

Continuous Measurement of the DCMU-Induced Fluorescence Response of Natural Phytoplankton Populations

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Abstract

The effect of 3×10^{-6} M DCMU [3-(3,4-dichlorophenyl)-1, 1-dimethylurea] on *in vivo* chlorophyll *a* fluorescence was observed in nearshore waters of the Southern California Bight. We compared fluorescence readings in the presence and absence of this inhibitor using parallel flow-through fluorometers. The increase in fluorescence induced by DCMU is expressed as the FRI (fluorescence response index). Theory and laboratory studies on batch cultures of phytoplankton suggest that the FRI is correlated with photosynthetic efficiency and/or physiological state, but other studies have produced results in apparent conflict with this interpretation. Although sufficient information does not exist to justify the use of fluorescence response as a precise physiological indicator in the field, we suggest that very low FRI values are a manifestation of photosynthetic debility in a sample. Vertical profiles showed a wide range of the fluorescence response index. At a station close to shore, low FRI values were observed well below the 1% light level, but the fluorescence response of the phytoplankton throughout the euphotic zone was similar to that of growing cultures. Farther offshore, the FRI was depressed near the surface, but increased in the enhanced nutrient conditions of the lower euphotic zone. The patterns observed were strong, and consistent with hypotheses which relate low values of the FRI to diminished photosynthetic capacity.

Introduction

A great deal of interest has been stimulated in the use of *in vivo* fluorescence response to study phytoplankton physiology. The findings of Samuelsson and Öquist (1977) were particularly encouraging: in unialgal batch cultures, photosynthetic capacity was strongly correlated with the increase of *in vivo* fluorescence induced by DCMU, a photosynthetic inhibitor.

The use of a fluorescence assay for the study of algal populations in the field is an attractive prospect due to the simplicity of methods and the ability to make continuous measurements. Unfortunately, we cannot interpret *in vivo* fluorescence data well because we do not understand the precise relationship between regulation of photosynthesis and *in vivo* fluorescence response.

In this paper, we present a method for the continuous measurement of DCMU-induced fluorescence increase and discuss patterns found in coastal waters

which are consistent with an interpretation relating fluorescence response to the photosynthetic activity of phytoplankton populations.

Materials and Methods

We studied the response of phytoplankton to DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea] in the laboratory, using a slightly supersaturated solution of DCMU in ethanol, added to the sample in 1:1000 dilution (final concentration, 3×10^{-6} M).

Coastal water was collected in polyethylene carboys at the Scripps Institution of Oceanography (SIO) pier. The water was pumped from the carboys at a rate of 1 l min^{-1} and DCMU was injected into the sample stream with a Technicon Model 1 proportioning pump. Fluorescence was read after a lag time by a Turner 111 fluorometer. Lag times between the DCMU addition and fluorescence reading were adjusted to 30, 60, 120, and 180

sec by inserting lengths of light-shielded tubing.

In similar experiments on DCMU-treated and untreated samples, the residence time in the fluorometer cuvette was varied between 0.36 and 2.5 sec by adjusting the flow rate. Flow was also stopped and fluorescence recorded for 3 min. Dark periods of 0 to 3 min were imposed before fluorescence reading.

The time course of fluorescence after DCMU addition was determined by using aliquots from log-phase cultures dispensed into a series of cuvettes. Initial fluorescence was measured on each with a Turner 111 fluorometer. DCMU was added and the samples were mixed. The cuvettes were placed in subdued light until fluorescence was determined. Each aliquot was used for one reading at incubation times from 15 sec to 5 min. Phytoplankton cultures for laboratory experiments were grown on 12 h light: 12 h dark cycles illuminated by cool-white fluorescent bulbs. Inocula were obtained from the Food Chain Research Group algal culture collection. *Gymnodinium splendens* (Clone W-E), and *Peridinium trochoideum* (72) were grown at 15°C. *Coccolithus huxleyi* (F-6) was grown at 20°C. Culture media was IMR/2 (Eppley *et al.*, 1967) with Si omitted for *C. huxleyi*.

Field data were collected on Cruise 12 of the Southern California Bight Survey of the Food Chain Research Group, aboard R.V. "E.B. Scripps", March 19-22, 1978. Two stations were occupied; S05, 8 km offshore from the San Onofre nuclear generating station (33°19.9'N; 117°37.9'W), and S010, 16 km offshore along the same line (33°20.0'N; 117°45.5'W). Chlorophyll *a* and phaeophytin were determined after extraction as by Strickland and Parsons (1972). Irradiance was measured with a quantum scalar irradiance meter (Booth, 1976). At Station S05, ammonium, phosphate and silicate were determined as in Strickland and Parsons (1972). Nitrate and nitrite were analyzed on a Technicon Autoanalyzer.

The system used for comparing the control *in vivo* fluorescence (F₁) with DCMU-treated fluorescence (F_{1DCMU}) is schematically diagrammed in Fig. 1. Water was pumped through 65 m of opaque $\frac{1}{2}$ inch inner diameter (ID) Gates Tufflex hose connected to a Little Giant LG-300 tubing pump with $\frac{1}{2}$ inch ID x $\frac{3}{4}$ inch outer diameter Tygon tubing. Under field conditions, this system provided water to a modified Turner 111 fluorometer (I in Fig. 1) with a lag time of 2 min as determined by dye injection. Water delivered by the pump (A) passed through a bubble trap (B). Part of the flow was diverted to a variable speed Masterflex

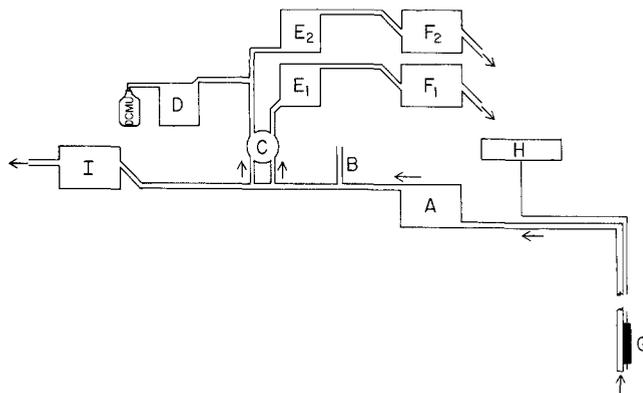


Fig. 1. Pumping system for comparison of DCMU-treated and control chlorophyll fluorescence; arrows indicate direction of water flow. A: Little Giant pump; B: bubble trap; C: Masterflex two-head pump; D: Technicon pump for DCMU addition; E₁, E₂: delay tubing; F₁: control fluorometer; F₂: DCMU-treatment fluorometer. Ancillary data provided by thermistor and depth transducer (G) and by data acquisition package (H). For conventional profiling, Pump C is not activated and fluorescence is read on Turner 111 fluorometer (I)

pump (Model 7020C) with two heads (7017, 7017-20) and Masterflex Tygon tubing No. 6408-06. This pump (C) delivered two parallel streams at the identical rate of 1.0 l min⁻¹.

The flow to the control fluorometer, F₁ (Turner Designs model 10-005 with continuous flow cuvette system 10-020) passed through enough light-shielded Tygon tubing (E₁) for an additional 30 sec delay. Another fluorometer (F₂) received an identical flow, except that slightly supersaturated DCMU in 95% ethanol was injected by a Technicon model 1 proportioning pump to a final dilution of 1:1000 (about 3 x 10⁻⁶ M). Residence time in the fluorometer cuvette was 0.54 sec.

Output from the fluorometers and temperature from a thermistor fastened near the hose inlet (Fig. 1, G) were documented on chart recorders. Depth of the hose inlet was determined with a pressure transducer.

Fluorometers were zeroed on the appropriate sensitivities with distilled water blanks. Seawater lines were shielded from sunlight.

After the span on each fluorometer had been adjusted to obtain agreement on the fluorescence reading for untreated surface water, a profile was performed using both fluorometers but no DCMU addition. The two records were essentially identical over the range encountered (8 to 78 units). The fluorometer outputs

were similarly matched several times in the course of the cruise.

When a small fluorescence increase in the presence of DCMU was encountered, we slowed the Masterflex pump flow by a factor of 2 in an attempt to assure ourselves that the inhibitor was fully effective. A decrease in pump flow increases the concentration of DCMU and prolongs the dark adaptation, residence time in the fluorometer cuvette, and exposure to DCMU before the fluorescence is measured. These changes had no effect on F_l or F_{lDCMU} .

Results

Laboratory Studies on Effect of DCMU

The natural sample from the SIO pier showed a maximum fluorescence increase of 110% after a 30 sec time delay between DCMU addition and fluorescence measurement. The increase was 105% after a 2 min delay. With a 3 min delay time, the increase was 83%. Dark delay times and residence times in the fluorometer had little effect (<10%) on F_l or F_{lDCMU} over the range of conditions.

The time course of fluorescence increase after DCMU addition for phytoplankton cultures showed that *Gymnodinium splendens* exhibited a maximum response within 30 sec after addition, as did *Coccolithus huxleyi*. *Peridinium trochoideum* showed 70 to 90% full response at 30 sec and maximum fluorescence increase within 1 min.

Field Measurements

Nine fluorescence response profiles were taken during the cruise. An example of one is given in Fig. 2. The control fluorescence and DCMU-treated fluorescence are shown, as well as extracted chlorophyll *a* (chl *a*) values taken from the profiling stream. It is of particular interest that the ratio of DCMU fluorescence to control fluorescence changed with depth in this profile.

A fluorescence response index (FRI),
$$FRI = \frac{F_{lDCMU} - F_l^{control}}{F_{lDCMU}}$$
 was calculated

at numerous depths in the profile, and results are plotted in Fig. 2B. This ratio was used by Kiefer and Hodson (1974) to represent the quantum efficiency of the photo-oxidation of water and is very similar to the "percent fluorescence increase" of Samuelsson *et al.* (1978). The index averaged ca. 0.15 near

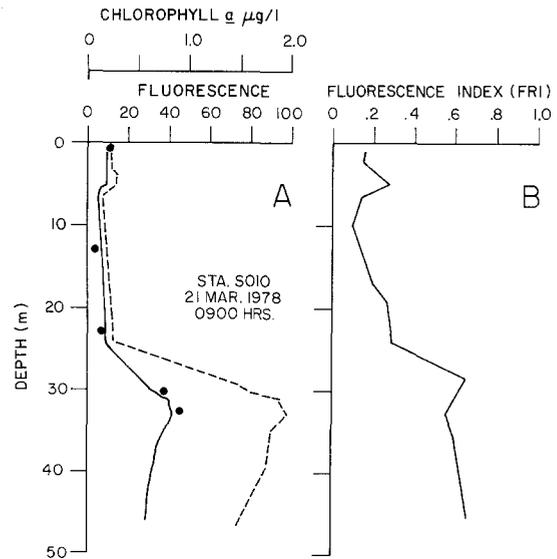


Fig. 2. Fluorescence parameters versus depth at Station SO10, March 21, 1978, 09.00 hrs. (A) Untreated control fluorescence, F_l , continuous line; DCMU-treated fluorescence, F_{lDCMU} , dashed line; discrete determination of chlorophyll *a* (circles). (B) $FRI = (F_{lDCMU} - F_l) / F_{lDCMU}$. Fluorescence units are arbitrary, but identical for F_l and F_{lDCMU} .

the surface and increased to 0.6 in the chlorophyll maximum layer. The euphotic depth (1% light level) at this station was 57 m.

Fig. 3 depicts profiles representative of fluorescence responses encountered during the sampling period. Station SO10, at 13.15 hrs on March 19, had a fluorescence profile similar to the same station at 09.00 hrs on March 21, shown in Fig. 2. The FRI was near zero in the upper several meters, where light intensity was $>0.2 \text{ ly min}^{-1}$. This is not an effect of illumination alone, since the insolation during the 09.00 hrs profile was higher than at 13.15 hrs two days before. The index in Fig. 3A increased at the chlorophyll maximum, and reached 0.6 at 52 m. The 1% light level was at 56 m.

The profiles from Station SO5 show the higher standing stock of phytoplankton at this nearshore location. An intense chlorophyll maximum layer composed primarily of *Gymnodinium splendens* (R. Lasker, personal communication) was present. At Station SO5, surface layer NO_3^- averaged $0.2 \mu\text{M}$. Although a very low concentration, it was the highest surface value found among the Southern California Bight grid stations that week. Surface layer NH_4^+ was less than $0.3 \mu\text{M}$, comparable with many other stations sampled.

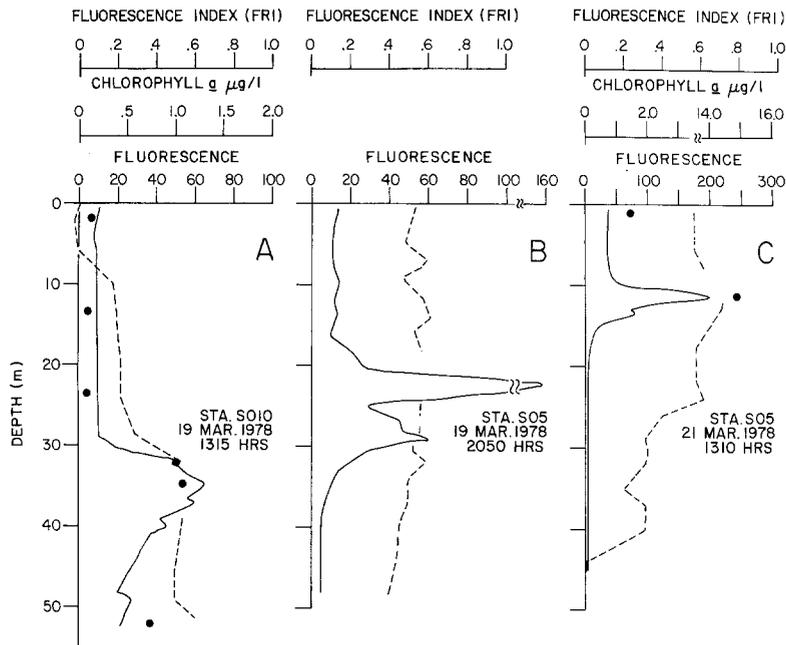


Fig. 3. Fluorescence parameters versus depth at Stations S010 and S05. Untreated control fluorescence, F_l , continuous lines; $FRI = (F_{lDCMU} - F_l) / F_{lDCMU}$, dashed lines; discrete determinations of chlorophyll *a*, circles. Gaps in FRI trace appear where F_{lDCMU} was off-scale

The FRI was high near the surface in both profiles at Station S05, but on March 21 it declined to zero at 45 m. An important difference in conditions at Station S05 on the 2 days was light penetration. The 1% light level on March 19 was 43 m. On March 21, it was 26 m. It should also be noted that the March 19 profile was taken at night; the profile from the same day at 13.10 hrs was very similar.

Discussion

Potential Artifacts

Before any attempt is made to relate patterns in the FRI to the physiology of the phytoplankton, we must determine that the fluorescence response is representative of the population *in situ* and not an artifact due to sampling methods or interference. For instance, if the permeability to DCMU varied between populations, a low FRI could be indicative only of incomplete inhibition of photosynthesis by DCMU. Our experiments on the time course of fluorescence increase after DCMU addition and supplemental measurements of oxygen evolution of phytoplankton cultures in the presence of 3×10^{-6} M DCMU (J.J. Cullen, unpublished data) support the assumption that most of the algal cells were fully affected by DCMU in our fluorescence assay.

Since fluorescence is in part a function of the proportion of Photosystem (PS) II reaction centers which are open (Papageorgiou, 1975), the state of dark adaptation of the phytoplankton at the time of the fluorescence assay can be important, especially if cells from different light levels in the vertical profiles are not in the same state of dark adaptation when they reach the fluorometer. Dark adaptation periods of 10 to 30 min are commonly used in laboratory experiments (Prézelin and Sweeney, 1977; Samuelsson and Öquist, 1977), but Bannister and Rice (1968) employed 3 min dark periods in their study with satisfactory results. Our experiments with varied flow rates showed that the FRI was insensitive to small increases in dark adaptation time. In the field, the FRI could be highly variable (Station S010) or essentially uniform (Station S05) at light levels greater than 1% surface irradiance. Also, night and day FRI profiles at Station S05 were very similar. These results indicate that our data are not seriously biased by differential dark adaptation in the vertical profiles. No night profiles were taken at Station S010.

The transient fluorescence changes after the onset of illumination which should be seen in dark-adapted algae but not in DCMU-treated samples could interact with our method of fluorescence measurement and confound our results. If

populations had different time scales for fluorescence induction (cf. Papageorgiou, 1975 with Samuelsson *et al.*, 1978), the fluorescence values recorded in the flow-through fluorometer could correspond to different stages of fluorescence response. Our determinations of F1 and FRI over a seven-fold range of residence time in the cuvette suggest that transient fluorescence response was not an important factor in our measurements. This is somewhat surprising, because transients are observed in laboratory studies and are prominent in theoretical discussions.

Figs. 2 and 3A show a strong correlation between chlorophyll and FRI. Fluorescence yields (F1:chl *a*, F1_{DCMU}:chl *a*) could be a function of chlorophyll concentration due to reabsorption of fluoresced light, but the ratio, F1:F1_{DCMU} should be insensitive to this type of interference unless the reabsorption efficiency of chlorophyll is altered in the presence of DCMU. Bannister and Rice (1968) observed no effect of DCMU on the fluorescence response of a mutant *Chlamydomonas reinhardtii* lacking cytochrome *f* (an electron transport link between PS II and PS I). We conclude that DCMU *per se* did not affect the light harvesting or fluorescence properties of the bulk chlorophyll of PS II in this organism. This observation alone is not conclusive. Our field results (Station S05) show an FRI of 0.6 over a wide range of chlorophyll concentrations, and the index can be steady while chlorophyll increases greatly in batch cultures (Samuelsson and Öquist, 1977). Thus, we do not have reason to suspect that the FRI is a direct function of chlorophyll concentration.

We cannot assess the contribution of soluble fluorescence (Herbland, 1978) in our profiles, but we were able to demonstrate that particulate phaeopigments did not contribute significantly to the FRI. The null hypothesis is that the ratio, phaeopigments: (chl *a* + phaeopigments), was unrelated to the FRI could not be rejected (Spearman's rank correlation test: $r_s = -0.11$, $N = 25$, $P > 0.25$). In the future, soluble fluorescence should be measured during sampling to estimate its correlation with fluorescence response.

Relationship Between in vivo Fluorescence and Photosynthetic Capacity

We have shown that the ratio of *in vivo* chlorophyll fluorescence to DCMU-treated fluorescence can vary systematically in vertical profiles and that this varia-

tion is probably not due to a number of potential artifacts. There are theoretical reasons for expecting the DCMU-induced increase in fluorescence to be proportional to photosynthetic efficiency, because DCMU blocks non-cyclic photosynthetic electron transport and eliminates fluorescence quenching by active Photosystem II (PSII) reaction centers (see Papageorgiou, 1975; and discussions in Samuelsson and Öquist, 1977; Samuelsson *et al.*, 1978; Slovacek and Hannan, 1977). We will refer to hypotheses which use this line of reasoning to relate fluorescence to photosynthesis as photosynthetic quenching hypotheses. In simple form, such hypotheses predict that fluorescence yield per unit chlorophyll should be inversely proportional to photosynthetic capacity and that the fluorescence yield of DCMU-poisoned cells is maximal and a constant.

Laboratory studies have related DCMU-induced fluorescence changes to algal physiological state or photosynthetic rates. This fluorescence increase is a good indicator of photosynthetic capacity through the growth stages of batch cultures (Samuelsson and Öquist, 1977; Samuelsson *et al.*, 1978), and reflects batch culture nutrient-depletion (Blasco and Dexter, 1972; Slovacek and Hannan, 1977). The FRI of *Gonyaulax polyedra* shows a circadian rhythm in phase with the rhythm of photosynthetic yield (Prézelin and Sweeney, 1977). A field study (B.B. Prézelin, unpublished data) demonstrates the same trends in natural phytoplankton off Southern California.

If a photosynthetic quenching hypothesis were applicable to our fluorescence assay, an FRI of 0 would be indicative of complete photosynthetic debility and an increase of the index would correspond to enhanced photosynthetic capacity. An index of 0.6 to 0.7 is about the highest observed in actively growing cultures (J.J. Cullen, unpublished data; Blasco and Dexter, 1972; Kiefer and Hodson, 1974). An FRI of zero was attained in a batch culture of *Skeletonema costatum* after 11 days of nitrogen depletion (Blasco and Dexter, 1972).

Although DCMU-induced fluorescence response has been shown to be related to photosynthetic capacity in a number of studies, several observations demonstrate the complexity of the photosynthesis-fluorescence relationship and are not in agreement with simple photosynthetic quenching hypotheses. Bannister and Rice (1968) have demonstrated slow changes in fluorescence parallel to oxygen evolution rate, not antiparallel as photosynthetic quenching hypothesis predict, and B.B. Prézelin (unpublished

data) observed a diurnal periodicity in Fl:chl *a* and Fl_{DCMU}:chl *a* in natural phytoplankton which was in direct proportion to photosynthesis (as mentioned, the FRI behaved in a manner consistent with a photosynthetic quenching hypothesis). Slovacek and Bannister (1973) found a large DCMU-induced fluorescence increase in *Chorella pyrenoidosa* deprived of CO₂ and photosynthetically disabled. Butler and Kitajima (1975) showed that variable fluorescence can experience a large change with little effect on photosynthetic capacity if the energy distribution in the photosynthetic apparatus is altered.

Particularly inconsistent with simple photosynthetic quenching hypotheses are the findings of Kiefer and Hodson (1974), who worked with nitrogen-limited chemostat cultures of the diatom *Thalassiosira pseudonana*. Using 4 dilution rates, they obtained a 10-fold range of assimilation number (maximum photosynthesis per unit chlorophyll) with no trend in the FRI, which was about 0.6. More chemostat work must be done to support this observation, but it seems that there may be a fundamental difference between fluorescence response in batch and in continuous cultures.

This is only speculation, but the data suggest that the FRI for any photosynthetically active cell is characteristically high (but not constant: see Prézelin and Sweeney, 1977) and that the index of cells which have stopped photosynthesizing is near zero. If this were true, the decrease of the FRI in batch cultures would be due to accumulation of autolysis products and/or cells which have stopped photosynthesizing. Such accumulation would not occur in continuous cultures because non-replicating material is diluted out. The occurrence of photosynthetically inactive algal cells in nature is apparently common. Unlabelled cells are visible in significant proportions in microautoradiographs of natural phytoplankton (Paerl, 1978; A.F. Carlucci, personal communication). We do not know if inactivation is permanent (death), or is reversible.

Fluorescence measurements made in the field could reflect the contributions of dissolved fluorescence (Herbland, 1978), phaeophytin, or photosynthetically inactive algae. Such fluorescence, not directly associated with photosynthesis and insensitive to DCMU, would affect the FRI. Clayton (1969) used the term "dead fluorescence" to describe the fluorescence contribution of cellular chlorophyll not functionally associated with the reaction centers of PS II, and discussed its effect on fluorescence re-

sponse in laboratory experiments. Butler (1978) could not substantiate the dead fluorescence hypothesis for chloroplasts at -196°C. As previously used, "dead fluorescence" is a rather specific term. We prefer a more general term, "inactive fluorescence" to describe any fluorescence contribution not directly associated with the photosynthetic activity of PS II (O₂ evolution). If a large proportion of the Fl measured were inactive fluorescence, the FRI would be depressed to a low value, although the photosynthetically active algae in the sample could still have an index near 0.6.

Interpretation of Field Results

With the information now available, we can only speculate on the precise nature of the FRI. We can say that a strong fluorescence response to DCMU is characteristic of growing phytoplankton and that the components of fluorescence in samples with low FRI's are unresponsive to DCMU. A low FRI probably indicates some degree of photosynthetic debility in a natural sample or at least a large contribution of inactive fluorescence. Low (<0.25) fluorescence response indices have only been observed under extreme conditions in the laboratory. We hypothesize that a low FRI measured in the field represents some sort of physiological stress on the population level. The manifestation of this stress which we measure would be an increase of the relative proportions of photosynthetically inactive fluorescence components.

This hypothesis requires rigorous testing before it can be accepted. In order to evaluate the patterns of FRI in relation to distributions of physical, chemical, and biological properties in our study, we will make the heuristic assumption that the hypothesis is valid and we will concentrate only on gross fluctuations of the FRI, fully realizing that other phenomena may have been instrumental in determining the fluorescence response.

Vertical profiles at Stations S010 and S05 show the full range of the FRI. A physiologically stressed population was indicated in the surface layer at the offshore station (Figs. 2B, 3A). Perhaps this is a manifestation of severe nutrient depletion. The zero value in the upper 5 m (Fig. 3A) may have been due to a mid-afternoon high-light effect. Phytoplankton in the surface layer at Station S05 did not show signs of severe stress (Fig. 3B, C). Although nitrogen levels were quite low, flux of nutrients into the surface-layer phytoplankton population was apparently sufficient to

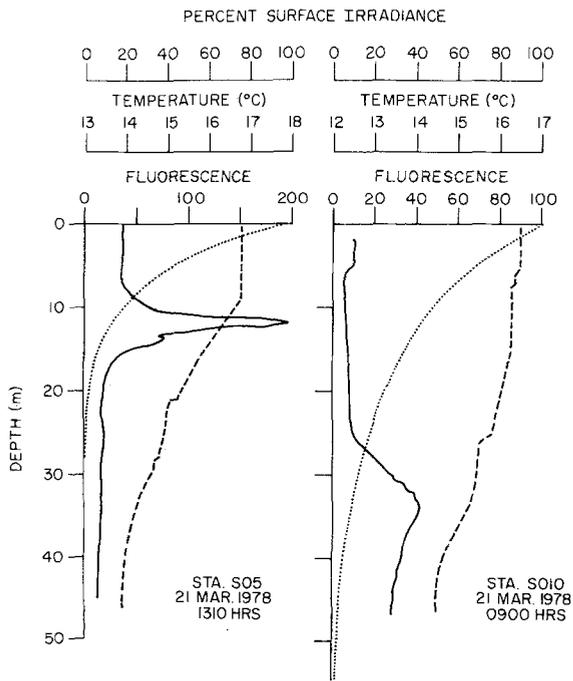


Fig. 4. Light (dotted lines), temperature (dashed lines), and fluorescence (continuous lines) versus depth at Stations S010 and S05

maintain efficient photosynthesis. This onshore-offshore contrast of the fluorescence response index is reminiscent of differences in phytoplankton carbon:chlorophyll ratios off La Jolla that were attributed to nutritional status of the algae (Eppley, 1968).

Due to the higher standing stock of phytoplankton at Station S05, especially on March 21, light penetration was diminished as compared to S010 (Fig. 4). Thus, our hose was able to reach strata which experienced low illumination. The fluorescence response index began to decrease at approximately the 1% light level on both occasions. In Fig. 3C, it reached zero just below the 0.1% light level. Phytoplankton sampled from these regions of low irradiance may be stressed due to inadequate light or the FRI may be depressed because most of the fluorescence is in inactive forms associated with particles sinking from the euphotic zone.

The patterns of FRI that we observed are very striking. The index is low where conditions appear to be non-supportive of vigorous phytoplankton growth and it is relatively high elsewhere. Unfortunately, correlation does not demonstrate causality. More work must be done to decide whether fluorescence response is an accurate indicator of algal physiological state or merely a provocative

parameter. We may not know exactly what we are measuring, but the patterns observed are too strong to ignore.

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