the fluorescence ratio was also usually observed at times corresponding to higher irradiances (between 10:00 and 16:00 h), suggesting a photoinhibition effect at the shallowest depths. The general shape of the fluorescence ratio profiles was also quite different from one station to another as were the σ_t profiles. In general, values of the ratio were almost uniform with depth during night-time, but showed vertical gradients during the day which were generally more pronounced at the stations characterized by a higher degree of stratification. Finally, the maximum night values of the fluorescence ratio were quite different from one station to another, and were also different at the same station for consecutive nights (see for example Stn 2). The higher night ratios always corresponded to sampling days with the lower irradiances (Table 1; Fig. 2).

DISCUSSION

There are 3 possible explanations for the temporal and vertical changes observed in the fluorescence ratio profiles of Fig. 2. The first and most evident is a photoadaptive response of phytoplankton to the diurnal light cycle in the mixed layer, as influenced by the combined effects of mixing and sinking, and dependent upon the relationship between the relative depths of the euphotic and mixed layers. The second explanation is a temperature dependence, since the photosynthetic behaviour (in particular fluorescence yield) of both laboratory and natural microalgae is influenced by interactions between temperature and conditions of illumination (e.g. Steeman-Nielsen & Hansen 1959, Murata 1975, Hitchcock 1980, Li 1980, Li et al. 1984). However, temperature differences in those studies were usually large (between 10 and 30 °C), compared to the vertical or diurnal variations observed during the present study (maximum differences of 6 °C). It is therefore unlikely that temperature could explain the variations observed in the fluorescence profiles of Fig. 2. This is supported by the fact that scatter diagrams (not shown) of fluorescence ratio versus temperature showed no consistent or significant relationship at any of the sampling stations, at any temporal (diel cycles) or spatial (depth) scales. The third explanation could be variations in fluorescence yield caused by changes in chlorophyll *a* and/or nutrient concentrations (e.g. Kiefer 1973, Loftus & Seliger 1975). However, there were no significant day and night differences in nutrient or chlorophyll concentrations (even on the vertical) that could explain the consistent variations observed in the F_a/F_b profiles. Also, nutrient concentrations were always well above recognized limitation thresholds. It follows that the most plausible explanation for the observed variations of the F_a/F_b profiles in Fig. 2 is a photoadaptive response of phytoplankton to diurnal light variations and to vertical excursions through an exponential light gradient.

Our results clearly indicate that the capacity of phytoplankton to cope with excess photosynthetic excitation energy depends on the previous light history of the cells in a systematic manner: phytoplankton which are adapted to lower irradiances (deep and night samples) exhibit higher fluorescence (higher F_a/F_b ratio) upon exposure to strong illumination than phytoplankton adapted to higher irradiances (surface and day samples). Vertical gradients in fluorescence ratio indicate

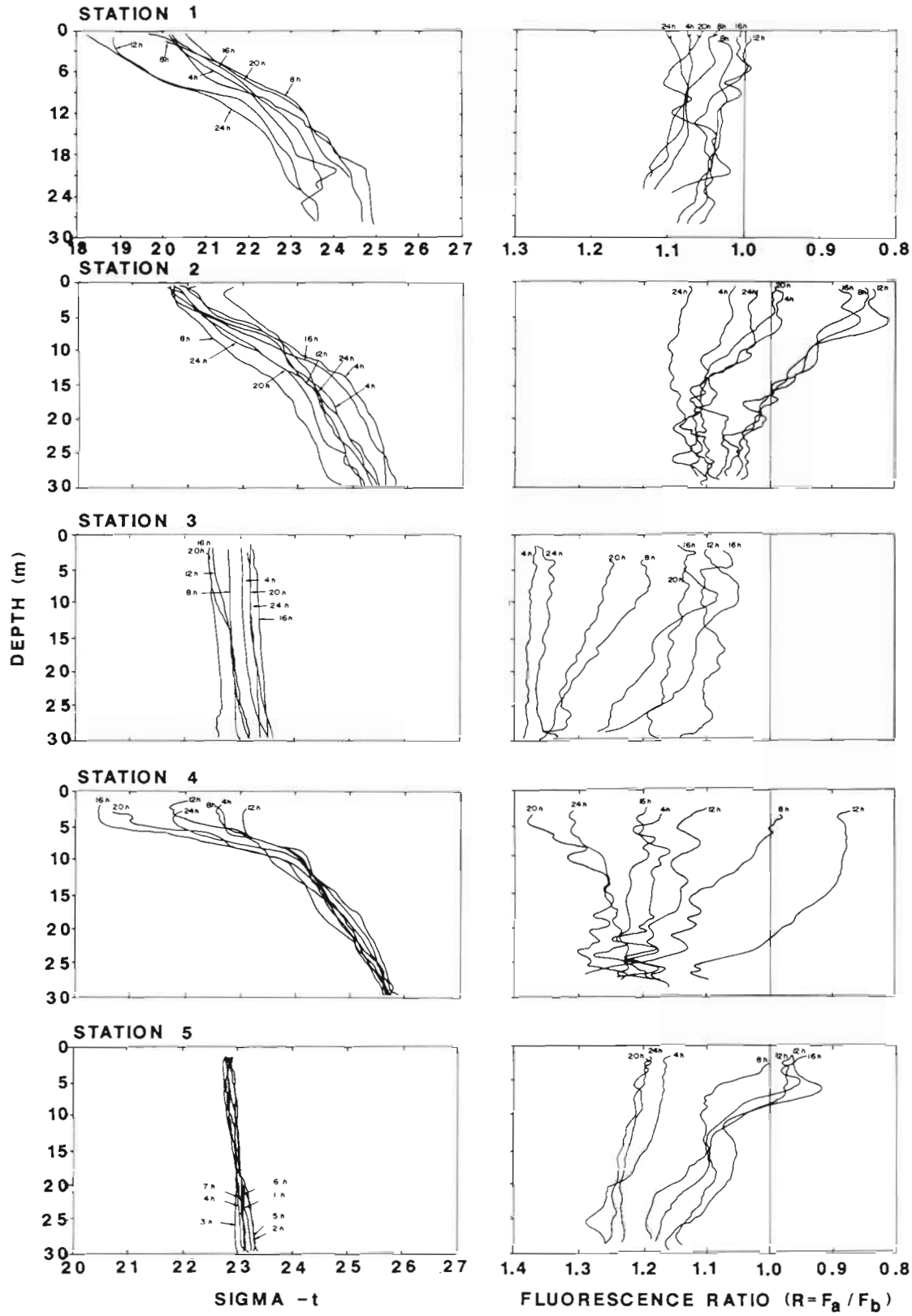

Fig. 2. Time series of vertical profiles of σ_t and fluorescence ratio at the 5 stations in the St. Lawrence Estuary

that phytoplankton adapted to high irradiance can cope with a strong light shock better (less fluorescence emitted) than phytoplankton adapted to low irradiance. However, when irradiance is strong and lasts for a significant period of time, phytoplankton cells can no longer cope with the light signal. This occurred at around noontime during days characterized by high irradiance (Table 1; Fig. 2), when more permanent effects resulted from photoinhibition. This is reflected in our results by an increased fluorescence ratio near the surface, at time of maximum irradiance. In other words, algal cells did lose their ability to cope with a strong light shock to a degree which depends on the intensity of photoinhibition and the duration of exposure to high irradiance. This led to a subsurface minimum in the fluorescence ratio at around 12:00 h (Fig. 2). Our results also suggest that higher irradiance during the preceding day resulted in a reduced capacity for fluorescence recovery at night. It has been suggested that there exists a threshold irradiance for the onset of photoinhibition in microalgae (Kyle & Ohad 1986), and that this threshold increases when algae have been grown under higher irradiance (Neale 1987). Alternatively, it has been demonstrated that algae may lose sensitivity to photoinhibition when adapted to higher irradiances (Steeman-Nielsen 1962, Takahashi et al. 1971, Belay & Fogg 1978, Lewis et al. 1984a). The fluorescence response to high irradiance is usually characterized by rapid photoinhibition (time scale of seconds to minutes), and recovery is not a mirror image of photoinhibition, being much slower (time scale of hours and days) and a function of the previous exposure (Belay 1981, Neale 1987). The kinetics of algal photoadaptation was examined by Cullen & Lewis (1988) in the context of vertical mixing.

Several authors have documented a strong correlation between light-saturated photosynthesis and fluorescence yield (e.g. Samuelson & Oquist 1977, Samuelson et al. 1978). This is consistent with the idea that phytoplankton adjust their photosynthetic machinery to maximize light-harvesting, when irradiance is low, and to minimize photo-oxidative damage to the photosynthetic machinery at high irradiance levels (Sukenik et al. 1987). As mentioned above, changes in photosynthetic capacity or in fluorescence yield in response to environmental fluctuations at a time scale consistent with this study ($t < 30$ min) have been attributed to cellular adjustment of the light-saturated rate of electron flow from PS II to PS I (Papageorgiou 1975) or to fluorescence quenching (Demmig & Björkman 1987, Demmig et al. 1987).

Therefore, we can conclude from our results that the fluorescence ratio of Demers et al. (1985) can be used to adequately describe diurnal and vertical fluctuations in fluorescence yield of phytoplankton. This ratio also indicated the frequent occurrence of fluorescence yield depression in the near surface waters (a photoinhibition response) when irradiance was at its highest day value. Our results, however, also suggest a significant influence of the prevailing environmental conditions (in particular vertical stability) in the water column at each station. This raises the possibility of using our fluorescence ratio results to characterize the vertical excursions of phytoplankton in the subsurface irradiance gradient.

Response of the ratio F_a/F_b to mixing and sinking: a model

All but one of the sampling stations were in the stratified region of the St. Lawrence Estuary, as indicated by the h/u^3 criterion (Simpson & Hunter 1974, Pingree & Griffith 1980). Stn 1 was the exception, but nevertheless did display a strong pycnocline (Fig. 2). Energy from tidal friction was, therefore, not sufficient to mix the entire water column, and mixing energy in the surface layer thus originated from the surface. Vertical displacements, or excursions due to near-surface mixing, can therefore be envisaged to be maximum near, but not at, the surface and to decrease with any increase in vertical density gradient.

To interpret the vertical profiles of F_a/F_b in terms of vertical mixing processes we use a simple conceptual model of the fluorescence response of phytoplankton to variations in irradiance when the cells are subject to vertical excursions in the water column. The model is based on the asymmetric fluorescence response of phytoplankton to changing irradiance, the response to increasing irradiance being very rapid (time scale of seconds to minutes), and the recovery being much slower (time scale of hours to days) depending on the level, intensity and duration of previous exposure (e.g. Belay 1981, Samuelson et al. 1985, Neale 1987). In other words, the fluorescence response to high irradiance being much faster than that to low irradiance or darkness, the photoadaptation status of phytoplankton should depend on the highest irradiance experienced in their recent light history (Sakshaug 1988). Also, studies such as those of Elser & Kimmel (1985) and Neale & Richerson (1987) have indicated that the fluorescence recovery in samples from field populations can be relatively slow. We have shown that the ratio F_a/F_b can be used as an adequate index of physiological adaptation. We can therefore assume that this physiological index at one depth respresents an ensemble average of all the cells present, and that it contains information about the most recent light history of the mixed phytoplankton populations. The value of the ratio $R = F_a/F_b$ is an indication of

the highest mean irradiance experienced by the cells in their recent past. It may, therefore, be hypothesized that this ratio depends on the highest irradiance experienced for some minimum interval of time, during an adaptation period P before measurement; e.g. R = G, where G is a function of the highest irradiance experienced during the adaptation period. The response time to high irradiance exposure is clearly much shorter than 30 min (our sampling frequency), since we have observed rapid changes of the fluorescence ratio with diurnal variations of irradiance (Fig. 2). Also, the time required for the cells to show a response to high irradiance must be shorter than the time scale of mixing or sinking processes in the top 30 m, otherwise vertical gradients would not be present in our data (Platt et al. 1982), and high fluorescence ratios below the euphotic zone could not be explained. Thus, for an irradiance profile that remains constant with time, the highest irradiance experienced by phytoplankton corresponds to the upper end of a vertical excursion. By considering cells already at the surface, we can thus estimate the function G:

$$R_0 = G\,(I_0), \qquad (2)$$

where I_0 is light intensity at the surface. In view of minimizing possible effects of between-station differences in species composition and long-term effects of different levels of photoinhibition, we used ΔR, the difference between daytime values of R and that observed during the previous night (which is almost depth independent; see Fig. 2). Vertical profiles of ΔR were extrapolated to the surface; light data were fitted to an exponential curve, and likewise, extrapolated to the surface. The results showed a significant linear correlation between ΔR_0 and $\ln(I_0)$ for each station (Table 2), as well as for all the stations combined (Table 2; Fig. 3). Using the combined data and neglecting the small positive offset (intercept) in order to force night values of ΔR to zero (variable shading by clouds or by the ship would produce a positive bias in ΔR, whereas photoinhibition would produce a negative bias), it follows that:

$$G(I) = 0.052 \ln(I), \qquad (3)$$

Table 2. Regression analyses of ΔR_0 on $\ln(I_0)$, for the 5 stations and all the stations combined

Station	Intercept	Slope	r^2 (%)	Prob. level
1	−0.046	0.073	70.3	≤ 0.001
2	−0.062	0.057	71.3	≤ 0.01
3	−0.123	0.091	62.1	< 0.01
4	0.157	0.038	40.8	< 0.02
5	0.062	0.043	58.6	< 0.01
Combined	0.056	0.052	46.4	< 0.001

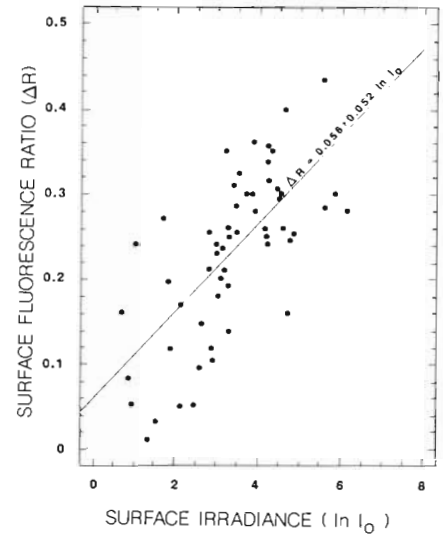

Fig. 3 Scatter diagram for the combined values of the surface fluorescence ratio and the logarithm of irradiance

where 0.052 is the slope of the regression (Table 2). Vertical profiles of G, based on fitted exponential light profiles, were plotted together with profiles of ΔR and σ_t (see some examples in Figs. 5, 6 and 9). In the absence of mixing or sinking, cells below the surface experience constant irradiance. In this case, profiles of ΔR and G are equivalent and linear with depth. Differences between profiles of G and ΔR are interpreted to indicate vertical motion caused by turbulent mixing and/or sinking.

The general concept of this interpretation is illustrated in Fig. 4, assuming that the phytoplankton cells do not differ physiologically with depth. If phytoplankton cells are vertically at rest, the curve ΔR should correspond to the curve G. If, on the other hand, vertical mixing alone affects the cells which are constrained within the mixed layer by the pycnocline, ΔR should exhibit a vertical profile as shown in Fig. 4a. In such a case, ΔR should have the same slope as G and the horizontal distance between the 2 curves is then representative of the intensity of vertical mixing. At depth B, for example, all cells should have recently experienced surface irradiance (A) during the adaptation period and the value of ΔR therefore reflects the highest irradiance experienced above. At depth D, cells show the averaged effect of exposure to the highest irradiance at the upper end of their vertical excursion which is represented by depth C. We will show later that vertical distances such as CD or AB can be used to estimate the vertical excursions of cells sampled at depths B, D, etc. However, the effects of mixing and sinking can rarely be separated. Sinking cells are also adapted to the highest irradiance of above. The value of ΔR for sinking without mixing depends on both the

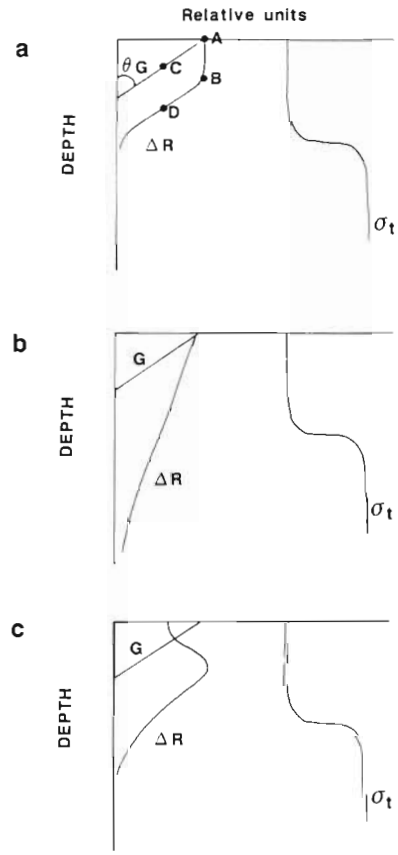

Fig. 4. Conceptual model of the vertical responses of the fluorescence ratio R = F_a/F_b to changes in irradiance and stratification

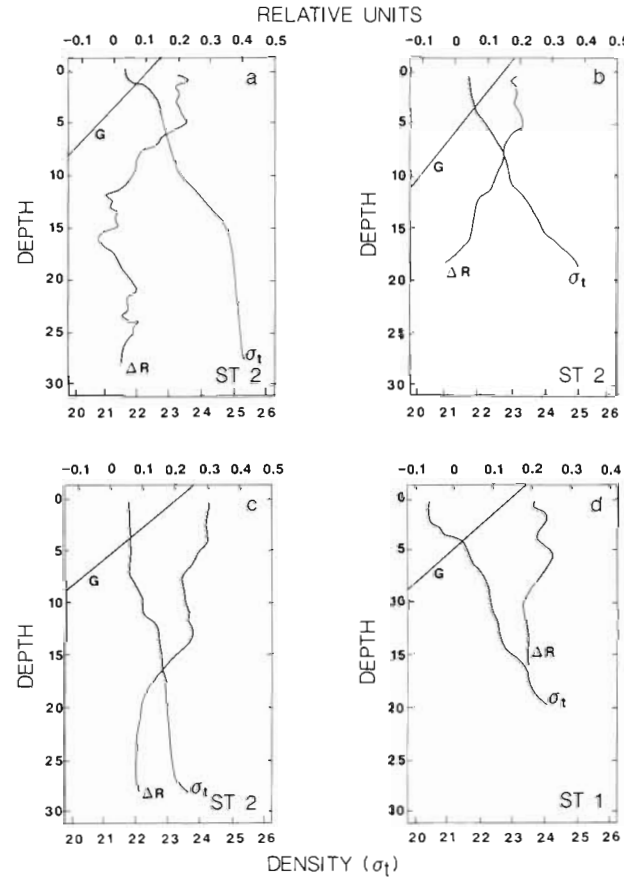

Fig. 5. Typical examples of profiles of ΔR, G and σ_t

rate of sinking and the relative numbers of sinking and healthy resident cells at the sampling depth. For the case of constant sinking rates and a uniform distribution of phytoplankton, the change in slope of the ΔR profile may be indicative of the intensity of sinking. This, however, remains to be tested under laboratory and field conditions. Finally, photoinhibition has the effect of reducing the value of ΔR near the surface to values less than G (Fig. 4c).

In our fluorescence profiles we can find many different examples to illustrate some of the features derived in Fig. 4. For example, Fig. 5a, b shows 2 profiles from Stn 2 which resemble Fig. 4a, where vertical mixing is the dominant factor. On the other hand, Fig. 6c, d shows examples from Stns 2 and 1, which resemble Fig. 4b where mixing and sinking are important factors. Fig. 6 shows examples of photoinhibition at 4 stations with different stratification conditions; Stns 2 and 4 (Figs. 6a, d) resemble Fig. 4c. However, there are also other cases such as the examples from Stns 3 and 5 (Figs. 6b, c) which showed photoinhibition in apparently well-mixed surface waters, as judged by the homogeneous vertical distributions of σ_t.

Physical estimate of mixing

To test the hypothesis that our fluorescence ratio can be used as a reasonable index of vertical excursion in the mixed layer, we need a reliable and independent estimate of the rate of vertical mixing. It can be argued that the gain in potential energy of the upper water column is proportional to the loss of energy by the wind (Kullenberg 1976). Thus:

$$k\ Rf_c = \frac{\rho_w\ K\ N^2\ H}{c_d\ \rho_a\ W^3} = \text{constant}, \qquad (4)$$

where the numerator represents the gain of potential energy and the denominator the loss of wind energy through friction, and where k = fraction of the wind energy transferred to turbulent kinetic energy; Rf_c = critical flux Richardson number; ρ_w = water density; K = coefficient of vertical turbulent diffusion of mass in the upper layer; N = Brünt Väisälä frequency in the upper layer; H = thickness of the upper layer; c_d = wind drag coefficient; ρ_a = air density; W = wind speed.

Dye dispersion studies in coastal waters (Kullenberg 1976) and studies of wind effect of summer stratifica-

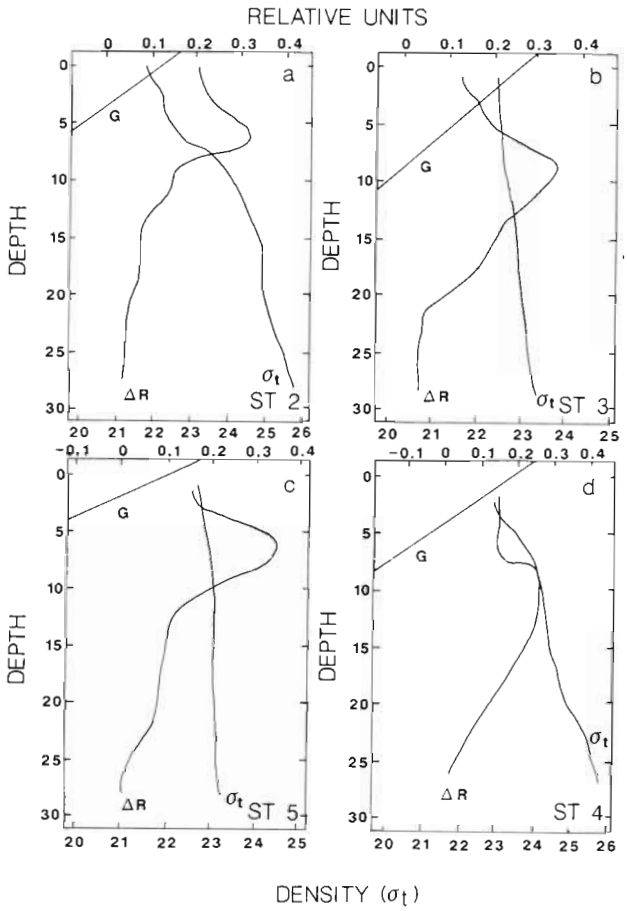

Fig. 6. Typical examples of profiles of ΔR, G and σ_t showing photoinhibition

tion on the European shelf (Simpson & Bowers 1981) both indicate that for a wind drag coefficient of 1.1×10^{-3}, k Rf_c lies between 0.1 and 0.2 % and is thus almost constant. Values of N^2 H, calculated as bulk values for the upper layer, varied by no more than a factor of 3, whereas the wind term, W^3, varied by a factor of more than 400. In comparison to the wind, therefore, N^2 H could be considered as uniform, as could the densities of air and water.

The vertical diffusion of mass depends on the turbulent motion and generally increases with the scale of the motion. We take K as being proportional to $\Delta Z^2/P$, where ΔZ is a vertical displacement and P is the adaptation period. Combining this with the equation above then gives $\Delta Z \propto \sqrt{W^3}$, giving a physical estimate of relative mixing excursion.

Cubed hourly estimates of the wind were averaged over 6 h periods prior to the sampling at sea. The period of 6 h was chosen in order to allow for downward diffusion of surface energy through the upper layer. The square root of this average can thus be taken as an estimate of the intensity of vertical mixing.

Comparison of biological and physical estimates of mixing

Biological estimates of mixing were calculated from the vertical profiles of ΔR and G. The profiles were first screened to choose those that satisfied the following criteria: (1) no evidence of photoinhibition, (2) at least 4 measurements in the euphotic layer, and (3) the uppermost data not deeper than 2.5 m from the surface. The mixing excursion, ΔZ, can be estimated from the horizontal distance between the curves ΔR and G multiplied by the slope of G. In order to avoid possible errors resulting from noise in the irradiance measurements and particularly from mismatches at the surface due to cloud or ship shadow, ΔZ was estimated by the following expression:

$$\Delta Z = Z - \frac{\Delta R_0 - \Delta R_z}{S}, \quad (5)$$

where Z = depth, S = tan θ (Fig. 4a), and the subscripts refer to the depth. Vertical excursion ΔZ was estimated for 5 and 10 m depths (Table 3) and was compared to $\sqrt{W^3}$ (Fig. 7). For both depths, minimum excursions increased with wind. Small excursions were not observed during strong winds. Thus, despite a large scatter in excursion estimates the results support the use of fluorescence ratio for estimating vertical mixing. In both cases, maximum excursions were limited by the surface.

A second estimate of the excursion caused by mixing was the depth at which vertical profiles of ΔR changed slope to become approximately parallel to the profile of G (point B in Fig. 4a). Thirty-three profiles, responding to our primary criteria above, displayed this feature (Table 3, Fig. 8), the depth of which showed a significant linear relationship with $\sqrt{W^3}$ (Fig. 8: $r = 0.72$; $p \leq 0.001$). Vertical excursions ranged up to 18 m, but were about 10 m for moderate winds. For similar moderate winds, gas bubbles created by breaking waves have been observed at depths greater than 5 m (Thorpe 1982). The adaptation period is considerably longer than the period of wind-waves, so that the present estimates of vertical excursion appear reasonable. Bah & Legendre (1985) have estimated vertical displacements up to 48 m in less than 10 min due to wind mixing in the Upper St. Lawrence Estuary. This supports our interpretation of ΔR profiles in terms of vertical mixing, which, therefore, confirms our earlier hypothesis that the fluorescence ratio of Demers et al. (1985) can be used as a valuable index of physical vertical movement in the natural environment. Other studies have demonstrated an influence of various physical factors on fluorescence yield and photosynthetic capacity in the natural environment (e.g. Harris 1980, 1984, Powell et al. 1984, Vincent et al.

Table 3. Parameter $\sqrt{W^3}$ used as a physical estimate of the mixing excursion, together with vertical excursions estimated from the ΔR profiles (ΔZ_5 = vertical excursion of cells sampled at 5 m, ΔZ_{10} = vertical excursion of cells sampled at 10 m, and ΔZ = depth at which profile of ΔR changed slope to become almost parallel to G). Average of wind cubed was taken on hourly wind values over a 6 h period prior to sampling at sea

Station	Date	Time (h)	$\sqrt{W^3}$ ($[m\ s^{-1}]^{3/2}$)	ΔZ_5 (m)	ΔZ_{10} (m)	ΔZ (m)
1	4 Sep	15:45	19.5	–	–	9
		16:15	30.4	–	–	6
		17:13	31.3	–	–	7
		17:48	31.3	–	–	9
		18:15	31.3	4.8	8.2	6
	5 Sep	06:45	12.9	4.7	9.4	–
		07:20	12.9	4.7	9.2	–
2	7 Sep	07:16	7.1	3.3	7.1	3
		07:44	7.8	4.0	9.0	2
		08:14	7.8	4.6	8.7	4
		08:40	9.2	3.9	8.3	4
		16:44	3.5	–	–	4
		17:44	2.1	–	7.8	6
		18:15	2.1	–	5.1	5
3	28 Aug	15:15	40.1	4.9	8.8	18
		16:46	38.8	5.0	9.3	–
		17:22	38.8	4.9	9.6	–
	29 Aug	–	30.4	–	–	14
		07:15	30.4	–	–	12
		09:44	30.1	–	–	9
		17:42	25.6	4.3	5.0	11
		18:17	25.6	4.3	6.5	6
		18:40	23.9	4.0	8.5	–
4	2 Sep	09:22	10.7	4.6	8.5	13
		09:44	12.4	4.7	8.1	6
		16:16	23.4	5.0	–	11
		16:48	21.9	–	–	6
	3 Sep	07:46	16.4	5.0	–	11
		08:16	16.4	4.0	7.1	–
		08:47	15.3	4.5	4.6	–
		12:46	14.1	4.6	6.8	–
		13:46	14.2	4.8	7.6	–
5	30 Aug	08:44	9.0	–	4.5	6
		09:23	9.0	4.1	6.0	5
		09:44	11.2	5.0	6.8	6
		16:41	6.8	5.0	8.6	–
		17:13	5.8	–	–	5
	31 Aug	07:14	12.7	4.7	–	–
		08:14	10.8	3.9	–	4
		08:44	8.1	0.8	4.4	4
		09:15	8.1	4.4	6.5	4
		10:14	11.55	5.0	4.5	5
		10:43	17.3	4.3	7.0	4
		11:15	17.3	4.8	7.4	7

1984, Elser & Kimmel 1985, Neale & Richerson 1987. Using a model, Falkowski (1983) has estimated vertical displacement rates of phytoplankton covering 2 orders of magnitude (from ca 3.8×10^{-3} cm s^{-1} to 1.1×10^{-1} cm s^{-1}) which are slightly lower than our values using P = 30 min.

Re-interpretation of fluorescence ratio profiles in light of the conceptual model

In light of our simple conceptual model, the easiest way to interpret the series of continuous vertical profiles of the fluorescence ratio $R = F_a/F_b$ is first to examine a

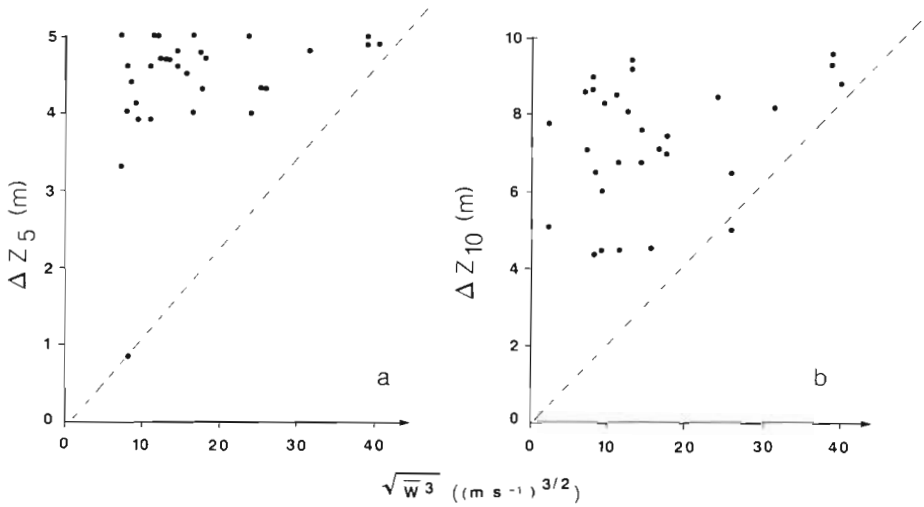

Fig. 7. Vertical excursions plotted against $\sqrt{\overline{W^3}}$ (a) ΔZ_5 = vertical excursion estimated at a depth of 5 m; dashed line in indicates $\Delta Z = 0.12 \sqrt{\overline{W^3}}$ (b) ΔZ_{10} = vertical excursion estimated at a depth of 10 m; dashed line indicates $\Delta Z_{10} = 0.21 \sqrt{\overline{W^3}}$

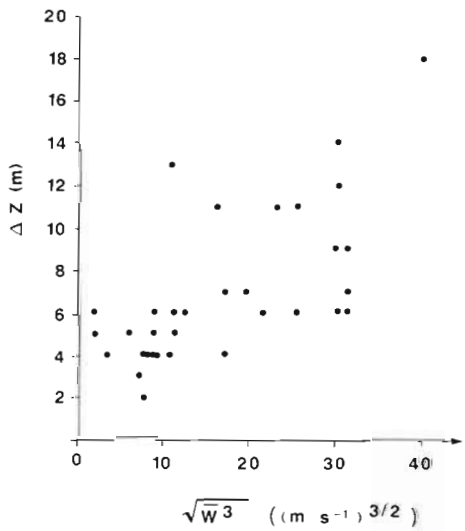

Fig. 8. Vertical excursion plotted against $\sqrt{\overline{W^3}}$, where ΔZ is the depth at which the profile of ΔR changed slope to become approximately parallel to G

station (Stn 2) which remained well stratified throughout the sampling period (Fig. 2) At this station, σ_t profiles and high Richardson numbers (Table 1: $Ri_0 > 1$) strongly suggest that there was little vertical exchanges across the pycnocline. Also, a strong subsurface maximum of phytoplankton (cell numbers, chlorophyll *a* and fluorescence) was located at the depth of this thermohaline boundary (Fig. 1). From the profiles of R in Fig. 2, we see that, starting at midnight (24 h) on the first day, the upper part of the R profile progressively decreased until noon (12 h), before increasing subsequently to another midnight maximum, in response to the diurnal irradiance cycle. Profiles at 12 and 16 h showed strong evidence of photoinhibition in the upper 5 m of the water column since this sampling day was particularly clear and bright (Table 1). As the depth of the euphotic layer reached only about 5 to 7 m at this station, deep phytoplankton cells should have remained unaffected by the solar light cycle since there was little or no vertical mixing. However, some phytoplankton cells which had experienced high irradiance in their recent history (say < 1 to 2 h) may have sunk through the pycnocline. This would be reflected by a small diurnal change in R at depths below the pycnocline, as was observed at Stn 2. Therefore, since mixing above the pycnocline was weak, variations in R profiles may be interpreted mainly as near-surface mixing, accompanied by relatively high sinking. The fact that the phytoplankton community was dominated by diatoms at that station (Table 1) lend more support to the sinking hypothesis. Thus, this is a station that can be associated to case b of our conceptual model (Fig. 4).

Stn 4 was also well stratified and showed temporal changes in the profiles of fluorescence ratios very similar to those of Stn 2 (Fig. 2). At Stn 4, however, the biomass maximum was located just under the pycnocline (Fig. 1). The range of variations of R was much wider at this station than at Stn 2. This suggests strong vertical mixing above the pycnocline, accompanied by high sinking of phytoplankton. Here again, diatom dominated the phytoplankton assemblage (Table 1) and we have another case b situation (Fig. 4). Photoinhibition was not very evident at this station as the sampling day was overcast (Table 1).

By contrast, the upper 30 m of Stn 3 remained relatively unstratified throughout the sampling period (Fig. 2). The depth of the euphotic layer varied between about 5 and 13 m, but the depth of the surface mixed layer was ca 48 m as opposed to ca 5 to 8 m for Stns 2 and 4 (Table 1). At Stn 3, the whole R profile down to 30 m moved back and forth during the day, as a function of the solar cycle (Fig. 2). The fact that the ΔR profiles

showed very little vertical gradients most of the time (e. g. Fig. 9c, d) can probably be interpreted as indicative of rapid vertical mixing of phytoplankton cells within the surface mixed layer. This means that cells within the mixed layer at that station were frequently

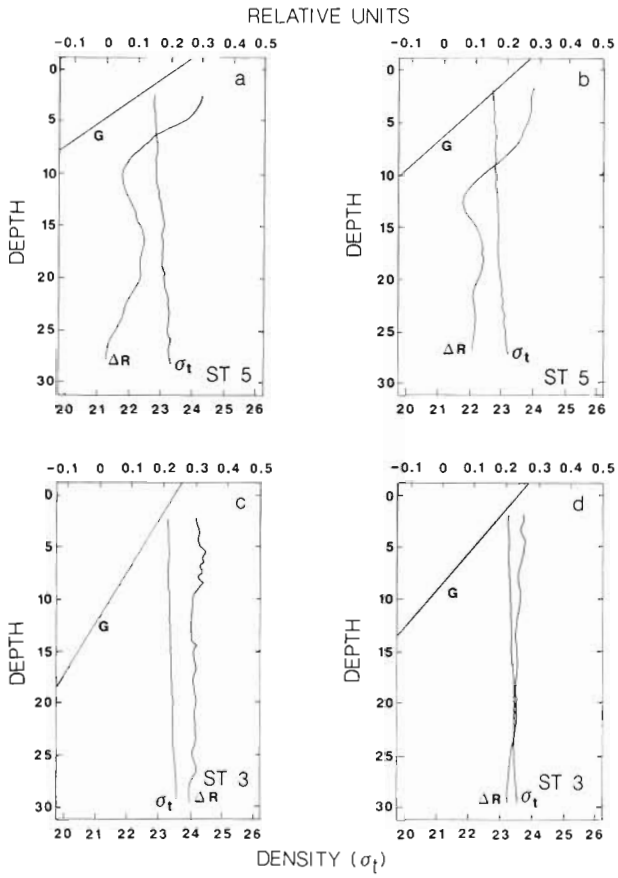

Fig. 9. Typical examples of profiles of ΔR, G and σ_t

exposed to surface irradiance, which implies vertical excursions greater than 30 m were being observed at this station over a time scale less than the time for adaptation. In fact, the straight vertical ΔR profile in the top 30 m at this station can probably be associated with the distance AB in Fig. 4a, and is thus indicative of the occurrence of large vertical excursions and, therefore, of fast vertical mixing. As a consequence, the fluorescence ratio at Stn 3, even under the photic zone, showed wide diurnal variations typical of surface waters. The absence of a subsurface biomass maximum (Fig. 1) would be consistent with this conclusion, as is also the fact that winds preceding sampling at this station were the strongest of the whole cruise (Table 3). Also, the fact that photoinhibition occurred at the surface (Figs. 2 and 6b), even with strong mixing, is an indication that the photoinhibition response of our ratio is very rapid, in accordance with the literature data.

Stratification characteristics at Stn 1 resemble those at Stn 2 with strong stability conditions, sufficient to prevent vertical mixing (Fig. 2). However, this was not reflected in the diurnal variations of the R profiles which were more characteristic of well-mixed conditions as observed at Stn 3. The presence of high frequency internal waves (2 to 6 h) encompassing the whole euphotic layer (observed in our data as well as by Muir 1979) might be invoked as another possible mechanism that would produce similar effects on our R profiles.

A slightly different situation occurred at Stn 5, where the vertical density structure (Fig. 2) and irradiance conditions (Table 1) were similar to those observed at Stn 3. However, close examination of the vertical profiles of R and ΔR (Figs. 2 and 9a, b) suggests that the cells were strongly responding to the light gradient. Strong photoinhibition also developed near the surface on this bright sampling day, and Table 3 indicates that winds were relatively weak during the 6 h period immediately preceding sampling, but were quite strong the preceding day. We interpret these data as reflecting a relaxation period (slow rate of vertical mixing) following the strong wind event that occurred the day before as documented in our wind data. This stresses the point that absence of density stratification in the water column does not necessarily imply strong vertical mixing.

CONCLUSION

In summary, this study shows that an extremely simple model can be used to explain the variations in vertical profiles of a fluorescence ratio in the natural environment. This approach may prove useful as a simple tool for identifying effects of vertical excursion (turbulent mixing, sinking, internal tides, etc.) on the photoadaptation dynamics of phytoplankton.

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