DCMU-Enhanced Fluorescence as an Indicator of Physiological Condition and Light History in Phytoplankton

By

Mary Elizabeth Putt, B.Sc.

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AUTHOR: Mary Elizabeth Putt, B.Sc. (University of Manitoba)

SUPERVISOR: Dr. G.P. Harris

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Abstract

Fluorescence (F), DCMU-enhanced fluorescence (F_{DCMU}) and a ratio of these two measurements (F ratio) were found to be useful indicators of light history but not physiological condition of natural phytoplankton assemblages.

Changes in the fluorescence properties of unialgal continuous and batch cultures at different growth rates and following nutrient addition were observed only during nutrient starvation. Nutrient deficiency in Lake Ontario was not revealed either by seasonal patterns of fluorescence or by short term changes in the F ratio following nutrient additions. This result however is not conclusive evidence of nutrient sufficiency because of the insensitivity of the fluorescence ratio as an indicator of growth rate.

The depression of F and F_{DCMU} observed in surface waters of Lake Ontario occurred during conditions of high light and low mixing rates. The result suggests that "photoinhibition" of photosynthesis as measured by conventional primary productivity techniques, may occur in nature only under these particular conditions.

A general relationship between temperature gradients or water column stability and the difference in fluorescence between 1 and 10 meters was observed. This relationship was due to both vertical structure in the assemblage and the physiological effect of light on fluorescence. Diurnal patterns of fluorescence were found to be due to the physiological effect of light on fluorescence rather than a circadian rhythm. The physiological effect was dependent on both the duration and intensity of exposure of the cells to light as well as the sensitivity of the assemblage to light. Differences were associated with seasonal changes in species composition with spring and winter populations exhibiting the greatest sensitivity.

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C	carbon
chl*a*	chlorophyll *a*
$cm\ s^{-1}$	centimeters per second
DMSO	dimethyl sulfoxide
DCMU	3-3(3,4-dichlorophenyl)-1,1-dimethyl urea
F	*in vivo* fluorescence
F/chl*a*	*in vivo* fluorescence per µg chlorophyll *a*
ΔF	the difference in F between two depths expressed as a percentage of the F at the lower depth
F_{DCMU}	DCMU-enhanced fluorescence
ΔF_{DCMU}	the difference in F_{DCMU} between two depths expressed as a percentage of the F_{DCMU} at the lower depth
hr	hours
mg	milligrams
mL	milliliters
L	liters
LST	local standard time
N^2	water column stability estimate, $10^6\ s^{-2}$
NH_4	ammonia nitrogen
NO_3^-	nitrate nitrogen
P_{max}	maximum photosynthetic rate
P_{opt}	optimum photosynthetic rate
$\mu E\ m^{-2}\ s^{-1}$	µEinsteins per square meter per second
µg	micrograms
$\mu g\ L^{-1}$	micrograms per liter
s	seconds
T	temperature (^oC)

1.Literature Review

1.1 General introduction

Light absorbed by photosynthetic pigments may be emitted in a process known as fluorescence. In limnology and oceanography, *in vitro* fluorescence may be used to quantify the amount of chlorophyll *a* in a polar solvent. *In vivo* fluorescence may also be used as a measure of algal biomass. Historically, continuous measurement of *in vivo* fluorescence in the water column has been instrumental in the measurement of vertical and horizontal patchiness of phytoplankton (Lorenzen 1966, Platt and Denman 1980). Current research involves the use of fluorescence in the investigation of both micro and mesocale processes. Both the characterisation of individual phytoplankton cells by flow cytometry (Yentsch 1982, Olson *et al* 1982) and the mapping of chlorophyll by means of ship and satellite remote sensing techniques (Smith *et al* 1982) involve its use.

The problem with the technique however is that the fluorescence of chlorophyll *a* of a natural assemblage is dependent on factors such as species composition, temperature, physiological condition, light history and innate rhythms (Kiefer 1973a,b; Harris 1978, Heaney 1978, Brand 1982). More recent investigators suggest the possibility of making use of the variability in fluorescence as a predictor of the

physiological status and light history of the phytoplankton (Vincent 1979, 1980, Harris 1983). As well, there are claims that the F ratio (a comparison of in vivo fluorescence and fluorescence yield following the addition of a photosynthetic inhibitor,DCMU) is a rapid means of measuring the photosynthetic potential of phytoplankton (Cullen and Renger 1979, Kimmel and White 1979, Roy and Legendre 1979,1980, Vincent 1980).

The broad objective of this thesis is to examine the validity of using in vivo fluorescence techniques to indicate physiological condition and light history of natural assemblages of phytoplankton.

1.2 Physiological basis of fluorescence

As an introduction to the topic of in vivo fluorescence in natural populations, the structure of the photosynthetic apparatus, a model of the primary photochemical events of photosynthesis, and the nature of in vitro and in vivo fluorescence in plant cells will be discussed. For more complete reviews on the subject see (Goedheer 1972, Mohanty 1972, Papageorgiou 1975, Harris 1978, Prèzelin 1981).

1.2.1 Chloroplast Structure

The photochemical pigments in plant cells are located on and

within the thylakoid membranes within a membrane bound organelle, the chloroplast. Only chlorophyll a molecules are necessary for photosynthesis to occur, however all plants have accessory pigments (other chlorophylls, carotenoids and phycobilins) which expand the spectral range of light which may be absorbed by the chloroplast. The photosynthetic pigments are associated with proteins and the protein-pigment complexes are, in turn, organized into two photosystems (PSI and PSII) (Prèzelin 1981). A photosystem may be thought of as an array of light harvesting pigments acting as antennae which surround specialized chlorophyll molecules or "reaction centers". This model was originally conceived from the work of Emerson and Arnold (1938) who showed that short flashes of light produced about one O_2 molecule per 2400 chlorophyll molecules. Since 8 quanta are required to produce one O_2 molecule, the number of chlorophyll molecules per trap is about 300.

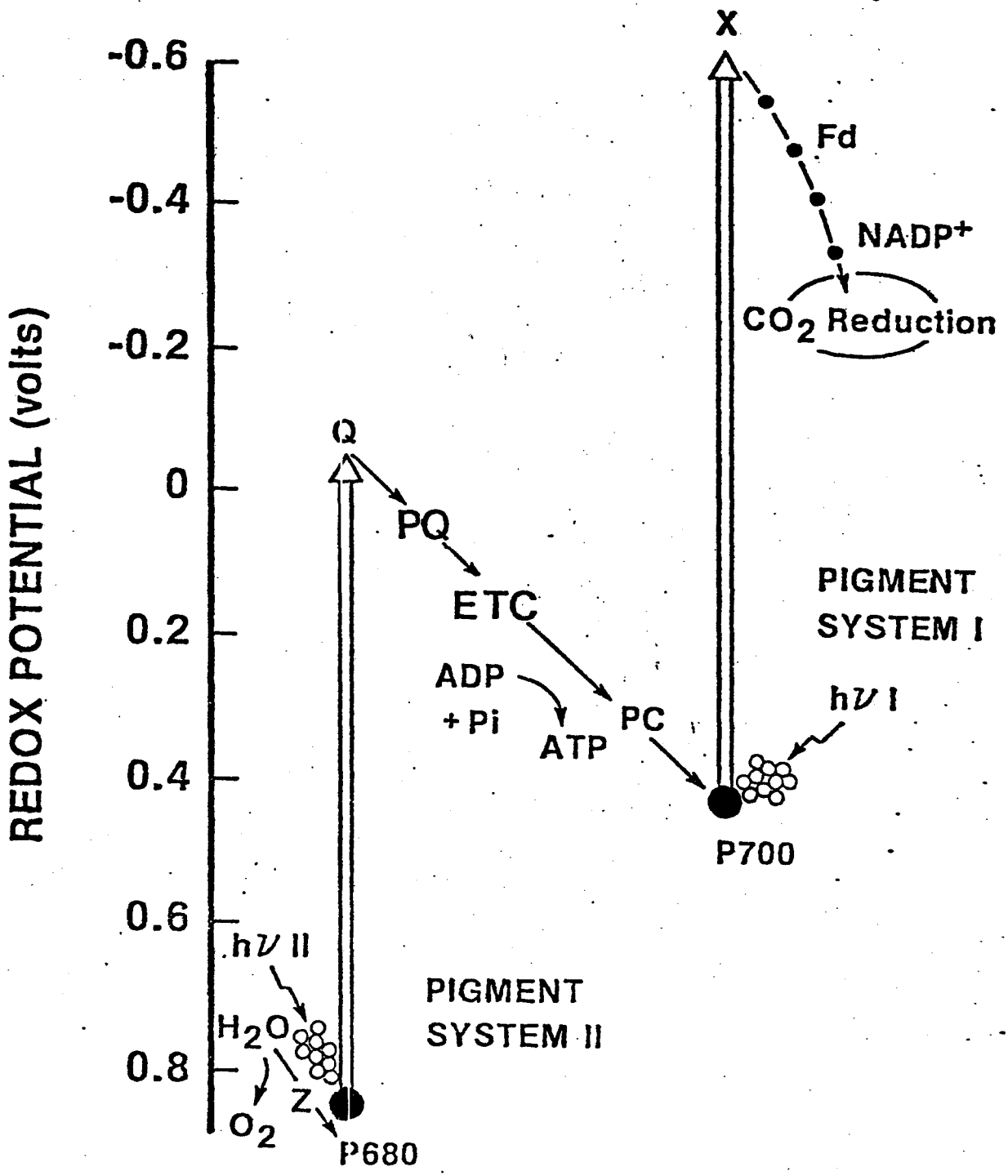
The existence of two "reaction centers" was indicated by the work of Emerson and Lewis (1943) and Emerson et al (1957) who found a drop in the quantum yield of O_2 evolution when red light (beyond a wavelength of 685 nm) was shone on the chloroplasts. When excitation light shorter than 685 nm was used simultaneously with excitation light of wavelenths greater than 685 nm the rate of O_2 evolution was higher than the sum of the rates given separately. The specialized molecules of the reaction centers (PSII and PSI) are known as P_{680} and P_{700}. PSII and PSI may be distinguished from each other by the physical separation of light (PSI) and heavy (PSII) particles (Papageorgiou 1975) as well as by their specific absorption

1.2.2 Primary Photochemical Events in Photosynthesis

The two photoacts are thought to be coupled together by a series of electron carriers. The most commonly used model for these events is the Z scheme (Figure 1.1). In this model water contributes an electron to a hypothetical electron donor (Z) located on the inside of the thylakoid membrane. This event liberates O_2 and releases protons into the interior compartment of the membrane. Light energy absorbed by accessory "antennae" pigments is transferred to P_{680}. The energy allows P_{680} to oxidize Z and in so doing raise the electron to a higher energy level, the excited singlet state. The electron reduces an electron acceptor (Q) which is located on the outside of the thylakoid membrane. A separation of charge and a membrane potential is thus created.

Q transfers the electron to plastoquinone (PQ) which in turn passes the electron to a series of electron acceptors (the cytochromes). The large amount of plastoquinone in the chloroplast suggests that it may serve some regulatory function. Plastoquinone is also important because its oxidation leads to the release of 2H+ into the intrathylakoid space. Along with the liberation of protons from the splitting of water, this establishes the proton gradient across the membrane necessary for photophosphorylation. Light energy drives a similar charge separation at PSI similar to that which occurs at PSII. The reduction of X (likely a ferredoxin or flavoprotein) in this case leads to the production of NADPH.

Figure 1.1 A simplified Hill and Bendall 'Z' scheme redrawn and adapted from Harris (1978). Broad arrows indicate light reactions. Abbreviations: Small circles, accessory photosynthetic pigments to the traps (P680 and P700) of photosystmes II and I respectively; Z, electron donor of PSII; Q, proposed electron acceptor of PSII, ETC, electron transport chain; PQ, plastoquinone; PC, plastocyanin; X, electron acceptor of PSI; Fd, ferredoxin; $NADP^+$, nicotinamide adenine dinucleotide phosphate; ADP, ATP, adenosine di-and tri-phosphate; P_i; inorganic phosphate; h , light energy.


REDOX POTENTIAL (volts)
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
X
Fd
NADP+
CO2 Reduction
Q
PQ
ETC
ADP
+ Pi
ATP
PC
PIGMENT
SYSTEM I
hν I
P700
hν II
H2O
Z
O2
P680
PIGMENT
SYSTEM II

One of the earliest problems with the Z scheme was due to what is now known as the "quantum yield anomaly" (Butler 1978). According to the Z scheme in its simplest form, maximal photosynthetic efficiency should be achieved when equal amounts of energy are absorbed by the two pigment systems. Emerson and Lewis (1943) reported that the quantum yield of photosynthesis was maximal and independent of wavelength from 570 to 685 nm. The findings suggested that there is a mechanism (the "spillover") which serves to redistribute excitation energy equally between the two systems. It has been suggested that a chlorophyll-protein complex serves to regulate the process (Anderson 1980). Whatever the mechanism it is now clear that not only are "dark" reactions of photosynthesis controlled by a complex feedback system but that also the distribution of excitation energy is regulated by membrane conformational changes and integrated into the photosynthetic process as a whole.

1.2.3 Chlorophyll absorbance and fluorescence in vitro

Light is absorbed at 680 and 430 nm by chlorophyll *a* *in vitro*. Chlorophyll *b* absorbs light at 453 and 643 nm while chlorophyll *c* absorbs at 441 and 629 nm (Goedheer 1972, Prèzelin 1981). Chlorophylls *b* and *c* are also distinguishable from chlorophyll *a* because the absorption of blue light is more intense than that of red light. *In vivo* the properties of free chlorophylls are modified by

conjugation of the molecules with proteins and their association with thylakoid membranes. The long wavelength absorption peaks are generally shifted 5 to 40 nm into the red region (Prèzelin 1981). In vivo, 3-10% of the light absorbed by PSII is emitted as fluorescence and this amounts to about 90% of the total in vivo fluorescence. Energy losses from PSI occur primarily as heat or radiationless transfer.

1.2.4 Factors affecting fluorescence yield

Light impinging upon a photosynthetic pigment raises an electron to a higher energy state--the excited singlet state. At this point three events may occur (Prèzelin 1981):

1) the energy may be transduced to neighboring pigment molecules and eventually to a reaction center. Most in vivo fluorescence comes from P_{680} which suggests that the energy transfer efficiency of accessory chlorophyll pigments is very high.

2) an unpairing of electron spin in the outer orbitals of the pigment molecule transforms the singlet state into the triplet state. Triplet-triplet state conversions may occur or the electron may return to

the ground state. The emitted light is known as delayed light emission.

3) The singlet state may return to the ground state. This is the phenomenon known as fluorescence.

The intensity of the emitted light is controlled by several factors. The equation of Lavorel and Etienne (1977)

$$1)\ F = I * \phi_f\ (I_t, t, \lambda_e, \lambda_f, \text{state})$$

states that F (fluorescence intensity) depends on the intensity of the absorbed light (I) and the quantum fluorescence yield ϕ_f. ϕ_f is a function of I_t (the intensity of illumination), t (time of illumination), λ_e (excitation wavelength), λ_f (fluorescence emission wavelength) and the state or prehistory of the cell.

The fluorescence yield may be written as (Mohanty 1972):

$$2)\ \phi_f = \frac{\text{emitted quanta}}{\text{absorbed quanta}} = \frac{k_f}{(k_f + k_p + k_h)}$$

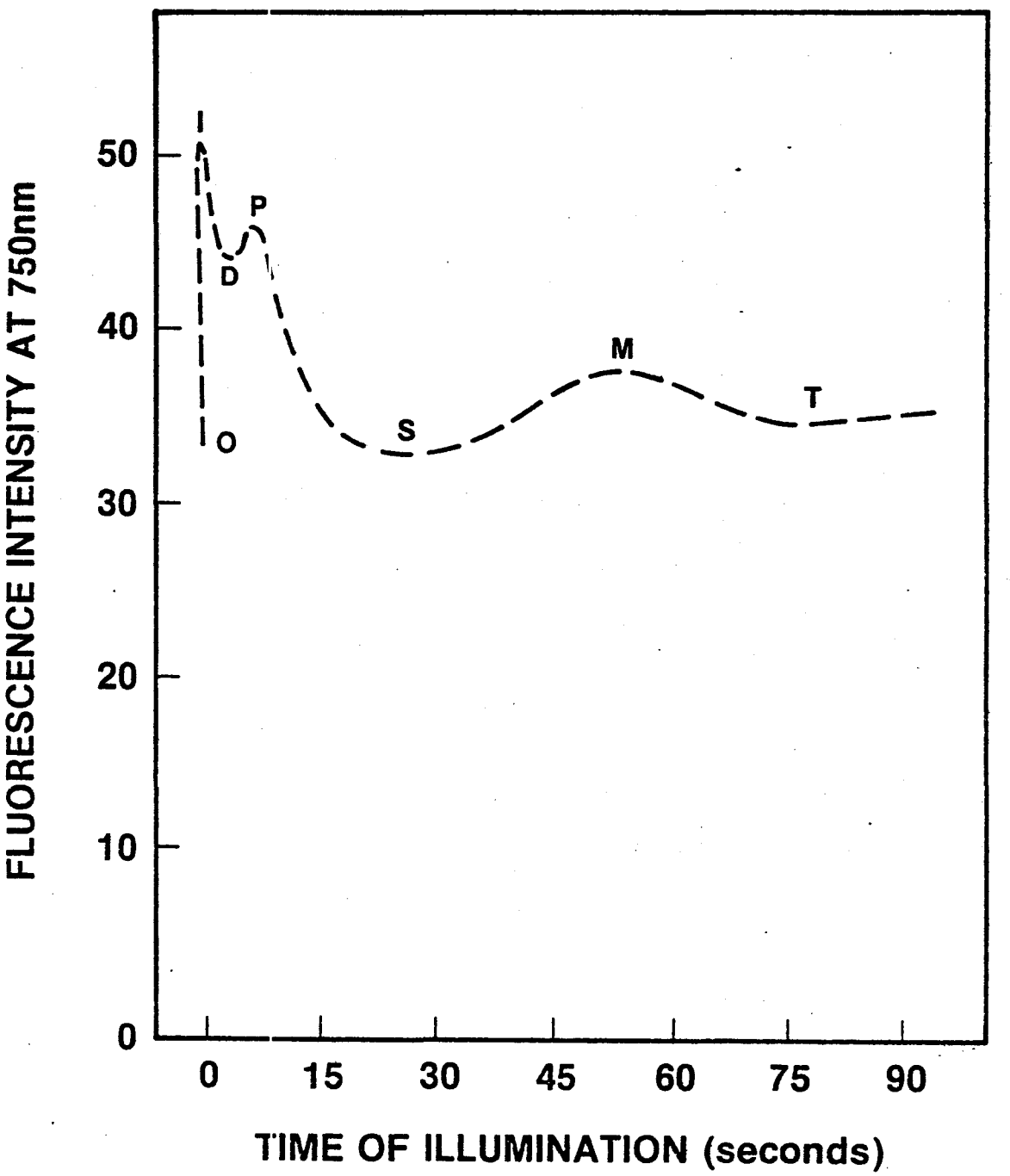
where k is a rate constant for k_f (fluorescence), k_p (photosynthesis), k_h (non-radiative de-excitation). k_p may be measured by the addition of the inhibitor DCMU which enhances fluorescence by blocking electron flow just after Q.

One might expect that fluorescence and photosynthesis as measured by O_2 evolution should mirror each other. High fluorescence values should be reflected by low O_2 evolution and vice versa (Mohanty 1972, Papageorgiou 1975, Harris 1978). It is at this point that the simple model becomes inadequate. The kinetics of fluorescence induction following exposure of dark adapted cells to bright illumination shows that a number of transients occur during the first few minutes of illumination. This phenomenon is known as the Kautsky induction effect (Figure 1.2).

During the initial 10 seconds of illumination the rate of oxygen evolution is essentially antiparallel to fluorescence except for the OI phase. The OIDPS phases are thought to correspond to the initial redox state of the primary acceptor of photosynthesis as electron flow is initiated. For example, initially, when Q is oxidized chlorophyll a fluorescence is quenched (e.g. the ID dip). When the PQ pool becomes filled, Q cannot be oxidized and fluorescence rises (the DP rise). Use of the inhibitor DCMU prevents the oxidation of Q and causes a rapid OI rise in fluorescence to a maximal level.

At longer time scales during the SMT phase oxygen evolution initially parallels fluorescence changes and eventually reaches a steady state. Thus photosynthesis does not mirror fluorescence; the SMT phases are rather thought to reflect the conformational changes associated with spillover.

Figure 1.2 Kautsky induction effects. Redrawn and adapted from Prezelin 1981.


FLUORESCENCE INTENSITY AT 750nm
50
40
30
20
10
0
O
D
P
S
M
T
0
15
30
45
60
75
90
TIME OF ILLUMINATION (seconds)

Evidence for this hypothesis comes from work with inhibitors and mutants. Slow changes in yield are sensitive to uncouplers of photosynthesis but not to energy transfer inhibitors (Papageorgiou and Govindjee 1968, Mohanty and Govindjee 1974). Mutant strains of Chlamydomonas reinhardii which lack PSI do not show the slow changes (Bannister and Rice 1968).

Illumination with bright light also causes macroscopic shrinkage of chloroplast membranes. This event is also reversed by uncouplers (Krause 1973) but corresponds to a longer time scale than does the SMT phase (Mohanty and Govindjee 1973).

1.3 Physiological condition

To decide whether the F ratio reflects the physiological status of phytoplankton a definition of "physiological status" is required. As an overall survival strategy in a rapidly changing environment, the ideal physiological condition for phytoplankton may be defined as one which allows the most rapid increase of either cell numbers or biomass (Harris 1980b). Growth rates are measurable in culture but an unequivocal method of determining the growth rate of natural phytoplankton assemblages does not exist (Eppley 1981). Several in situ methods are discussed here briefly.

Photosynthesis (measured by ^{14}C uptake) is often confused with

growth rates. The existence of large pools of storage photosynthate and high photosynthetic rates in non-dividing cells is evidence that growth may be uncoupled from photosynthesis (Healey 1979).

The kinetics of nutrient uptake, in particular phosphorus in lakes and nitrogen in the oceans, is often used to indicate the degree to which these elements "limit" growth. Growth rate however is dependent on both internal and external nutrient concentrations (Rhee 1979). In an environment such as a lake in which both of these values are variable, it may be hard to predict if and when the two rates are closely coupled. Determination of the cellular distribution of both carbon and nutrients may eventually result in a better understanding of these processes (Morris 1981, Eppley 1981).

Changes in concentration of extracted chlorophyll _a_ are measurable but the influence of accessory pigments, changes in the PSU in response to light conditions and the contribution of degradation products make this an unreliable parameter for measuring growth as well.

Enrichment experiments are an alternate means of attempting to identify the nutrients most limiting to algal growth (Vollenweider 1969). These experiments are based on the idea that growth (generally measured as ^{14}C uptake) should be enhanced upon the addition of a limiting nutrient. It is increasingly clear that this view is too

simplistic and that experiments of this nature may give misleading results (Harris 1978, Lean and Pick 1982, Lean et al 1983, Turpin 1983).

1.4 Nutrient metabolism

While emphasis has been placed on the role of phosphorus as the element which controls the biomass of phytoplankton in freshwater lakes there is also evidence that nitrogen may be an important nutrient in the lower Great Lakes (Murphy 1980). Since the metabolism of nitrogen is more closely associated with photosynthesis than is phosphorus a more thorough investigation of the relationship between nitrogen status and fluorescence was conducted. A brief summary of the metabolism of both nitrogen and phosphorus metabolism follows.

1.4.1 Nitrogen metabolism

The assimilation of NO_3^- involves reduction to NO_2^- outside the chloroplast by nitrate reductase (NR) and transport of NO_2^- into the chloroplast where it is reduced to NH_4^+. It is thought that NH_4^+ is assimilated by a combination of two enzymes—glutamine synthetase (GS) and glutamate synthase (GOGAT). The reduction of NO_3^- to NH_4^+ requires 8 electrons; the assimilation of NH_4^+ by the GS:GOGAT system requires 2 more electrons and an ATP molecule (Syrett 1981).

The interaction of NO_3^- and NH_4 metabolism, photosynthesis and respiration is complicated and not well understood. NH_4 has been shown to be preferentially assimilated in cultures grown on NO_3^- as an N source. Both inhibition of NO_3^- uptake and loss of NR and nitrite reductase are involved (Conway 1977, Serra et al 1978b, Hipkin et al 1980). In the ocean, McCarthy et al (1977) found that NO_3^- uptake was only 7% of the nitrogen ration of the phytoplankton when NH_4 concentration exceeded 1 $\mu g\ L^{-1}$. Conway (1977) showed that the addition of NH_4 suppressed NO_3^- uptake within 30 minutes in the field. The preference for NH_4 over NO_3^- has been observed in both oceanic (McCarthy et al 1977, McCarthy 1980) and freshwater (Prochazkova et al 1970) systems. The addition of NH_4 has been found to suppress the uptake of NO_3^- in some systems (Conway 1977) and to have no effect in others (Conover 1975).

The assimilation of either NO_3^- or NH_4^+ by normal Chlamydomonas cells requires both light and CO_2. Similar rates of uptake of either form of N alone have been observed. Removal of either CO_2 or light prevents assimilation. In N-starved Chlamydomonas cells NH_4^+ is assimilated faster than NO_3^-, and both processes may occur in the dark. Light enhances the rate of uptake of both species and has the greatest effect on the rate of NO_3^- assimilation Syrett (1981).

As summarized by Syrett (1981) the relationship between light and nitrogen metabolism is due primarily to the generation of reduced

ferredoxin and ATP. Reduced ferredoxin may be used to

1) reduce NO_2^-
2) reduce NAD(P)H and hence NO_3^- in eukaryotic algae
3) drive the GOGAT reaction
4) activate/inactivate enzymes via thioredixin

ATP is used to:

1) drive uptake mechanisms of NO_3^-, NO_2^-, and NH_4^+
2) drive the GS reaction of NH_4 assimilation
3) stop reoxidation of mitochondrial NADH by O_2 thus making NADH available for NO_3^- reduction

It should however be emphasized that these are potential interactions; to date there is no general view of the mechanism of nitrogen assimilation (Morris 1974).

1.4.2 Phosophorus metabolism

Like nitrogen, phosphorus metabolism involves active uptake by a specific transport mechanism (Blum 1966, Nalewajko and Lean 1980). Unlike nitrogen however the uptake of phosphorus is not closely linked to photosynthesis. While phosphorus uptake requires energy, it may be supplied by either respiration or photosynthesis and in some lakes no

difference between uptake in the light and in the dark is observed.

Unlike nitrogen which is segregated within the cell in the amino and nucleic acid fractions, a large portion of cellular phosphorus is found in an extremely labile cellular fraction—the phosphate esters and ATP. These compounds have turnover times in the order of minutes. RNA, DNA and lipid phosphorus comprise the remainder of the organic fraction of cellular phosphorus (Nalewajko and Lean 1980).

Algal cells have a minimum cell content (cell quota) below which the cell cannot grow (Droop 1973) however under optimal conditions the cellular phosphorus content may exceed the minimum by an order of magnitude (Lund 1950, Mackereth 1953, Nalewajko and Lean 1980). Much of the surplus phosphorus accumulates in the form of polyphosphates (condensed phosphates of various chain lengths) (Nalewajko and Lean 1980). In chemostat culture, the rate of assimilation of phosphorus is related to the cell quota which is in turn related to the growth rate of the phytoplankton (Rhee 1979). This relationship has led to the use of the P turnover time (i.e. the amount of time required for an amount of phosphate equivalent to that present in the system to be taken up) as an indicator of the nutrient status of phytoplankton (Lean and Nalewajko 1979, Lean et al 1983a,b). In addition to cell quota, P turnover times in the field are related to the amount of phosphate present and the biomass in the system (Lean et al 1983a).

Natural systems, unlike chemostat cultures, are not steady state systems (Jannasch 1974, Rhee 1979, Harris 1980b). It is thus not appropriate to simply apply the interpretation of the results observed in culture to those observed in the field (Harris 1983, 1981). In a perturbed system, short term nutrient uptake may be uncoupled from growth. There is evidence that most phytoplankton actually grow at a rate near μ_{max} (Goldman et al 1979, Eppley 1981, Harris 1980b, 1983). If this is true then nutrient kinetic data are of little use in determining physiological condition in the field.

1.5 Nutrient Status--Observed Trends in Culture

In photosynthetically efficient cells, if the simplest model is accurate, minimal light energy should be emitted as fluorescence and maximal amounts transported down the electron transport chain. The F ratio should be high due to high F_{DCMU} and low F. This should be translated into high carbon fixation (as measured by ^{14}C uptake) in the dark reactions of photosynthesis.

In chemostat cultures F/chla for any single dilution rate is constant over time (Blasco and Dexter 1972). Kiefer (1973b) however showed that F/chla was higher at lower dilution rates in N-limited cultures. No change in F ratio however was observed (Kiefer and Hodson 1974).

In batch culture, during balanced growth, cell numbers and fluorescence increase at the same rate. The rate of increase in F is thus a good indicator of growth rate (Sakshaug and Holm-Hansen 1977, Fukazawa et al 1980, Brand and Guillard 1981). As predicted by the model F/chla rises following nutrient depletion, presumably because the photosynthetic apparatus becomes less efficient (Sakshaug and Holm-Hansen 1972, Blasco 1973, Samuelsson and Oquist 1977, Samuelsson et al 1978). The rise occurred within one day of depletion of N but a lag of 6 days preceded the rise in the case of P depletion in cultures of Skeletonema (Blasco 1973). F/chla was also found to be larger in N-limited than in P-limited media (Slovacek and Hannan 1977). Roy and Legendre (1979) found that DCMU-enhanced fluorescence also declines following nutrient depletion in batch culture.

Both the F ratio and P_{max} rise during exponential phase and fall during stationary phase (Samuelsson and Oquist 1977, Samuelsson et al 1978, Roy and Legendre 1979). During exponential growth increases in both F and F_{DCMU} parallel changes in the photosynthetic rate. The inverse relationship predicted by the model occurs only during stationary phase (Prèzelin 1981).

1.6 Observed Trends in the Field

Changes in absolute value of F and F_{DCMU} are less useful as a measure of growth in the field because of the variability in fluorescence per unit chlorophyll between different species. Expressed on a relative scale however, the fluorescence measurements may give some information about population dynamics. Vincent (1981) found that a large variation in cellular photosynthetic capacity (roughly equivalent to the F ratio) was correlated to a major shift in species composition. Fukazawa et al (1980) found that a large increase in F ratio preceded a dinoflagellate bloom and did suggest that the method was suitable for estimating growth rates of natural populations. Low DCMU-enhanced fluorescence ratios are often observed in aphotic populations (Cullen and Renger 1979, Vincent 1979, Harris 1980). It may be that this is a result of an accumulation of inactive fluorescence (Cullen and Renger 1979, Vincent 1981). The F ratio would thus give an indication of the proportion of "active" cells in a sample rather than a measure of the average physiological condition.

Vincent (1980) states that "considerable uncertainty plagues the relationship between DCMU-induced fluorescence and algal productivity". A survey of various attempts at correlating photosynthesis to the F ratio in natural populations shows this to be indeed true. Kimmel and White (1979) found that the two measurements

followed similar trends but made only four observations. Roy and Legendre (1979, 1980) report significant correlations between these parameters in the field, however a closer examination of their data shows that in no case did r^2 exceed .53. Both Vincent (1981) and Prèzelin and Ley (1980) observed a closer relationship between the two parameters in natural populations. In contrast, Harris (1980) and Sephton (1980) found no correspondence between F ratios and P_{max}. Harris (1980) suggests that this is the expected result in a rapidly changing environment in which one parameter (fluorescence) reflects changes occurring at scales of minutes while the other (P_{max}) is variable at scales of hours and days.

1.7 Nutrient enrichment in culture

Enrichment experiments may be conducted over a period of hours or days (Vollenweider 1969). Both have their problems. Long term enrichments are affected by alterations of light, grazing, natural loadings and regeneration of nutrients as well as by the prolonged enclosure of small volumes of water (Healey 1979).

A review of the problems associated with short term bottle assays may be found in Harris (1978), Healey (1979), Lean and Pick (1982), Lean et al (1983a,b). Nutrient deficient algae are rich in carbohydrate or lipid, have low photosynthetic and growth rates, and are low in RNA and protein (Healey 1979, Rhee 1979, Syrett 1981).

It might be expected that metabolism and energy flow would be directed toward nutrient uptake prior to any increases in photosynthesis. Lean and Pick (1982) suggest that a better indicator than simple ^{14}C uptake is some ratio of optimum photosynthetic rate and maximum nutrient uptake rates.

Both photosynthesis and respiration have been shown to be affected by nutrient additions to nutrient depleted cultures. Healey (1979) found elevated rates of respiration in Scendesmus while Turpin (1983) and Healey (1979) found depressed ^{14}C uptake following nutrient addition. On the other hand Goldman et al (1981, 1982) found no short term interactions of photosynthesis and nitrogen uptake in marine phytoplankton.

More direct evidence for the involvement of the primary events of photosynthesis comes from the work of Kessler and Zumft (1973), who found decreased fluorescence induction in the first few seconds following additions of nitrite to Chlorella. Larsson, Ingemarsson and Larsson (1982) found increased O_2 evolution following addition of NO_3^- to Scendesmus for cells which were grown at P_{max}. The capacity for non-cyclic electron flow allowed simultaneous reduction of NO_3^- and CO_2.

1.8 Nutrient enrichment in the field

On the basis of two experiments (one over a period of 6 hours, the other over a period of 5 days) conducted in New Zealand lakes, Vincent (1981) suggests that enhanced F ratios following nutrient addition are indicative of increased photosynthetic potential in these populations. Both reduced carbon uptake in natural populations (Falkowski and Stone 1975, Lean and Pick 1981, Lean et al 1982) and enhancement of ^{14}C uptake (Goldman 1972) have been observed in natural populations following nutrient additions.

1.9 Diurnal patterns of fluorescence

Diurnal patterns of fluorescence are known to exist in both freshwater and marine systems (Loftus and Seliger 1975, Karabashev and Solov'yev 1976, Prèzelin and Ley 1981, Vincent 1981). Diurnal patterns of photosynthesis have been shown to exist in Lake Ontario using both ^{14}C uptake and O_2 electrode techniques (Harris 1973a, Stadlemann et al 1974). The ability to predict daily photosynthesis is affected by the variance imposed on any model by diel photosynthetic rhythms (MacCaull and Platt 1975, Prèzelin and Ley 1980). In simplistic terms, there are two explanations for the existence of these rhythms of photosynthesis in phytoplankton:

1) the rhythm is controlled by some intrinsic "biological clock"

2) some environmental variable "forces" the rhythm according to the oscillation of the external factor (MacCaull and Platt 1975).

The two observations are not independent in that a clock-controlled rhythm is also entrainable to the period of the exogenous oscillator. For example, a light-dark period may not create a rhythm in photosynthesis but rather provide a cue to the cell such that the biological and environmental events are coupled (Chisholm 1981).

There is also much evidence that light alone is a strong forcing factor of photosynthetic rates. Terrestrial plants are exposed to a light regime which is relatively constant (depending on latitude) in terms of length but variable in accord with local weather conditions. The light regime of the phytoplankton is dependent on the mixing rate of the water column in which it is suspended. Depending on the speed of circulation the "average" light period which a cell experiences may vary from seconds to hours. Depending on the depth of the mixing zone that period may occur once, less than once or more than one time per day (Harris 1973b, Marra 1980).

Accurate modelling requires a knowledge of which of these two factors, an intrinsic circadian rhythm or a response to light, is responsible for the diurnal patterns of photosynthesis.

1.10 Circadian rhythms in culture

A method of detecting circadian rhythms in algal cultures is to determine whether a rhythm which exists in cultures grown under a day-night cycle persists under conditions of constant light. In this way, laboratory experiments have shown the existence of clock-controlled patterns of both light limited and maximal photosynthesis as measured by ^{14}C uptake in marine dinoflagellates and diatoms (Prèzelin and Sweeney 1977, Harding et al 1982). Brand (1982) found evidence of circadian rhythms of chlorophyll fluorescence and DCMU-enhanced fluorescence in 24 algal species of widely varying phylogenetic background.

1.11 Circadian rhythms in the field

Prèzelin and Ley (1980) found that the daily periodicity of photosynthesis observed in natural populations dominated by marine diatoms was largely due to a circadian rhythm of photosynthesis and that the natural periodicity in ^{14}C uptake correlated well to the periodicity in F and F_{DCMU}. Diurnal periodicities of photosynthesis have also been observed in Lake Ontario (Harris 1973a, Harris and Lott 1973, Stadlemann et al 1974). Vincent (1979) observed diurnal rhythms in F in several lakes in New Zealand. The persistence of the rhythm under continuous low light suggested the existence of a circadian component to the observed rhythms.

1.12 Light effects in culture

The fluorescence of both marine and freshwater diatoms is depressed within 5 minutes exposure to light of high intensity (Kiefer 1973b, Heaney 1978). In cultures of Lauderia borealis Kiefer (1973b) found that bright light (approximately 1000 μE M^{-2} sec^{-1}) caused a depression in fluorescence which could be separated into a fast (approximately two minutes) and a slow response (thirty minutes). The response was dependent on nutrient status of the cells (see below).

The fast component was not accompanied by a change in the absorption properties of the cell at 440nm. Microscopic examination, however, showed that the chloroplasts had undergone visible contraction (Kiefer 1973b). Mohanty and Govindjee (1973) and Krause (1973) did find alteration in absorbance character at 535 nm for isolated spinach chloroplasts and cells of Anacystis nidulans within the first minutes of illumination. [This effect was also observed in natural populations by Harris (1980).] The differences between their results and those of Kiefer (1973b) may have been due to a species or a treatment effect. Mohanty and Govindjee (1973) found that the time course of slow fluorescence changes was more rapid than for absorbance changes. They suggested that "microscopic" changes in the chloroplast appear to precede changes at the macroscopic level.

The slow decrease in fluorescence in diatoms appears to

correspond to a redistribution of the chloroplasts to the valvar ends of the cell (Kiefer 1973b, Harris and Piccinin 1977). Gross morphological changes in chloroplasts of other species have not been well described. Loftus and Seliger (1975) however noted that large diel changes in fluorescence only occurred when diatoms were present in the species assemblage. Harris (1980) noted differences in the Kautsky induction curves of individual cells of green and diatom species from Hamilton Harbor.

The physiological status of the cell may also affect the response to bright light. Kiefer (1973b) and Kiefer and Hodson (1974) noted that the amount of decrease was much larger in the more nutrient deficient cells. Loftus and Seliger (1975), on the other hand, found that the final fluorescence yield in stationary and log phase cultures of Phaeodactylum was similar. When expressed as a percent the results of Roy and Legendre (1979) also suggest that there is no difference between the response to light of nutrient stressed and nutrient sufficient green algae. It should be mentioned that the light intensity used by Roy and Legendre (1979) (160 $\mu E\ m^{-2}\ s^{-1}$) was less than that used by Kiefer (1973b) (near 1000 $\mu E\ m^{-2}\ s^{-1}$). This may have contributed to the difference in the results.

1.13 The effect of light on natural assemblages

The sensitivity of a population to light appears to be variable. Loftus and Seliger (1975) found diurnal changes occurred in populations of diatoms but not in populations dominated by dinoflagellates or diatoms. Harris and Piccinin (1977) and Harris (1980) found differences between the response to photoinhibiting light intensities of diatoms and green algae fromn Hamilton Harbor.

Various intensities have been observed as threshold irradiances i.e. the irradiance below which light has no effect when compared to a sample maintained in the dark. Heaney (1978) and Vincent (1981) found threshold values for populations of phytoplankton in lakes in England and New Zealand of around 100 $\mu E\ m^{-2}\ s^{-1}$ while Kiefer (1973a) recorded 475 $\mu E\ m^{-2}\ s^{-1}$ for marine populations.

Light history, as well, as nutrient status, is known to affect the structure of the chloroplast and the photosynthetic efficiency of algae. "Shade adapted" species are known to have a large complement of chlorophyll and saturate photosynthesis at lower light intensities than sun species (Harris 1978). Prèzelin (1981) suggests four mechanisms by which "adaptation" may occur:

1)changes in the size of the photosynthetic unit

2)density of photosynthetic units

3)changes in levels of enzymes

4)efficiency of coupling

It is interesting to consider which of these mechanisms might affect the fluorescence response. Neither changes in enzymatic rates nor an increase in the density of photosynthetic units (assuming the units have a similar structure) should affect the F ratio. On the other hand an increase in the size of the PSU should result in a decreased $F_{DCMU}/chla$. A change in the state of the thylakoid membranes and the efficiency of photosynthetic coupling might also be reflected by fluorescence properties to fixed light. Kulandaivelu and Senger (1976) found decreased efficiencies of photosynthetic coupling attributable to decreased levels of plastoquinone after maintaining Scenedesmus for 2 to 4 days in the dark. The recovery of efficient photosynthetic coupling occurred within hours but was inhibited by bright light.

Evidence of the close coupling of photosynthesis to light and the influence of variable light comes from measurements of photosynthesis made at short time intervals with an O_2 electrode (Harris 1973a,b, Harris and Lott 1973, Marra 1978a,b, Marra 1980, Marra and Heinemann 1983). Marra (1978b) found enhancement of integral photosynthesis in samples which were moved vertically through the water column as compared to bottles suspended at fixed depths. The effect was not totally explained by an inhibition of the maximum rate of photosynthesis. In this experiment, as in Harris (1973a), the data suggests a non-linear response of photosynthesis to light intensity.

In laboratory experiments photosynthesis was found to track irradiance closely on cloudy days and on days of variable light intensity but not on sunny days (Marra and Heinemann 1983). Extremely rapid changes (seconds) in fluorescence attributed to changes in the re-dox level of plastoquinone have been recorded in natural populations using more sophisticated fluorometers (Vincent 1979, Abbot et al 1982).

The depression of fluorescence at the surface in vertical profiles of the water column is usually attributed to light (Kiefer 1973b, Loftus and Seliger 1975, Cullen and Renger 1979, Harris 1980, Vincent 1980). This is confirmed by experiments in which short incubations (minutes) at high light intensity cause rapid depressions in the fluorescence of natural populations (Kiefer 1973b, Loftus and Seliger 1975, Harris 1980). The recovery of fluorescence in the dark following these types of light treatment was somewhat slower than the rate of decrease in the light.

Because of the variability in the rate at which phytoplankton are moved through the water column there is no simple empirical relationship between light intensity and the depression of surface fluorescence. Instead Harris (1980) and Sephton (1980) suggest that depression in F ratio only occurs when wind speeds are low. This relationship may be more precisely expressed by relating the change in F ratio to the Richardson number. The Richardson number, is a ratio of buoyancy to turbulent kinetic energy induced by wind and thus should indicate the relative rate of mixing within the water column (Harris 1983).

2. The Thesis Problem

It is the broad objective of this thesis to examine the use of fluorescence and DCMU-enhanced fluorescence as an indicator of the physiological status and light history of natural assemblages of phytoplankton.

Changes in the fluorescence parameters of batch cultures of Chlamydomonas and Synnechococcus will be compared to other physiological indicators. The response of the F ratio and ^{14}C uptake following nitrogen additions to batch cultures during exponential and stationary phase growth will also be examined. A comparison of seasonal changes in F ratio, P turnover time and ^{14}C uptake in the phytoplankton will be made. The potential use of the F ratio as an indicator of physiological condition in the field is discussed in the context of these results.

Diurnal patterns of fluorescence are described. The possibility that these patterns are due to an intrinsic biological rhythm is tested. The response of fluorescence to light (in particular, the rate at which fluorescence responds to light and changes in the response to light over the season) are examined in detail. The hypothesis (section 1.13) that it is the mixing rate of the water column which determines whether fluorescence will be affected by light is tested. The general relationship between the physical character of the water column (as measured by temperature and density gradients) and fluorescence is described.

3. Materials and Methods

3.1 Measurement of physiological condition in culture

3.1.1 Culture Methods

Batch cultures of Chlamydomonas reinhardii or Synechococcus leopoliensis were maintained in 1 liter flasks on a shaker using Chu 10 media with added Wood's Hole micronutrients (Nichols 1973). Cultures were inoculated with 20 milliliters of cells maintained in the same media and in stationary phase. The pH of the original media was 6.4 and rose to a pH of 7.0-7.5 during growth. Growth limitation by nitrogen was achieved by reducing the nitrogen in the media to 1% (30 μg NO_3-N L^{-1}) of the original concentration. C:N:P ratios in ug-atoms in the media were:

1) for complete media 130:190:100

2) for N-limited media 130:2:100

Light intensity was 160 μE m^{-2} s^{-1} under a 12:12 LD regime. Photosynthetically active radiation within the 400-700 nm waveband was measured with a quantum light sensor (LICOR).

Measurements were made daily 9 hours after the lights came on. Cell number was measured on a Coulter Counter (Model F). Diel growth rate was calculated according to Fukazawa et al (1980). Chlorophyll a was measured using the modified acetone extraction technique of Burnison (1980). Filtration at this level of chlorophyll is not necessary. 2.1 milliliters of culture was frozen and then thawed prior to the addition of 4 milliliters of DMSO. The solution was made up to a final concentration of 90% acetone and the fluorescence of the extract determined fluorometrically (see below). Fluorescence measurements were calibrated to the concentration of chlorophyll a measured spectrophotometrically using the equations of Jeffrey and Humphrey (1975) and expressed in micrograms per milliliter of culture ($\mu g mL^{-1}$).

Continuous cultures of Chlamydomonas were maintained in 1 liter round-bottom flasks using Basic Bold's media (Nichols 1973). The concentration of N was reduced to 170 $\mu g\ L^{-1}$ and C (2000 $\mu g\ L^{-1}$) in the form of $NaHCO_3$ added such that the final C:N:P ratio in the media was 78:70:10 and 78:7:10 as µg-atoms.

3.1.2 Fluorescence measurements

25 milliliter samples were poured into 25X148 mm pyrex test-tubes and fluorescence measured using a Turner Designs Model 10 fluorometer equipped with a red-sensitive photomultiplier. The filters and lamp were as recommended by the manufacturer (Blue lamp; excitiation filter, Corning 5-60; reference filter, Corning 3-66; emission filter, Corning 2-64) for use with *in vivo* fluorescence of chlorophyll *a*. DCMU (3-(3,4-dichlorophenyl)-1,1-dimethyl urea) was added to a final concentration of $1X10^{-5}$M and a second fluorescence measurement (F_{DCMU}) made after 10 minutes.

3.1.3 ^{14}C Uptake

^{14}C uptake in cultures was measured according to the method of Lean and Burnison (1979) by adding 100λ 50 μCi mL^{-1} $NaH^{14}CO_3$ to 25 milliliter samples and incubating for 10 minutes beside the stock cultures. Duplicate 10 milliliter samples were filtered onto .45 μ Millipore filters. The filters were acidified with 100λ 1N HCl and left for at least 2 hours before dissolving in ACS (aqueous counting scintillant--Amersham). Samples were counted using a Beckman LS230 and corrected for colour quenching using the channels ratio method (Wang *et al* 1975). Controls (either samples treated with DCMU before addition of $H^{14}CO_3^-$ or those left in the dark) were subtracted from all sampes.

3.2 Measurement of physiological condition in the field

3.2.1 Study Site

Field work was conducted at two shallow and one deep station on Lake Ontario, Ontario, Canada. Their characteristics are described in Table 3.1. Several experiments were also conducted in Jack's Lake, Ontario, Canada (44°41'N, 78°03'W). This lake is 60 kilometers northeast of Peterborough on the edge of the Canadian shield.

3.2.2 Fluorescence Measurements

Fluorescence was measured as described above (section 3.1). The fluorometer was set to zero using lake surface water filtered through .2 μ Nucleopore filters. There was negligible difference between the standardization obtained with this water and with distilled water throughout the season. Consistency of measurement was tested by measuring the fluorescence of 25 milliliters of PCS (aqueous counting scintillant). The filtrate was checked periodically for dissolved fluorescence.

Table 3.1 Characteristics of Lake Ontario study sites

STATION	LATITUDE	LONGITUDE	DEPTH (meters)
401	43°53'48''N	78°17'00''W	38
403	43°35'50''N	78°13'48''W	176
405	43°23'56''N	78°11'50''W	38

3.2.3 Sensitivity of the method

An estimate of the errors associated with sampling and measurement was made by determining the difference between duplicate samples at each depth for the profiles discussed in section 3.3. The differences between replicates from all depths were averaged for each date. This average was used to determine when real differences existed between treatments and samples. A difference between two numbers greater than twice the error estimate was considered real.

Error estimates ranged from .02 to .12 relative units for F; .03 to .15 relative units for F_{DCMU}; .02 to .08 for the F ratio. Because biomass (expressed as F or F_{DCMU}) changed over an order of magnitude over the season results will often be expressed on a relative basis. On this basis error estimates ranged from 3 to 16% for F; 3 to 6% for F_{DCMU}; 4 to 16% for the F ratio. Thus a difference between two values greater than .30 relative units (30% for F), (15% for F_{DCMU}) is considered real. A value greater than .16 (30%) is considered to represent a real difference for the F ratio. Differences stated to be significant in the text do not refer to true statistical significance but refer to differences which exceed twice the estimated errors of measurement and sampling.

3.2.4 Chlorophyll a

Chlorophyll a was measured spectrophotometrically by the Water Quality Division CCIW (Burlington) (Environment Canada 1979).

Chlorophyll a was measured using a hot DMSO/acetone extraction technique (Burnison 1980). In the field 100 milliliters of lake water was filtered onto GF/F filters and frozen for storage. These samples were later thawed and 4 milliliters DMSO was added to the samples prior to heating for 10 minutes at 65°C. Field samples were refiltered through a GF/C filter and the filtrate made up to 25 milliliters total volume with 90% acetone. The fluorescence of the extract was measured using the Turner Designs Fluorometer described above and calibrated using regression analysis (Parker 1976) to the chlorophyll a values obtained by Canada Center for Inland Waters (Burlington). r^2 values ranged from .77 to .98 and except for the Octboer sample, correlations were significant at the p=.001 level. The correlation coefficient for the October sampling was significant at p=.01.

3.2.5 Fluorescence Indices

The fluorescence indices used were:

$$1)\ \text{F ratio} = \frac{F_{DCMU} - F}{F_{DCMU}}$$

This ratio is equivalent to the FRI of Cullen and Renger (1979) or the F ratio of Kimmel and White (1973). It is roughly equivalent to the CPC used by VIncent (1979, 1980) except that no dark pretreatment was used. This ratio is convenient to use in that it scales from 0.0 to 1.0. Other workers often express DCMU-enhanced fluorescence as a ratio of F_{DCMU}/F (c.f. Blasco and Dexter 1973, Samuelsson and Oquist 1977, Harris 1980).

$$2)\ F_{DCMU}/\text{chl}\underline{a} = \frac{F_{DCMU}}{[\text{chlorophyll } \underline{a}]}$$

$$3)\ F/\text{chl}\underline{a} = \frac{F}{[\text{chlorophyll } \underline{a}]}$$

wwhere chlorophyll _a_ is expressed in $\mu g\ L^{-1}$. F/chl _a_ is equivalent to the R value used by Heaney (1978) and Harris (1980).

3.2.6 Indices of the physical character of the water column

Estimates of water column stability (as N^2) were obtained by determining density differences between surface and 5 meter or 10 meter water.

$$N^2 = \frac{(P_{d1} - P_{d2})}{d} \times g$$

(Phillips 1966)

where P_{d1} and P_{d2} are the densities of the two depths; d is the distance between the two depths in centimeters, and g is the acceleration due to gravity. The density values were obtained by obtaining the specific temperature of the depths in question and using the equation of state for pure water:

$$P_d = P_{4^oC} \times 1 - [7.11 \times 10^{-6} (T - 4)^2]$$

where T is the temperature in degrees celsius of the water and P_{4^oC} is the density of water at 4^oC. N^2 estimates ranged from 0 to $10^3 \times 10^{-6}$ s^{-2}.

Temperature was measured to .1 ^oC using the EBT aboard C.S.S. Limnos. The difference between the temperature at any two depths is the ΔT.

3.2.7 ^{14}C uptake

In situ ^{14}C measurements were made according to the method of Lean and Burnison (1979). R.L. Cuhel and D.R.S. Lean conducted most of the experiments. 30 μCi $H^{14}CO_3^-$ were added to 300 milliliter glass BOD bottles filled with lake water. Samples were collected as described above from 2.5 m intervals through the photic zone, at three times during the day (approximately 06:00, 10:00, 14:00) and incubated at the depth from which they had been removed.

Incubations lasted four hours except for a duplicate set from 06:00 which was incubated until dusk. Darkened bottles from each depth, at each time, were incubated either *in situ* or in the incubator maintained at a temperature close to that of the epilimnion. Fluorescence measurements were made before and after each incubation.

Following incubation 100 milliliters was filtered onto a GF/F filter, acidified with 200λ1N HCl for several hours, and placed in PCS (aqueous counting scintillant). Samples were counted in liquid scintillation counters. Determination of P_{max} was made at fixed light in a rotating wheel type incubator as described below (section 3.3). Differences in values between two depths greater than 15% are considered significant.

3.3 Comparison of seasonal patterns of P turnover time and fluorescence

P turnover times were provided by D.R.S. Lean using the method of Lean and Nalewajko (1979). Representative fluorescence measurements were chosen from a series of diurnal profiles for which the effect of light was thought to be minimal. Except for 3 occasions [22 September (station 403), 30 June (station 401), and 19 October (station 403)], measurements were made either after sunset or before sunrise (between 18:00 and 06:30).

3.4 Nutrient additions to cultures

Cell number, F ratio and chlorophyll *a* were monitored daily as described above (section 3.1). Cessation of cell division as opposed to the end of the increase in any of the fluorescence properties was arbitrarily chosen to indicate the onset of the stationary phase in culture.

30 $\mu g\ N\ L^{-1}$ were added either in the form of NO_3^- or NH_4SO_4 several minutes prior to the lights coming on. Replicate cultures were used on two occasions as described in the text. Duplicate subsamples were removed and ^{14}C uptake measured as described above

(section 3.1.3). Fluorescence was determined prior to the incubation. DCMU was used to block ^{14}C uptake after 20 minutes. DCMU-enhanced fluorescence was measured 10 minutes after addition of DCMU. ^{14}C uptake was measured prior to nutrient additions, at 40 minute intervals for the first 1.5 hours, and then at approximately 2.5 hour intervals for the next 10.5 hours. The data is expressed as the fraction incorporated into cellular material relative to the total added ^{14}C.

3.5 Nutrient additions in the field

Throughout the season either thrice rinsed 300 milliliter BOD bottles or clear plastic 250 milliliter polycarbonate bottles were filled with lake water and placed in two rotating wheel type incubators. Temperature was maintained at a level similar to that of the original water (except as noted for individual experiments). 4 or 5 light levels (L1, L2, L3, L4, L5) ranging from 5 to 1680 $\mu E\ m^{-2}\ s^{-1}$ (depending on the incubator) were used. The measured intensities are indicated in Table 3.2. 10 to 100 $\mu g\ L^{-1}$ N in the form of $(NH_4)_2SO_4$ or KNO_3 were added to one bottle at each light level. Length of incubation was four to sixteen hours (see individual experiments). 10 to 100 $\mu g\ L^{-1}$ P was added in the form of KH_2PO_4 at L_3. The length of these incubations was 3 hours. F and F_{DCMU} were determined immediately prior to and after incubation. The length of time that samples were exposed to room light was less than 10 minutes.

Table 3.2 Light intensity in $\mu E\ m^{-2}\ s^{-1}$ at the 5 light levels (L1 to L5) used in experiments in the incubator

Date	L1	L2	L3	L4	L5
28 April	900	230	105	8	
18 May	1330	450	112	7	
1 July	770	259	88	4	
2 July	1890	756	336	140	53
3 July	1680	756	336	140	53
18 August	1680	756	336	140	53
19 October	1350	510	200		55
22 November	1300	750	250		85

Water chemistry analyses were carried out by the Water Quality Division (CCIW Burlington) according to the Analytical Methods Manual (Environment Canada 1979). Measurements of NO_3^- and NH_4, and the ratio of particulate C to particulate N are described in section 4.4.

3.6 Diurnal patterns of fluorescence

F and F_{DCMU} were measured as described above (3.2) at intervals ranging from 3 to 6 hours during a 24 hour period. The sampling interval was varied in order to ensure that on each occasion at least one measurement was made shortly after sunrise, at mid-day, shortly after sunset and during the middle of the night. On occasion subsamples were removed and placed in a dark incubator at a temperature similar to that of the water column ($\pm 2^{o}C$) for 1 hour prior to a second measurement of F and F_{DCMU}.

3.7 Circadian rhythms

Water from 5 meters was placed in rinsed 300 milliliter BOD bottles and placed in an incubator at 40 $\mu E\ m^{-2}\ s^{-1}$ at a temperature within $2^{o}C$ of that in the water column for a period of 24 hours.

3.8 Time Course of Fluorescence Changes

To examine the time course of fluorescence changes water was incubated at constant light intensity as described above (3.4). Samples were taken at intervals ranging from 10 minutes to several hours (see individual experiments). The length of time that samples were exposed to room level light was about 5 minutes.

3.9 Recovery in the dark

Water was incubated at constant light intensity as described above (section 3.4). The lights in the incubator were then either shut off or the samples were moved into another darkened incubator. Fluorescence was measured prior to and after placement in the dark.

3.10 Seasonal Response to Fixed Light

Water was incubated without nutrient additions as described above (section 3.4). Duration and intensity of light for each incubation is described in chapter 4.

3.11 The effect of light and mixing

300 milliliter glass BOD bottles were rinsed three times and filled with lake water sampled from 2.5 meter intervals through the water column to a depth of 20 meters. The bottles were suspended in the water column for periods of time ranging from 3 to 15 hours. ^{14}C uptake was measured at this time. F and F_{DCMU} were measured prior to and after incubation and compared to the fluorescence profile in the water column made near the end of the incubation.

3.12 Light response from different depths

Water from different depths was incubated as described above (3.4); see text for details of depths sampled.

4. Results

4.1 Observed patterns of physiological condition and fluorescence in culture

4.1.1 Continuous Cultures

Table 4.1 describes the fluorescence characteristics of Chlamydomonas grown in "cyclostat" culture in modified Bold's media (C:N:P 78:70:10) under a 16:8 LD cycle. Both biomass (F) and F/chla were highest at the lowest dilution rate. There were no significant differences in F ratio at the different dilution rates.

The concentration of N was reduced by a factor of 10 in order to ensure nitrogen-limitation (as opposed to carbon or light limitation). At this level of nitrogen (17 $\mu g\ L^{-1}$) washout occurred at the highest dilution rate and biomass (F) decreased at the lower dilution rates. F/chla and the F ratio were similar at both levels of nitrogen.

Table 4.1 Comparison of fluorescence properties of "cyclostat" cultures of Chlamydomonas at different dilution rates and nitrogen levels; values represent the means and standard errors of the mean; dilution rate expressed day^{-1}; C:N:P expressed as µg-atoms in the media.

DILUTION RATE	C:N:P	F	F/chl*a*	F ratio
1.2	78:70:10	.105±.15	.12±.04	.68±.02
.6		.177±.29	.14±.03	.67±.03
.23		.320±.38	.24±.05	.67±.02
-	78:7:10	-	-	-
.6		.095±.007	.14±.03	.67±.02
.23		.231±.051	.16±.06	.67±.02

4.1.2 Batch Culture

Figure 4.1 shows that Chlamydomonas in N-limited media underwent only a short exponential phase. As is evident from the difference in the slopes of the lines, cell number and in vivo fluorescence (F) increased at different rates. Thus growth could not be described as balanced in these cultures. If 4 days is considered the end of exponential phase, it may be seen that F and F_{DCMU} (and hence the F ratio) changed little during the first 3 days of stationary phase. This is also evident from Figure 4.2a in which growth rate is compared to F ratio. F ratios greater than .70 occurred both before and after the onset of stationary "phase" on day 4. F ratios of .45-.55 occurred only during later stages of stationary growth. Even after 7 days in stationary phase the F ratio had only declined to a value of .48 (64% of the highest observed value).

Synechococcus underwent a 5 day exponential growth phase in which all four parameters F, F_{DCMU}, cell number and chlorophyll a increased at a similar rate (Figure 4.3). Following the onset of stationary phase (day 6), chlorophyll a decreased in these cells. The F ratio tended to be lower in Synechococcus than in Chlamydomonas. The highest value observed was .64 while in Chlamydomonas values greater than .70 occurred even during stationary phase. The decline

Figure 4.1 Growth curve for Chlamydomonas grown in batch culture on Chu 10 with reduced nitrogen.

△—△ cells mL^{-1} (X 10^7)

□--□ chlorphyll *a* ($\mu g\ mL^{-1}$)

●—● F

○—○ F_{DCMU}

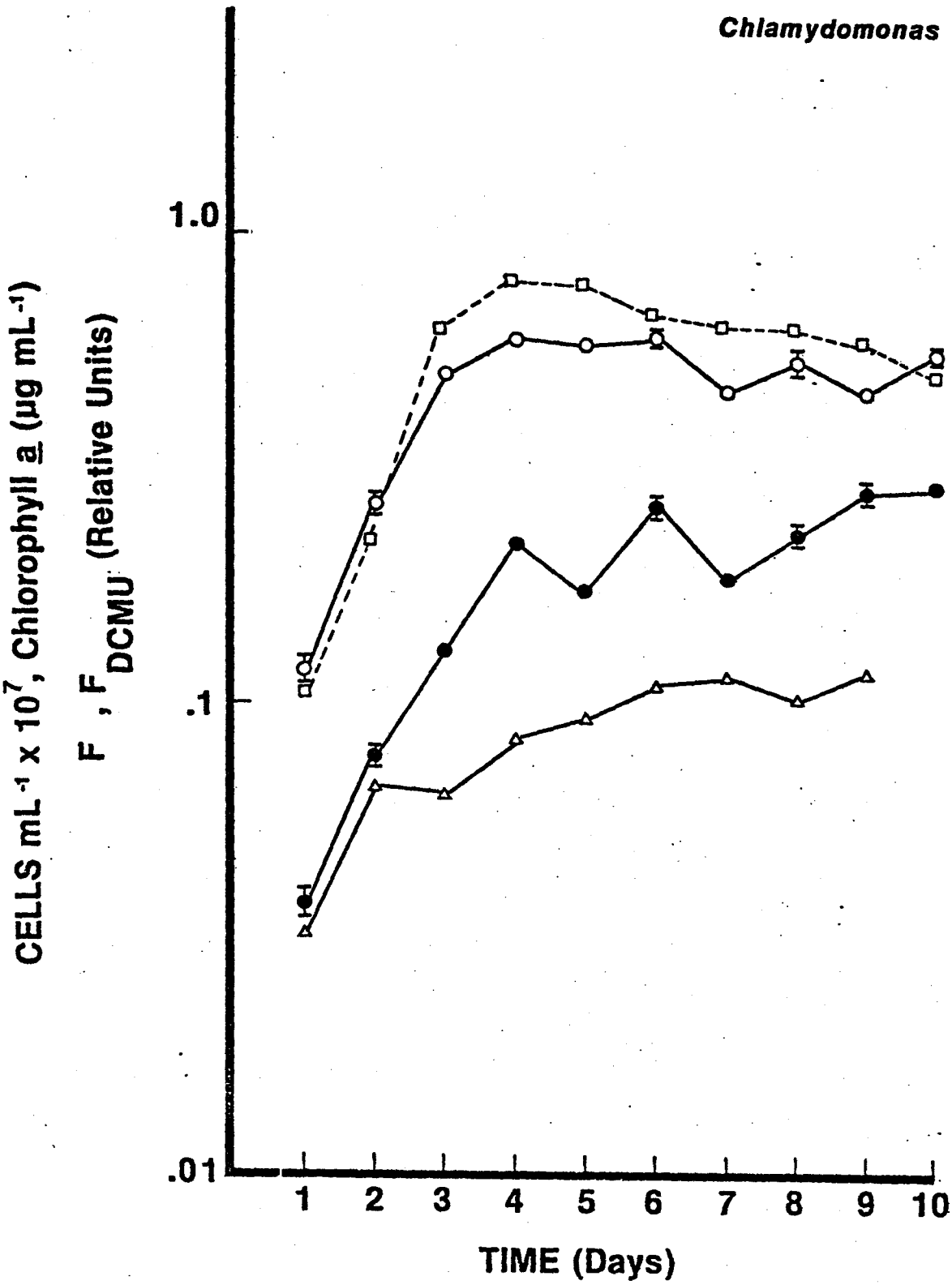

Chlamydomonas
CELLS mL-1 x 10^7, Chlorophyll a (µg mL-1)
F , F DCMU (Relative Units)
1.0
.1
.01
1
2
3
4
5
6
7
8
9
10
TIME (Days)

Figure 4.2 Comparison of growth rate and F ratio in
a) Chlamydomonas and b) Synechococcus

●—● F ratio

o—o Diel growth rate (determined by the change in cell number)

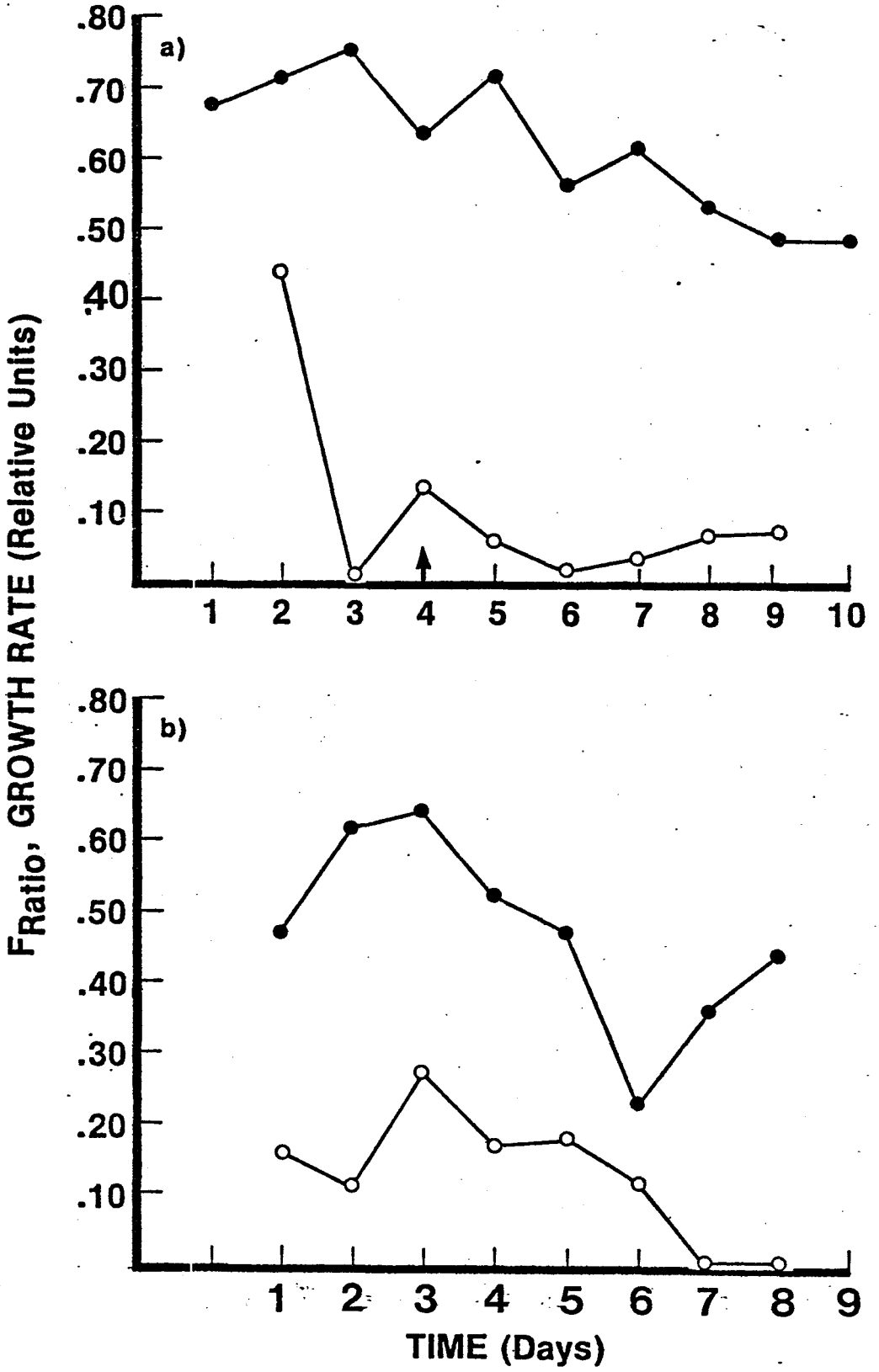
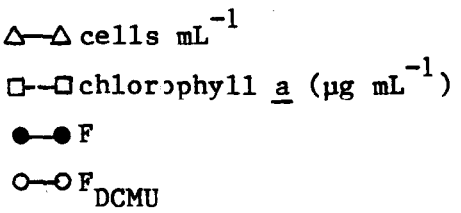
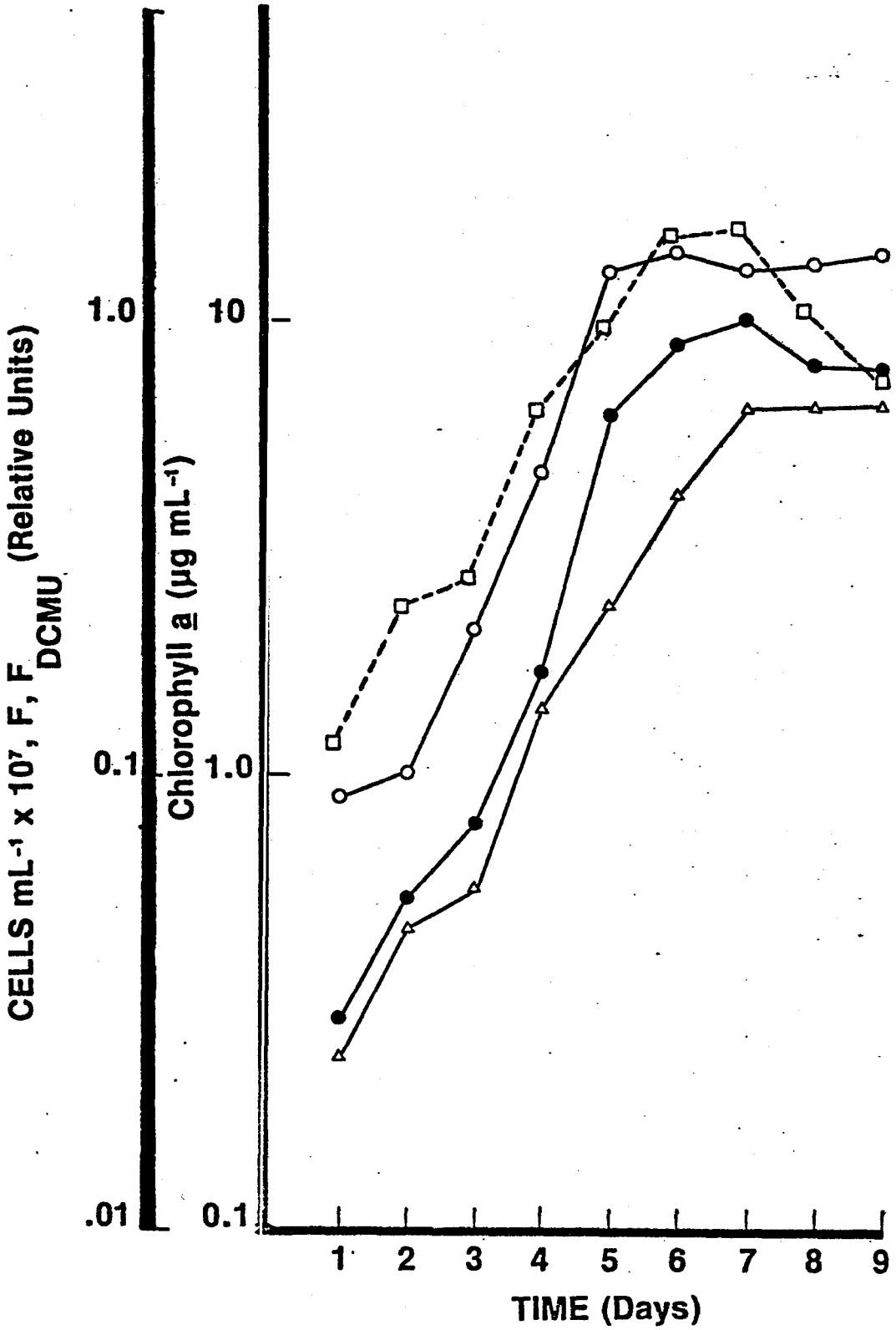

a)
b)
.80
.70
.60
.50
.40
.30
.20
.10
1
2
3
4
5
6
7
8
9
10
F_{Ratio}, GROWTH RATE (Relative Units)
TIME (Days)

Figure 4.3 Growth curve for Synechococcus grown in batch culture on Chu 10 (complete media).




CELLS mL-1 x 10^7, F, F DCMU (Relative Units)
1.0
0.1
.01
Chlorophyll a (µg mL-1)
10
1.0
0.1
1
2
3
4
5
6
7
8
9
TIME (Days)

in F ratio in Synechococcus preceded the cessation of cell division Figure 4.2b) as compared to Chlamydomonas in which the F ratio remained high even after cell division stopped (Figure 4.2a).

Figure 4.4 shows that in Chlamydomonas ^{14}C uptake on both a per cell and per chlorophyll basis decreased exponentially during the later stages of batch growth while the F ratio declined by about 30% from its maximal value.

In Synechococcus there was more variability in ^{14}C uptake however this variability did not correspond to that observed for the F ratio (Figure 4.5). Low rates of ^{14}C uptake were associated with lower F ratios at the end of batch growth but again the change in ^{14}C uptake was larger than that of the F ratio. In both species F/chl*a* increased gradually over the course of growth (Figure 4.4, 4.5). The absolute value of F/chl*a* was higher in Chlamydomonas than in Synechococcus. The absolute value of F/chl*a* for Chlamydomonas was higher in batch than in continuous culture.

Figure 4.4 Changes in photosynthesis and fluorescence during batch growth of Chlamydomonas on Chu 10 with reduced nitrogen. indicates the onset of stationary phase

O—O C uptake (μg C μg chl a^{-1} hr^{-1})

Δ--Δ C uptake (μg C $cell^{-1}$ hr^{-1})

▲—▲ F ratio

●—● F/chl a

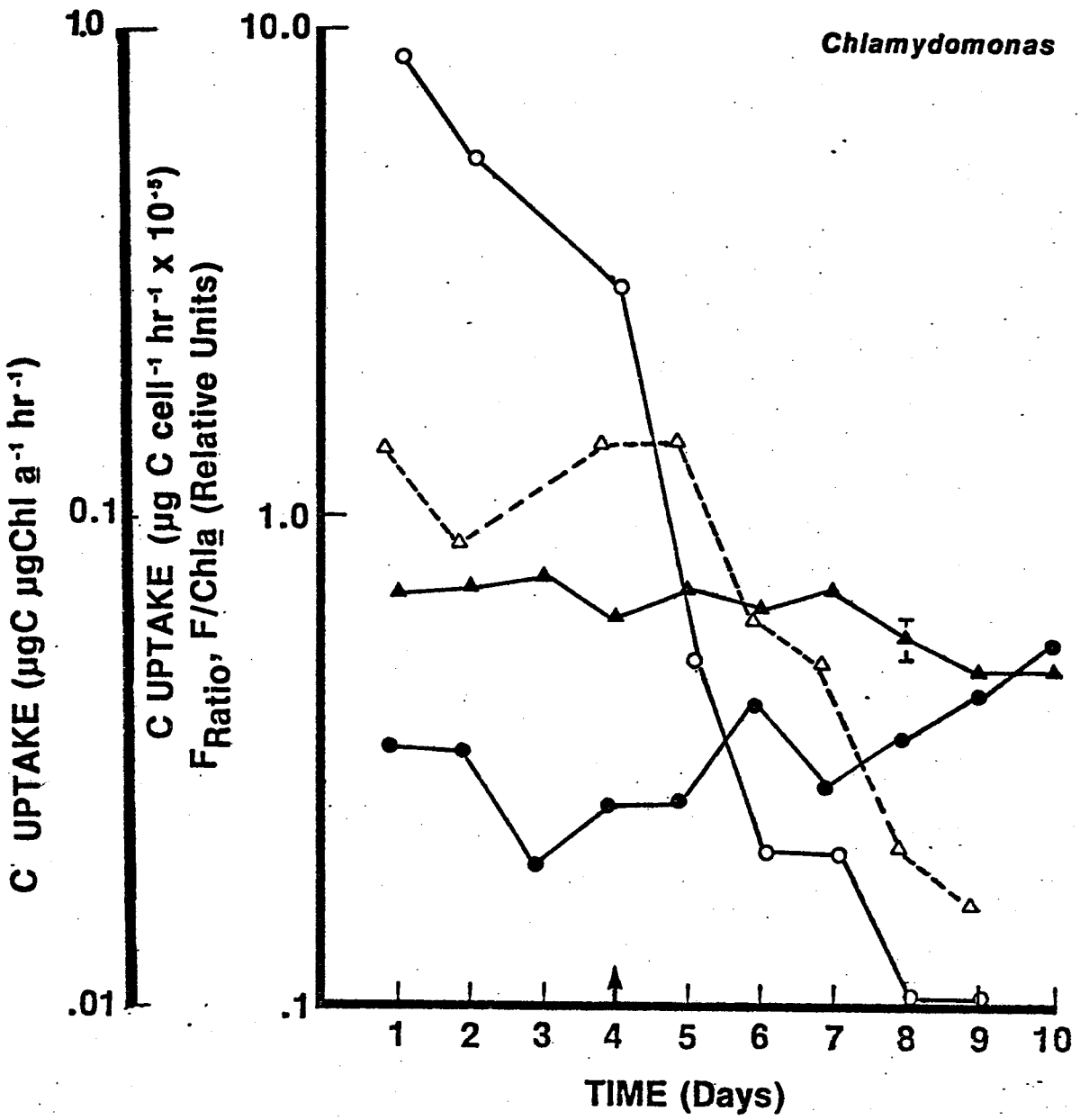
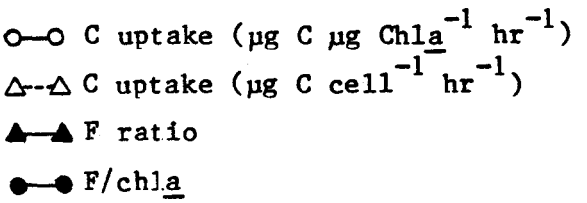
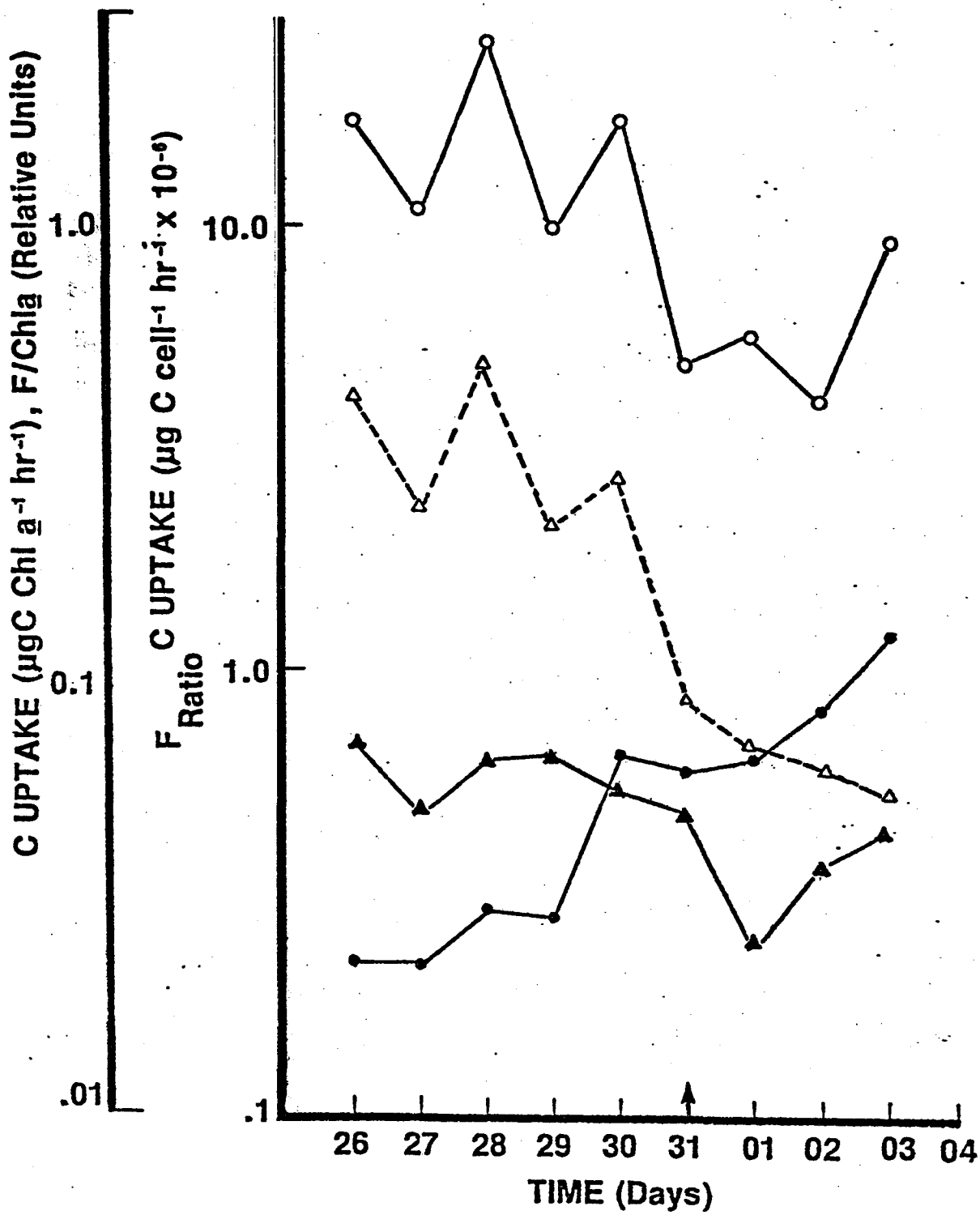

Chlamydomonas
C UPTAKE (µgC µgChl a⁻¹ hr⁻¹)
C UPTAKE (µg C cell⁻¹ hr⁻¹ x 10⁻⁵)
F_Ratio, F/Chla (Relative Units)
1.0
0.1
.01
10.0
1.0
.1
1
2
3
4
5
6
7
8
9
10
TIME (Days)

Figure 4.5 Changes in photosynthesis and fluorescence during batch growth of Synechococcus on Chu 10. ↓ indicates the onset of stationary phase




C UPTAKE (µgC Chl a⁻¹ hr⁻¹), F/Chla (Relative Units)
1.0
0.1
.01
F Ratio
C UPTAKE (µg C cell⁻¹ hr⁻¹ x 10⁻⁶)
10.0
1.0
.1
26
27
28
29
30
31
01
02
03
04
TIME (Days)

4.2 Observed patterns of fluorescence and physiological condition in the field

4.2.1 Seasonal and vertical patterns of chlorophyll a

The greatest biomass of chlorophyll a (7-8 $\mu g\ L^{-1}$) observed at station 403 occurred in late June and mid September. A "bloom" (9 $\mu g\ L^{-1}$) of chlorophyll a occurred at station 401 in September but not in June. The data is included in Appendices 1.1 and 1.2.

Vertical profiles of F, F_{DCMU} and temperature are included in Appendix 1.3 (station 401) and Appendix 1.4 (station 403). These profiles were made prior to dawn or after sunset when light was not expected to have an effect on fluorescence. Visual examination suggests that distinct vertical structures of F or F_{DCMU} occur primarily during periods of vertical structure in the temperature profiles in the water column (e.g 8 June, 11 August, 23 September at station 401).

The relationship between ΔT (the difference in temperature between 1 and 10 meters) and the ΔF_{DCMU} or ΔF (the difference in F_{DCMU} or F between 1 and 10 meters expresed as a percentage of the value at 10 meters) is shown in Figure 4.6 and Figure 4.7. The dashed line is an arbitrarily chosen upper boundary line. The pattern was similar for both ΔF and ΔF_{DCMU}. At high values of ΔT (4-6^{o}C) values of ΔF or

Figure 4.6 Relationship between ΔT and ΔF_{DCMU} (%) where ○ indicates data from a profile made between 20:00 and 06:30; ● indicates data from all other profiles. The dashed line is an arbitrarily chosen upper boundary. ΔT is the difference in temperature between 1 and 10 meters; ΔF_{DCMU} is the absolute difference in F_{DCMU} between 1 and 10 meters expressed as a percentage of F_{DCMU} at 10 meters. Note that when ΔT is small most points are smaller than 15% i.e. the level above which differences are considered real.

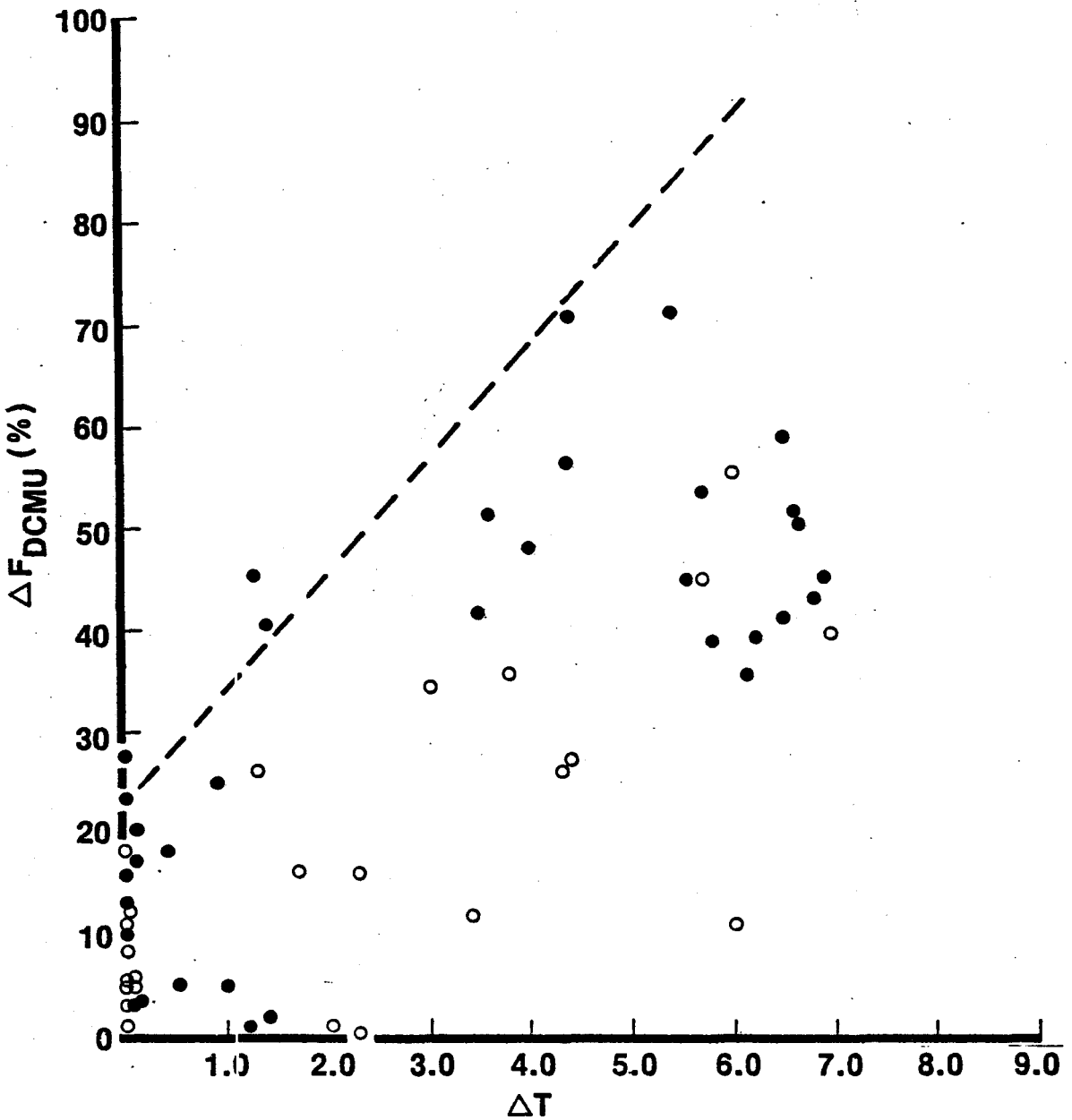
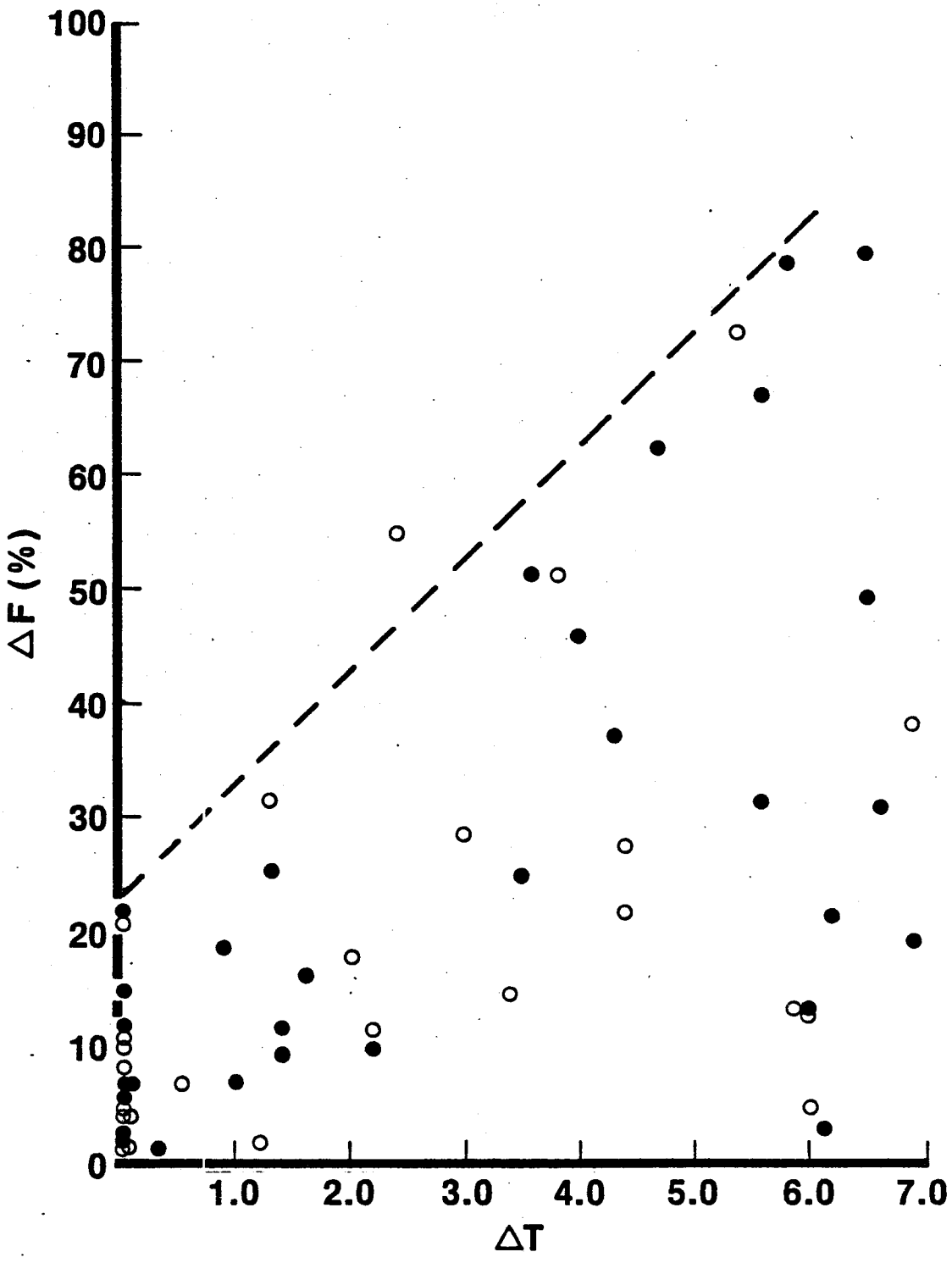

100
90
80
70
60
50
40
30
20
10
0
ΔF DCMU (%)
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
ΔT

Figure 4.7 Relationship between ΔT and $\Delta F(\%)$ where ○ indicates data from a profile made between 20:00 and 06:30; ● indicates data from all other profiles. The dashed line is an arbitrarily chosen upper boundary. ΔT is the difference in temperature between 1 and 10 meters; ΔF is the absolute difference in F between 1 and 10 meters expressed as a percentage of F at 10 meters. Note that when ΔT is small most points are smaller than 30% (the level above which differences are considered real.)


100
90
80
70
60
50
40
30
20
10
0
ΔF (%)
1.0
2.0
3.0
4.0
5.0
6.0
7.0
ΔT

F_{DCMU} up to about 80% were possible. Values not considered significant (i.e. less than 15%) were also possible at large ΔT. At low ΔT (1.0-2.0°C) however, the boundary line did not rise much above 40% and the majority of points were found to be lower than the levels considered to represent significant differences for both ΔF and ΔF_{DCMU}.

The distribution of the ΔF_{DCMU} and ΔF present in night samples (from profiles made between 20:00--06:30) is also shown in Figure 4.6 As indicated by vertical profiles (Appendices 1.3, 1.4) both large and small values of ΔF or ΔF_{DCMU} could occur at night.

The relationship between N^2 and ΔF or ΔF_{DMCU} appeared to be less well defined (Appendices 1.5, 1.6).

4.2.2 Comparison of fluorescence and P turnover time

A comparison of P turnover time and fluorescence at the two stations was made in order to determine whether the two parameters followed the same trends. At the offshore station (403) P turnover time remained high (180 hours) until late June when stratification occurred (D.R.S. Lean unpub. data) (Figure 4.8). Turnover times in the order of minutes were then observed until late October. Neither the F ratio nor F/chla showed a consistent pattern over the season compared to P turnover time. In fact the lowest value observed (.38)

occurred in April when turnover times were long.

At the shallow station (401) stratification had occurred by May and turnover times were already rapid when the first fluorescence measurements were made (Figure 4.9). The values obtained here were generally lower than those observed at the deepwater station (403). Again there was no consistent relationship between P turnover time and the fluorescence measurements. Low F ratios were observed in both October and November samples despite high P turnover times. The October sample was made during an upwelling event and water at this time showed a large component of dissolved fluorescence and contained little chlorophyll _a_. The highest F/chl_a_ for the season was observed under these conditions.

The range of values of F ratio obtained in Jack's Lake, where P turnover time was an order of magnitude more rapid than in Lake Ontario (D.R.S. Lean unpub. data), was similar to that observed in Lake Ontario (data not shown).

Figure 4.8 Comparison of P turnover time, F ratio and F/chl*a* at station 403 (Lake Ontario, 1982).

O—O P turnover time (hours) (D.R.S. Lean unpub. data)

●—● F ratio (relative units)

▲—▲ F/chl*a* (relative units)

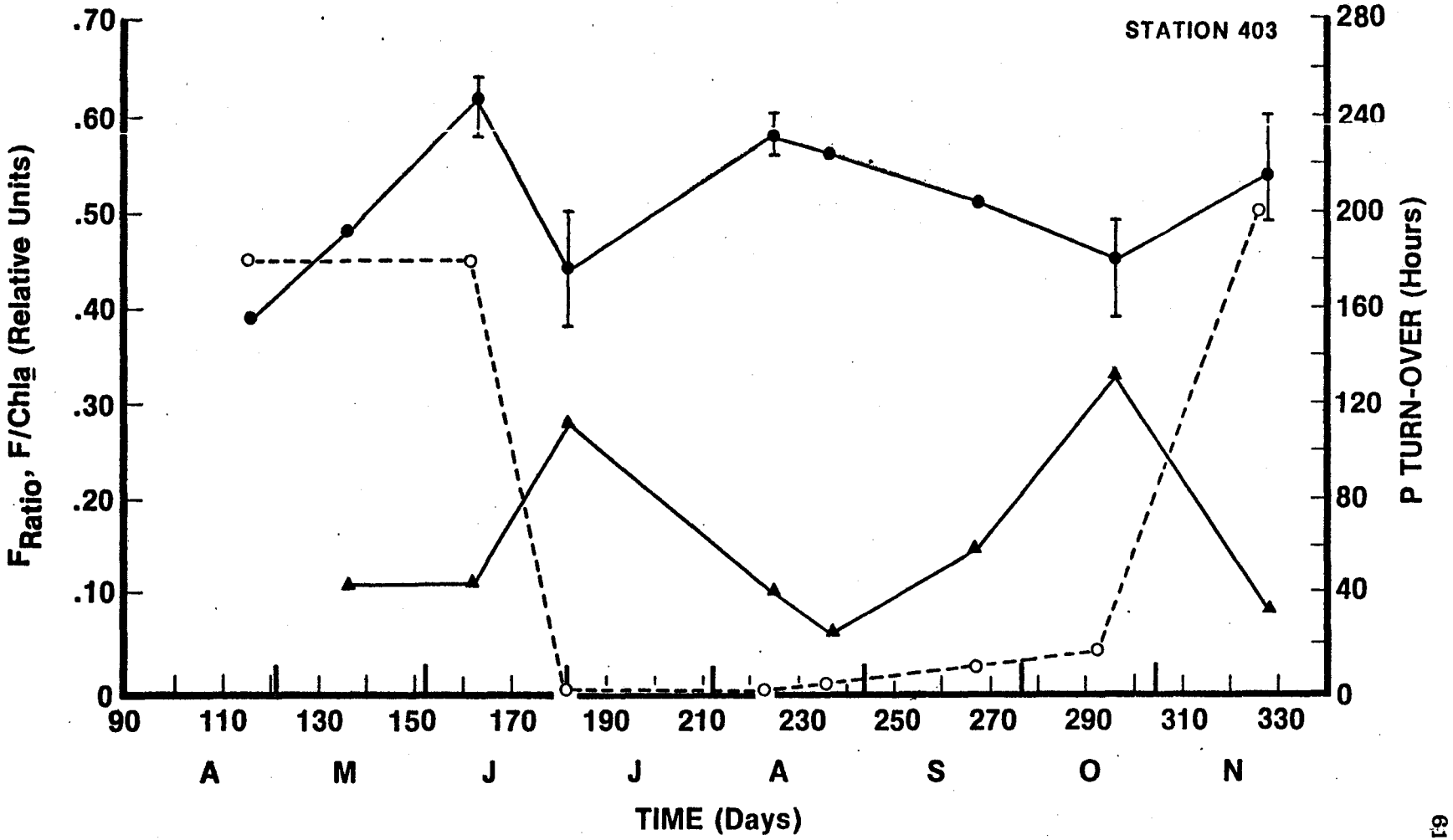
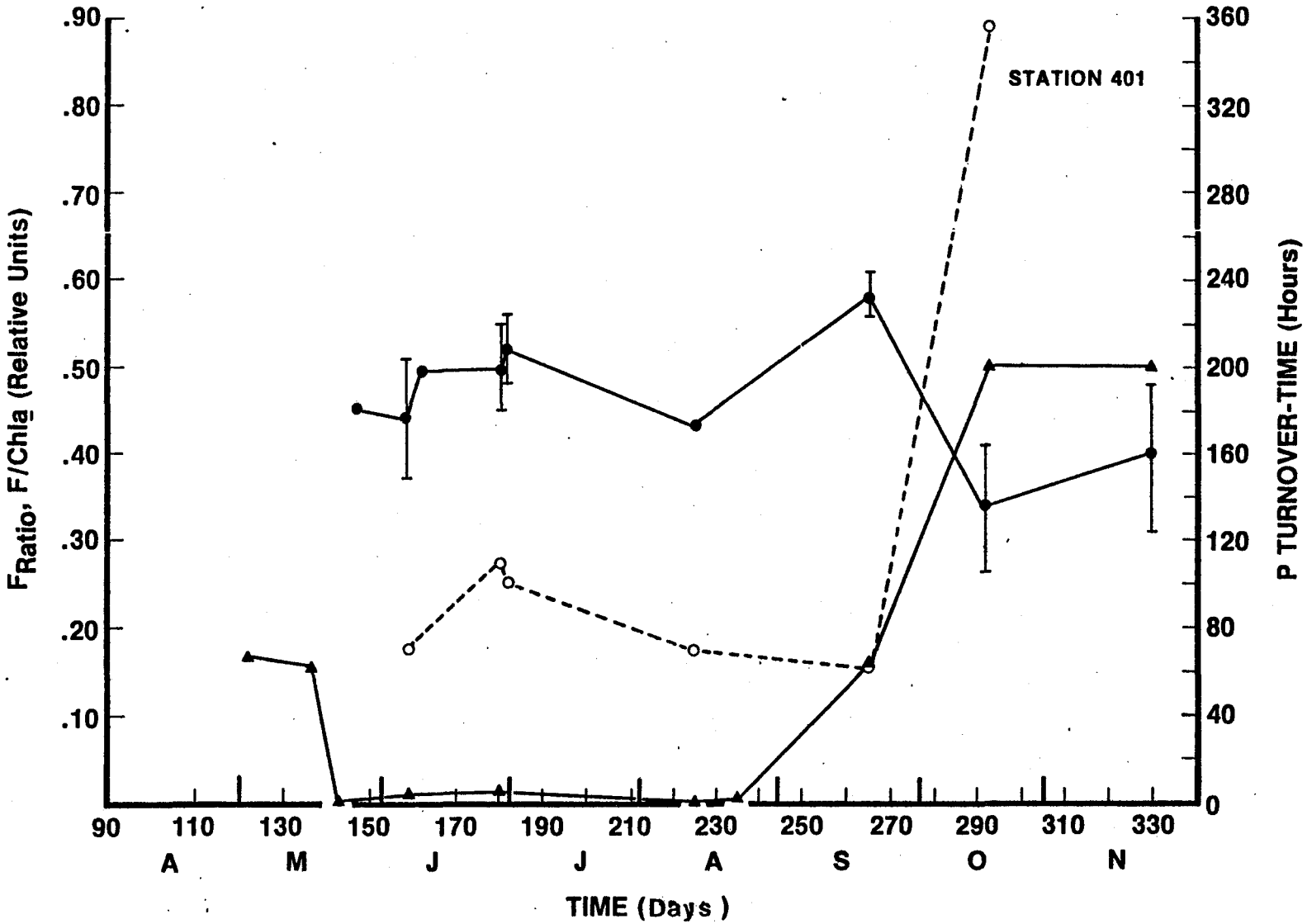

STATION 403
.70
.60
.50
.40
.30
.20
.10
0
280
240
200
160
120
80
40
0
F Ratio, F/Chla (Relative Units)
P TURN-OVER (Hours)
90
110
130
150
170
190
210
230
250
270
290
310
330
A
M
J
J
A
S
O
N
TIME (Days)

Figure 4.9 Comparison of seasonal P turnover time, F ratio and F/chl*a* at station 401 (Lake Ontario 1982).

▲—▲ P turnover time (hours) (D.R.S. Lean unpub. data)

●—● F ratio (relative units)

○-○ F/chl*a* (relative units)


STATION 401
F_{Ratio}, F/Chla (Relative Units)
P TURNOVER-TIME (Hours)
.90
.80
.70
.60
.50
.40
.30
.20
.10
360
320
280
240
200
160
120
80
40
0
90
110
130
150
170
190
210
230
250
270
290
310
330
A
M
J
J
A
S
O
N
TIME (Days)

4.2.3 Comparison of the F ratio in the field and in culture

The absolute value of the F ratio observed in 5 meter water was .34 to .60 (Figures 4.8, 4.9). Values of the F ratio as high as those occurring during exponential growth of Chlamydomonas (.70) in batch culture were not observed; values of the F ratio as low as those which occurred during stationary phase of Synechococcus (.23) were also not observed in epilimnetic waters.

4.2.4 Comparison of fluorescence and ^{14}C uptake

Figure 4.10 shows that there was no relationship between the F ratio and primary productivity expressed per unit chlorophyll a. Vincent (1981) suggests that the F ratio multiplied by the concentration of chlorophyll a should indicate the amount of "active" chlorophyll. No relationship between this ratio and ^{14}C uptake expressed per unit chlorophyll a was observed for this data set (data not shown).

4.2.5 Seasonal patterns and vertical profiles of the F ratio

Figure 4.11 shows the changes in F ratio which occurred at fixed depths in the water column throughout the season at station 403. Vertical profiles are included in Appendix 1.7. 5 meter populations

Figure 4.10 Comparison of seasonal primary productivity and F ratio at station 403 (Lake Ontario 1982). Regression analysis showed there to be no significant relationship between the two parameters.

O--O C uptake (mg C mg Chl $\underline{a}^{-1}$ hr^{-1}) (D.R.S. Lean unpub. data)

●—● F ratio (relative units)

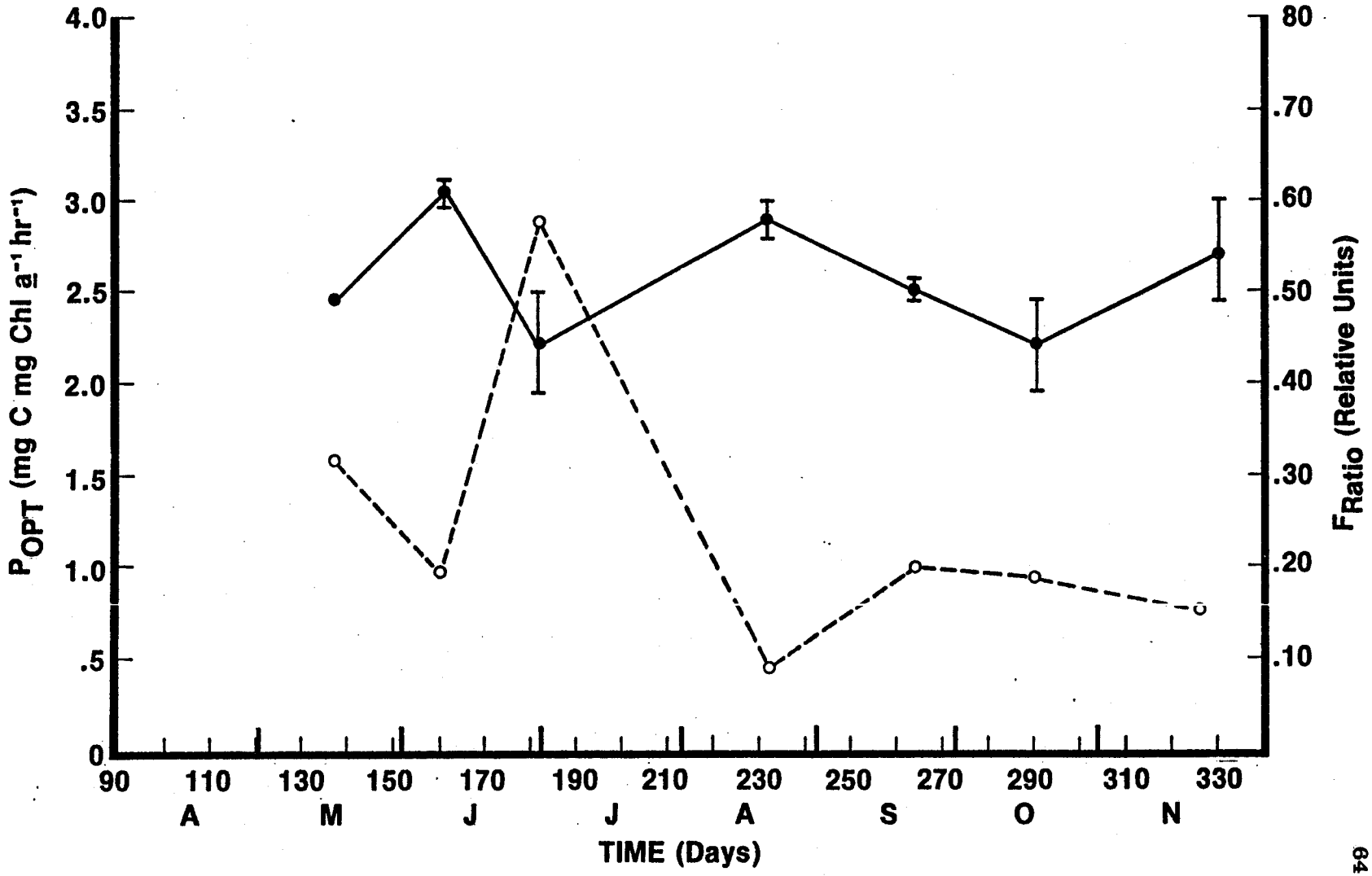
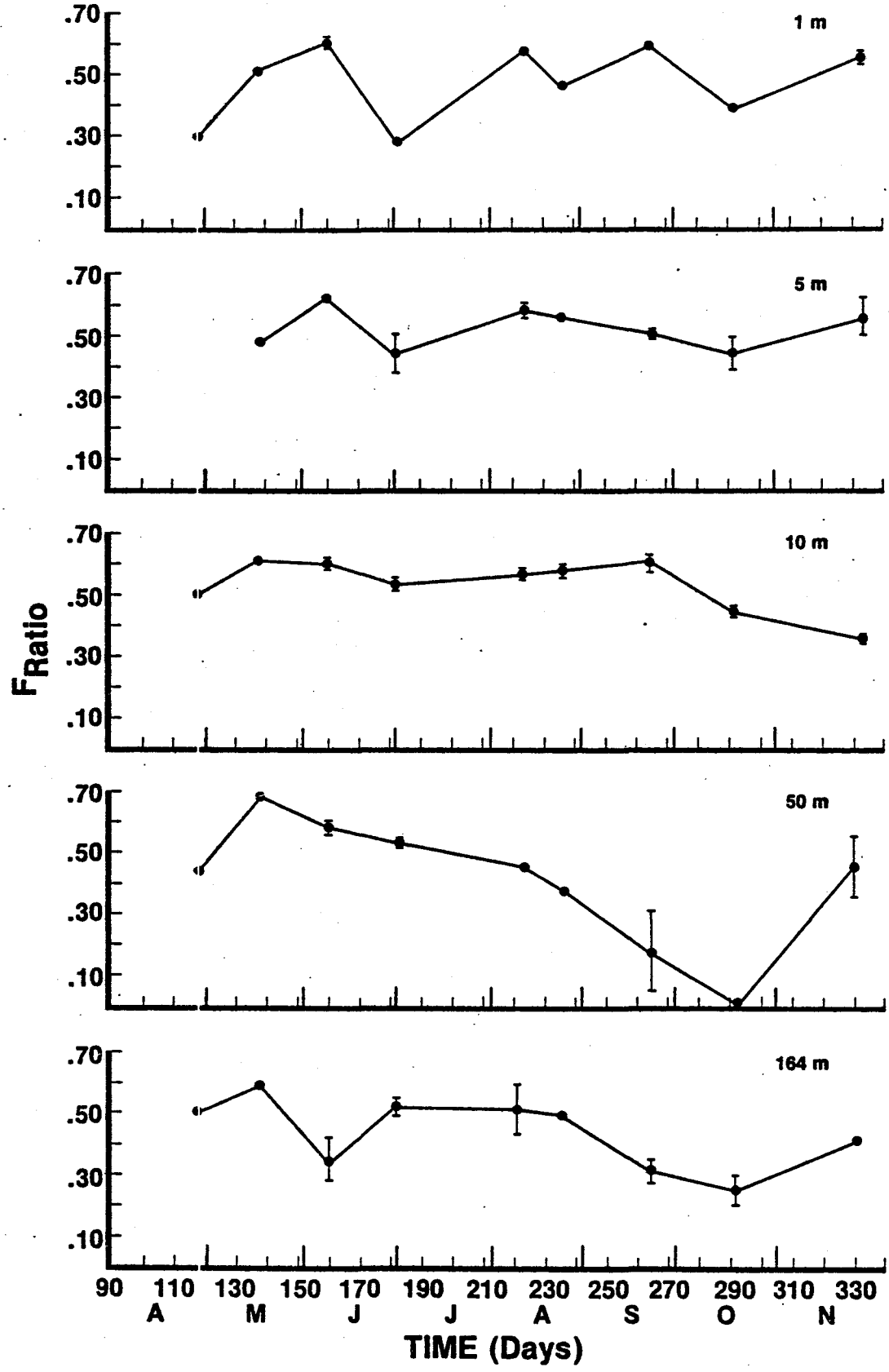

4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
0
P_{OPT} (mg C mg Chl a^{-1} hr^{-1})
80
.70
.60
.50
.40
.30
.20
.10
F_{Ratio} (Relative Units)
90
110
130
150
170
190
210
230
250
270
290
310
330
A
M
J
J
A
S
O
N
TIME (Days)

Figure 4.11 Seasonal changes in the F ratio at fixed depths in the water column at station 401 (Lake Ontario 1982)


.70
.50
.30
.10
1 m
5 m
10 m
50 m
164 m
F Ratio
90 110 130 150 170 190 210 230 250 270 290 310 330
A M J J A S O N
TIME (Days)

are representative of epilimnetic populations while 50 and 164 meter populations are representative of hypolimnetic populations during the period of stratification. The 10 meter population was representative of the metalimnion except for the October and September samples.

Although the data was selected from profiles made at night in order to minimize the effect of light, there was still more variability in the F ratio of the surface sample than in the water from 5 and 10 meters (Figure 4.11). Until July, when the water column became stratified, the F ratio of deep water samples was not significantly different to that of water near the surface (1,5, and 10 meters). Following stratification (July 2) the F ratio of samples at 50 and 164 meters underwent a gradual decline until by October (Day 292) there was no DCMU-enhanced fluorescence at 50 meters and the F ratio at 164 meters had fallen to .25. This decline was very gradual, however. Even after 42 days in the dark there was little difference between epilimnetic and hypolimnetic populations. After 83 days, clear differences were apparent between epilimnetic and hypolimnetic populations. Following the breakdown of the thermal structure the F ratio at these depths rose but remained somewhat lower than those at 1 and 5 meters.

The other observation of interest is that on July 2 the F ratio of water at 1 and 5 meters was lower than that of water from lower depths (Appendix 1.7). This station was isothermal 2 days

previously and the fluorescence properties of the two layers on July 2 were dramatically different. These observations are in agreement with microscopic observation at the time (W. Taylor pers. comm.) which suggested that the epilimnion in this case had not resulted from surface heating in the water column but was more likely the result of the advection of a layer of water onto the sample site.

Figure 4.12 and Appendix 1.8 show the seasonal changes at 4 depths and vertical profiles of F ratio and temperature respectively for the inshore station (401). Except for 23 September, the epilimnion was not as well developed as at the offshore station. Figure 4.12 shows that except for the May and October samples the F ratio remained above .45 throughout the season. The reason for the low values early in May is not apparent. The low values observed in October were correlated to the upwelling of deep water as described above in section 4.2.3. In contrast to the offshore station, the F ratio of samples from below the thermocline at the inshore station showed no consistent decline over the course of the season.

Vertical profiles (Appendix 1.7, 1.8) show that vertical structure in the F ratio was also present in the water column at certain times of the year. A similar pattern to ΔF or ΔF_{DCMU} was observed for the ΔF ratio and the ΔT (Figure 4.13). At low values of ΔT (up to $2.0^{o}C$), the boundary line shows that only two points were greater than .15 (the level considered to represent a significant difference). When ΔT was 0 the majority of points were clustered

Figure 4.12 Seasonal changes in the F ratio at fixed depths in the water column at station 403 (Lake Ontario 1982)

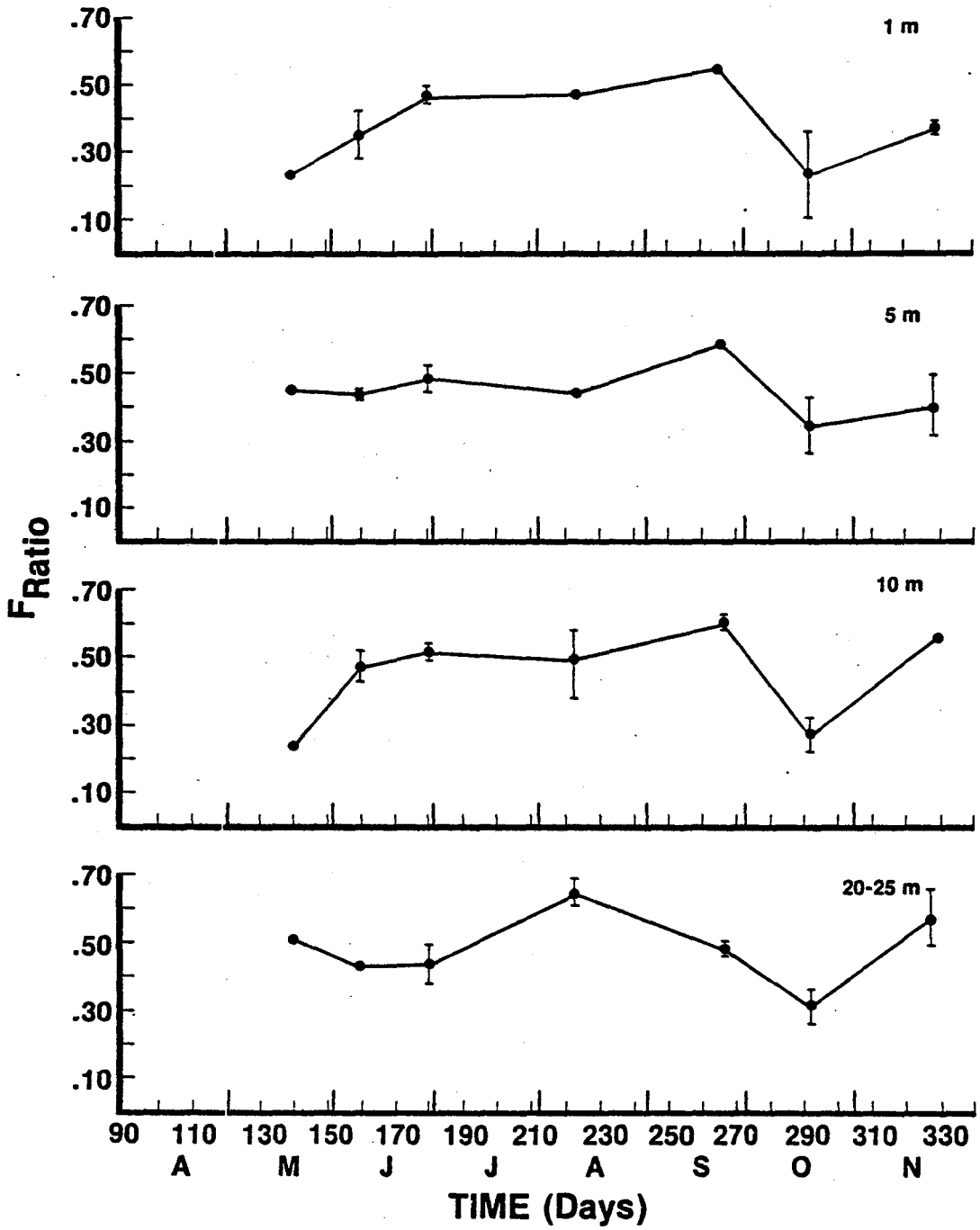
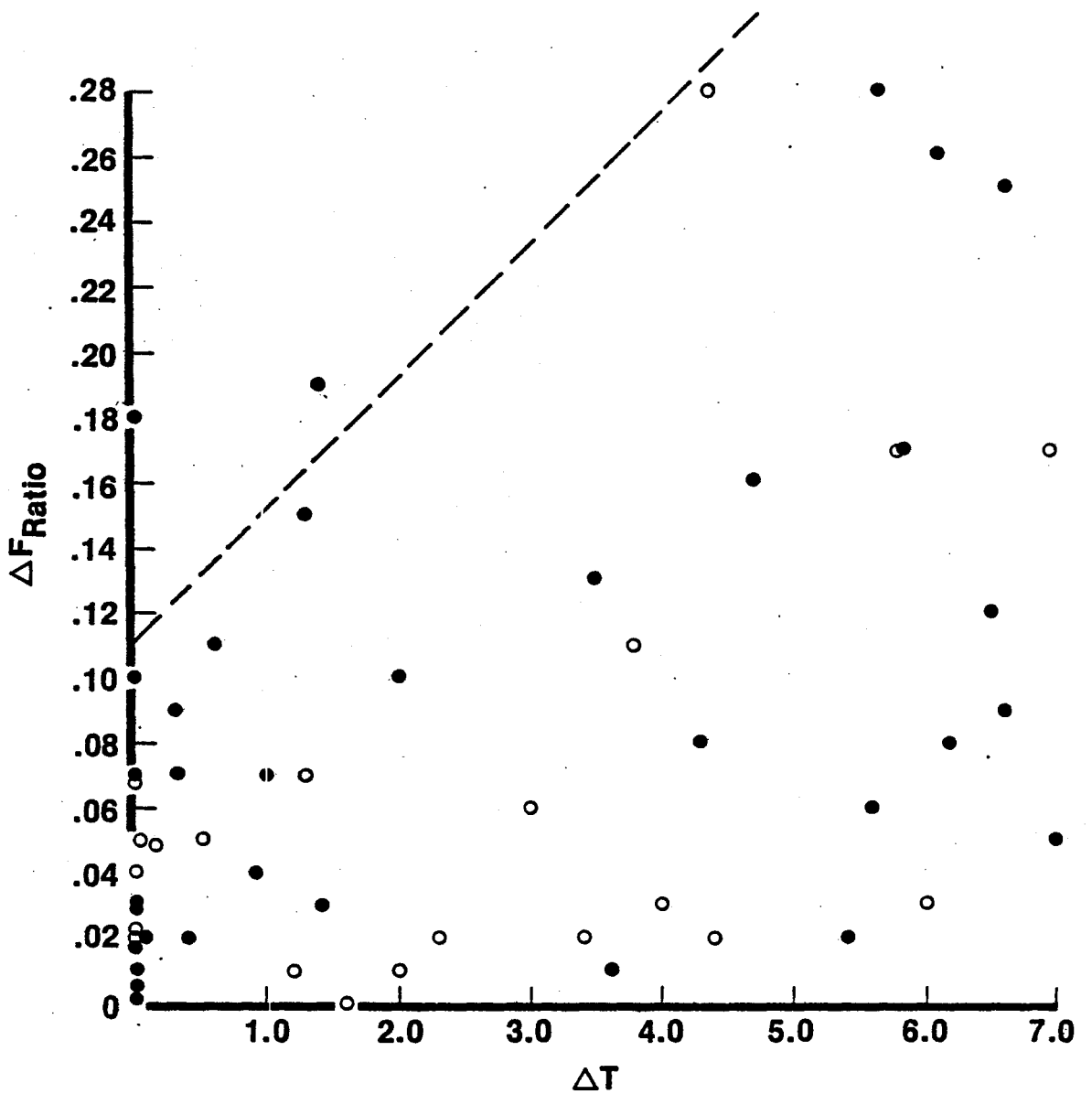

1 m
5 m
10 m
20-25 m
.70
.50
.30
.10
F Ratio
90 110 130 150 170 190 210 230 250 270 290 310 330
A M J J A S O N
TIME (Days)

Figure 4.13 Relationship between ΔF ratio and ΔT where ○ indicates data from profiles made between the hours of 20:00 and 06:30; ● indicates data from all other profiles. The dashed line indicates an arbitrarily chosen upper boundary. ΔT is the difference in temperature between 1 and 10 meters; ΔF ratio is the difference in F ratio between 1 and 10 meters. Note that at low ΔT few points were larger than .15 (the level above which differences are considered to be real).


.28
.26
.24
.22
.20
.18
.16
.14
.12
.10
.08
.06
.04
.02
0
ΔF Ratio
1.0
2.0
3.0
4.0
5.0
6.0
7.0
ΔT

below .10. At high values of ΔT the values of the ΔF ratio were observed to be as high as .28 although low values were still possible. The relationship between N^2 and the ΔF ratio was similar (Appendix 1.8).

4.3 Nitrogen enrichment in culture

Table 4.2 shows the mean and standard error of the mean for the fluorescence and photosynthetic parameters of batch cultures of Chlamydomonas prior to nutrient addition. The largest standard error for the fluorescence parameters was about 7% thus a difference in a fluorescence value larger than 15% will be considered significant here. Similarly, values greater than 30% (twice the largest observed standard error of the mean) are considered to represent a significant difference in ^{14}C uptake.

Table 4.2 shows that the F ratio and ^{14}C uptake in these cultures are representative of the "typical" batch culture described above (section 4.1). The F ratio and ^{14}C uptake were highest in exponentially growing cells and fell during stationary phase. Although the F in 1 day stationary phase cells was not significantly higher, the number of cells in this experiment (5.4×10^6 cells mL^{-1}) was approximately five times that used in all other experiments.

Table 4.2 Initial fluorescence and photosynthetic uptake in batch cultures of Chlamydomonas prior to nutrient additions. Stated values are means of all measurements and standard errors of the mean, prior to nutrient addition. Values in parentheses are the standard error of the mean expressed as a percentage of the mean. ^{14}C uptake expressed as the fraction incorporated expressed as a percent.

CULTURE GROWTH STAGE	F	F_{DCMU}	F ratio	^{14}C UPTAKE
Exponential	.088±.002 (2.3)	.244±.014 (5.7)	.63±.02 (3.2)	5.87±.11 (3.7)
1 Day Stationary	.148±.005 (3.4)	.322±.020 (6.2)	.53±.02 (3.8)	2.31±.32 (13.8)
3 Day Stationary	.294±.004 (1.4)	.475±.011 (2.3)	.41±.02 (4.9)	1.56±.14 (9.0)
6 Day Stationary	.270±.012 (4.4)	.560±.015 (2.7)	.51±.01 (2.0)	.80±.12 (15.0)
7 Day Stationary	.180±.009 (7.6)	.201±.014 (7.0)	.41±.03 (7.3)	.19±.01 (5.3)

4.3.1 Effect of nitrogen enrichment on the F ratio

There was no effect during 16 hour experiments of either NH_4 or NO_3^--N addition to the F ratio of cells grown at 1 to .2 μ_{max} in continuous cultures (data not shown).

The changes in ^{14}C uptake and fluorescence which occurred in batch cultures during the exponential and 7 day stationary phase of growth over the course of the 12 hour experiment are shown in Figure 4.14 for NH_4 addition and in Figure 4.15 for NO_3^- addition. The remainder of the data from experiments conducted on cultures in 1, 3, and 6 day stationary phase cultures is included in Appendix 2.1. A source of variability which must be considered in these experiments is that associated with the intrinsic diurnal rhythm of photosynthesis and fluorescence in this organism. Table 4.3 is a comparison of the difference between the final fluorescence and ^{14}C uptake measured after twelve hours to that measured prior to nutrient addition. The difference is expressed as a percentage of the value observed prior to nutrient addition. Table 4.3 shows that there were significant changes in the final F ratio of the control culture in all but the 6 day stationary phase cells. An examination of the control cultures shows that the observed pattern was not consistent from experiment to experiment. There was a tendency for the F ratio to increase over the 12 hour period in exponential, 1 day stationary and 3 day stationary phase cells. There was an overall trend toward a decreased F ratio

Figure 4.14 The effect of NH_4 enrichment on Chlamydomonas for a) the F ratio in exponential phase b) the F ratio in 7 day stationary phase, c) ^{14}C uptake in exponential phase and d) ^{14}C uptake in 7 day stationary phase cells.

▲—▲ F ratio (in the control culture)

△—△ (in the culture following NH_4 addition)

▲—▲ ^{14}C uptake (% ^{14}C incorporated in the control culture)

△—△ (% ^{14}C incorporated following NH_4 addition)

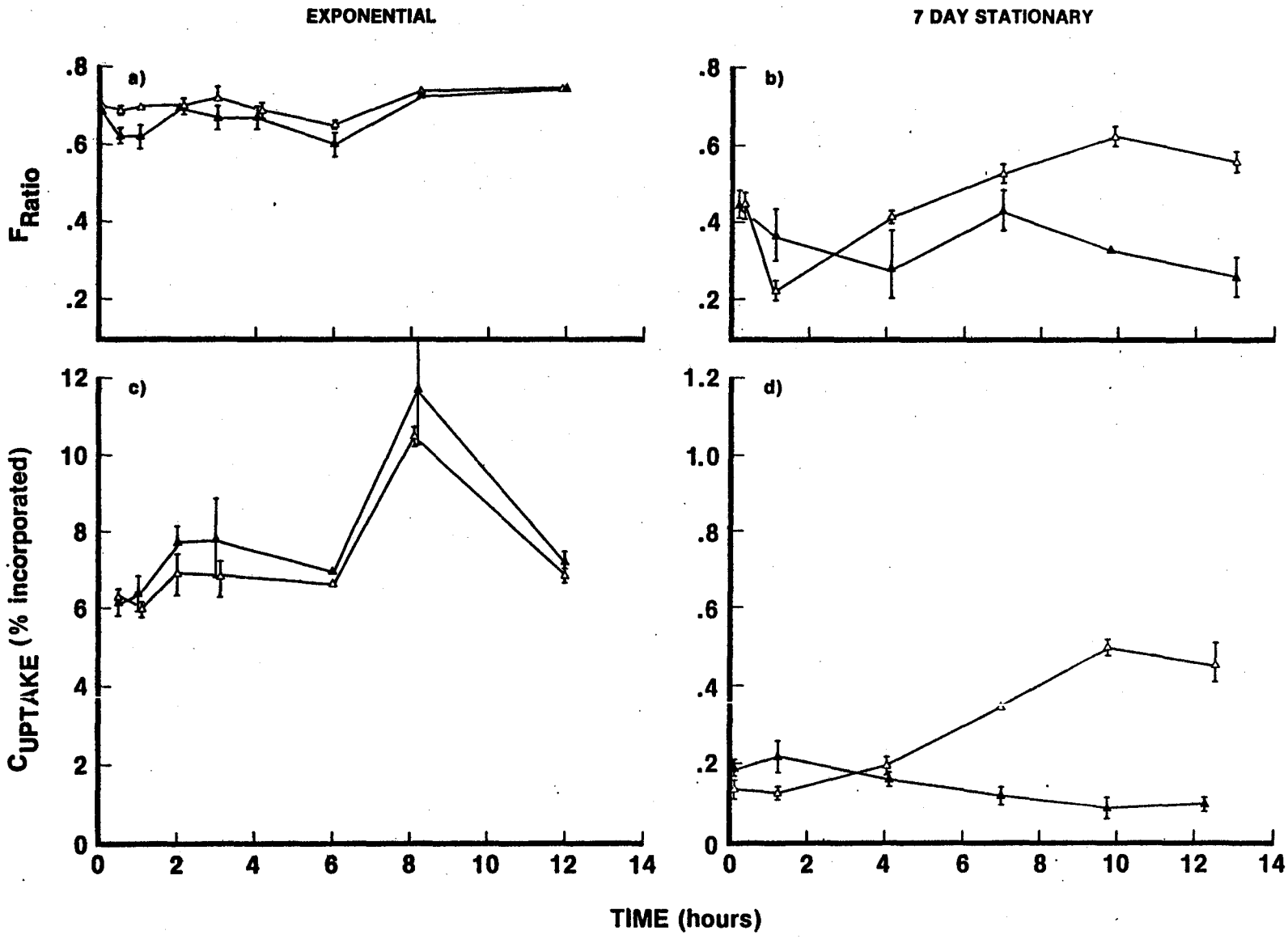
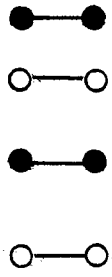

EXPONENTIAL
7 DAY STATIONARY
a)
b)
c)
d)
F Ratio
C UPTAKE (% incorporated)
TIME (hours)
.8
.6
.4
.2
12
10
8
6
4
2
0
1.2
1.0
0
2
4
6
8
10
12
14

Figure 4.15 The effect of NO_3^- enrichment on Chlamydomonas for a) the F ratio in exponential phase b) the F ratio in 7 day stationary phase c) ^{14}C uptake in exponential phase d) ^{14}C uptake in 7 day stationary phase cells



F ratio (in the control culture)

(in the culture following NH_4 addition)

^{14}C uptake (% ^{14}C incorporated in the control culture)

(% ^{14}C incorporated following NH_4 addition)

Note the similarity in ^{14}C uptake between replicate cultures in 7 day stationary phase cells, and the similarity between treated and untreated cultures in exponential phase growth. This was in contrast to the variability observed between replicate cultures in 1 day stationary phase growth (Appendix 2.1.1, 2.1.4)

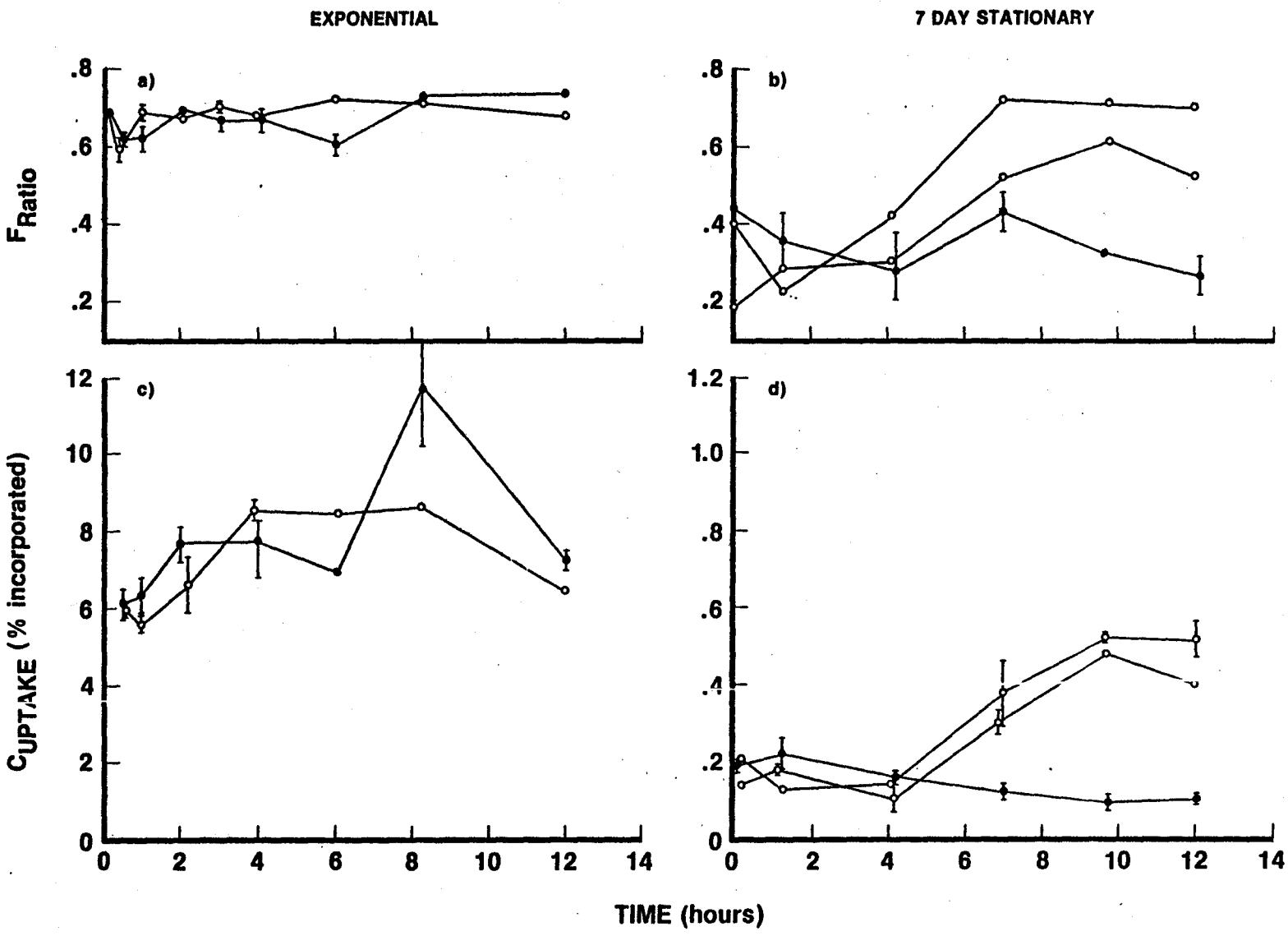

EXPONENTIAL
7 DAY STATIONARY
a)
b)
c)
d)
F Ratio
C UPTAKE (% incorporated)
TIME (hours)
.8
.6
.4
.2
12
10
8
6
4
2
0
1.2
1.0
0
2
4
6
8
10
12
14

Table 4.3 Difference in fluorescence and photosynthesis following a 12 hour incubation with NH_4 and NO_3 additions; each difference is expressed as a percentage of the initial value; * denotes a value which is significantly different to that observed prior to nutrient addition.

CULTURE GROWTH	TREATMENT	^{14}C	F ratio	F_{DCMU}	F
Exponential	Control	+15	+19*	+78*	+35*
	NH_4	+58*	+7	+40*	+17*
	NO_3	+46*	+15*	+67*	+29*
1 Day Stationary	Control	+37*	+54*	+49*	-13
	NH_4	-11	-6	+52*	+70*
	NO_3	+286*	+27*	+153*	+72*
3 Day Stationary	Control	-41*	+26*	-1	-22*
	NH_4	-2	+33*	+3	-43*
	NO_3	+43*	+48*	+32*	-14
6 Day Stationary	Control	-70*	-6	-22*	-17*
	NH_4	+25	+12	-2	-17*
	NO_3	+53*	+38*	+23*	-22*
7 Day Stationary	Control	-33*	-40*	-11	+17*
	NH_4	+272*	+26*	+14	-10
	NO_3	+170*	+129*	+44*	-21*

Note: Controls frequently show significance differences. This is likely due to an intrinsic rhythm (see text for details.)

over the course of the experiment in 6 and 7 day stationary phase cells (Table 4.3). There were significant decreases in ^{14}C uptake in control cultures in all but exponentially growing and "1 day stationary" phase cells. In order to consider both the variability associated with sampling errors and that due to diurnal patterns, the effect of nitrogen additions will be examined both in terms of how the treated sample differed from its initial value (Table 4.3) and how the treated sample differed to the control (Table 4.4).

There was no effect during 16 hour experiments of either NH_4 or NO_3^- addition to the F ratio of cells grown at 1 to .2 μ_{max} in cyclostat culture (data not shown).

In exponential, 1 day stationary and 3 day stationary phase cultures, no increase in the F ratio following NH_4 addition was observed compared to the control culture (Table 4.4, Figure 4.14a). The addition of NH_4-N resulted in an increased F ratio as compared to the control only after 6 to 7 days in stationary phase (Table 4.4, Figure 4.14b). Differences between the F ratio of treated and control cultures were not observed prior to the measurement made 4 hours after the addition of NH_4 (Figure 4.14b, Appendix 2.1.2, 2.1.3). The F ratio may increase either as a result of increased F_{DCMU} or decreased F. Table 4.4 shows that in the case of NH_4-N addition the cause of the rise was not consistent for the two experiments. In 6 day stationary cultures it was due to an increase in F_{DCMU} while in 7 day cultures it was due to decreased F (Table 4.4).

Table 4.4 Fluorescence and ^{14}C uptake in Chlamydomonas following a 12 hour incubation with added N (30 $\mu g\ L^{-1}$) compared to untreated cultures; all values expressed as a percentage of the control at the end of the experiment; * denotes a significant values

CULTURE GROWTH STAGE	TREATMENT	F ratio	F	F_{DCMU}	^{14}C uptake
Exponential	NH_4	100	100	101	94
	NO_3	92	109	90	89
1 Day stationary	NH_4	82*	236*	170*	180*
	NO_3	97	202*	196*	539*
3 Day Stationary	NH_4	114	99	147*	162*
	NO_3	133*	90	129*	148*
6 Day Stationary	NH_4	126*	100	135*	171*
	NO_3	136*	120*	190*	404*
7 Day Stationary	NH_4	235*	62*	104	442*
	NO_3	234*	78*	147*	375*

Significant enhancement of the F ratio compared to the control cultures was not evident in exponentially growing or 1 day stationary phase cells following NO_3^- addition (Table 4.4, Figure 4.15a) when compared to the control culture. In contrast to NH_4 there was an effect of the addition of NO_3^- on 3 day stationary phase cells (Table 4.4). There was also an enhancement of the F ratio in 6 and 7 day stationary phase cultures (Table 4.4 Figure 4.15). As with NH_4 there was a lag period of between 4 and 6 hours prior to enhancement with NO_3^- (Figure 4.15, Appendix 2.1.5, 2.1.6). In the case of NO_3^- the enhancement of the F ratio was consistently due, in large part, to an increase in F_{DCMU}. Table 4.3 shows that F_{DCMU} at the end of the experiment was significantly larger than that measured prior to nutrient addition in all experiments. Table 4.4 shows that F_{DCMU} was also significantly larger than the control in all stationary phase cultures treated with NO_3^-.

There was no effect of PO_4-P addition in either 7 day stationary or 1 day stationary phase cells (Appendix 2.1.1).

4.3.2 Effect of nitrogen enrichment on ^{14}C uptake

^{14}C uptake in NH_4 treated cultures was not significantly different to untreated cultures in exponentially growing cells (Figure 4.14c). NH_4 addition did result in an enhancement of ^{14}C uptake when

compared to control cultures in all stationary phase cells (Table 4.4). ^{14}C uptake was 160 to 440 percent of that observed in untreated cultures. Table 4.3 shows that this was not necessarily due to an increase in ^{14}C uptake in the treated cells over the course of the experiment. Rather in 1 day, 3 day and 6 day stationary phase cultures the ^{14}C uptake was not significantly different 12 hours after addition to that observed prior to addition. The "enhancement" of ^{14}C uptake in treated as compared to untreated cultures was actually due to a decrease in ^{14}C uptake over the course of the experiment in the control culture.

^{14}C uptake was not enhanced when compared to control cultures following addition of NO_3^- to exponentially growing cells (Figure 4.15). Enhancement of ^{14}C uptake of 150 to 540 percent of that observed in the control cultures occurred in all stationary phase cells (Table 4.4). While the apparent "enhancement" of ^{14}C uptake in NH_4 treated cells was largely due to a decrease in ^{14}C uptake in the control culture, there did appear to be a real increase in the ^{14}C uptake of the NO_3^- treated cells over the course of the experiment. Table 4.3 shows that ^{14}C uptake in stationary phase cells following NO_3^- addition was 43 to 286% larger than that measured prior to nutrient addition.

4.3.3 Relationship between fluorescence and ^{14}C uptake

No consistent relationship between ^{14}C uptake and the F ratio was observed for the data set as a whole. Table 4.3 shows that in NO_3^- treated samples an increase in the F ratio over the course of the experiment was accompanied by a significant increase in ^{14}C uptake in all experiments. In NH_4 treated cultures however an increase in the F ratio in 3 day stationary phase cells was not accompanied by a significant increase in ^{14}C uptake while in the control cultures an increase in the F ratio in 3 day stationary phase cultures was accompanied by a significant decrease in ^{14}C uptake (Table 4.3)

4.4 Nutrient enrichment in the field

4.4.1 Nitrogen enrichment

A summary of the nitrogen addition experiments is shown in Table 4.5. The data is included in Appendix 2.2. The addition of NH_4 (100 $\mu g\ L^{-1}$) to 5m samples from both inshore and offshore stations in Lake Ontario during the June cruise appeared to result in slightly enhanced F ratios following a 4 hour incubation (Appendix 2.2.1). The enhancement was more pronounced in water from 1m and 150m following a 16 hour incubation on 12 June (Appendix 2.2.2).

Table 4.5 Summary of nutrient levels and mixing regime on the occasions when NH_4 additions were made. + indicates a positive effect of NH_4 addition; ? indicates a possible effect of NH_4 addition; -indicates no effect of NH_4 addition. C:N is the ratio of masses of these two elements in the particulate fraction.

DATE	STA	DEPTH (meters)	MIXING REGIME	LENGTH OF INCUBATION (hours)	C:N	AMBIENT NUTRIENTS NO_3 ($\mu g\ L^{-1}$)	NH_4 ($\mu g\ L^{-1}$)	ADDITION ($\mu g\ L^{-1}$)	EFFECT
8 June	401	5	stratified	4	5.5	282	2	100	?
10 June	403	5	mixing	4	6.8	360	<1	100	?
12 June	403	1	mixing	16	6.2	360	2	100	+
		150	"	"	7.2	"	"	100	+
30 June	401	5	stratified	16	7.1	291	3	100	-
1 July	405	50	stratified	8	6.5	205	2	50	-
2 July	403	150	stratified	16	6.9	185	3	10	-
13 August	403	10	stratified	8	4.8	105	5	100	-
		150	"	"	6.4	328	1	"	-

No consistent enhancement of F ratio following NH_4 addition was observed at any other time during the summer (Table 4.5, Appendix 2.2.1). Enhancement at L_2 and L_3 but not L_1 appeared to occur on 2 July. The addition of NO_3^- (100 µg L^{-1}) had no effect on the F ratio at any time.

Table 4.5 shows that while the concentration of NH_4 was near the limits of detection, NO_3^- levels ranged from 105 to 360 µg L^{-1} (24 to 81 µg N L^{-1}) and the C:N ratio in the particulate fraction ranged from 4.8 to 7.2. There was no apparent relationship between the light history of the cells as indicated by the mixing regime, and the response to nitrogen.

4.4.2 Phosphorus enrichment

The addition of phosphorus in a series of experiments during the summer cruises in which the P turnover time ranged from minutes to hours (D.R.S. Lean unpub. data) resulted in no change in the F ratio (data not shown).

4.5 Observations of diurnal changes of fluorescence in the water column

Diurnal patterns of fluorescence properties of water samples from 1,5, and 10 meters at station 401 on 8 June 1982 and 12-13 August 1982 will be examined in detail. These profiles were selected because while both days were bright and sunny (Table 4.6), the physical character of the water column (based on N^2 and ΔT) was dramatically different on the two occasions. F_{DCMU}/chl*a* for profiles not discussed here or in section 4.6 are included in Appendix 3.1.

On 8 June, N^2 was high, indicating high stability in the water column. On 12 August the buoyancy function was relatively high between one and ten meters but low between 1 and 5 meters (Table 4.6). Thus fluorescence should be more affected on 8 June than on 12 August especially between 1 and 5 meters.

Three aspects of fluorescence patterns will be examined:

1) the change in F_{DCMU} from the initial measurement following a 1 hour dark treatment after sampling

2) the change in F_{DCMU}/chl*a* during the 24 hour period at one depth and between depths

Table 4.6 Environmental data for June and August. Light is expressed in langleys day^{-1}. N^2 is the buoyancy function between 1 and 5 meters, and 1 and 10 meters in $s^{-2} \times 10^{-6}$. ΔT is the average difference in temperature in ^{o}C between 1 and 5 meters, and 1 and 10 meters from all samplings at which fluorescence measurements were made.

Date	Sta	Light	N^2 (1 and 5)	N^2 (1 and 10)	ΔT (1 and 5)	ΔT (1 and 10)
8 June	401	686	330	330	1.8	4.0
12 August	403	567	4	480	.2	2.6
26 August	403	521	0.0	0.0	0.0	0.0
29-30 June	405	358	630	460	3.5	5.0

3) changes in the F ratio during the 24 hour period at one depth and between depths

Figure 4.16a shows that a dramatic change in F_{DCMU} occurred in the 1 meter water on 8 June after placing the sample in the dark. The effect was present up until 20:30 although solar radiation had decreased by this time. The differences in F_{DCMU} of 5 or 10 meter water followed no such consistent pattern (Figure 4.16b,c).

On 12 August the F_{DCMU} of only one sample removed during the daylight hours (1 meter at 18:00) showed any significant change following a 1 hour incubation in the dark (Figure 4.17a). 5m and 10m water showed no significant change when placed in the dark (Figure 4.17b,c).

Figure 4.18 and Figure 4.19 compare the F_{DCMU}/chl*a* of 1 meter water to that of the deeper water (5m and 10 m) on these two occasions. On 8 June the F_{DCMU}/chl*a* of 1m water showed a depression in 2 out of 3 samples taken during daylight hours (Figure 4.18a). Curiously in 2 out of 3 profiles taken during daylight hours the F_{DCMU}/chl*a* of 5 meter water was higher than in the corresponding samples removed at night. The 10 meter water sample showed little change with time (Figure 4.18b). Figure 4.19a shows that on 12 August a small depression in F_{DCMU}/chl*a* was apparent in the 1 meter sample during the day. Changes in F_{DCMU}/chl*a* were less consistent at 5

Figure 4.16 The change in FDCMU following a 1 hour dark treatment on 8 June (station 401, Lake Ontario 1982) for water from:

a) 1m

●—◐ F_{DCMU} prior to dark treatment
○--○ F_{DCMU} after dark treatment

b) 5m

■—■ F_{DCMU} prior to dark treatment
□--□ F_{DCMU} after dark treatment

c) 10m

▲—▲ F_{DCMU} prior to dark treatment
△--△ F_{DCMU} after dark treatment

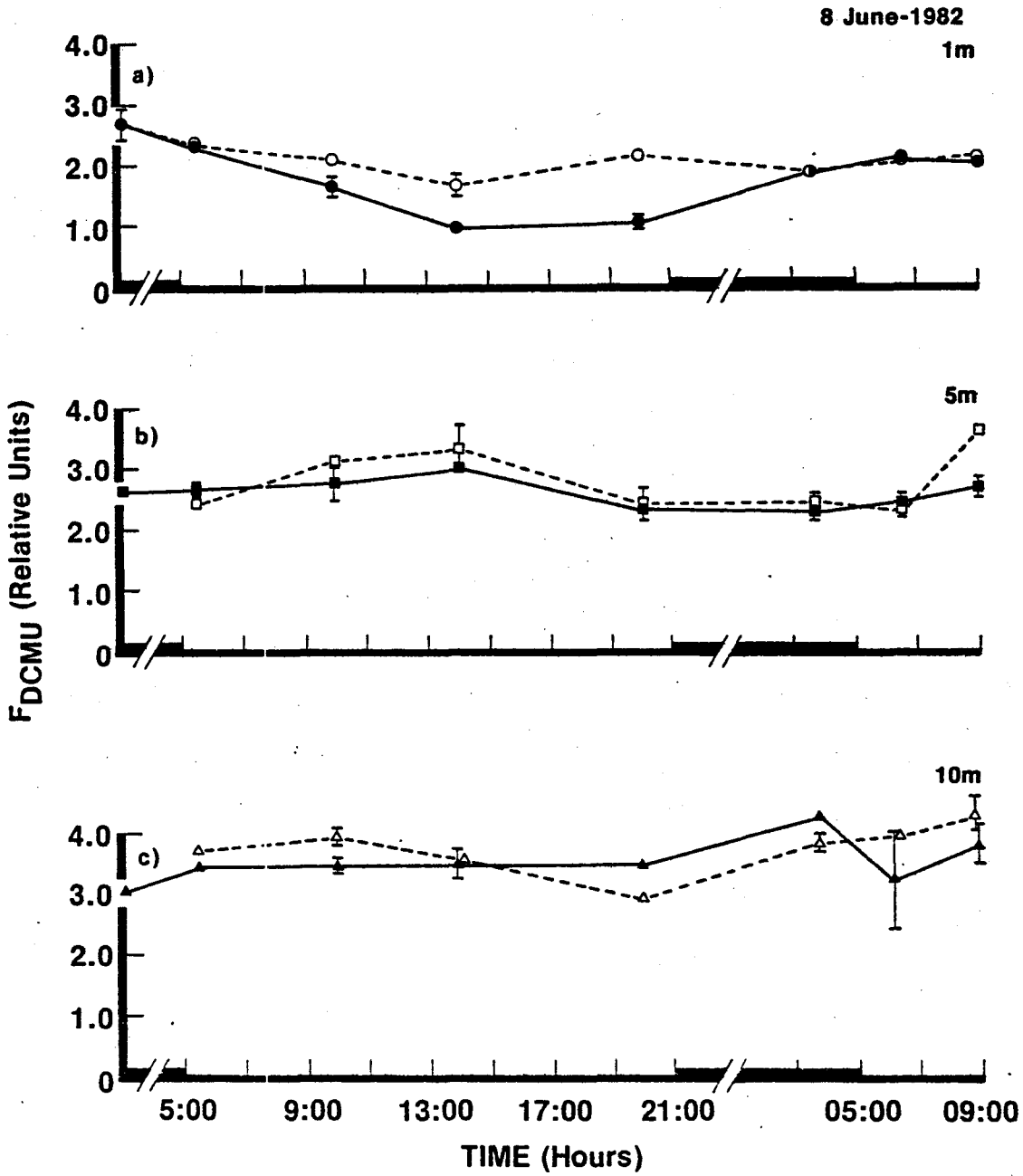

8 June-1982
1m
a)
4.0
3.0
2.0
1.0
0
5m
b)
4.0
3.0
2.0
1.0
0
10m
c)
4.0
3.0
2.0
1.0
0
F_{DCMU} (Relative Units)
5:00
9:00
13:00
17:00
21:00
05:00
09:00
TIME (Hours)

Figure 4.17 The change in FDCMU following a 1 hour dark treatment on 12 August (station 403, Lake Ontario 1982) for water from:

a) 1m

●—● F_{DCMU} prior to dark treatment
○–○ F_{DCMU} after dark treatment

b) 5m

■—■ F_{DCMU} prior to dark treatment
□–□ F_{DCMU} after dark treatment

c) 10m

▲–▲ F_{DCMU} prior to dark treatment
△—△ F_{DCMU} after dark treatment

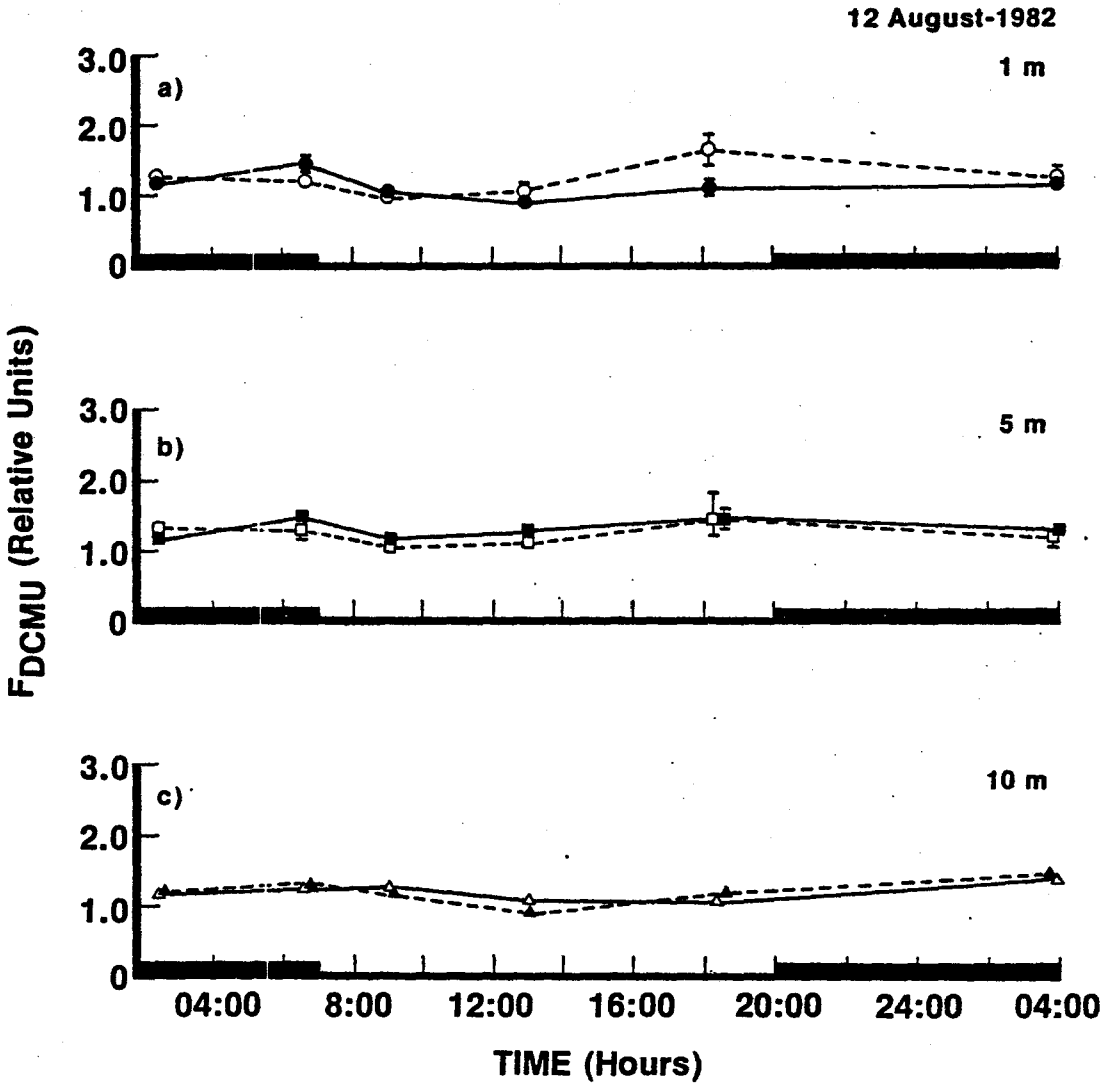

12 August-1982
1 m
5 m
10 m
a)
b)
c)
3.0
2.0
1.0
0
F_{DCMU} (Relative Units)
04:00
8:00
12:00
16:00
20:00
24:00
04:00
TIME (Hours)

Figure 4.18 The difference in F_{DCMU}/chl*a* on 8 June (station 401, Lake Ontario 1982) between:

a) ●—●1m and ○-○5m

b) ●—●1m and ▲-▲10m

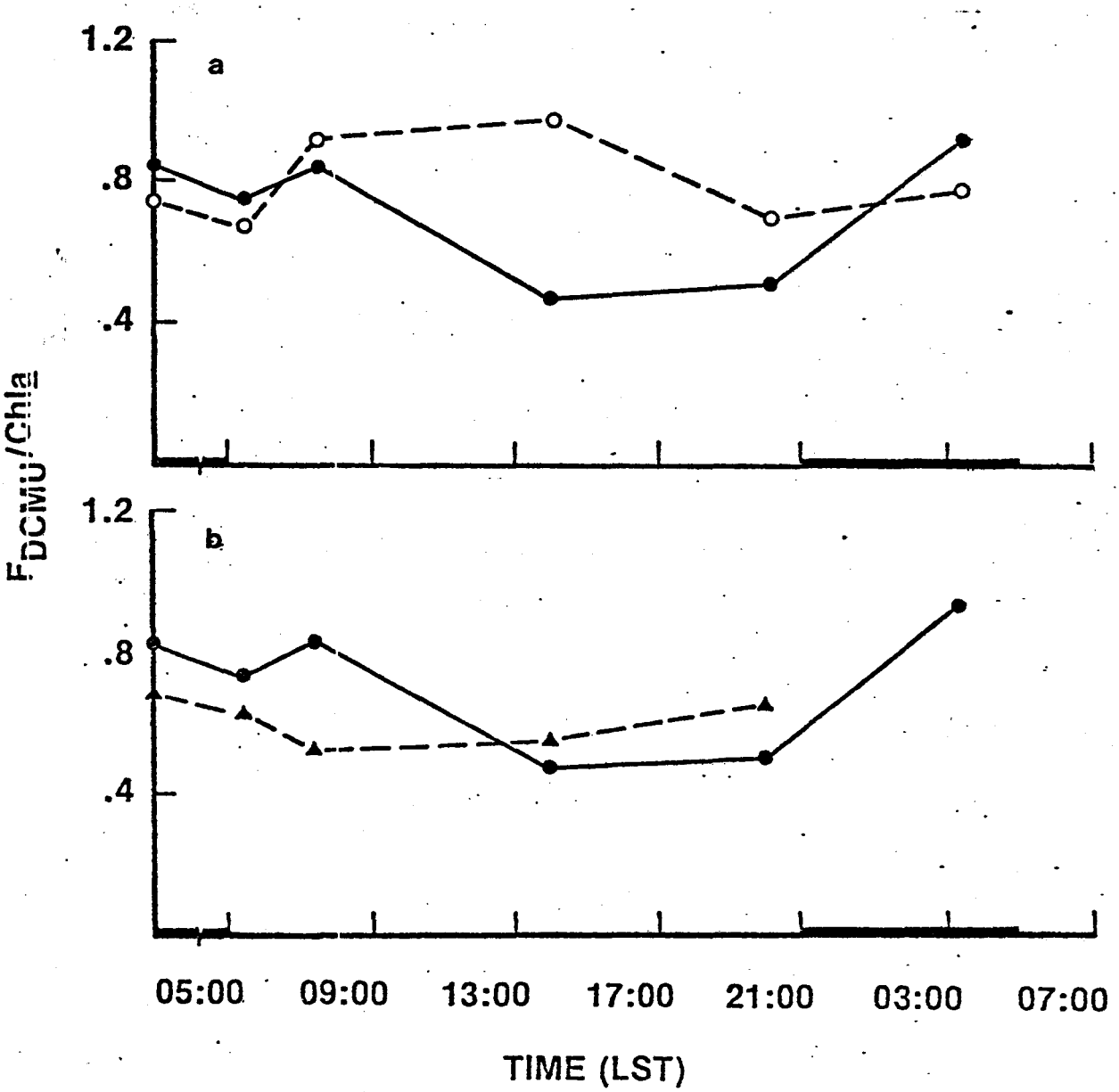
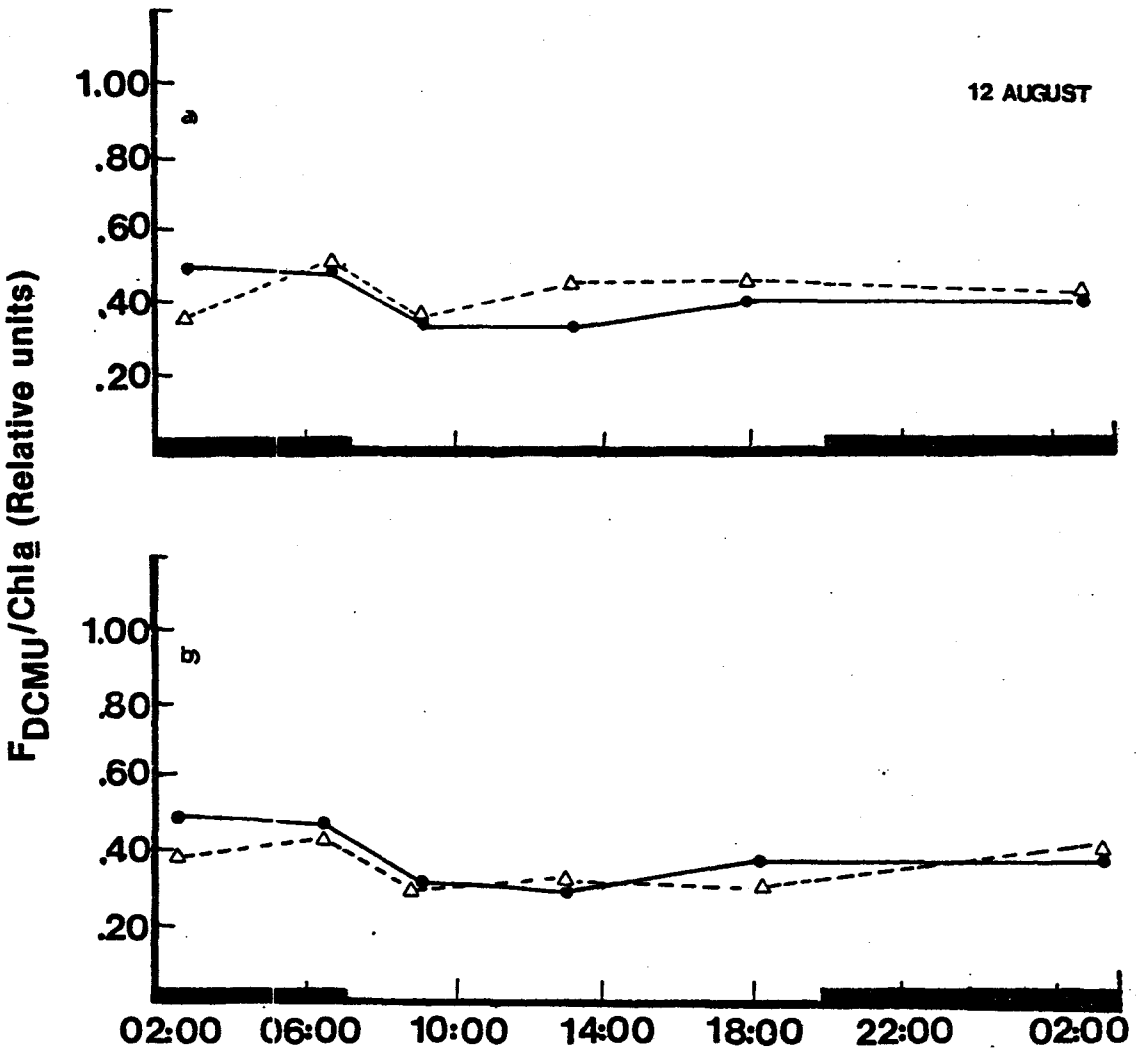

1.2
a
.8
.4
1.2
b
.8
.4
$F_{DCMU}/Chla$
05:00
09:00
13:00
17:00
21:00
03:00
07:00
TIME (LST)

Figure 4.19 The difference in F_{DCMU}/chl*a* on 12 August (station 403, Lake Ontario) between:

a) ●–●1m and Δ–Δ5m

b) ●–●1m and Δ–Δ10m


12 AUGUST
a
b
1.00
.80
.60
.40
.20
$F_{DCMU}/Chla$ (Relative units)
02:00
06:00
10:00
14:00
18:00
22:00
02:00

meters but the absolute value was generally higher at this depth than at 1 meter. The F_{DCMU}/chl*a* of the 10 meter water sample showed little change with time.

The F ratio data was not wholly consistent with the two other parameters. 1 meter water on 8 June showed no significant decline in F ratio until after 20:00 but the low value persisted into the night (Figure 4.20a). 5 meter and 10 meter water showed little consistent change. There was a depression of the F ratio at both of these depths (but not at 1 meter) on the morning of 9 June. On 11-12 August there were few significant differences between the F ratio at 1 and either 5 or 10 meters (Figure 4.21a,b).

A comparison of the differences in fluorescence parameters in a vertical profile gave similar information. F_{DCMU}/chl*a* was significantly depressed in surface waters on 8 June (Appendix 3.2). An increase in FDCMU/chl*a* at 5 meters was noted. Similar increases were found to occur during some incubations at low light (c.f. section 4.7). While DCMU-enhanced fluorescence increased during the day in samples from 10 meters and above which were placed in the dark, samples taken during the night and from deeper waters showed little change in DCMU-enhanced fluorescence when placed in the dark (Appendix 3.3). The pattern in the F ratio from daylight samples

Figure 4.20 The difference in F ratio on 8 June (station 401, Lake Ontario 1982) between:

a) ●—●1m and △-△5m

b) ●—●1m and □-□10m

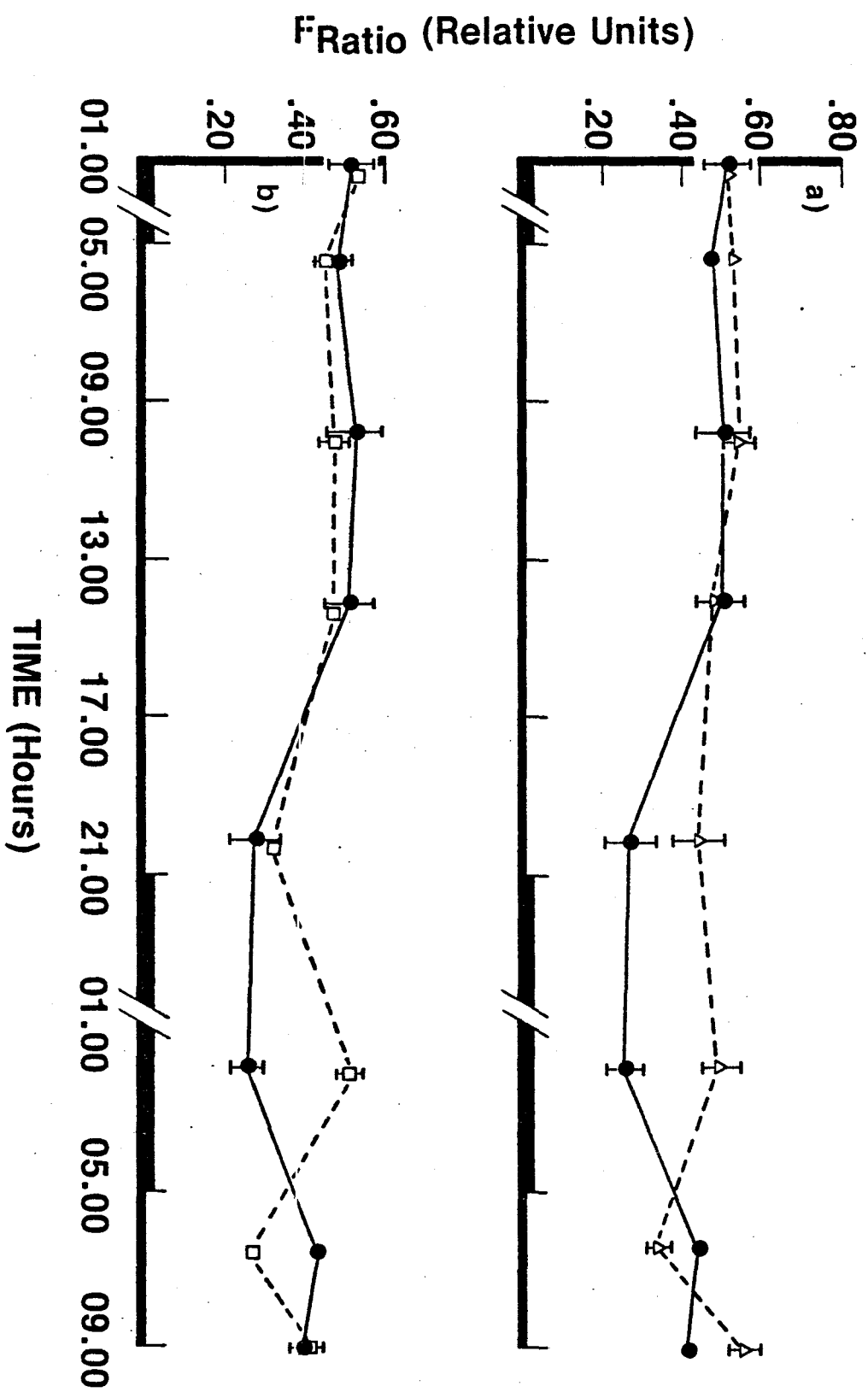
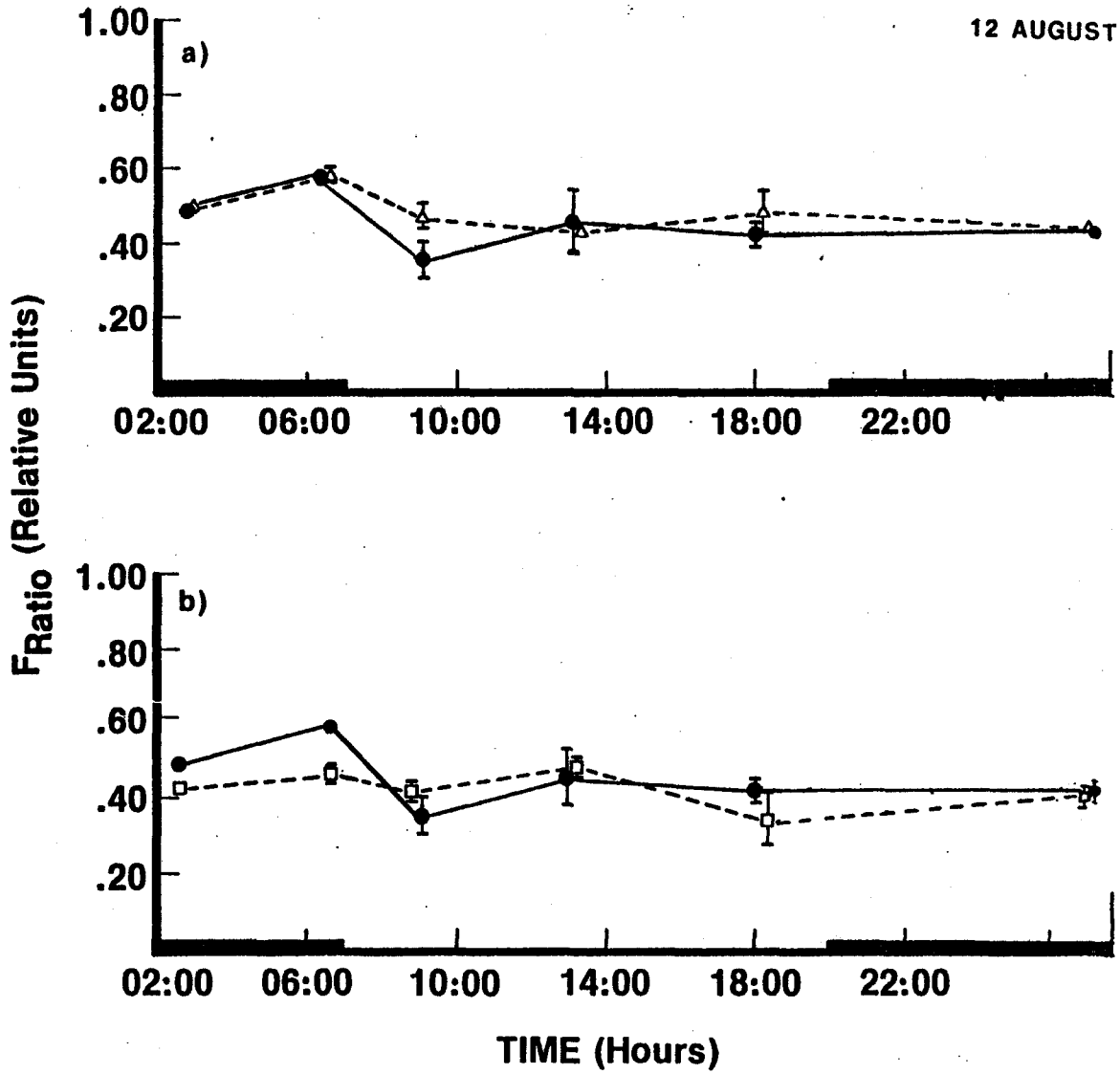

F Ratio (Relative Units)
.80
.60
.40
.20
a)
b)
01.00
05.00
09.00
13.00
17.00
21.00
01.00
05.00
09.00
TIME (Hours)

Figure 4.21 The difference in F ratio on 12 August (station 403, Lake Ontario 1982) between:

a) ●-●1m and △-△5m

b) ●-●1m and □-□10m


12 AUGUST
a)
b)
1.00
.80
.60
.40
.20
02:00
06:00
10:00
14:00
18:00
22:00
F Ratio (Relative Units)
TIME (Hours)

did not show consistent inhibition when compared to deeper water samples and there was more scatter in the depth profile (Appendix 3.4).

In the samples from the water column which was mixing more rapidly on August 12, F_{DCMU}/chl*a* showed some surface depression in comparison to other depths at 13:00 and 18:10 and was slightly enhanced in 5 meter water (Appendix 3.5). DCMU-enhanced fluorescence was little affected by dark treatment except at the surface at 18:10 (Appendix 3.6). The F ratio data again showed little surface depression (Appendix 3.7).

4.6 Circadian Rhythms

In order to examine the possibility of the existence of circadian rhythms in the fluorescence of phytoplankton in Lake Ontario, two types of observations were made. Samples were incubated under low light for periods greater than 24 hours, and the *in situ* fluorescence of populations on two occasions when light intensity and mixing rates were low was examined.

There was little variability in the F ratio in samples incubated at low light over the 24 hour period on either 30 June at station 401 or station 403 on 10 June 1982 (Appendix 4.1.1). Both periods coincided with days on which incident light was low and the *in situ*

profiles corresponded to the incubator experiments. While some oscillations, especially at 5 meters occurred in the F ratio on 29 June, no distinct pattern was evident over the 24 hour period. The data is included in Appendix 4.1.2 and 4.1.3.

4.7 Time course of fluorescence changes at fixed light

An examination of the short term (minutes) changes in fluorescence were made in order to determine how rapidly and at what intensity, light induced changes in fluorescence could be measured with this system. The experiments were also intended to show which of the three measurements (F, F_{DCMU}, or the F ratio) was most responsive to short term light exposure.

At Jack's Lake there were decreases in F_{DCMU} when compared to the initial value within the first five minutes of exposure to light of 770 and 240 $\mu E\ m^{-2}\ s^{-1}$ (Figure 4.22a,b). There were differences between the initial F_{DCMU} and the first measurement after placement in the incubator. The most rapid rate of change for F_{DCMU} occurred within the first 5 to 25 minutes. In contrast there was no consistent trend in F for the first 50 to 70 minutes at either light intensity (Figure 4.22c,d). After this time fluorescence showed a fairly steady decline. Because of this variability in F, the F ratio was also variable and there was often no clear distinction between the two light levels (Figure 4.23a,b).

Figure 4.22 Fluorescence during a 280 minute incubation of water from (●——●) 2m and (○--○) 8m at Jack's Lake (18 August, 1982):

a) F_{DCMU} at high light (770 $\mu E\ m^{-2}\ s^{-1}$)
b) F_{DCMU} at low light (240 $\mu E\ m^{-2}\ s^{-1}$)
c) F at high light (770 $\mu E\ m^{-2}\ s^{-1}$)
d) F at low light (240 $\mu E\ m^{-2}\ s^{-1}$)

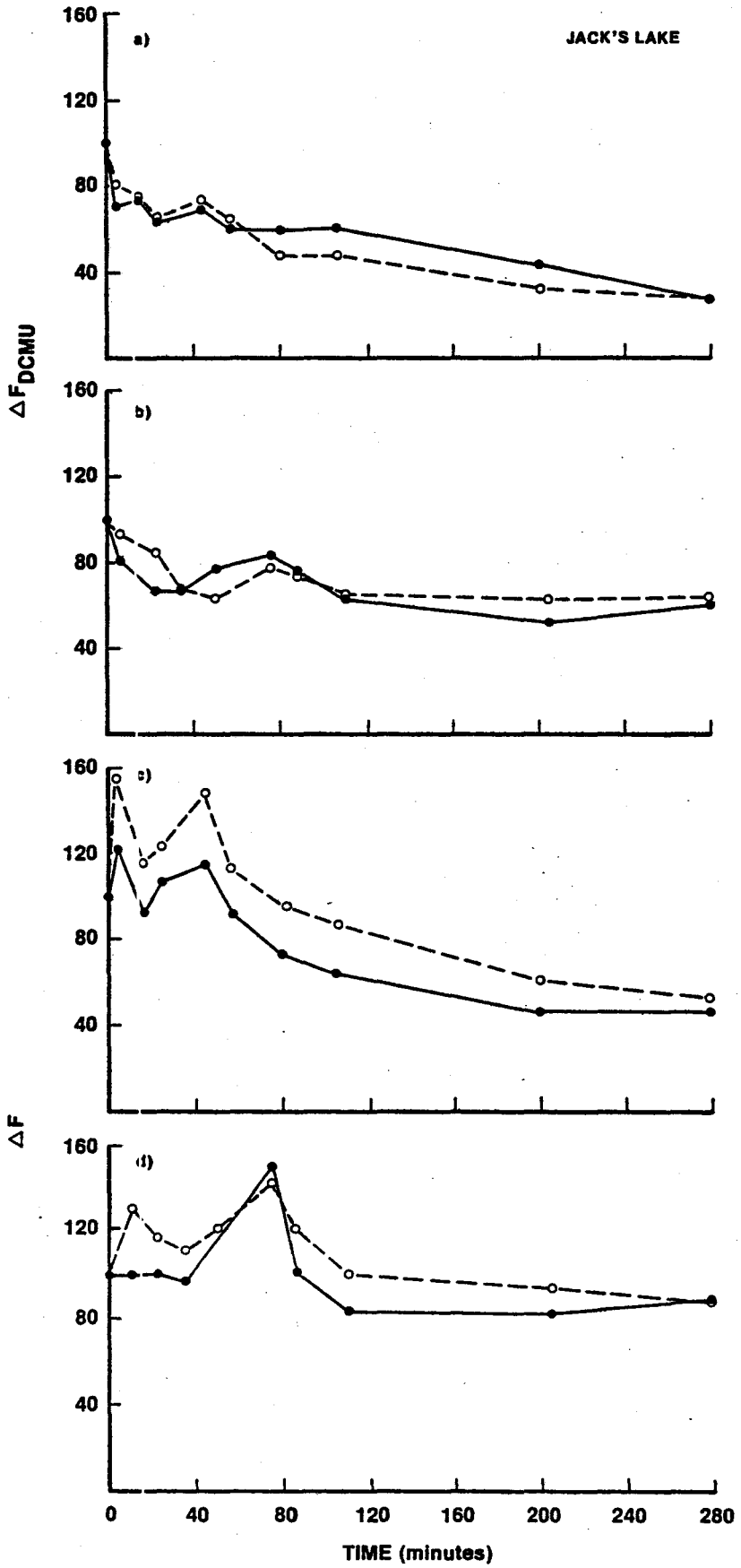
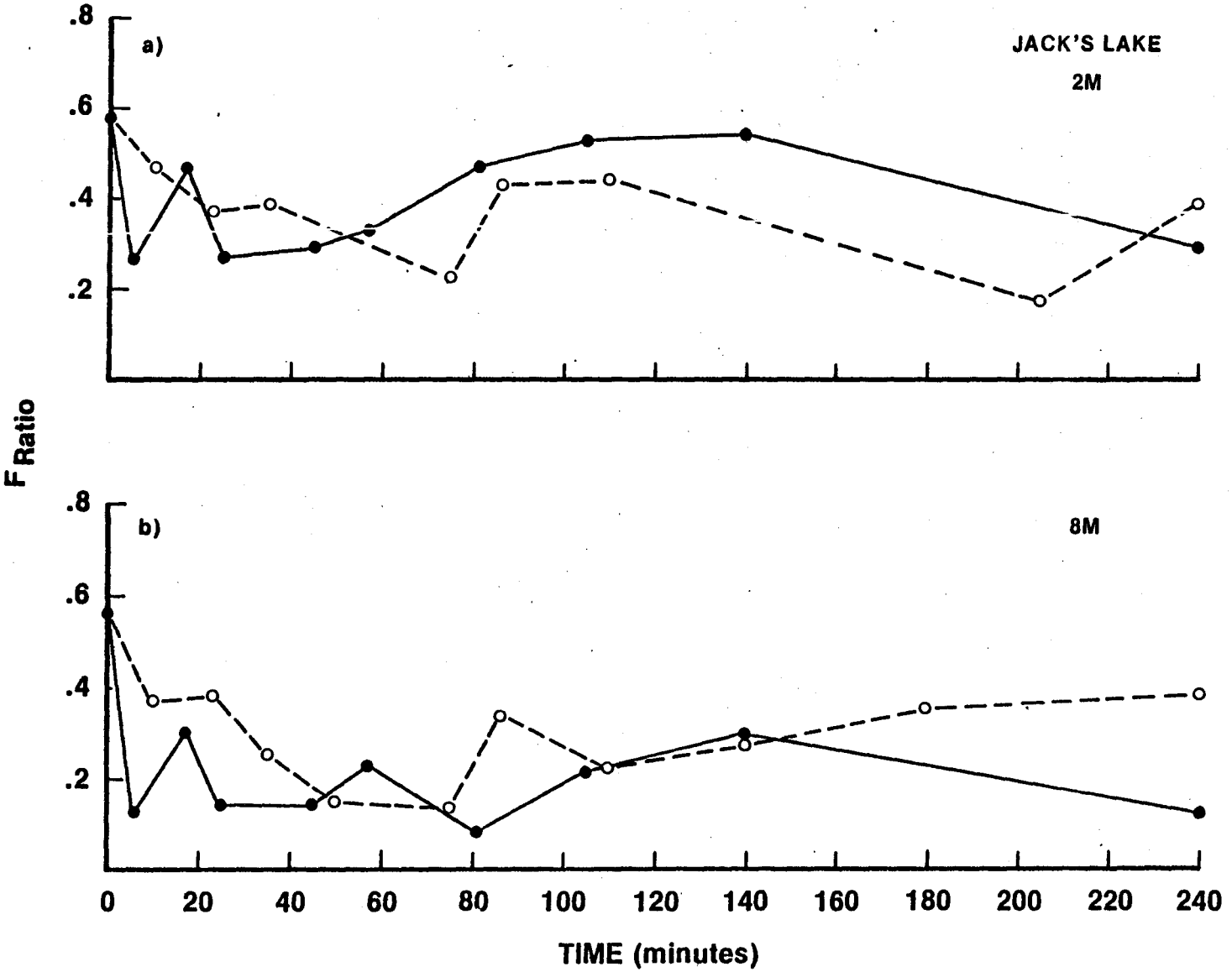

JACK'S LAKE
a)
b)
c)
d)
160
120
80
40
ΔF DCMU
ΔF
0
40
80
120
160
200
240
280
TIME (minutes)

Figure 4.23 The F ratio in water from Jack's Lake (18 August, 1982) incubated at (●—●) 770 μE m^{-2} s^{-1} and (○--○) 240 μE m^{-2} s^{-1}

a) 2 meter water

b) 8 meter water


a)
JACK'S LAKE
2M
.8
.6
.4
.2
F Ratio
b)
8M
.8
.6
.4
.2
0
20
40
60
80
100
120
140
160
180
200
220
240
TIME (minutes)

On both 23 September and 19 October the general pattern for water sampled from 1m and 20m was similar to that of Jack's Lake. There was an effect of bright light on F_{DCMU} at the first sampling i.e. within 10 minutes on 23 September (Figure 24a,b) and within 20 minutes on 19 October (Figure 24c,d). Both samples treated with high (1300 $\mu E\ m-2\ s^{-1}$) and moderately high (670 $\mu E\ m^{-2}\ s^{-1}$) light showed definite differences when compared to the dark treatments by 1 hour. By 2 hours the rate of decline was very slow. There were differences between stations in the response to lower light. On 23 September for both depths the differences between the dark control and the samples treated with 200 or 60 $\mu E\ m^{-2}\ s^{-1}$ were small and inconsistent (Figure 25a,b). On 19 October the differences were again small but consistently lower than those of the dark treated samples (Figure 25c,d).

In contrast to F_{DCMU} consistent differences in F between light and dark treatments were not apparent within the first hour of incubation. At very high light (1300 $\mu E\ m^{-2}\ s^{-1}$) there was eventually a significant decrease in F on both dates at both depths (Figure 4.26). At moderately high light intensity (670 $\mu E\ m^{-2}\ s^{-1}$) the results were more variable. 20 meter water was clearly different from the dark on both dates (Figure 4.26b,d) but the difference between 1 meter water and the dark was smaller and of questionable significance (Figure 4.26a,c). No difference between the F on 23 September or 19 October for either depth at 200 $\mu E\ m^{-2}\ s^{-1}$ and the dark control was observed (data not shown).

Figure 4.24 Comparison of the F_{DCMU} of water from a) 1m, b) 20m on 23 September (station 405, Lake Ontario 1982) and c) 1m, d) 20m on 19 October (station 405, Lake Ontario 1982) during an 8 hour incubation at high light and in the dark. All values expressed as a percent relative to the initial value. ↓ indicates the onset of darkness. Light intensity of incubation indicated by:

(1300 $\mu E\ m^{-2}\ s^{-1}$)
(670 $\mu E\ m^{-2}\ s^{-1}$)
(Dark)

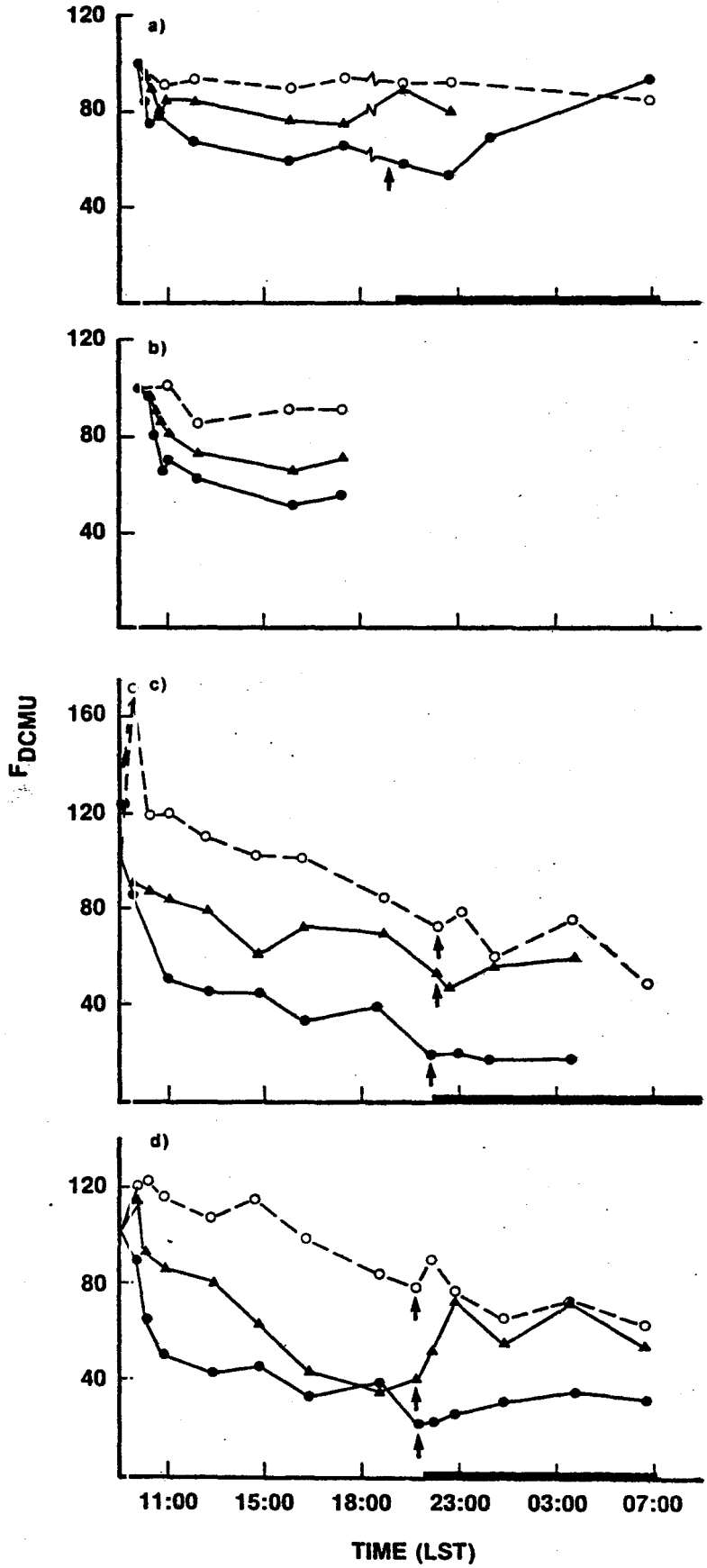

a)
b)
c)
d)
120
80
40
160
F_{DCMU}
11:00
15:00
18:00
23:00
03:00
07:00
TIME (LST)

Figure 4.25 Comparison of the F_{DCMU} of water from a) 1m, b) 20m on 23 September, (station 405, Lake Ontario 1982) c) 1m, d) 20m on 19 October, during an 8 hour incubation at moderate light and in the dark. All values expressed as a percent relative to the initial value. ↑ indicates the onset of darkness. Intensity of incubation indicated by:

(200 $\mu E\ m^{-2}\ s^{-1}$)
(60 $\mu E\ m^{-2}\ s^{-1}$)
(Dark)

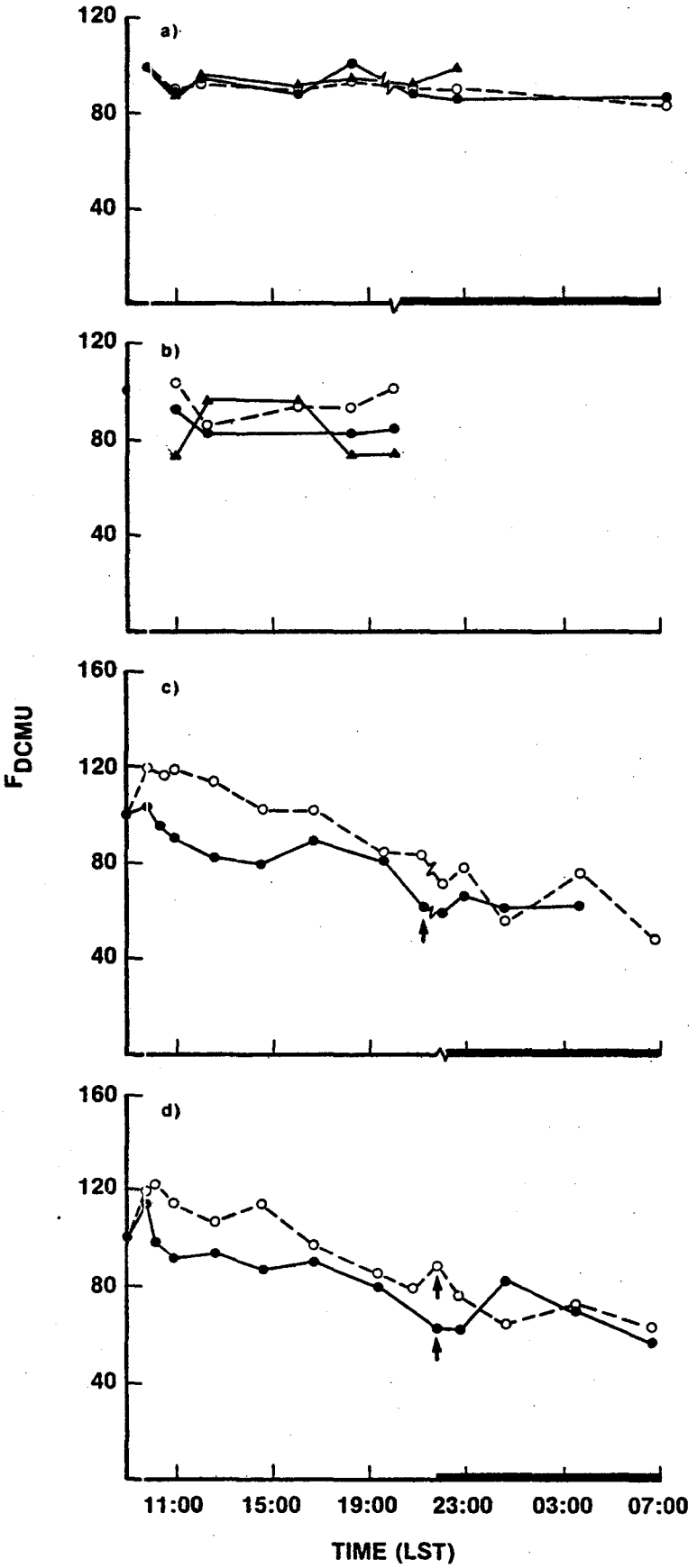
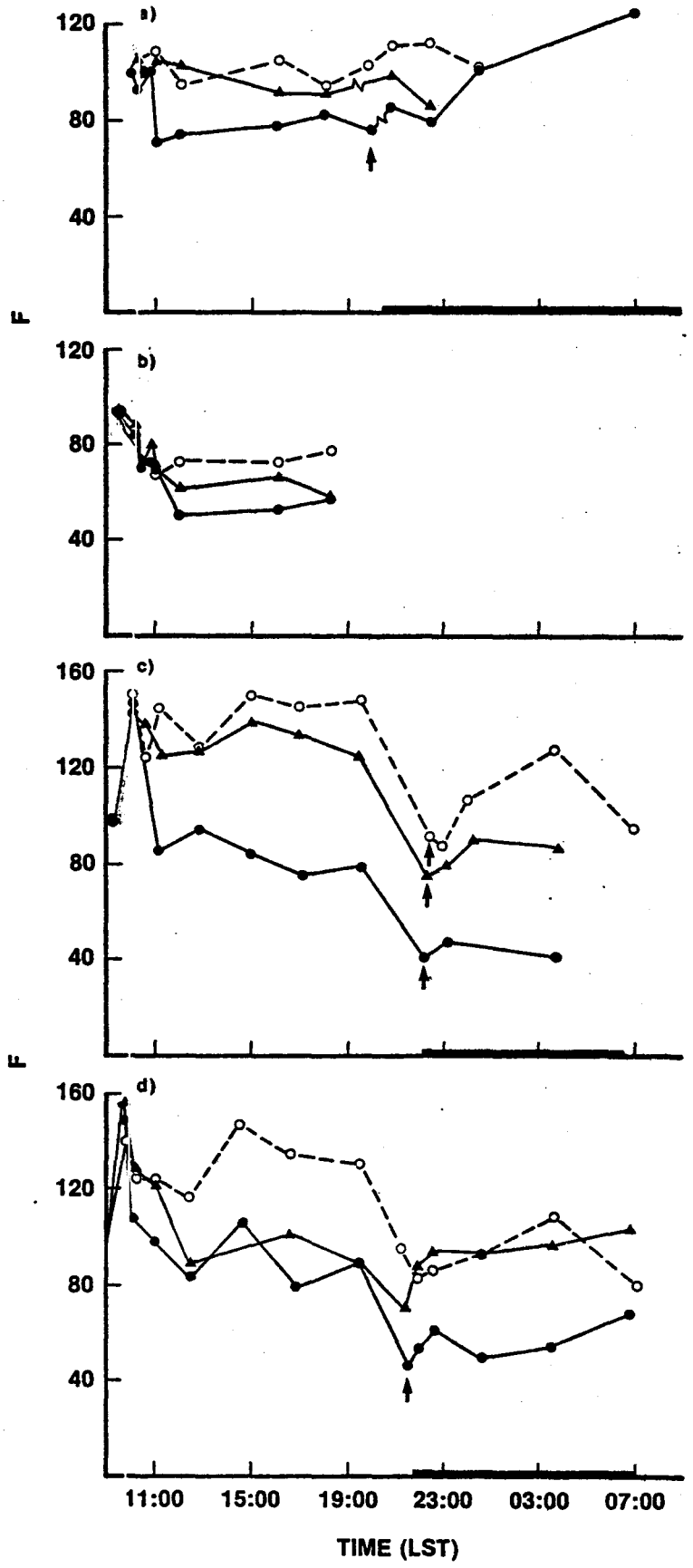

a)
b)
c)
d)
120
80
40
160
120
80
40
160
120
80
40
F_{DCMU}
11:00
15:00
19:00
23:00
03:00
07:00
TIME (LST)

Figure 4.26 Comparison of the F of water from a) 1m, b) 20m on 23 September, (station 405, Lake Ontario 1982) c) 1m, d) 20m on 19 October, (station 405, Lake Ontario 1982) during an 8 hour incubation at moderate light and in the dark. All values expressed as a percent relative to the initial value. ↑ indicates the onset of darkness. Intensity of incbation indicated by:

(1300 $\mu E\ m^{-2}\ s^{-1}$)
(670 $\mu E\ m^{-2}\ s^{-1}$)
(Dark)


a)
b)
c)
d)
120
80
40
160
120
80
40
F
11:00
15:00
19:00
23:00
03:00
07:00
TIME (LST)

On both 23 September and 19 October, the F and F_{DCMU} of the dark controls showed a large amount of variability. The decrease in F_{DCMU} in both 1m (Figure 4.25c) and 20m (Figure 4.25d) water was particularly large.

As in Jack's Lake no consistent pattern of decrease in the F ratio over the course of the experiment was observed on 23 September (data included in Appendix 5.1.1). A consistent trend of decreasing F ratio on 19 October was apparent at 1300 and 200 $\mu E\ m^{-2}\ s^{-1}$ within the first hour of incubation but the differences between the F ratio in samples incubated at low light and in those incubated in the dark had disappeared by the end of 8 hours (data included in Appendix 5.1.2).

The fluorescence response of the phytoplankton in late November at station 403 was dramatically different to that of the phytoplankton in September and October. F_{DCMU} showed differences from the control within 10 minutes of exposure to light at 1300 and 470 $\mu E\ m^{-2}\ s^{-1}$ and was only 25% of the initial value following a 3 hour incubation at 1300 $\mu E\ m^{-2}\ s^{-1}$ (Figure 4.27a).

During another experiment with water from the nearshore station (401) there were again significant differences within 10 minutes between F_{DCMU} from the water incubated at 470 $\mu E\ m^{-2}\ s^{-1}$ and the dark control. There were significant differences in F_{DCMU} between the water incubated at 150 $\mu E\ m^{-2}\ s^{-1}$ and the dark control after 40 minutes (Figure 4.28a).

Figure 4.27 Comparison of the a) F_{DCMU} b) F c) F ratio in 5m water from station 403 on 22 November,1982 (Lake Ontario) incubated in the light and in the dark for 200 minutes. All values expressed as a percent relative to the initial value. Intensity of incubation indicated by:

(1300 $\mu E\ m^{-2}\ s^{-1}$)
(470 $\mu E\ m^{-2}\ s^{-1}$)
(Dark)

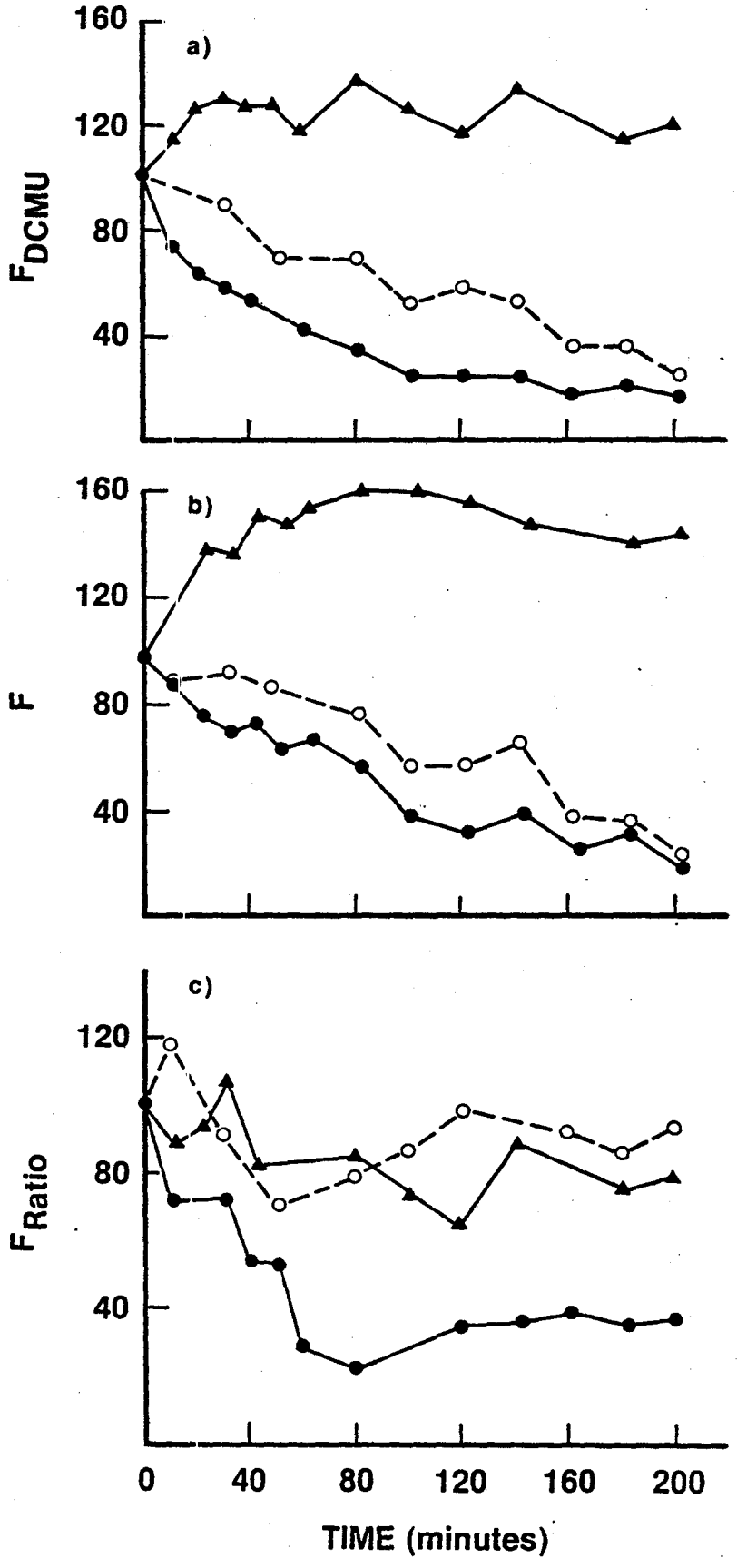
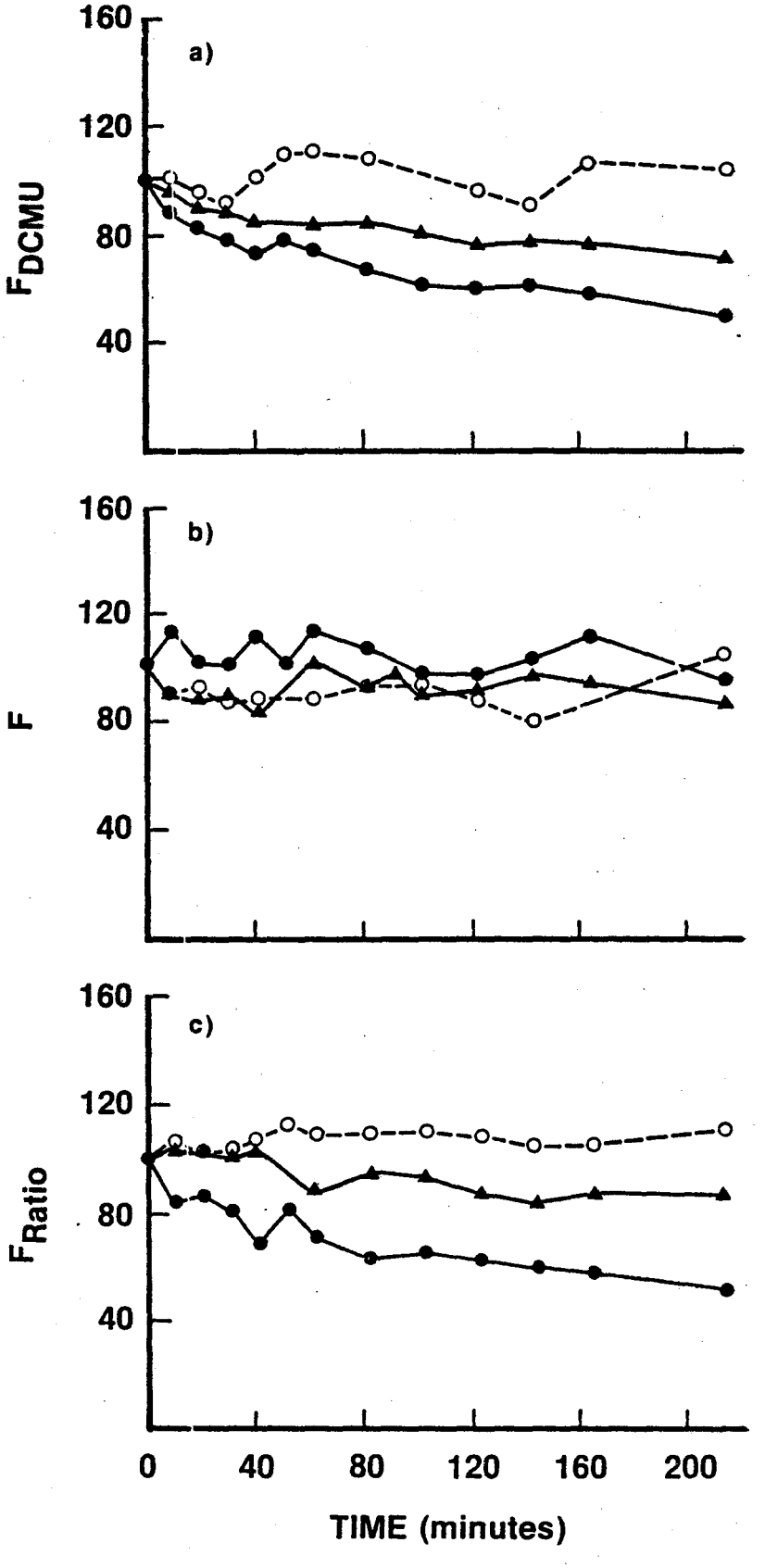

a)
b)
c)
160
120
80
40
F_{DCMU}
F
F_{Ratio}
0
40
80
120
160
200
TIME (minutes)

Figure 4.28 Comparison of the a) F_{DCMU}, b) F c) F ratio in 5m water from station 401 on 23 November,1982 (Lake Ontario) incubated in the light and in the dark for 220 minutes. All values expressed as a precent relative to the initial value. Intensity of incubation indicated by:

(470 $\mu E\ m^{-2}\ s^{-1}$)
(150 $\mu E\ m^{-2}\ s^{-1}$)
(Dark)


160
120
80
40
a)
F_{DCMU}
160
120
80
40
b)
F
160
120
80
40
c)
F_{Ratio}
0
40
80
120
160
200
TIME (minutes)

The response of F was again less predictable. The fluorescence of samples incubated at bright light (1300 and 500 $\mu E\ m^{-2}\ s^{-1}$) on 22 November (Figure 4.27b) was much lower than that of the dark incubated samples while the in vivo fluorescence of samples from station 403 incubated at 470 $\mu E\ m^{-2}\ s^{-1}$ was greater than that of the control (Figure 4.28b).

While there was a trend for F ratios to decrease in the light and increase in the dark there was obviously more variability in this ratio than in either F_{DCMU} or F alone (Figure 4.27c,4.28c).

4.8 Recovery of fluorescence in the dark following incubation in the light

4.8.1 Recovery following short incubation in the light

The results from three experiments performed near the end of the year were similar. Following a 1 hour incubation at 1300 $\mu E\ m^{-2}\ s^{-1}$ some recovery of F_{DCMU} was apparent after one half hour and complete recovery (to the level of the dark control) had occurred by the end of 1 hour in the dark (Figure 4.29a). Because the fluorescence of plankton on 22 November was more light sensitive, a shorter incubation was performed. Figure 4.30a shows that, there were increases in F_{DCMU} within the first 20 minutes of dark treatment following a 15 minute incubation at 1300 $\mu E\ m^{-2}\ s^{-1}$. Full recovery of

Figure 4.29 Comparison of a) F_{DCMU} b) F and c) the F ratio of water from 5m on 23 October,1982 (Lake Ontario) incubated in the light at 1300 $\mu E\ m^{-2}\ s^{-1}$ for 60 minutes and then placed in the dark for 60 minutes to that of water incubated in the dark for 120 minutes; the ↑ indicates the times at which the lights were turned off in the incubator. All values expressed as a percent relative to the initial value.

(Light treated samples)

(Dark treated samples)

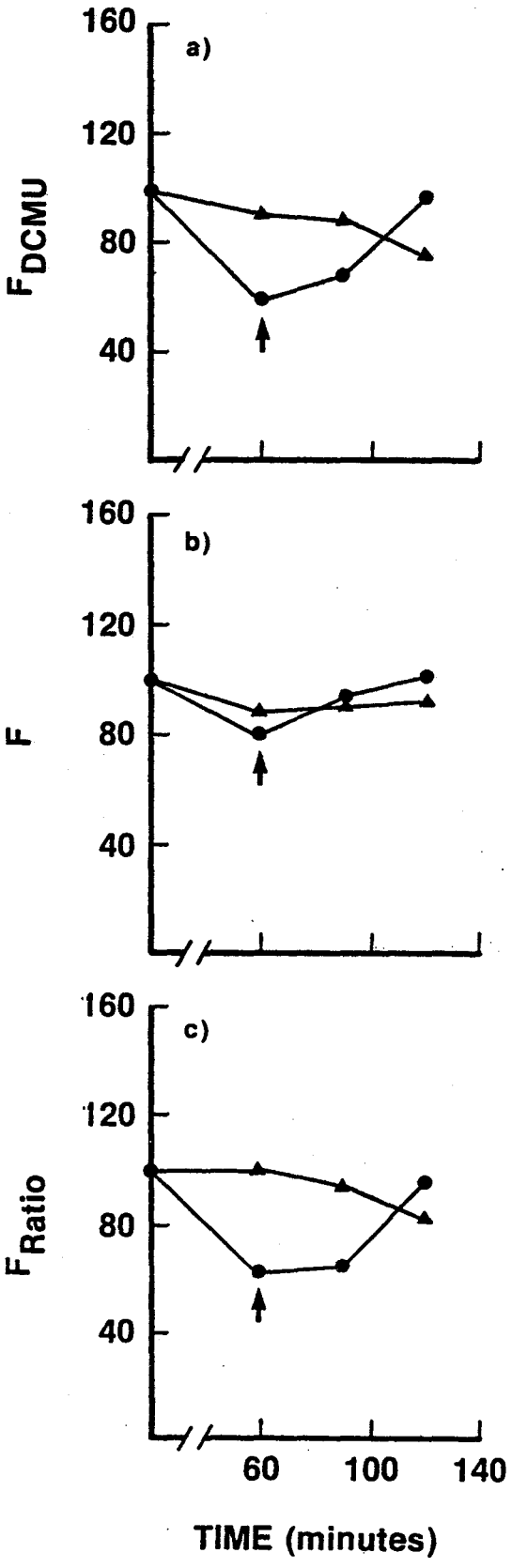
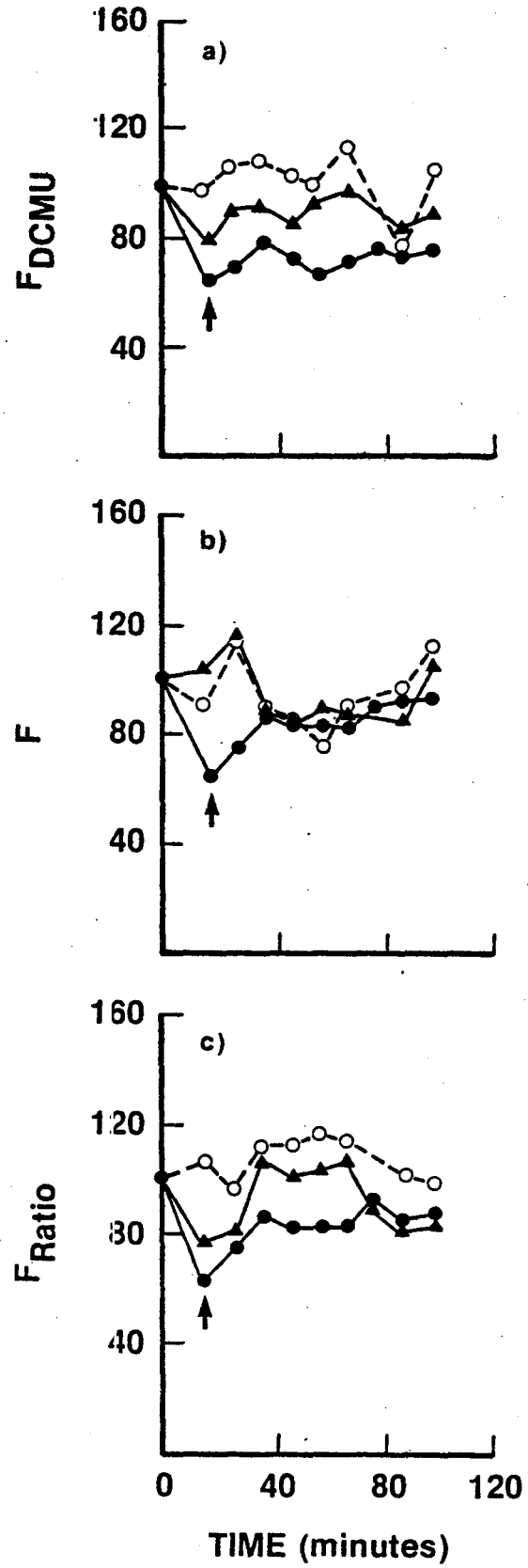

160
120
80
40
a)
F_{DCMU}
160
120
80
40
b)
F
160
120
80
40
c)
F_{Ratio}
60
100
140
TIME (minutes)

Figure 4.30 Comparison of a) F_{DCMU} b) F c) the F ratio of water from 5m at station 403 (Lake Ontario) on 22 November,1982 incubated in the light at 1300 $\mu E\ m^{-2}\ s^{-1}$ and 470 $\mu E\ m^{-2}\ s^{-1}$ for 15 minutes and then placed in the dark for 85 minutes to that of water incubated in the dark for 100 minutes; the ↓ indicates the times at which the lights were turned off in the incubator. All values expressed as a percent relative to the initial value.

(Water treated at 1300 $\mu E\ m^{-2}\ s^{-1}$)
(Water treated at 470 $\mu E\ m^{-2}\ s^{-1}$)
(Dark treated sample)


160
120
80
40
a)
F_{DCMU}
160
120
80
40
b)
F
160
120
80
40
c)
F_{Ratio}
0
40
80
120
TIME (minutes)

F_{DCMU} to the level of the dark control, had not occurred by the end of 40 minutes. There appeared to be significant differences, as well, between the dark control and the sample treated with 470 $\mu E\ m^{-2}\ s^{-1}$ after 40 minutes in the dark. Following another incubation at 470 $\mu E\ m^{-2}\ s^{-1}$ (for forty minutes) there were increases in F_{DCMU} within the first 20 minutes of dark treatment (Figure 4.31a). Full recovery, however was not complete within 3 hours. At 150 $\mu E\ m^{-2}\ s^{-1}$ "recovery" of F_{DCMU} was complete within 10 minutes (Figure 4.31a).

Recovery of F was more rapid. In all of the experiments discussed above there were no differences between light-treated samples and dark controls within 20 minutes following placement in the dark (Figures 4.29b,4.30b,4.31b).

The F ratio did not recover within 20 minutes dark exposure in any of these experiments (Figures 4.29c,4.30c,4.31c).

A comparison of the rate of recovery of fluorescence following incubations of different lengths of time is shown in Figure 4.32. On this occasion water from the same sampling on 22 November was incubated for 15 minutes and 40 minutes at 1300 $\mu E\ m^{-2}\ s^{-1}$. <u>In vivo</u> fluorescence recovered fully following a 15 minute incubation but not a 40 minute incubation (Figure 4.32b). Figure 4.32 shows that F_{DCMU} also recovered less rapidly following the 40 minute incubation.

Figure 4.31 Comparison of a) F_{DCMU} b) F and c) the F ratio of water from 5m at station 401 (Lake Ontario) on 23 November,1982 incubated in the light at 470 μE m^{-2} s^{-1} and 150 μE m^{-2} sec^{-1} for 40 minutes and then placed in the dark for 160 minutes to that of water incubated in the dark for 200 minutes; the ↑ indicates the time at which the lights were turned off in the incubator. All values expressed as a percent relative to the initial value.

●—● (Water treated at 470 uE m^{-2} s^{-1})
▲—▲ (Water treated at 150 uE m^{-2} s^{-1})
O-O (Dark treated samples)

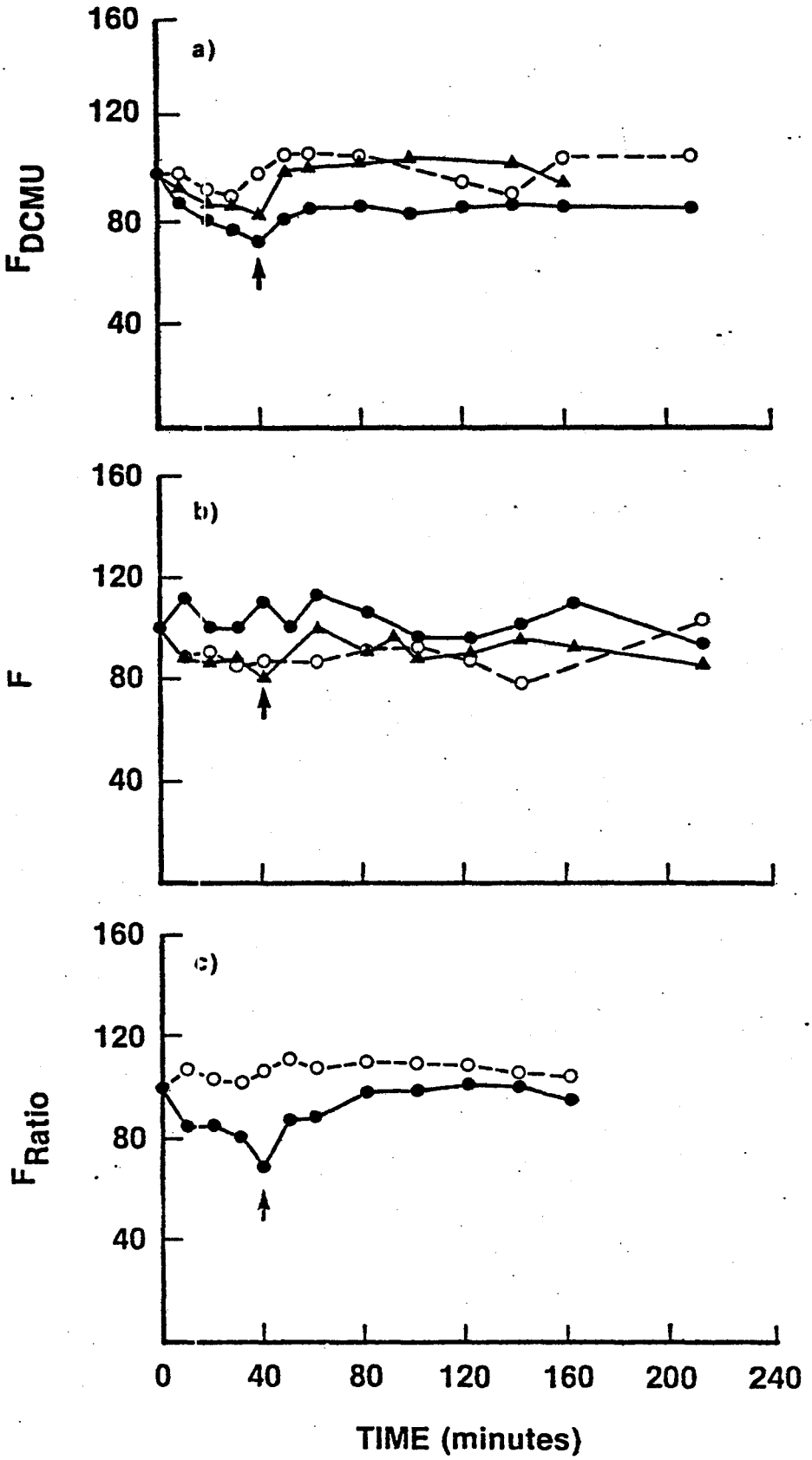
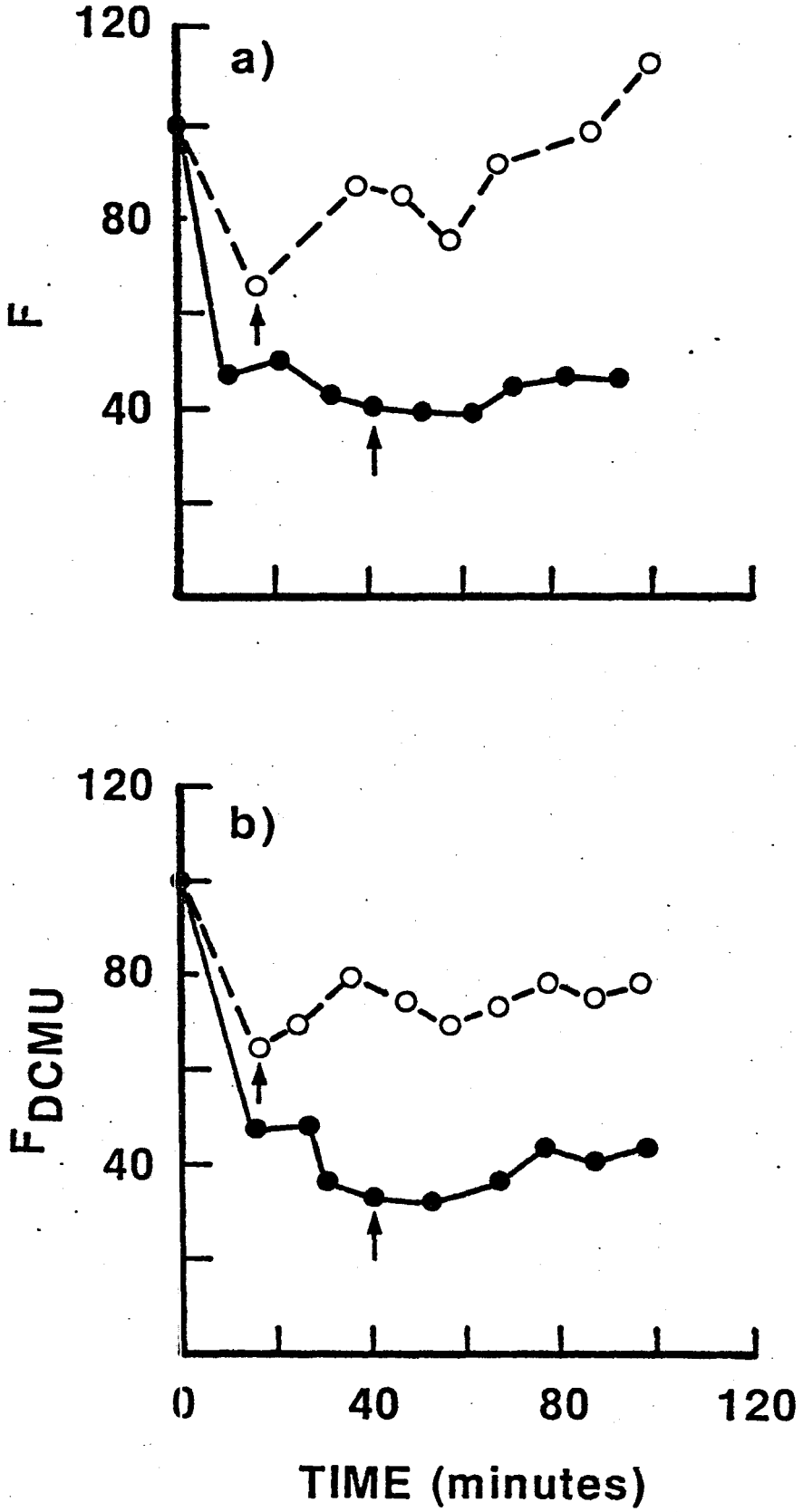

160
120
80
40
a)
F_{DCMU}
160
120
80
40
b)
F
160
120
80
40
c)
F_{Ratio}
0
40
80
120
160
200
240
TIME (minutes)

Figure 4.32 Comparison of a) F_{DCMU} and b) F of water from 5m at station 403 (Lake Ontario) on 23 October, 1982 incubated in the light at 1300 $\mu E\ m^{-2}\ s^{-1}$ for 15 minutes (O---O) and 40 minutes (●—●) and then placed in the dark for 85 minutes and 60 minutes; the ↑ indicates the time at which the lights were turned off in the incubator. All values expressed as a percent relative to the initial value.


120
a)
80
F
40
120
b)
80
F_{DCMU}
40
0
40
80
120
TIME (minutes)

4.8.2 Recovery following long term incubation in the light

Table 4.7 shows that in 20 out of 30 observations (67%) the F and F_{DCMU} of samples incubated either continuously in the dark or at low light intensity (90-150 $\mu E\ m^{-2}\ s^{-1}$) for a period of hours followed by an incubation in the dark were not significantly different to the initial value. In contrast, F and F_{DCMU} were significantly less than their initial values after continuous incubation at high light followed by a dark treatment in 9 out of 18 observations. F_{DCMU} showed less recovery in the dark than did the F of the same sample in all experiments following treatment at high ligh intensity. F_{DCMU} was affected in 7 out of 9 observations.

4.9 Seasonal response to fixed light

Table 4.8 is a summary of the fluorescence of samples kept in the dark for three hours to that of the fluorescence measured at the start of the experiment over the course of the season. Only 5 out of 22 measurements of F and F_{DCMU} were significantly different to their initial value. Samples were dark adapted before the experiment and further fluorescence changes over the course of the experiment were generally minimal. The fluorescence properties of the dark treated samples will be used as a control to which the light treated samples may be compared.

Table 4.7 Recovery of fluorescence in the dark following incubation at high and low light compared to samples maintained continuously in the dark for the same period of time. L_1 is 700-1000 uE m^{-2} s^{-1}; L_2 is 90-150 uE m^{-2} s^{-1}. Fluorescence is expressed as a % of the initial value. * denotes a significant value. Length of incubation is expressed in hours; depth in meters.

DATE	DEPTH	LENGTH OF INCUBATION		F_{L_1}			F_{DCMU}		
		LIGHT	DARK	L_1	L_2	DARK	L_1	L_2	DARK
1 July	5	12	8	77		70*	80		82*
11 June	1	16	8	76	96	104	43*	88	124*
	150	16	8	57*	95	115	36*	97	100
30 June	5			92	89	95	88	130	94
2 July	5			73	93	76*	47*	90	59*
23 September		12	4	101	100	102	69*	86	87
19 October	1	12	4	84	94	127*	58*	84*	76*
22 November	1	.7	8	58*		112	46*		85*
22 November	1	1.3	2	80		142	72*		128*

Table 4.8 Fluorescence of samples removed over the course of the season and incubated in the dark. All fluorescence values expressed as a percentage of the initial value. * denotes a significant value. Depth expressed in meters.

DATE	STA	DEPTH	F	F_{DCMU}	F ratio
18 May	403	5	77	81*	103
8 June	401	5	108	104	105
10 June	403	5	90	100	88
30 June	401	5	79	91	115
13 August	403	150	138*	116*	86
24 August	401	5	112	114	101
23 September	401	1	95	90	97
19 October	405	1	102	100	98
19 October	401	1	117	106	111
22 November	403	1	142*	90	64*
18 August	Jack's Lake metalimnetic Chrysophyte community		78	83*	83

Tables 4.9, 4.10, 4.11 compare fluorescence parameters of lake water incubated at light intensities of about 1000 $\mu E\ m^{-2}\ s^{-1}$, 300 $\mu E\ m^{-2}\ s^{-1}$, and 100 $\mu E\ m^{-2}\ s^{-1}$ for about three hours to that of lake water incubated in the dark for the same period of time. The exact light intensity for each incubation is indicated in each table. The largest effect on F and F_{DCMU} was observed at the highest light intensity. F_{DCMU} was 31 to 77% of the dark value following incubation at high light (Table 4.9); 71 to 92% of the dark value following incubation at moderate light (Table 4.10); 67 to 124% of the dark value following incubation at low light (Table 4.11). Only 7 out of 22 fluorescence values were not significantly lower than that of the dark following incubation at high light (Table 4.9). All values of F_{DCMU} were significantly decreased following incubation at high light. The F of spring (18 May, 8 June) and early winter (22 November) samples also decreased significantly.

The response of F was more variable than F_{DCMU}. Samples treated with greater than 1000 $\mu E\ m^{-2}\ s^{-1}$ of light generally showed some decrease in fluorescence however the pattern was not always consistent to that observed for F_{DCMU}. For example F_{DCMU} in water from station 401 was similar on 8 and 30 June. But, while untreated fluorescence (F) was similar to the control on 30 June, it was significantly decreased as compared to the dark on 8 June (Table 4.9).

Table 4.9 Fluorescence of samples removed over the course of the season and incubated at high light. All values expressed as a percentage of the dark value. Light expressed in $\mu E\ m^{-2}\ s^{-1}$; depth in meters. * indicates a significant value. ' indicates an approximate value. Incubations were approximatley 3 hours except on 13 August when the length of incubation was 4 hours and on 18 August when the length of incubation was 2 hours.

DATE	STA	DEPTH	PHYSICAL CHARACTER	LIGHT	F	F_{DCMU}	F ratio
18 May	403	5	mixed	1330	54*	52*	96
8 June	401	5	stratified	770'	63*	72*	128*
10 June	403	5	mixing	770'	82	68*	100
30 June	401	5	stratified	770	91	77*	83*
24 August	401	5	stratified	770	76	53*	56*
23 September	401	1	stratified	510	78	74*	97
19 October	405	1	stratified	510	95	57*	31*
19 October	401	1	mixing	510	100	67*	57*
22 November	403	1	stratified	750'	27*	31*	14*
13 August	403	150	stratified	750'	34*	31*	88
18 August	Jack's Lake metalimnetic Chrysophyte community			750'	96	73*	78*

Table 4.10 Fluorescence of samples removed over the course of the season and incubated at moderate light. All fluorescence values expressed as a percentage of the dark value. * indicates a significant value. ' indicate an approximate value. Light expressed in $\mu E\ m^{-2}\ s^{-1}$; depth in meters. Length of incubation as in Table 4.9

DATE	STA	DEPTH	PHYSICAL CHARACTER	LIGHT	F	F_{DCMU}	F ratio
18 May	403	5	mixing	450	83	71*	86
8 June	401	5	stratified	260'	75	89	150*
10 June	403	5	mixing	260'	111	92	98
30 June	401	5	stratified	250	97	92	94
24 August	401	5	stratified	350	82	94	110
23 September	401	5	stratified	250'	87	97	110
19 October	405	1	stratified	250	106	72*	59*
19 October	403	1	stratified	250	102	88	87
22 November	403	1	stratified	150	56*	67*	142*
13 August	403	150	stratified	250'	72	72*	100
18 August	Jack's Lake metalimnetic Chrysophyte community			330	109	84*	79

Table 4.11 Fluorescence of samples removed over the course of the season and incubated at low light. All fluorescence values expressed as a percentage of the dark value. * indicates a significant value. Light expressed in $\mu E\ m^{-2}\ s^{-1}$ (' is an approximate value); depth in meters. Length of incubation as in Table 4.9.

DATE	STA	DEPTH	PHYSICAL CHARACTER	LIGHT	F	F_{DCMU}	F ratio
18 May	403	5	mixing	112	105	124	112
8 June	401	5	stratified	100'	101	108	116
10 June	403	5	mixing	100'	136	104	90
24 August	401	5	stratified	91	88	104	105
22 September	401	5	stratified	250	87	102	92
22 November	403	1	stratified	150	56*	67*	144*
18 August	Jack's Lake		metalimnetic Chrysophyte community	140'	86	97	96

Because both F and F_{DCMU} were affected by light treatment, and because the extent to which each was affected varied seasonally, no consistent change in the F ratio was observed. In samples where the change in F_{DCMU} was larger than the change in F e.g. 19 October and 22 November or in the Chrysophyte population, there was also a decrease in F ratio when compared to the dark treatment. Spring samples however which exhibited large changes in both F and F_{DCMU} showed little difference or actually showed an increased F ratio over dark treated samples. The largest depression in F_{DCMU} occurred consistently at all light intensities in the winter (22 November) and in the 150 meter sample (13 August). Summer (June) through early fall (September) samples were least responsive to long term exposure to bright light (Table 4.9).

Because of the existence of a thermal bar in the spring in Lake Ontario, the inshore station (401) was stratified while the mid-lake station (403) was fully mixed at the first June sampling. The ^{14}C data showed that maximum ^{14}C uptake occurred at 950 $\mu E\ m^{-2}\ s^{-1}$ in the stratified population (station 401) while rates on 10 June 1982 (403) were somewhat depressed at this intensity. Absolute rates of photosynthesis expressed on a per chlorophyll basis were also lower in the deeply mixed population (D.R.S. Lean unpub. data). The data suggests that the deepwater population was "shade adapted". The P turnover time at the inshore station in early June, was 5 minutes while at the off-shore station it was 200 hours (D.R.S. Lean unpub. data). Thus the physiology of the phytoplankton in terms of P status and light history should differ at the two stations. The response of

F_{DCMU} to bright light was similar while the value observed for F in the "sun" population at station 401 was actually lower than that observed for the "shade" algae at station 403 (Table 4.9).

4.10 The effect of different mixing regimes on a single population

In order to test the effect of vertical mixing alone it is necessary to compare the response of a single community under the two regimes. Profiles from three days will be examined as illustrations of the three possible combinations of light and mixing regimes which may occur in the water column.

On 26 August, high light intensities occurred along with high mixing rates as indicated by low N^2 (Table 4.12). The F_{DCMU}/chl*a* in the water column showed little change between the surface and deeper water in either morning or afternoon samples (Figure 4.33a,b). In contrast, the F_{DCMU}/chl*a* of water incubated in the bottles at 1 meter showed a large depression when compared to 5 meter water. The F ratio showed a similar pattern (Appendix 6.1)

Table 4.12 shows that light conditions were bright and little mixing occurred on 8 June. As on 26 August the F_{DCMU}/chl*a* was depressed in samples incubated in bottles at the surface (Figure 4.34a,b,c). On this occasion, however the F_{DCMU}/chl*a* at the surface of the water column was also depressed especially in the latter two incubations. F_{DCMU} may have been higher in the water from the bottles than in the water column because of a longer dark treatment during

Table 4.12 Comparison of ^{14}C uptake and F_{DCMU} in the water column and following incubation in bottles at incident light during different physical regimes. F_{DCMU} expressed as a ratio of the fluorescence measurement after bottle incubation or in the water column to that prior to incubation. * indicates a significantly lower value. The large enhancement of F_{DCMU} on 8 June was due to a depression of the initial fluorescence measurement. ^{14}C is the ratio of ^{14}C uptake (mg C mg chla^{-1} hr^{-1}) at 1m and 5m. Values less than 1 indicate surface inhibition; values greater than 1 indicate no surface inhibition of photosynthesis. Length of incubation in hours; solar radiation in langleys day^{-1} (data for Jack's Lake not available); N^2 between 1 and 5 meters is expressed in $s^{-2} \times 10^{-4}$.

DATE	TIME	STA	LENGTH INCUBATION	N^2	SOLAR RADIATION	^{14}C	F_{DCMU} WATER	F_{DCMU} BOTTLES
8 June	AM	401	4.3	171	686	.87	.72*	.79*
	NOON		3.8	778		.51*	.65*	.83*
	PM		6.8	686		.48*	1.10	1.90
29 June	ALL DAY	401	16.1	630	358	1.50	1.00	1.25
1 July	AM	405	4.9	0	735	1.45	.87	.84*
	PM		4.9	290		.54*	.71*	.63*
26 August	AM	403	4.0	0	521	.75*	1.02	.66*
	PM		4.8	101		.31*	.98	.80*
22 September	PM	403	4.4	0	243	1.53	1.02	1.04
	ALL DAY		10.2	0		2.13	1.05	1.12
Jack's Lake	ALL DAY		13.5	0	N/A	2.00	.98	.90

Figure 4.33 Comparison of the F_{DCMU}/chl*a* in water incubated in bottles (▲--▲) to that in the water column (O—O) on 26 August, 1982 station 403 (Lake Ontario):

a) 06:50--10:50

b) 10:55--15:41

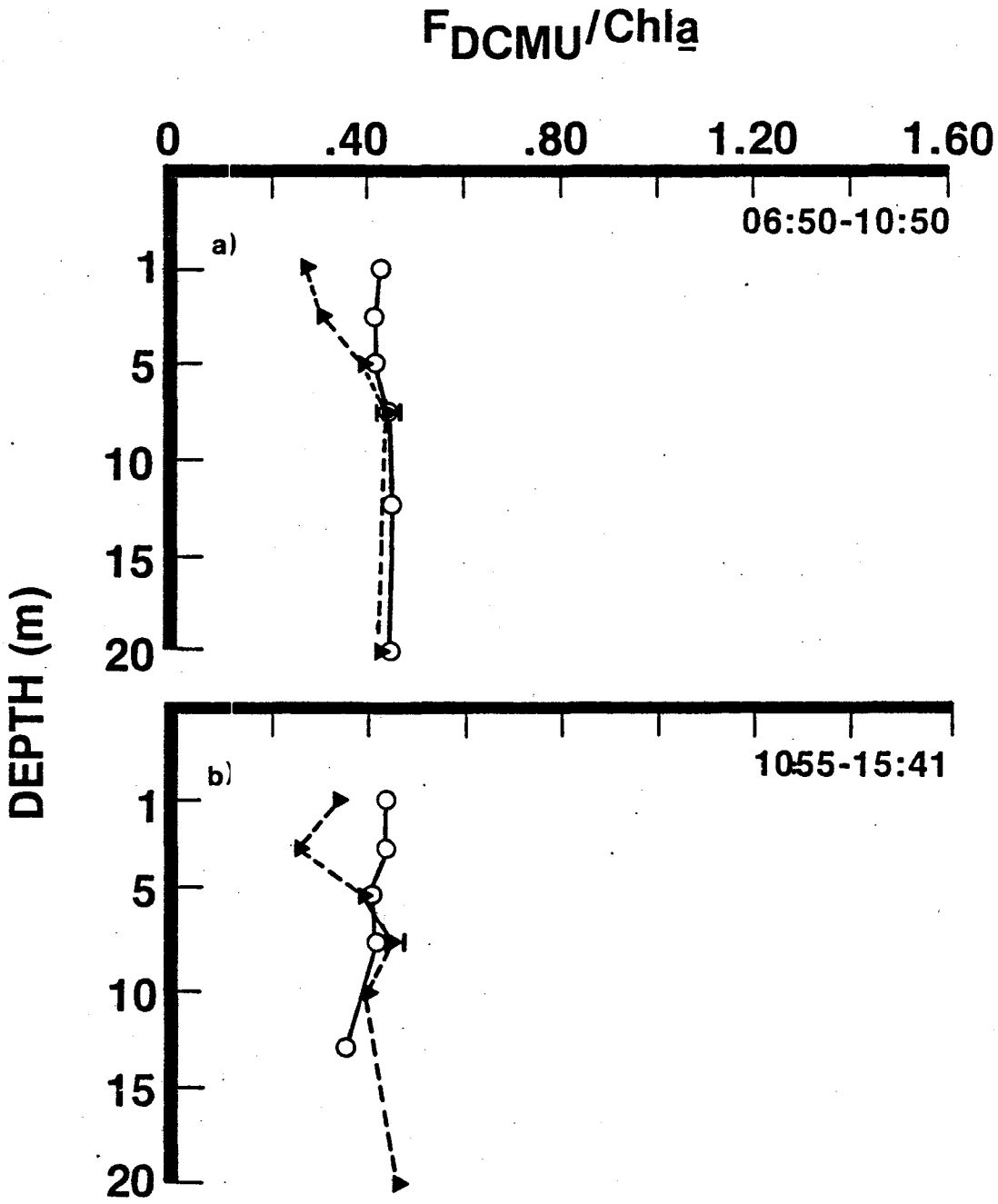
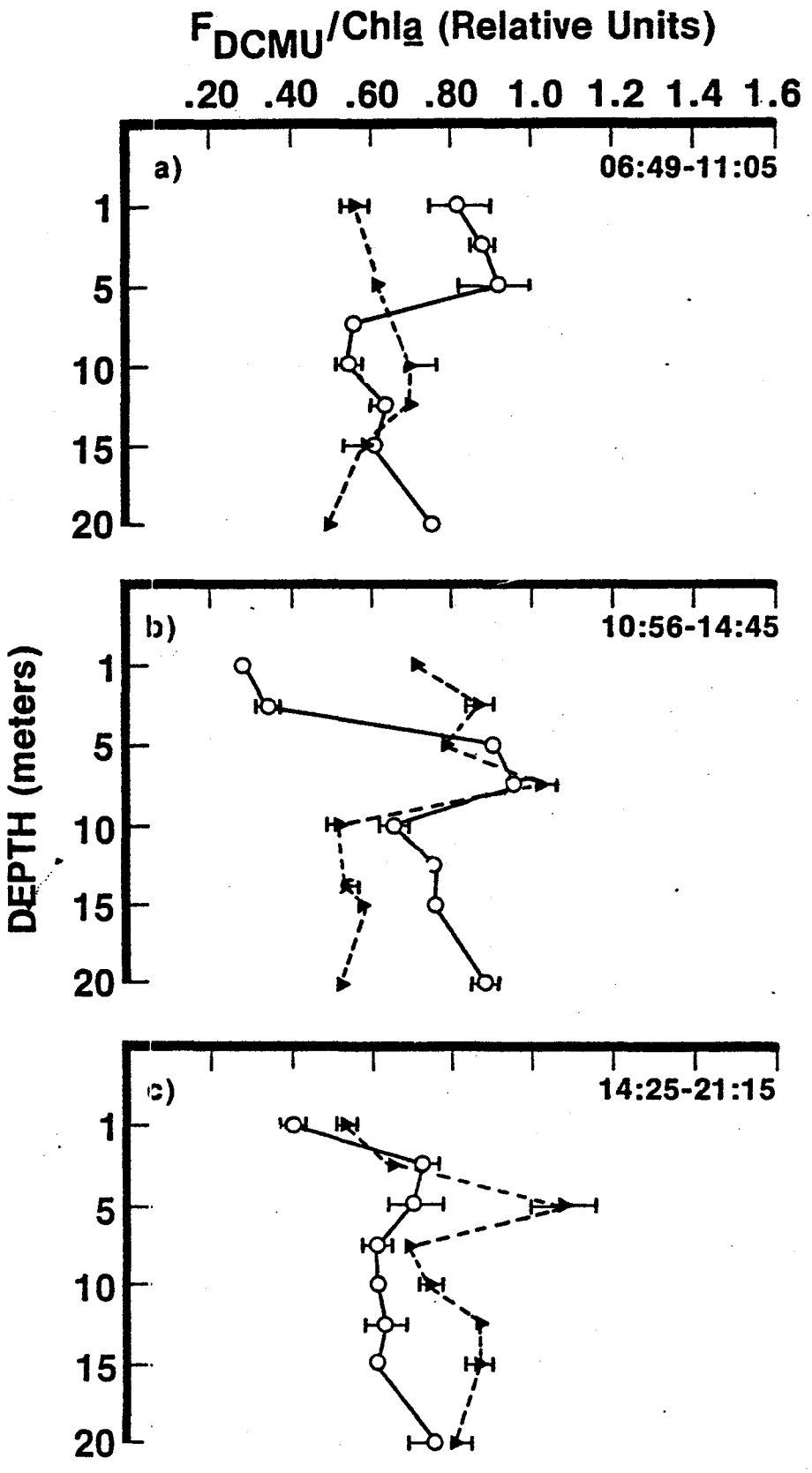

$F_{DCMU}/Chl\underline{a}$
0
.40
.80
1.20
1.60
06:50-10:50
a)
1
5
10
15
20
DEPTH (m)
10:55-15:41
b)
1
5
10
15
20

Figure 4.34 Comparison of the F_{DCMU}/chl*a* in water incubated in bottles (▶--▶) to that in the water column (O—O) on 8 June, 1982 at station 401 (Lake Ontario):

a) 06:49--11:05
b) 10:56--14:45
c) 14:25--21:15

Note the occurence of a higher F_{DCMU}/chl*a* in the bottles. This may be due to a longer dark treatment during transport from the site of incubation to the ship in these samples than in those sampled directly from the water column.


F_{DCMU}/Chla (Relative Units)
.20 .40 .60 .80 1.0 1.2 1.4 1.6
a)
06:49-11:05
b)
10:56-14:45
c)
14:25-21:15
DEPTH (meters)
1
5
10
15
20

transport from the site of incubation to the ship. The F ratio data is included in Appendix 6.2. The general pattern observed was the same although, as discussed in section 4.5 and 4.8, this parameter was less valuable as an indicator of light history on this date.

Light conditions on 29 June were low, however the F ratio from surface bottles and at the surface of the water column were slightly depressed. F_{DCMU}/chl*a* declined at lower depths in the vertical profiles as well (Figure 4.35).

4.11 Comparison of surface depression of ^{14}C uptake and F_{DCMU} in bottles and in the water column

Figures 4.36 and 4.37 show ^{14}C uptake along with the F_{DCMU} data discussed in section 4.10. Depression of ^{14}C uptake in bottles occurred on both 26 August (Figure 4.36) and 8 June (Figure 4.37) during high levels of incident light but not on 29 June (Figure 4.35) when solar radiation was low. This pattern of surface depression of ^{14}C uptake corresponded to that observed for the F_{DCMU}/chl*a* of the water incubated in bottles. On 8 June when water column stability was high it also corresponded to the pattern observed in the water column (Figure 4.37). No correlation was observed however on 26 August when water column stability was low (Figure 4.36).

The data from all experiments is summarized in Table 4.12. A significant decrease between the ^{14}C uptake at 1 and 5 meters was accompanied by a significant decrease in the F_{DCMU} measured after incubation in the bottles to that measured prior to incubation on all

Figure 4.35 Comparison of a) the F ratio b) F_{DCMU}/chl*a* in water incubated in bottles (●---▶), and in the water column (○—○) and (●—●) ^{14}C uptake on 29 June, 1982 at station 401 (Lake Ontario).

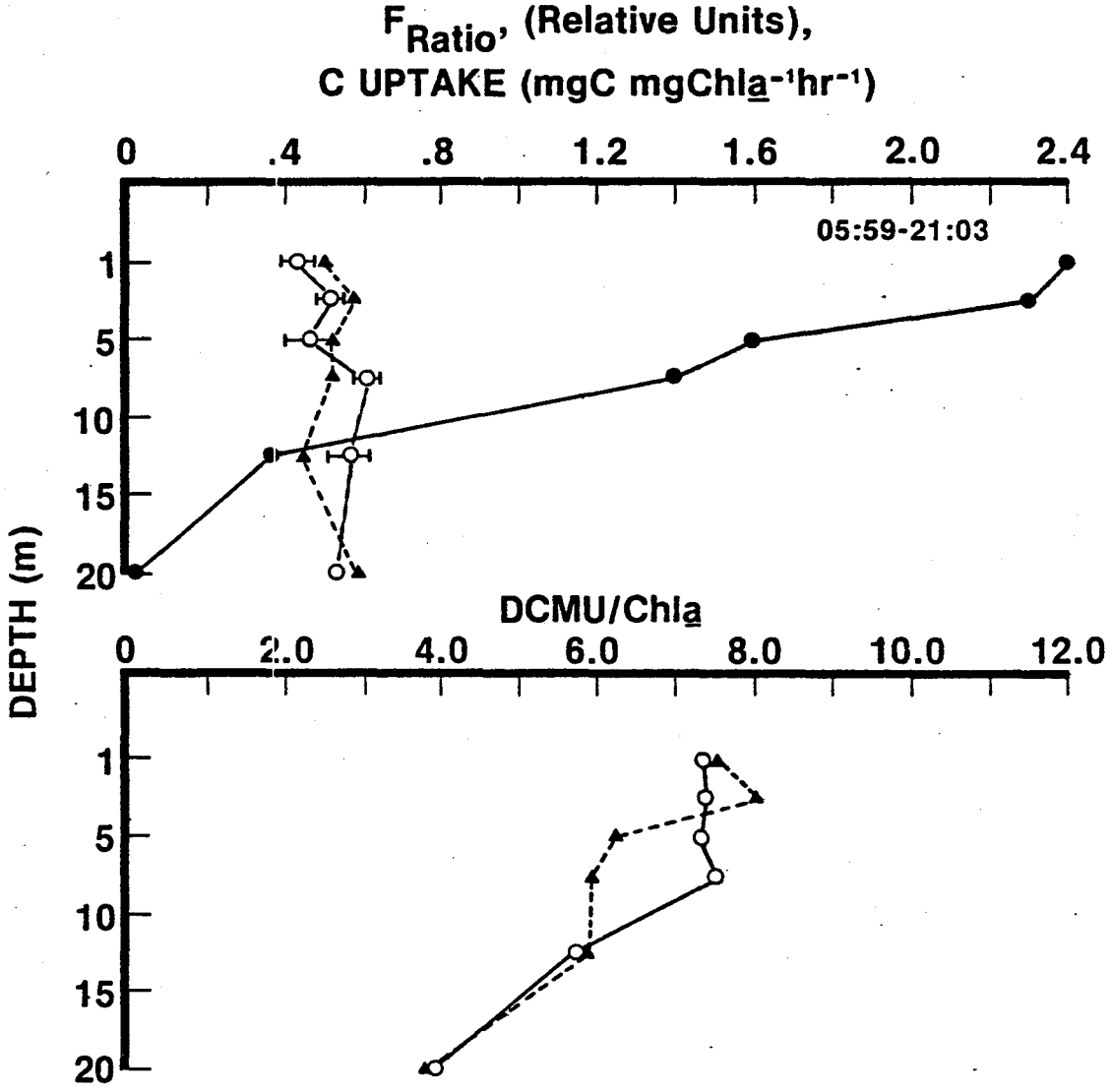

$F_{Ratio'}$ (Relative Units),
C UPTAKE (mgC mgChla^{-1}hr^{-1})
0
.4
.8
1.2
1.6
2.0
2.4
05:59-21:03
1
5
10
15
20
DEPTH (m)
DCMU/Chla
0
2.0
4.0
6.0
8.0
10.0
12.0
1
5
10
15
20

Figure 4.36 Comparison of ^{14}C uptake (●——●) and the F_{DCMU}/chl*a* in water incubated in bottles (▶--▶) to the F_{DCMU}/chl*a* in the water column (○—○) on 26 August, 1982 at station 403 (Lake Ontario):

a) 06:50--10:50
b) 10:55--15:41

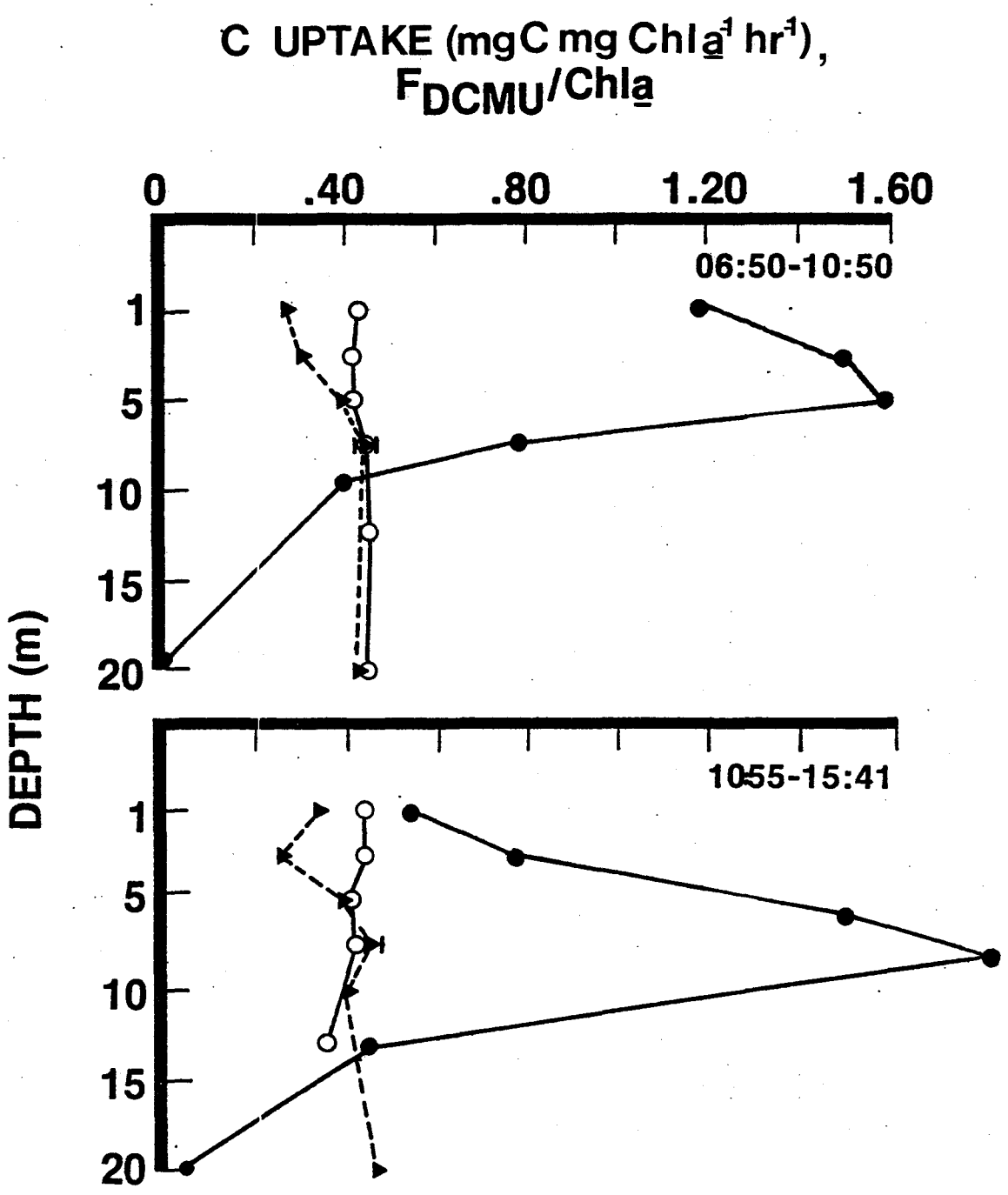
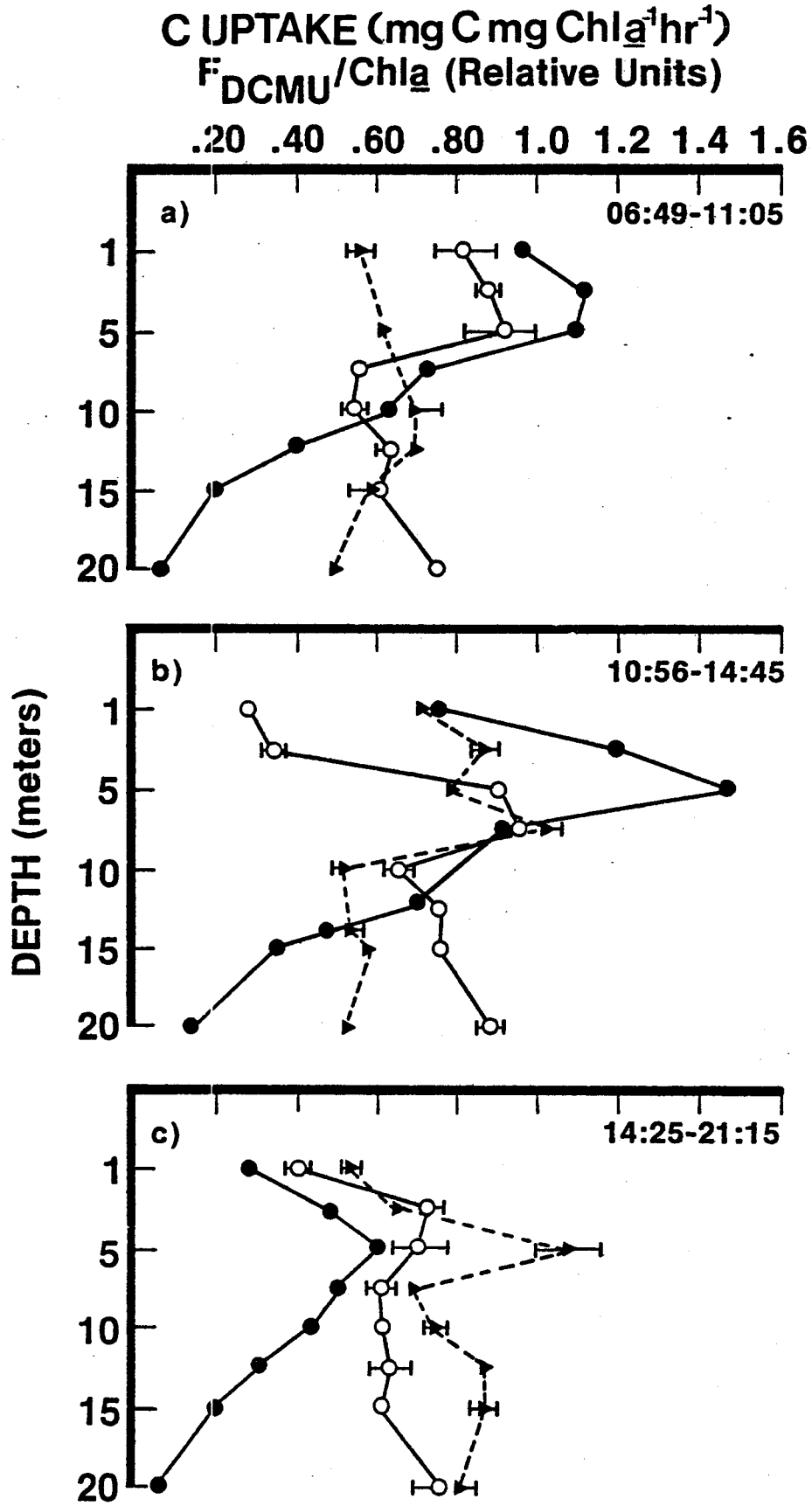

C UPTAKE (mgC mg Chl a⁻¹ hr⁻¹),
F_DCMU/Chl a
0
.40
.80
1.20
1.60
06:50-10:50
1055-15:41
DEPTH (m)
1
5
10
15
20

Figure 4.37 Comparison of ^{14}C uptake (●—●) and the F_{DCMU}/chl*a* of water incubated in bottles (▶--▶) to the F ratio in the water column (○—○) on 8 June, 1982 at station 401 (Lake Ontario):

a) 06:49--11:05
b) 10:56--14:45
c) 14:25--21:15


C UPTAKE (mg C mg Chl a^{-1} hr^{-1})
F'_{DCMU}/Chl a (Relative Units)
.20 .40 .60 .80 1.0 1.2 1.4 1.6
a)
06:49-11:05
b)
10:56-14:45
c)
14:25-21:15
DEPTH (meters)
1
5
10
15
20

occasions except for the final incubation on 8 June. On occasions when solar radiation and N^2 were high (e.g. 8 June, 1 July) there were also significant depressions of F_{DCMU} in the water column. As shown above, when N^2 was low (26 August) and solar radiation high no depression was observed.

4.12 Comparison of the fluorescence response of different depths to constant light

Table 4.14 shows the fluorescence measurements following incubation of water from different depths expressed as a percentage of the fluorescence measured at the beginning of the experiment. Two experiments were conducted during isothermal conditions in the water column. There were no consistent differences between the fluorescence of 1m and 150m water on 11 June at station 403 or between 1m and 30m water on 19 October at station 405. ^{14}C uptake was also similar at both depths on 11 June, 1982 (data not shown).

Experiments between 1 July and 23 September were conducted during periods of stratification. On 2 July, 1982 water from immediately below the thermocline at 20m and from 150m was compared. It was believed that the phytoplankton from 150 meters might be more affected by the light treatment if they had been maintained at low light for a longer period of time, however the opposite result was observed. F and F_{DCMU} decreased more in 20 meter water. Rates of carbon uptake per chlorophyll were similar at both depths (R.Cuhel unpub. data).

Table 4.18 Comparison of the fluorescence characteristics of water from different depths incubated under continuous light. F and F_{DCMU} are expressed as a % of the initial value following incubation at a light gradient. Intensity of L_1 ranged from 770 to 1300 $\mu E\ m^{-2}\ s^{-1}$; L_2 is 34% of L1; L_3 is 11% of L_1; L_4 is 1% of L_1. * indicates significant differences between the response of two depths e.g on 2 July there were significant differences between the response at all light intensities except L_1.

DATE	STA	DEPTH (meters)	LENGTH OF INCUBATION (hours)	F L_1	F L_2	F L_3	F L_4	F DARK	F_{DCMU} L_1	F_{DCMU} L_2	F_{DCMU} L_3	F_{DCMU} L_4	F_{DCMU} DARK
11 June	403	1	16	35	40*	92	100		22	27*	80	107	
		150		37	96*	91	85		20	66*	81	76	
1 July	405	1	10	86	98	96	90	117*	87	102*	105*	106*	91*
		30		62	76	92	73	44*	56	86*	71*	64*	44*
2 July	403	20	16	20	38*	59*	87*	81*	14	36	58	100	87
		150		31	84*	125*	107*	101*	17	46	68	98	89
18 August	Jack's Lake		2	44	89				27	61			
			8	54	89				27	64			
23 September	403	1	10	74	103*		128*	95	67	84		96	90
		20		50	60*		96*	73	63	72		85	97
19 October	405	1	12	74	133	95	100		31	71	86	86	
		20		78	101	97	104		33	43	79	84	

On 18 August 1982 a subsurface (2 meters) and an 8 meter sample from the thermocline in Jack's Lake were incubated for 3 hours at 18° C. The original fluorescence (F) of the 8 meter sample was approximately twice that of the 2 meter sample thus indicating the presence of a small metalimnetic peak. If the organisms making up this "peak" had been exposed to the light conditions found there for some period of time their fluorescence characteristics might be expected to differ from those at 2 meters. This was not found to be the case. The decrease in F and F_{DCMU} was remarkably similar in 2 and 8 meter samples.

On the two other occasions there did appear to be significant differences between the response of epilimnetic populations and metalimnetic or hypomlimnetic populations. On 1 July 1982, samples from above and below the thermocline were incubated for 10 hours in the light at 13.5° C. The 30 meter sample was thus warmed about 7° C while the 1 meter sample was incubated at its initial temperature. 30 meter water showed a larger relative decrease in F and F_{DCMU} compared to its initial value than did the 1 meter sample. The 30 meter sample incubated in the dark however also showed a significant decrease in F and F_{DCMU} over time. Thus, on this occasion, the response may not be attributed conclusively to light alone.

On 23 September the F of water from within the thermocline (20 meters) was lower than that of surface water (1 meter) following a 12 hour incubation. The temperature of the 20 meter sample was increased 4°C however the dark control was not significantly affected.

5.Discussion

5.1 Use of fluorescence as a physiological indicator in culture

The values of F ratio (.64-.70) observed in continuous culture of Chlamydomonas were comparable to those obtained in batch culture during the exponential phase of growth and are similar to those cited by Blasco and Dexter (1972) and Kiefer and Hodson (1974) for continuous cultures of marine diatoms. The observation that the F ratio did not decrease when the level of nitrogen was reduced might be expected. In a chemostat culture, it is the dilution rate which controls the growth rate of the organism. The concentration of limiting nutrient should affect only the biomass in the system (Goldman et al 1979). Thus at a lower level of nitrogen the number of cells should decrease but the growth rate, physiological condition (and hence F ratio) should remain the same.

In batch culture of Chlamydomonas, the F ratio remained high even when growth rate was zero. In Synechococcus there was more variability in the pattern of change of the F ratio. The occurrence of transient changes in the fluorescence of a sample in the cuvette during measurement may have affected the values obtained. The

fluorescence of blue-green algae is known to undergo a larger SMT transition than that of red or green algae (Mohanty 1972). Lower overall F ratios may be the result of the prokaryotic structure of the photosynthetic membrane of the cyanobacteria.

Higher levels of F/chl*a* in batch culture of *Chlamydomonas* as compared to chemostat culture are in agreement with the results of Kiefer (1973b). As observed by Heaney (1978) the absolute value of F/chl*a* was higher in the green (*Chlamydomonas*) than in the blue green species (*Synechococcus*). F/chl*a* was higher in stationary than in exponential phase in both species. Because of differences between species in absolute value of this ratio and because there was no precise point at which F/chl*a* began to increase, this parameter would not give consistent information about the onset of nutrient stress in natural populations.

The absence in chemostat culture of the low values of the F ratio which characterise late stationary phase in batch culture is indicative of a fundamental difference between the two systems. Stationary phase in batch culture is induced by nutrient depletion and/or accumulation of inhibitory products released during growth while in a chemostat there is always a dynamic equilibrium between nutrient input and growth (Rhee 1979). Thus nutrient deficiency should be associated with batch culture and nutrient limitation with chemostat systems. Morris *et al* (1974) suggest that the difference

between limitation and deficiency is that the former does not markedly affect physiological condition. The data presented here suggest that a low F ratio at least in Chlamydonomas reflects nutrient deficiency rather than nutrient limitation.

The general trend toward both decreased F ratios and ^{14}C uptake during stationary phase growth is in agreement with the pattern observed by Samuelsson and Oquist (1977) and Roy and Legendre (1979). These authors however suggest that there is a closer relationship between the two measurements than what was observed here. The decrease in ^{14}C uptake during stationary phase in both Chlamydomonas and Synechococcus in this data set was much greater than the decrease in F ratio. Substantial changes in ^{14}C uptake were also possible without change in the F ratio. Samuelsson and Oquist (1977) and Samuelsson et al (1978) measured fluorescence during the first 5 seconds following excitation at a light intensity high enough to induce Kautsky induction effects. This measurement may be more closely related to photosynthesis than the fluorescence measured using a Turner Design fluorometer (Harris 1980).

That ^{14}C uptake should not decrease in direct proportion to the F ratio is perhaps not surprising (Harris 1980). The F ratio reflects the capacity of the electron transport in the thylakoid membranes. Much of this capacity is used to generate ATP and NADPH much of which is used in the dark reactions of photosynthesis. ATP and reductant

produced during non-cyclic electron flow are also used for other biosynthetic, reductive, and transport processes which are essential for cellular survival (c.f. section 1.4) (Raven 1976).

5.2 Observed patterns in the field

5.2.1 Use of fluorescence as a physiological indicator in the field

Even a preliminary examination of the fluorescence characteristics in culture suggests that the use of fluorescence as a physiological indicator may be restricted to situations in which nutrient starvation occurs. As discussed in section 1.4 the determination of physiological condition and growth rates is far more complex in a nonsteady state system such as a lake than under controlled culture conditions. At this point it becomes necessary to redefine the objective of this thesis. Rather than determining whether the F ratio is a good indicator of physiological condition in the field, the data in this thesis may only be used to ask whether the F ratio gives similar information to that of other physiological indicators currently used in the field.

The answer is no. If P turnover times and fluorescence are to give similar information about the nutrient status of phytoplankton then F/chl*a* should rise while the F ratio should fall as P turnover

time becomes more rapid. No such pattern was observed. This is perhaps not surprising in light of literature reports suggesting that photosynthesis and P metabolism are not closely linked (Nalewajko and Lean 1980).

The random pattern observed over the season in the F/chl*a* of 5m water suggests that the major factor affecting this parameter may have been due to changes in the species assemblage. Differences in F/chl*a* between species have been previously reported (Heaney 1978, Loftus and Seliger 1975, Kiefer 1973b) and attributed to distribution in the chloroplast of the chlorophyll *a* molecules and the amount of interference in the measurement due to accessory pigments (Heaney 1978). Samuelsson *et al* (1978) suggest that there is no difference between the F ratio of different phylogenetic groups but give data only for *Chlorella* and *Scenedesmus*. The differences observed here between *Chlamydomonas* and *Synechococcus* indicate that there are significant species differences in the F ratio as well.

Fluorescence did not appear to be a useful indicator of Popt over the season in Lake Ontario either. This is in agreement with the results observed by Harris (1980) and Roy and Legendre (1980) during a survey of the St. Lawrence River. Literature reports showing high correlations between the F ratio and photosynthesis have involved either cultured material (Samuelsson and Oquist 1977) or natural populations from a single sampling (Vincent 1981, Prèzelin and Ley

1980). It appears that the F ratio has potential as an indicator of photosynthetic capacity only for populations in which species composition remains relatively uniform.

Species variability may also account for the observation that the F ratio in lake water was never as high as that observed during exponential growth of Chlamydomonas. Chlorophyll degradation products did not appear to be a contributing factor since the acidification ratio observed in the epilimnion was generally higher than that observed in culture (data not shown). Since the acidification ratio measured fluorometrically is dependent on the amount of chlorophyll c in the acetone extract (Holm-Hansen et al 1970), this conclusion remains tentative.

The low F ratios from the deepwater samples at the offshore station are likely indicative of the photosynthetic debility of plankton which become trapped in the dark below the thermocline for long periods of time. A large component of dissolved fluorescence was observed but not quantified in these samples. Any conclusions about the photosynthetic capacity of cells sedimenting from the epilimnion would require careful measurements of this component as suggested by Tunzi et al (1975). The absence of these low ratios at the inshore station is likely due to upwelling events which prevent water from being maintained in the dark for a sufficient length of time.

The data shows that real differences in the F ratio between two depths were more likely to occur when there was a temperature or density gradient in the water column. Since large differences in F ratio were possible at night, the data suggests that the relationship was not due entirely to surface depression of the F ratio by high light intensity (c.f. sections 5.7, 5.9). It is suggested that these differences may be largely due to vertical differences in the species composition of the assemblage.

5.2.2 Relationship between fluorescence and physical character of the water column

Large differences in F_{DCMU} or F may be caused by differences in chloropyll *a* (associated with differences in the biomass or species composition of the community) or by an effect of light on fluorescence (c.f. sections 5.5, 5.7, 5.13). In order to test the hypothesis that large values of F_{DCMU} or F are due solely to depression of fluorescence at the surface by high light, it is necessary to examine profiles made at night. The observation that large differences in F and F_{DCMU} occur in the absence of light is evidence that the relationship observed here is at least partially due to vertical structure of chlorophyll *a* in the water column.

The results show that significant differences in F or F_{DCMU} between two depths were more likely to occur when there were also differences in temperature between the two depths or when water column stability was high. As observed by Harris (1973b), vertical structure of the physical character of the water column was accompanied by vertical structure of biomass.

5.3 Use of fluorescence as a physiological indicator following nutrient addition in culture

It might be expected that fluorescence would be more responsive to changes in nitrogen status than to changes in phosphorus status because of the close relationship between photosynthesis and nitrogen metabolism (section 1.4).

The observation that the F ratio was enhanced following N additions only in Chlamydomonas which had been in stationary phase for some time (3 days for NO_3^- addition, 6 days for NH_4 additions) however, suggests that changes in this parameter following N addition are sensitive only to severe nitrogen deficiency. This conclusion is consistent with the observations made over the course of growth in batch culture for this organism (section 5.1). It is also consistent with the suggestion of Kiefer and Hodson (1974) that cells tend to conserve their photosynthetic apparatus under nutrient stress. It is suggested that the technique described here is likely not sensitive enough to measure

any N deficiency which might occur in freshwater systems. These effects are discussed more fully in section 5.4. It appears also that the relationship between ^{14}C uptake and the F ratio is not sufficiently strong to allow predictive modelling of the two physiological indicators following nutrient additions.

The existence of a lag period of several hours prior to the enhancement of the F ratio suggests some synthesis or repair of the photosynthetic apparatus following the addition of N to N-deficient cells. The results also suggest that the effect is greater following addition of NO_3^- than following NH_4 addition. The addition of NO_3-N might stimulate the production of electron chain capacity in order to supply the reductant and energy necessary for NO_3^- metabolism. This would be reflected in an enhanced F ratio and in increased ^{14}C uptake. Since the metabolism of NH_4 is less energetically demanding, a smaller effect on the electron transport chain and consequently the F ratio is expected. Reports in the literature of the uptake of NH_4^+ in the dark suggests that respiration at least has the potential to replace photosynthetic electron transport as a source of energy for this process (Syrett 1981).

The nutrient additions were made to replicate cultures rather than to sub-cultures of a single batch culture. This procedure avoided bacterial and N-contamination from the air or on the glassware which might have arisen during transfer of the cells. Despite

standardization of the inoculation technique there was clearly culture to culture variability which might be reduced using the latter technique. Variability in duplicate ^{14}C measurements may have been due, in part to the phototactic response of Chlamydomonas. Cells were frequently observed to congregate near the surface of the incubation tube and if the suspension was not agitated prior to filtration large sample to sample variability might have arisen.

A light:dark cycle was used in these experiments in order to approximate the light regime experienced in nature. Chlamydomonas however has a distinct cellular clock (Bruce 1970). This clock mechanism appeared to be present in the fluorescence and photosynthetic patterns of the control cultures in these experiments. This pattern however was not consistent through all stages of growth. In order to determine the effect of N additions more precisely it would be necessary to either characterise the intrinsic rhythm at the various growth stages more thoroughly or to remove the source of variability by using cultures grown under continuous light.

5.4 Use of fluorescence as a physiological indicator following nutrient addition in the field

The existence of ambient NO_3^- and C:N ratios characteristic of healthy phytoplankton (Healey 1979) gives no indication of nitrogen deficiency in these Lake Ontario samples. Thus it does not appear

that the changes observed in the F ratio are indicative of nitrogen deficiency in these populations. Since phytoplankton from various depths during both deep mixing and stratified physical regimes exhibited the effect, it appears that light limitation was not a factor in these results. The concentration of $(NH_4)_2SO_4$ in the experiment (1.6 μM) was well below the concentration of NH_4Cl (100 μM) which Mohanty (1972) used to demonstrate the effect of ionic concentration on fluorescence yield.

Vincent (1980) suggested that an enhancement of the F ratio following N additions in 2 experiments in a New Zealand lake was indicative of N-limitation. Whether the response indicated a change in physiological condition or an increase in the abundance of species with an intrinsically higher F ratio in response to the shift in N:P ratio in the bottles is not clear.

While the experiments here were not extensive both field and lab work (section 5.3) suggest a need for caution in the interpretation of the response of the F ratio following nutrient addition as an indicator of nutrient deficiency. Phposphorus deficiency is certainly not reflected by enhancement of the F ratio following phosphorus additions. Lab work suggests that an enhancement of the F ratio following nitrogen addition is indicative of the nutrient starvation observed in stationary phase in batch culture rather than the nutrient limitation induced in continuous culture.

Natural situations, however, (although not steady state) are more similar to chemostat than to batch cultures. Thus the behavior of the F ratio in nature would be expected to be more similar to that observed in a continuous than a batch culture. In short, no effect as a result of nitrogen addition might be expected in all but the most severely "stressed" lakes. A more conclusive interpretation of the field data following nitrogen additions in this situation is not possible.

5.5 Observations of diurnal patterns of fluorescence in the water column

As discussed above (section 1.9) diurnal patterns of fluorescence may reflect a response to an exogenous "forcing factor" or an intrinsic biological rhythm. The observation that distinct diurnal patterns of fluorescence occurred only on occasions when solar irradiance was high suggests that the basis of any diurnal pattern of fluorescence observed in Lake Ontario is due to light. On 8 June, the depression of F_{DCMU}/chl*a*, the depression of the F ratio at the surface in vertical profiles and the increase in F_{DCMU} from the original measurement following a dark treatment all suggest that the photosynthetic apparatus of the phytoplankton in the water column during the day were exposed to bright light sufficiently long enough to become "photoinhibited". This result is consistent with the

environmental data which showed that on 8 June 1982 both solar radiation and water column stability were high. Because both F and F_{DCMU} changed over the course of the day, the F ratio was not consistently depressed at the surface during the day. Results of a single profile made at mid-day using such information would be misleading.

On 12 August, although solar radiation was again high, there was no consistent depression of any of the fluorescence parameters at the surface of the water column. It is suggested that mixing rates were rapid enough on this date to prevent bright light from affecting the photochemical apparatus. The sampling technique used here involves a 10 minute dark treatment. The possibility that recovery in the dark was rapid enough to prevent light induced changes from being detectable using this method is discussed below (section 5.11).

The remainder of this thesis will examine:

1) the evidence that circadian rhythms are involved in the diurnal patterns of fluorescence in Lake Ontario

2) the nature of the response of fluorescence to light

3) the interaction of mixing rates in the water column and the effect of light on fluorescence

5.6 Circadian Rhythms

The results of incubations at continuous low light and the examination of the changes in fluorescence at the surface in the water column on two occasions when environmental light levels were low are evidence that circadian rhythms did not contribute to the depression of fluorescence observed in surface waters in Lake Ontario. The two populations were chosen in order to test for circadian rhythms in populations which were expected to have different light histories. The population from station 403 was undergoing deep mixing while that of station 401 was sampled under conditions of stratification. Thus the population from station 401 is more likely to experience a "constant" light-dark regime and hence should be more likely to exhibit circadian rhythms in photosynthesis than the deep mixing population (Chisholm 1981).

That circadian rhythms of photosynthesis were not found does not prove that such rhythms do not exist within the Lake Ontario population. Different species or size classes may exhibit different fluorescence patterns such that a sample of whole lake water exhibits no distinct pattern of its own. In addition, measurements were made on only two populations. Harris (1973) found diurnal patterns of photosynthesis in net hauls of diatoms. While microscopic examination showed that diatoms were present,in the summer of 1982, a large number of unidentified nanoplankton also occurred in these samples. Passing

the sample through a 5 μ nucleopore filter showed that approximately 85% of the fluorescence remained in the filtrate. The importance of the small size fraction may explain the disagreement with previous results.

5.7 Time course of fluorescence changes in the light

The effect of light exposure on fluorescence is obviously dependent on the intensity of light and the duration of exposure. As discussed by Vincent (1980) populations have ben found to exhibit "threshold" irradiances of 100 to 475 $\mu E\ m^{-2}\ s^{-1}$. These values are within the range which was observed to have a significant effect on the fluorescence of the Lake Ontario phytoplankton.

F_{DCMU} and F responded to light at different rates. In agreement with the results of Harris (1980), F_{DCMU} was more responsive than F. The most rapid response for F_{DCMU} occurred within the first 5 to 25 minutes of exposure while the response of F often became apparent only after a 1 to 2 hour treatment. Thus on a sunny day, if there is no decrease in F_{DCMU}/chl*a* in surface water, then the water must be moving rapidly enough to prevent the phytoplankton from being at the surface for more than about one half hour. (This conclusion presumes that the period in the dark, which was about 5 minutes shorter for the incubator experiments than in the field, and confinement to bottles did not affect the results).

These results differed from the diatom populatons of Kiefer (1973) and Loftus and Seliger (1975) in that in vivo fluorescence (F) did not respond within the first minutes of exposure to bright light. This may be an artifact of the technique if a depression in in vivo fluorescence underwent recovery during the first five minutes in the dark. Alternatively the result may reflect fewer diatoms in these communities.

Two other observations which were of interest in these experiments were the enhancement of F_{DCMU} at low light intensity and the difference in the response to light of winter and fall populations. The enhancement of F_{DCMU} at low light may explain the enhancement of F_{DCMU} often observed in vertical profiles at about 5 meters (section 5.5). Seasonal difference in the response to light are discussed more thoroughly in section 5.9.

5.8 Recovery of fluorescence in the dark following incubation in the light

An examination of the recovery in the dark following incubation in the light is important in these experiments because the technique involved a short dark treatment. Recovery is obviously dependent on the duration and intensity of the preceding light treatment. As reported by Kiefer (1973b) and Heaney (1978) the length of time

required for "recovery" of fluorescence was greater than that required for inhibition. Following incubation at high light, increases in both F and F_{DCMU} were apparent within 10 to 20 minutes. The F of these samples was similar to that of the dark control within this time period. It would be interesting to perform this experiment on spring populations in which untreated fluorescence was more sensitive to light treatment. Full "recovery" of F_{DCMU} took longer and was not complete in the November samples after several hours.

The length of time that samples from the euphotic zone were in the dark in the Niskin bottles following sampling was about 10 minutes. If the phytoplankton in the water column had been exposed to a light intensity of about 1000 $\mu E\ m^{-2}\ s^{-1}$, this response would certainly be reflected by a depressed F_{DCMU}. However, the in vivo fluorescence (F) of samples (as measured by this technique) which have undergone only brief exposure to bright light would likely not appear significantly different to those maintained in the dark for long periods of time. Longer exposures at lower irradiances would likely produce a similar answer however it is difficult to make further generalizations because of seasonal variability in the fluorescence response (c.f. section 5.9). Future experiments should include a more complete study of both the rapidity of the response to light and the recovery in the dark.

The experiment also suggests an explanation for some of the

variability which is apparent in vertical fluorescence profiles through the euphotic zone. This variability may be a reflection of the existence of "parcels" of water which have not yet fully recovered from a light treatment but which have mixed with water having a longer dark history.

Confinement to bottles at low light or in the dark for periods of time equivalent to a diurnal cycle did not significantly affect the *in vivo* fluorescence or F_{DCMU} of the Lake Ontario phytoplankton in at least 65% of the experiments. Irreversible changes in fluorescence and F_{DCMU} did occur during long term exposure to bright light. This suggests that exposure to high light intensities damages the electron transport chain as well the structure of individual chlorophyll *a* molecules.

5.9 Seasonal differences in the response to light

The response to light of F_{DCMU} in epilimnetic populations was very similar from June through September while early spring and winter populations were more "light sensitive". Because the response of untreated fluorescence to an extended light period was more variable even in the summer, the F ratio did not show a consistent decrease to a fixed light incubation. Thus the change in F_{DCMU} rather than the change in F ratio appears to be a more consistent indicator of prolonged exposure to light.

Microscopic examination showed large abundances of Melosira and cryptomonads in the spring (I. Gray pers.comm.) while October samples contained a diverse community of greens and cryptomonad species. The summer community was dominated by smaller (<5μ) cells which could not be positively identified but which were likely a combination of small blue-green, chrysophyte and green species (F. Pick pers. comm.).

Differences in the ^{14}C uptake and P turnover times suggest that the physiology of the organisms was different at the two stations in early June. Since the responses of the populations to fixed light were not different, the mechanism of "adaptation" likely did not involve a change in the efficiency of coupling between the two photosystems. Similarity of F/chl*a* and F_{DCMU}/chl*a* between the two communities also suggests that increased size of the PSU is not involved. However, since a large number of small cells (probably cyanobacteria or chrysophytes) were present at this time, "shade adaptation" may have involved synthesis of pigments other than chlorophyll *a*. In contrast to the response observed in nitrogen-stressed populations (Kiefer and Hodson 1974, Kiefer 1973b) there did not appear to be increased sensitivity to light in P-stressed populations at this time.

Increased sensitivity to bright light was observed in the samples from the aphotic zone. The sensitivity of F_{DCMU} may be due to

a decreased efficiency of the coupling of photosynthesis in these cells. This was the suggestion of Kulandaivelu and Senger (1976a,b), who found decreased efficiency of photosynthetic coupling after two days of maintaining batch cultures of Scendesmus in the dark. Exposure to low light induced a maximal recovery of the efficiency of coupling in these experiments. At high light less recovery occurred and the authors suggest autooxidation as a possible cause.

5.10 The effect of different mixing regimes on the fluorescence of a single population

While the preceding discussion (sections 5.7, 5.9) and literature reports (sections1.12, 1.13) show that fluorescence is very sensitive to light, the extent to which natural populations are affected by light should be dependent on the stability of the water column and the rate at which the phytoplankton circulate through the water column (Harris 1983, 1980). A comparison of the fluorescence response in bottles and in the water column on 26 August shows that vertical mixing processes prevented the light-induced depression of fluorescence in surface waters of the water column on this occasion. It is likely that this was also the case on 12 August (section 5.5). On the other hand, when high light was combined with high stability (8 June) or when light levels were low (29 June), the fluorescence measurements made in bottles were comparable to those made in the water column.

Comparison of the fluorescence in bottles and in the water column is subject to error in that the bottles underwent a longer dark pretreatment during transport from the water to the ship thaan did samples in which fluorescence was measured directly. Hence there is greater chance of "recovery" from a depression of fluorescence. This may account for higher F ratios or $F_{DCMU}/chl\underline{a}$ in water from bottles as compared to that in the water column.

5.11 Relationship between depression of ^{14}C uptake and fluorescence

The similarity between depression of $F_{DCMU}/chl\underline{a}$ in bottles and depression of ^{14}C uptake suggests that it is possible to make inferences about primary productivity estimates from the fluorescence data. (This statement is not meant to imply a predictive relationship between P_{opt} and the F ratio c.f. section 5.2.1) On 8 June, the depression of ^{14}C uptake observed in bottles appeared to reflect real events in the water column; on 26 August the depression of ^{14}C uptake was likely an artifact.

5.11 Relationship between depression of ^{14}C uptake and fluorescence

The similarity between depression of $F_{DCMU}/chl\underline{a}$ in bottles and depression of ^{14}C uptake suggests that it is possible to make inferences about primary productivity estimates from the fluorescence data. (This statement is not meant to imply a predictive relationship between P_{opt} and the F ratio c.f. section 5.2.1). On 8 June, the depression of ^{14}C uptake observed in bottles appeared to reflect real events in the water column; on 26 August the depression of ^{14}C uptake was likely an artifact of the bottle technique.

Modellers of primary productivity have often debated the validity of including surface depression of photosynthesis in integrated estimates of primary productivity (Vollenweider 1969, Harris 1978). It is suggested that a more extensive study of the type of experiments described here may offer a simple method of at least estimating the occasions on which surface inhibition is likely to be a real phenomenon.

5.12 Difference in the fluorescence response of phytoplankton from different depths

Since differences in the stability of the water column apppear to allow vertical stratification of the community (section 5.2.2) it might be expected that differences in the fluorescence response to light of the community might also arise during conditions of strong thermal stratification. Kulandaivelau and Senger (1976) found decreased efficiencies of photosynthetic coupling after maintaining Scendesmus for 2 to 4 days in the dark and that the recovery of efficient photosynthetic coupling was inhibited by bright light. The decrease of F and F_{DCMU} of cells from the aphotic zone which have been in the dark for some time might thus be expected to be larger than the decrease observed in cells from the euphotic zone. Alternatively, during periods of active mixing there should be no difference between the fluorescence response even in samples from widely separated depths.

During isothermal conditions on 11 June 1982, and 19 October the data suggests that the lake was mixing rapidly enough even at the deep station (403) to prevent populations having differing fluorescence responses to long-term light incubations from developing. It was more difficult to conduct controlled experiments under thermally stratified conditions because temperature gradients in nature could not be replicated in a single incubator. Under

stratified conditions some differences between the response of samples from within and below the thermocline as compared to epilimnetic samples were noted. These differences may be attributed to prolonged exposure to darkness.

The difference between the fluorescence response of 20 and 150 meter water on 1 July was interesting and unexpected. As described above (section 4.2.5) it appeared that the epilimnion had developed from water advected into the region rather than by surface heating. These physical events appear to have resulted in a vertical structure of the water colum which was reflected in the fluorescence properties of the water from 20 and 150 meters.

The similarity of response between epilimnetic and metalimnetic water at Jack's lake was also somewhat surprising. Pick (1982) found that metalimnetic populations such as this are "shade adapted". As with the inshore and offshore popultation in early June (section 5.9), light adaptation did not result in differences in the response of fluorescence to light.

Cullen and Renger (1979) suggest that low F ratios in nature may be due to an accumulation of cells which have stopped photosynthesizing and which have an F ratio near zero. They suggest that a small proportion of photosynthetically active cells would retain an F ratio of about .6. The F ratio would thus give an

indication of the proportion of "active" cells in a sample rather than an overall measure of the physiological condition of the population. The response of individual "active" cells to light should be similar regardless of their abundance in the water sample. If the theory of Cullen and Renger (1979) is correct the response of lake water to light (when expressed on a relative basis) should be similar regardless of the original F and F_{DCMU}. Since this was not the case, it appears that fluorescence parameters do give information about the average condition of the whole population as well as the proportion of "active" cells.

5.13 General Discussion

A survey of the literature suggests that fluorescence is variable at time scales ranging from days to minutes. At a scale of days F and F_{DCMU} reflect the physiological condition and species composition of the phytoplankton assemblage; at scales of minutes F and F_{DCMU} reflect the light history. The use of fluorescence and DCMU-enhanced fluorescence as a bioassay is appealing for several reasons. Firstly, the technique avoids the problems associated with the confinement of phytoplankton to bottles. Secondly, an F ratio is much less costly in terms of time and materials than bioassays involving the use of radiotracers (e.g. ^{14}C uptake or ^{32}P turnover times).

Unfortunately it must be the conclusion of this thesis that the F ratio is not a useful indicator of physiological condition in natural assemblages of phytoplankton. An examination of the F ratio of Synechococcus and Chlamydomonas over the course of growth in batch culture shows that a high F ratio may be observed for growth rates between zero and near μ_{max} and over a wide range of photosynthetic capacity. In a comparison of seasonal patterns of F ratio and P turnover time, it was not possible to distinguish changes in the F ratio due to physiological condition from those due to changes in species composition. Nitrogen additions to nitrogen deficient cultures produced a response only under conditions of severe stress not likely encountered in the field. Nitrogen additions in the field produced confusing results while phosphorus additions produced a consistently negative result even in P-stressed communities. These results suggest that any conclusions about physiological status or photosynthetic capacity in natural assemblages based on the F ratio (e.g. Cullen and Renger 1979, Vincent 1980, 1981, Goldman and Priscu 1983) should be treated with skeptiscism.

The observation that distinct fluorescence patterns in the water column occurred only on occasions when solar radiation was high suggests that these patterns are due to a response to an exogenous factor (light) rather than a biological rhythm. This was confirmed by incubation of water under continuous low light in which no change in the fluorescence properties (and hence no circadian rhythm) was

observed. The response of fluorescence to light was complicated. It was dependent on the duration and intensity of the light exposure and varied with differences in species assemblage. Physiological condition did not appear to affect the response to light although cells which had been in the dark for long periods of time were more sensitive to high light.

In agreement with Harris (1983), a relationship between the physical character of the water column and the difference in F or F_{DCMU} between the surface and deeper water was observed. These differences are potentially due to two factors: 1) differences in biomass between the two depths and 2) the effect of light on the photosynthetic apparatus of surface populations. An examination of the data from fluorescence profiles made at night and from diurnal patterns of fluorescence confirms the importance of both factors during conditions of high water column stability.

While it was not possible to separate these two effects, it was clear that a reduction in water column stability prevented both processes from occurring. On occasions when there was no temperature gradient in the water column (e.g. during spring and fall mixing) there was little structure in vertical fluorescence profiles. Vertical mixing was shown to prevent the surface depression of flurescence in a comparison of the water incubated in bottles to that in the water column.

In conclusion, while fluorescence does not appear to be a reliable indicator of physiological condition it may have practical applications in assessing the recent light history of natural assemblages of phytoplankton. A comparison of the DCMU-enhanced fluorescence response of phytoplankton following an incubation *in situ* at fixed depths, to that in a depth profile should indicate whether mixing rates are high enough to prevent photoinhibition of flurescence. In turn, the occasions on which ^{14}C estimates of productivity are most likely to be representative of *in situ* rates may be inferred and this information used to improve the data base required for models of primary productivity.

6. Summary

1. The F ratio showed little change over a wide range of growth rates in continuous and batch cultures of Chlamydomonas. The F ratio of a cyanobacteria (Synnechococcus) was lower than that of Chlamydomonas during exponential growth.

2. No relationship between P turnover time or ^{14}C uptake and the F ratio in natural populations was observed. Differences in the F ratio due to changes in the species composition of the community could not be differentiated from the effect of physiological condition.

3. Addition of NO_3-N and NH_4-N to N-deficient Chlamydomonas resulted in a change in F ratio relative to the control only in severely stressed cultures. The addition of NO_3^- resulted in a larger change in F ratio and ^{14}C uptake than did the addition of NH_4.

4. The measurement of the fluorescence ratio following either phosphorus or nitrogen addition to natural populations is not a useful bioassay of nutrient stress in natural populations.

5. Diurnal patterns of fluorescence were due to a response to light rather than a circadian rhythm. F_{DCMU} responded more rapidly and recovered less rapidly following exposure to high light than did F. Because of the variability in the response of F and F_{DCMU} to bright light, the F ratio was the least consistent indicator of light history.

6. There were differences in the response of the different assemblages to light at different times of the year. Differences in light response were not due to a rapid sun-shade type adaptation although prolonged dark treatment did result in increased light sensitivity.

7. Depressions of F and F_{DCMU} occurred in surface waters only during conditions of high light and low mixing rates. Comparisons of F and F_{DCMU} in bottles and in the water column suggest that the ^{14}C method is likely a better approximation of primary productivity under these conditions or when light levels are low than when the water column is mixing rapidly.

8. A general relationship between the relative change in F, F_{DCMU}, or F ratio and temperature or density gradients in the water column was observed. The relationship was likely a result of vertical stratification of biomass, community composition and light-induced depression of fluorescence when water column stability was high enough to prevent vertical mixing.

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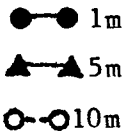
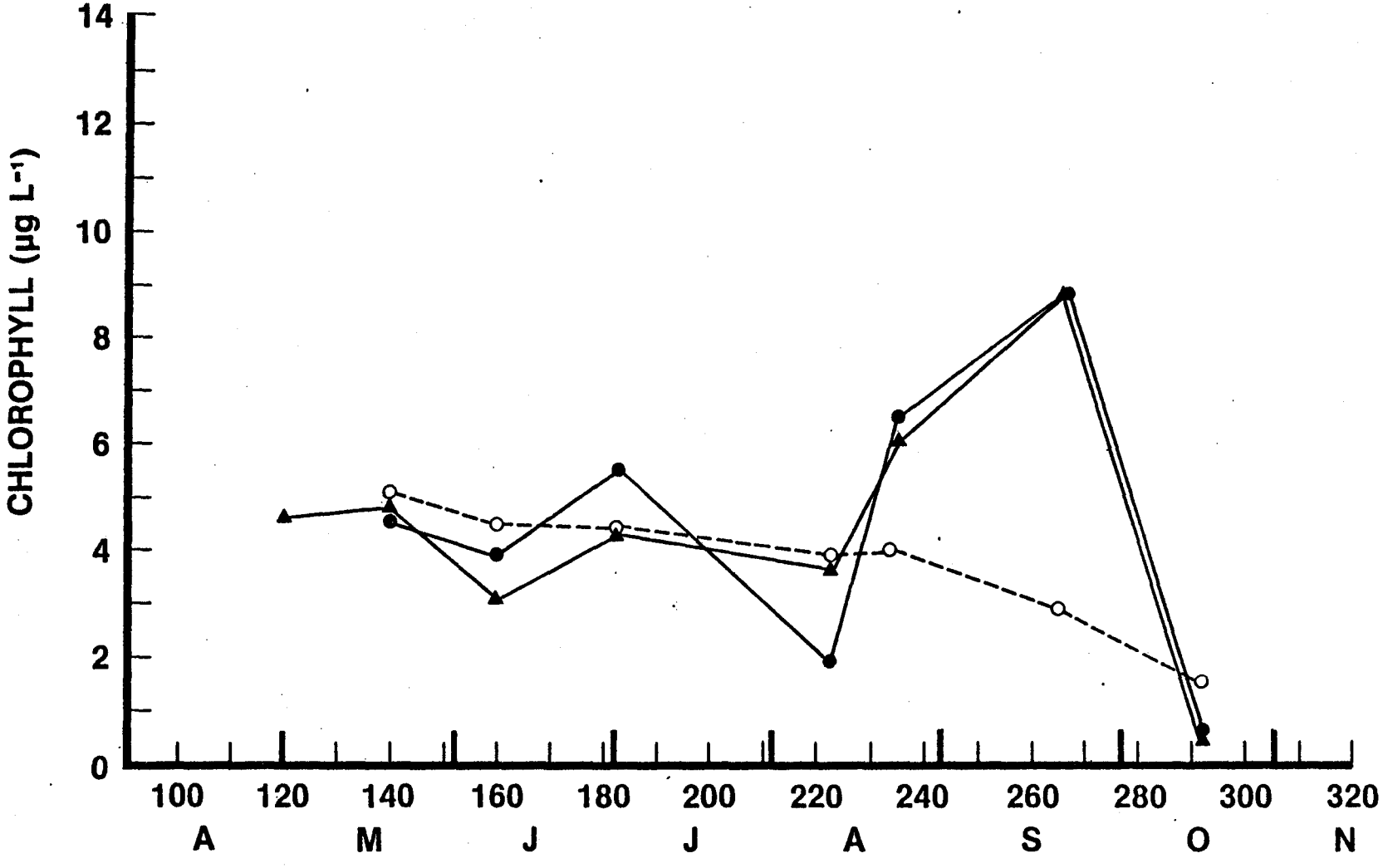
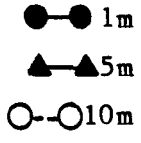
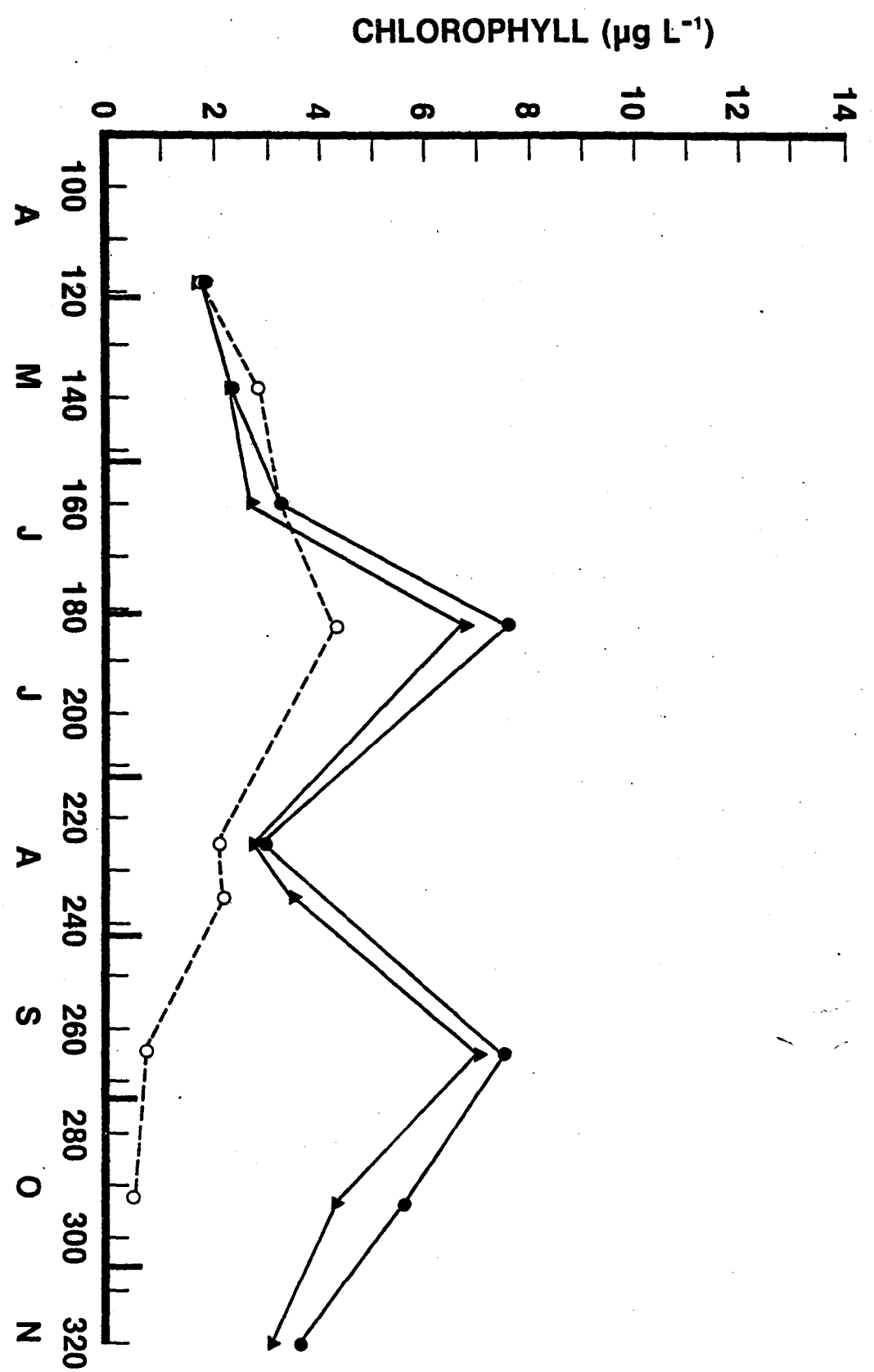
Appendix 1.1 Seasonal changes in chlorophyll *a* ($\mu g\ L^{-1}$) at station 401 (Lake Ontario, 1982).



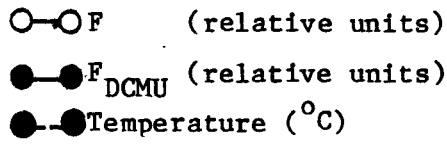

CHLOROPHYLL (µg L⁻¹)
14
12
10
8
6
4
2
0
100
120
140
160
180
200
220
240
260
280
300
320
A
M
J
J
A
S
O
N

Appendix 1.2 Seasonal changes in chlorophyll *a* ($\mu g\ L^{-1}$) at station 403 (Lake Ontario, 1982).

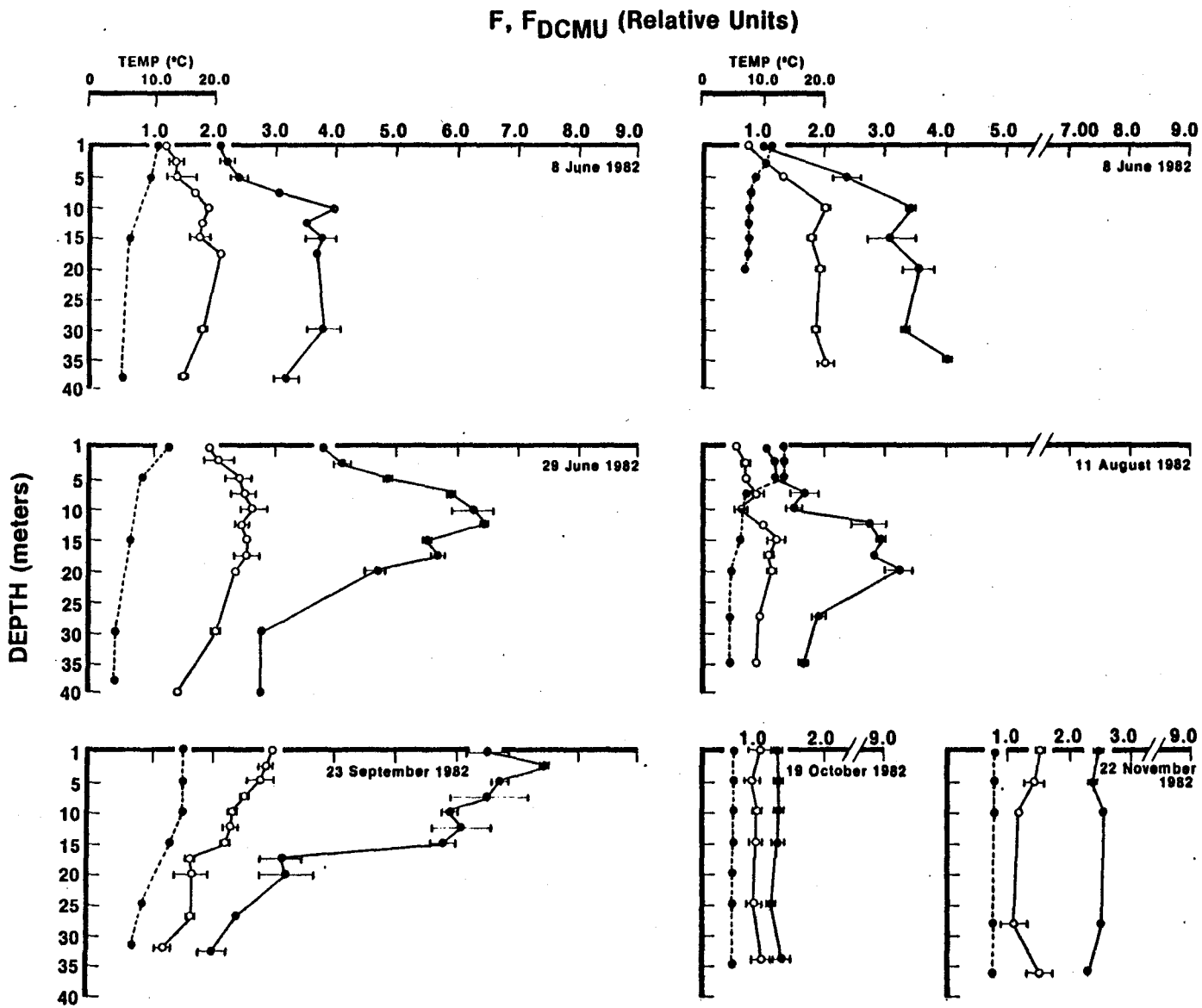



CHLOROPHYLL (µg L⁻¹)
0
2
4
6
8
10
12
14
100
120
140
160
180
200
220
240
260
280
300
320
A
M
J
J
A
S
O
N

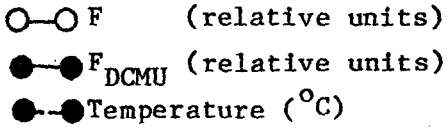
Appendix 1.3 Vertical profiles of F, F_{DCMU} and temperature throughout the season at station 401 (Lake Ontario 1982).



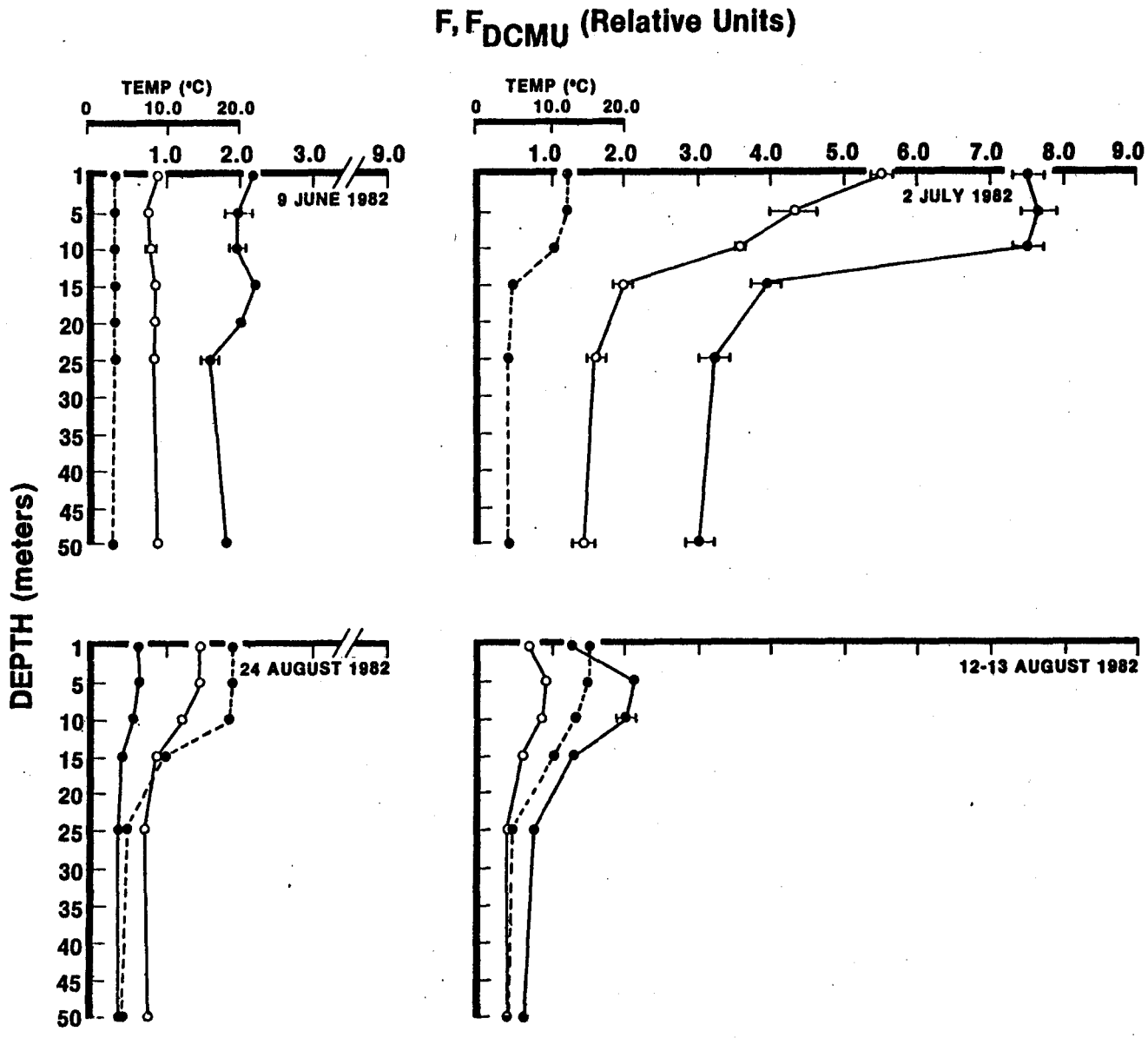
Note the absence of vertical stratification of either temperature or fluorescence on 19 October and 22 November.


F, F_{DCMU} (Relative Units)
DEPTH (meters)
TEMP (°C)
0
10.0
20.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
7.00
8.0
9.0
1
5
10
15
20
25
30
35
40
8 June 1982
8 June 1982
29 June 1982
11 August 1982
23 September 1982
19 October 1982
22 November 1982

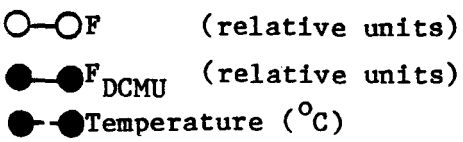
Appendix 1.4.1 Vertical profiles of F, F_{DCMU} and temperature (^{o}C) at station 403 (Lake Ontario) for June and August (1982).



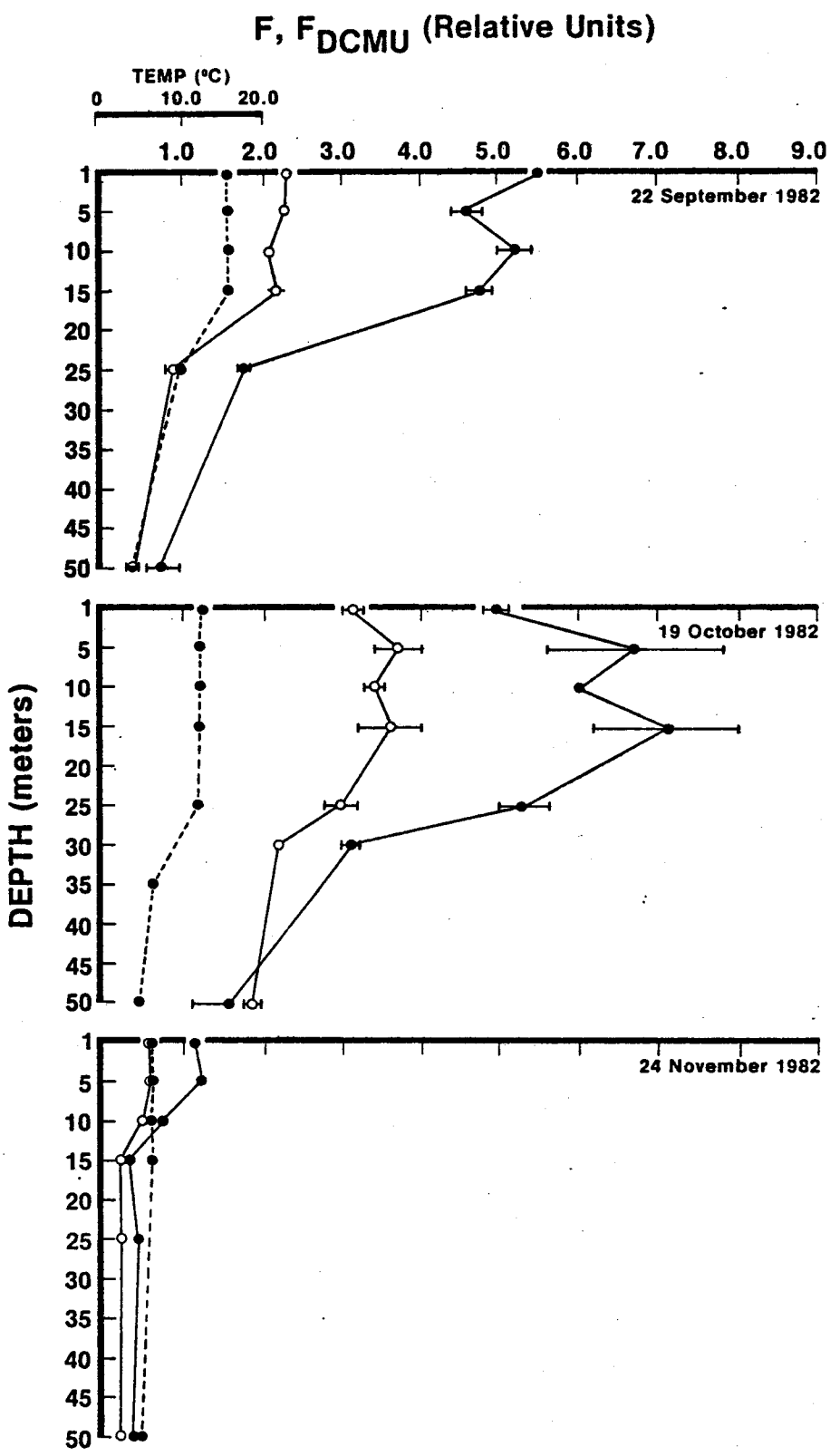
Note the absence of vertical stratification of either fluorescence or temperature on 9 June.


F, F_DCMU (Relative Units)
TEMP (°C)
0
10.0
20.0
1.0
2.0
3.0
9.0
4.0
5.0
6.0
7.0
8.0
DEPTH (meters)
1
5
10
15
20
25
30
35
40
45
50
9 JUNE 1982
2 JULY 1982
24 AUGUST 1982
12-13 AUGUST 1982

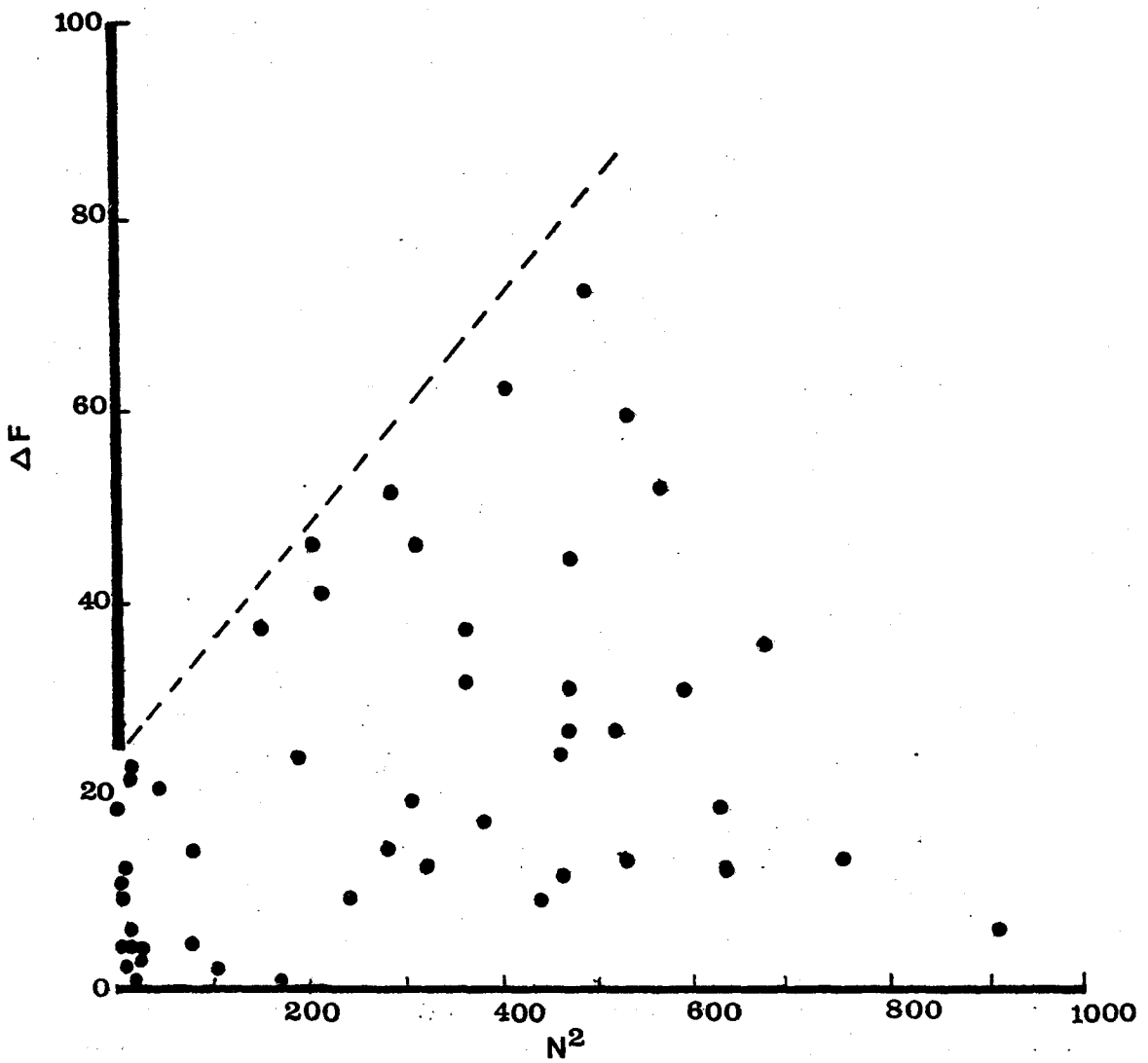
Appendix 1.4.2 Vertical profiles of F, F_{DCMU} and temperature (^{o}C) at station 403 (Lake Ontario) for September to November 1982.



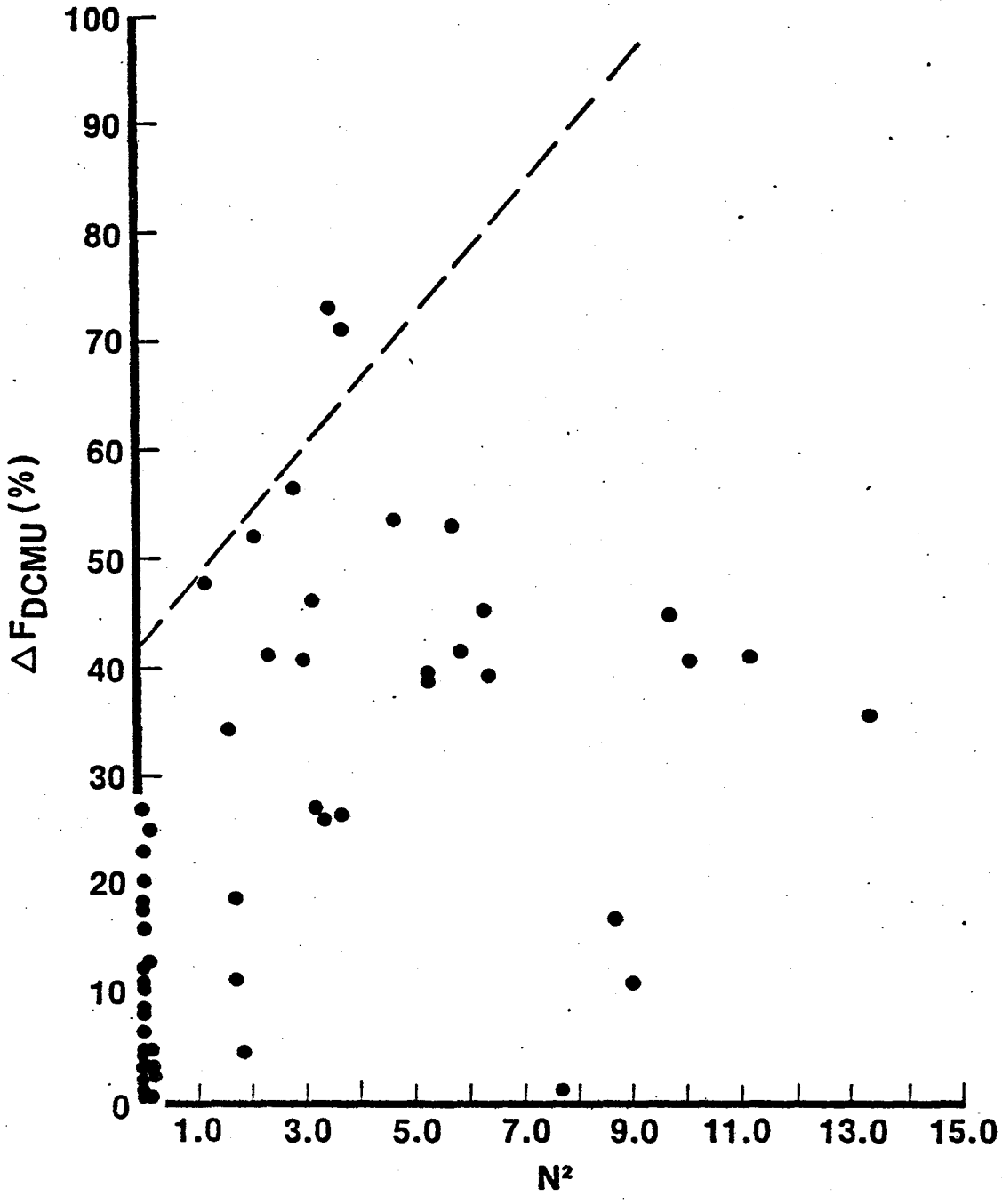
Note the absence of vertical stratification of either fluorescence or temperature on 24 November.


F, F_{DCMU} (Relative Units)
TEMP (°C)
0
10.0
20.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
22 September 1982
19 October 1982
24 November 1982
DEPTH (meters)
1
5
10
15
20
25
30
35
40
45
50

Appendix 1.5 Relationship between N^2 and $\Delta F . \Delta F$ (%) is the absolute difference in F between 1 and 10 meters expressed as a percentage of F at 10 meters. N^2 is expressed in s^{-2} X 10^{-6}. The dashed line is an arbitrarily chosen upper boundary line. The relationship appears less well defined than that between ΔT and ΔF(%) (Figure 4.7).


100
80
60
40
20
0
ΔF
200
400
600
800
1000
N2

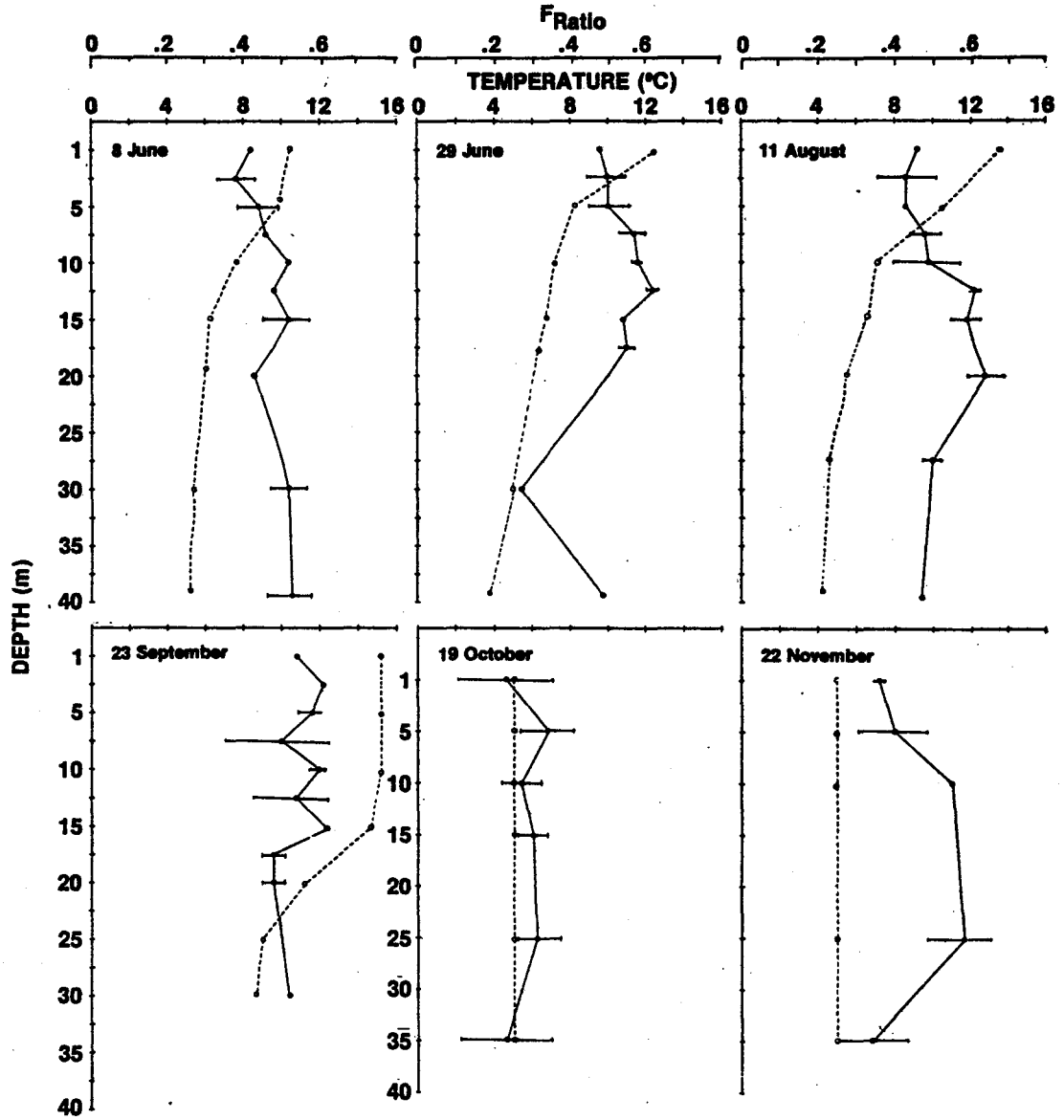
Appendix 1.6 Relationship between N^2 and $\Delta F_{DCMU} \cdot \Delta F_{DCMU}$ (%) is the absolute difference in F_{DCMU} between 1 and 10 meters expressed as a percentage of F_{DCMU} at 10 meters. N^2 is expressed in $s^{-2} \times 10^{-6}$. The dashed line is an arbitrarily chosen upper boundary line. The relationship appears less well defined than that between ΔT and ΔF_{DCMU} (%) (Figure 4.8).


100
90
80
70
60
50
40
30
20
10
0
ΔF_DCMU (%)
1.0
3.0
5.0
7.0
9.0
11.0
13.0
15.0
N²

Appendix 1.7 Seasonal changes in vertical profiles of the F ratio at station 401.

●—● F ratio

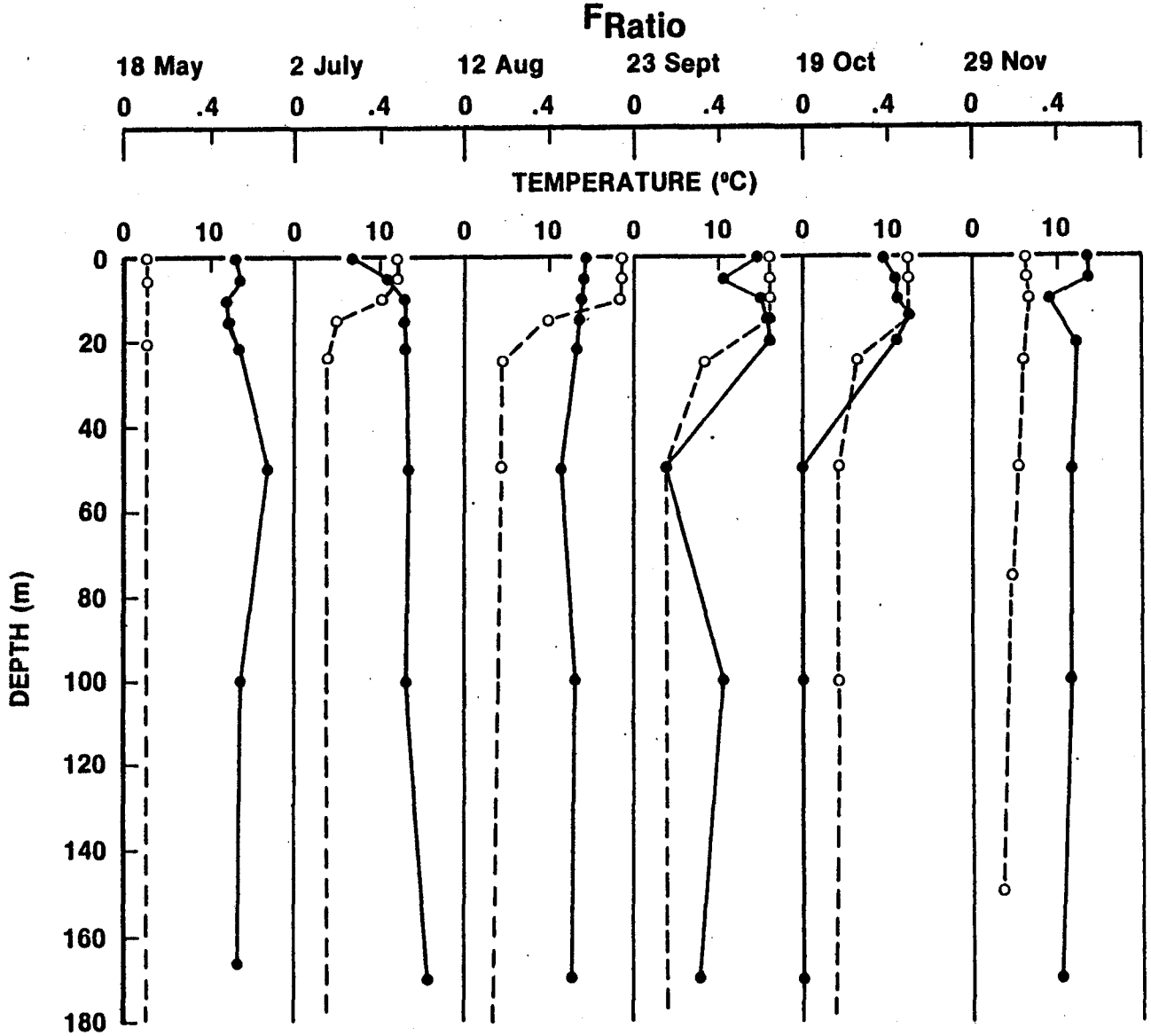
o—o Temperature (^{o}C)


F Ratio
0 .2 .4 .6
TEMPERATURE (°C)
0 4 8 12 16
DEPTH (m)
1 5 10 15 20 25 30 35 40
8 June
29 June
11 August
23 September
19 October
22 November

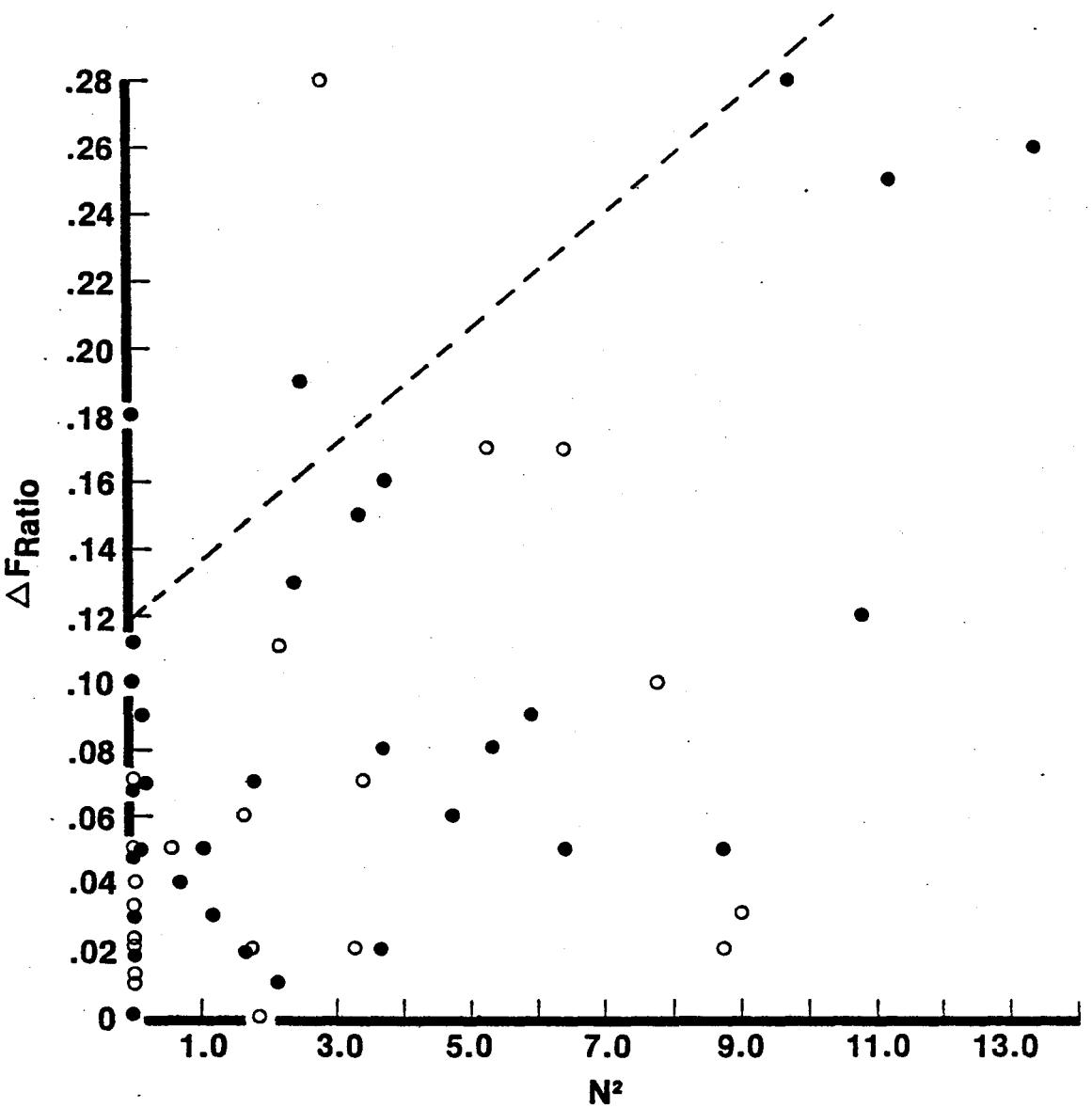
Appendix 1.8 Seasonal changes in vertical profiles of the F ratio at station 403.

●—● F ratio

o--o Temperature (^{o}C)


FRatio
18 May
2 July
12 Aug
23 Sept
19 Oct
29 Nov
0
.4
TEMPERATURE (°C)
0
10
DEPTH (m)
0
20
40
60
80
100
120
140
160
180

Appendix 1.9 Relationship between ΔF ratio and N^2 (Lake Ontario, 1982) No significant correlation was observed between the two variables. ΔF ratio is the difference in F ratio between 1 and 10 meters. N^2 is expressed as s^{-2} X 10^{-6}. The dashed line is an arbitrarily chosen upper boundary line.


.28
.26
.24
.22
.20
.18
.16
.14
.12
.10
.08
.06
.04
.02
0
ΔF Ratio
1.0
3.0
5.0
7.0
9.0
11.0
13.0
N²

Appendix 2.1.1 The effect of NH_4 enrichment on a) the F ratio and b) ^{14}C uptake for Chlamydomonas in 1 day stationary phase. Replicate treatment cultures were used on this occasion; the results of each culture are plotted separately. The "control" in this case is the average of the values obtained for an untreated culture and a culture treated with 30 ug L^{-1} PO_4.

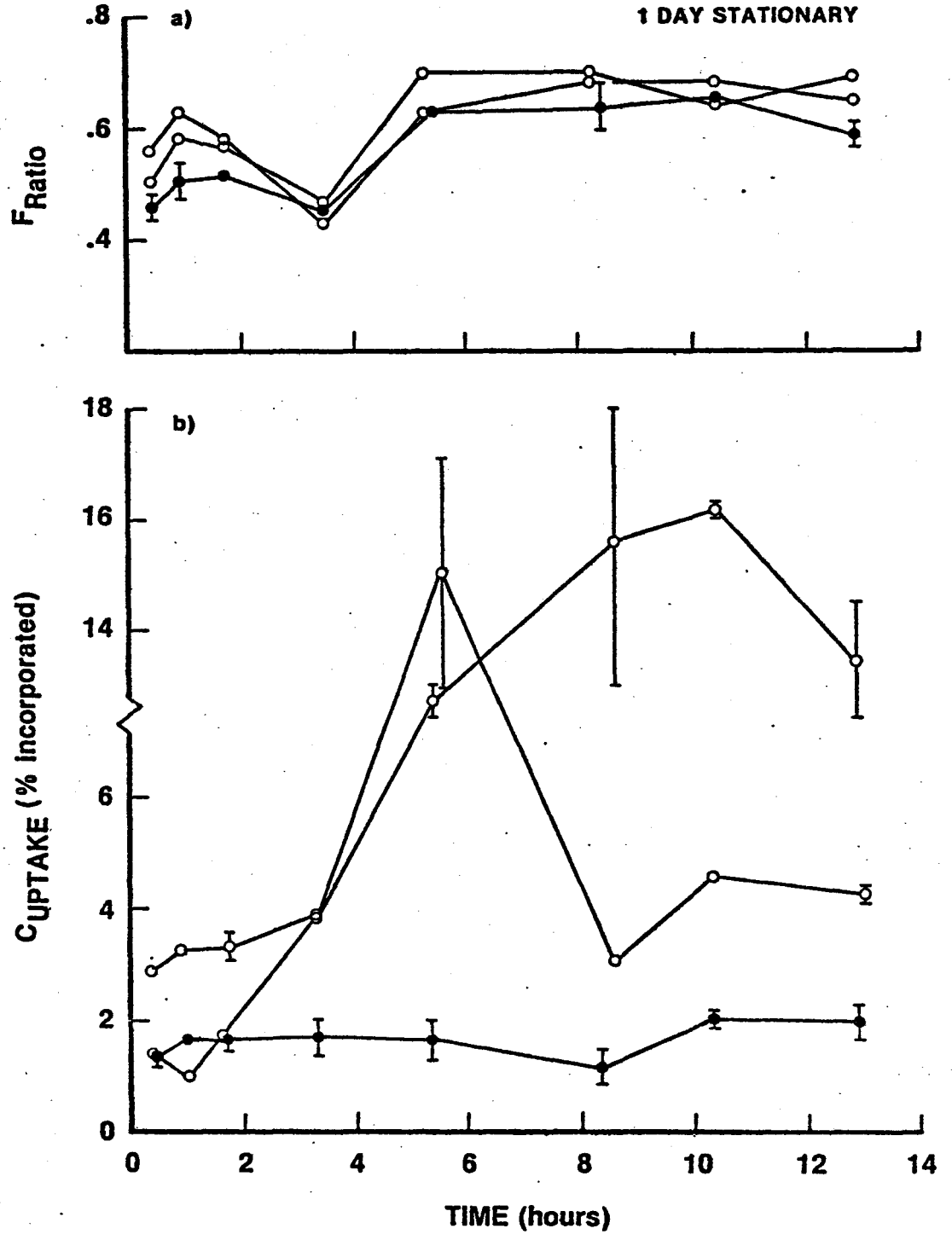
●—● F ratio (in the control culture)

○—○ (in the culture following NH_4 addition)

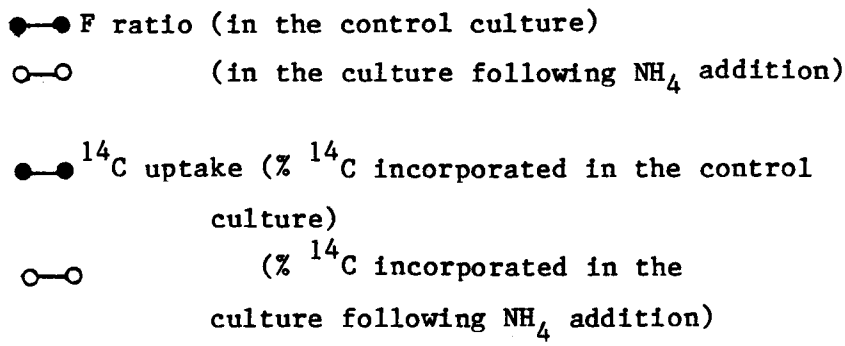
●—● ^{14}C uptake ($\%^{14}C$ incorporated in the control culture)

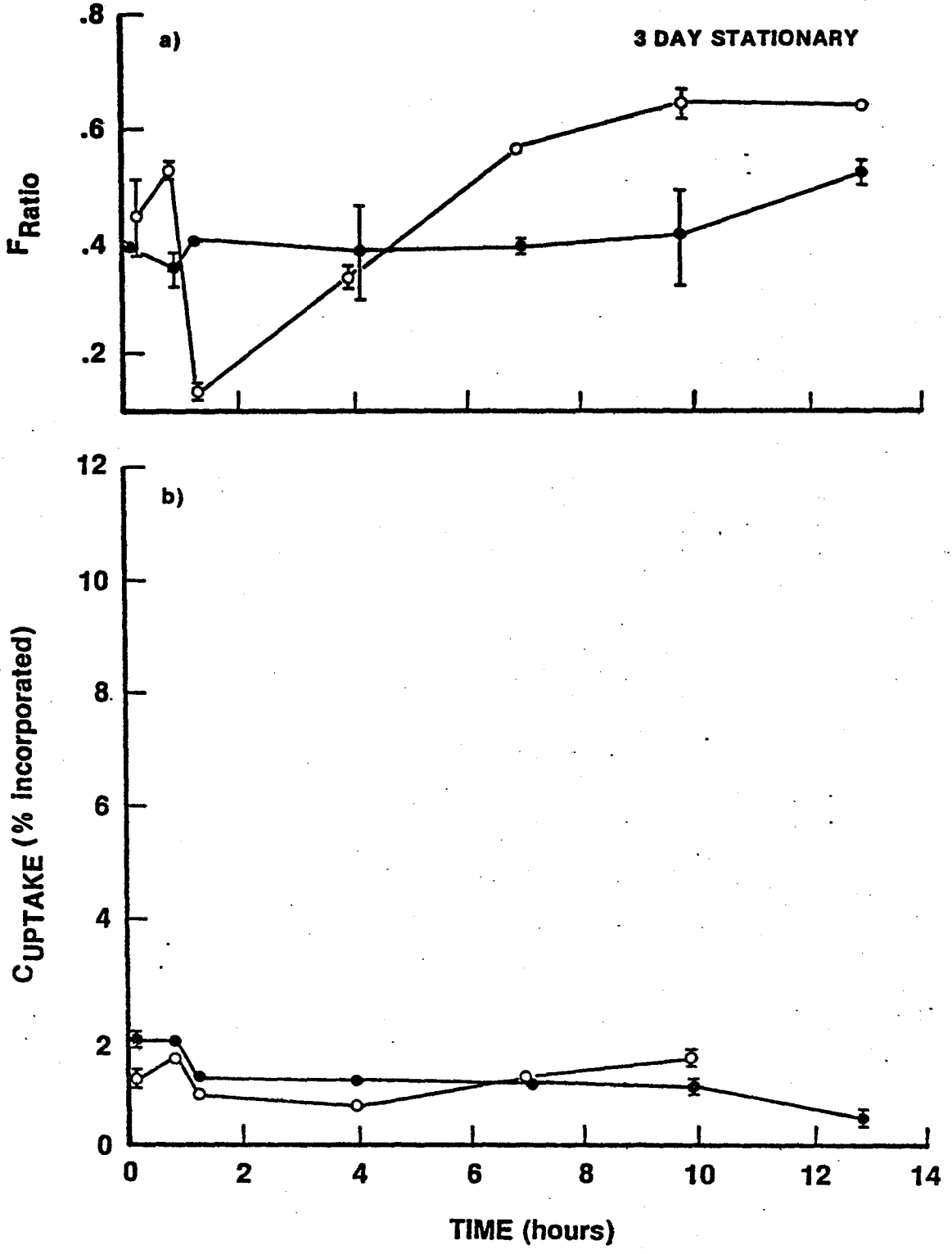
○—○ ($\%^{14}c$ incorporated in the culture following NH_4 addition)

Note that there were significant differences between the response of the replicate cultures on this occasion.

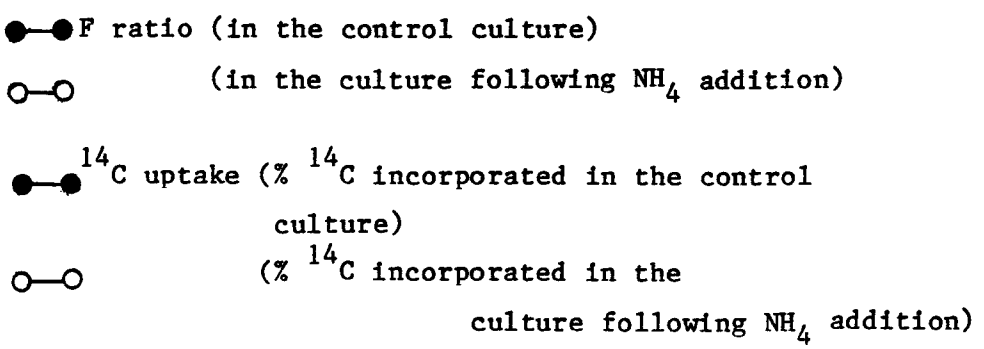

a)
1 DAY STATIONARY
.8
.6
.4
F_{Ratio}
b)
18
16
14
6
4
2
0
C_{UPTAKE} (% Incorporated)
0
2
4
6
8
10
12
14
TIME (hours)

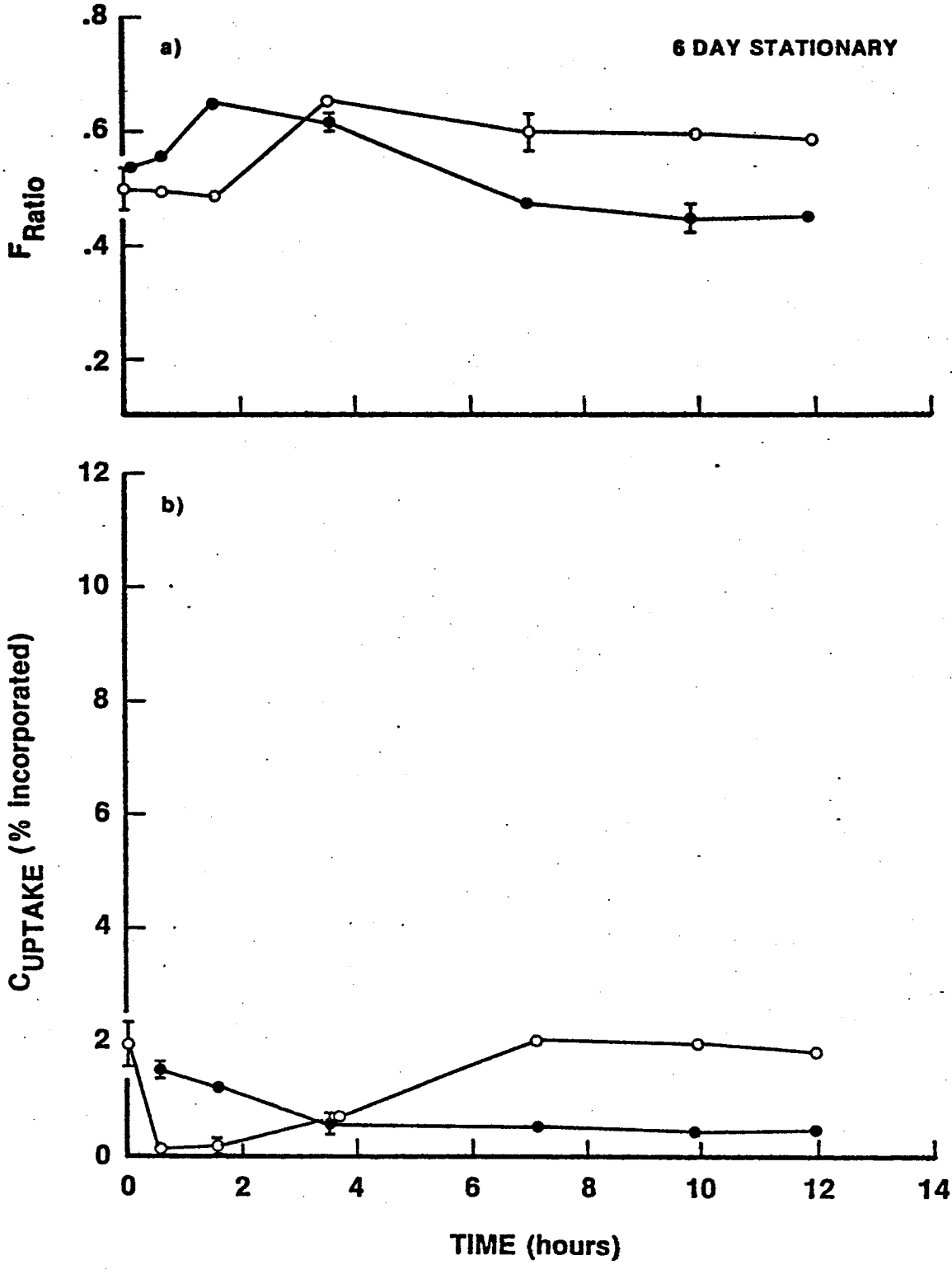
Appendix 2.1.2 The effect of NH_4 enrichment on a) the F ratio and b) ^{14}C uptake for Chlamydomonas in 3 day stationary phase.




a)
3 DAY STATIONARY
.8
.6
.4
.2
F Ratio
b)
12
10
8
6
4
2
0
C UPTAKE (% Incorporated)
0
2
4
6
8
10
12
14
TIME (hours)

Appendix 2.1.3 The effect of NH_4 enrichment on a) the F ratio and b) ^{14}C uptake for Chlamydomonas in 6 day stationary phase.




a)
6 DAY STATIONARY
.8
.6
.4
.2
F Ratio
b)
12
10
8
6
4
2
0
C UPTAKE (% Incorporated)
0
2
4
6
8
10
12
14
TIME (hours)

Appendix 2.1.4 The effect of NO_3^- enrichment on a) the F ratio and b) ^{14}C uptake for Chlamydomonas in 1 day stationary phase. The results of replicate cultures are plotted separately.

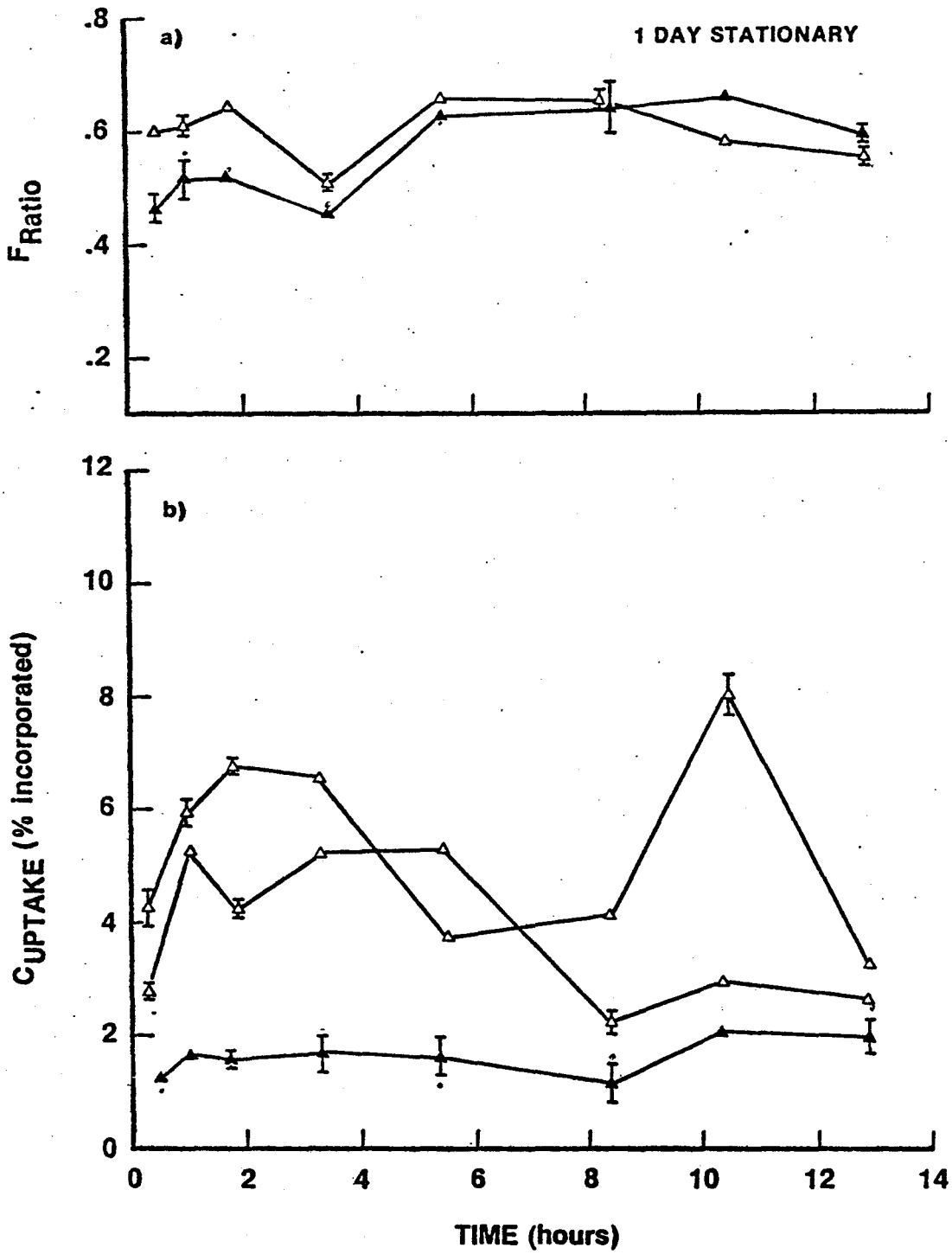
▲—▲ F ratio (in the control culture)

△—△ (in the culture following NH_4 addition)

▲—▲ ^{14}C uptake (% ^{14}C incorporated in the control culture)

△—△ (% ^{14}C incorporated in the culture following NH_4 addition)

Note that there were significant differences between the replicate cultures on this occasion.


a)
1 DAY STATIONARY
.8
.6
.4
.2
F Ratio
b)
12
10
8
6
4
2
0
C UPTAKE (% incorporated)
0
2
4
6
8
10
12
14
TIME (hours)

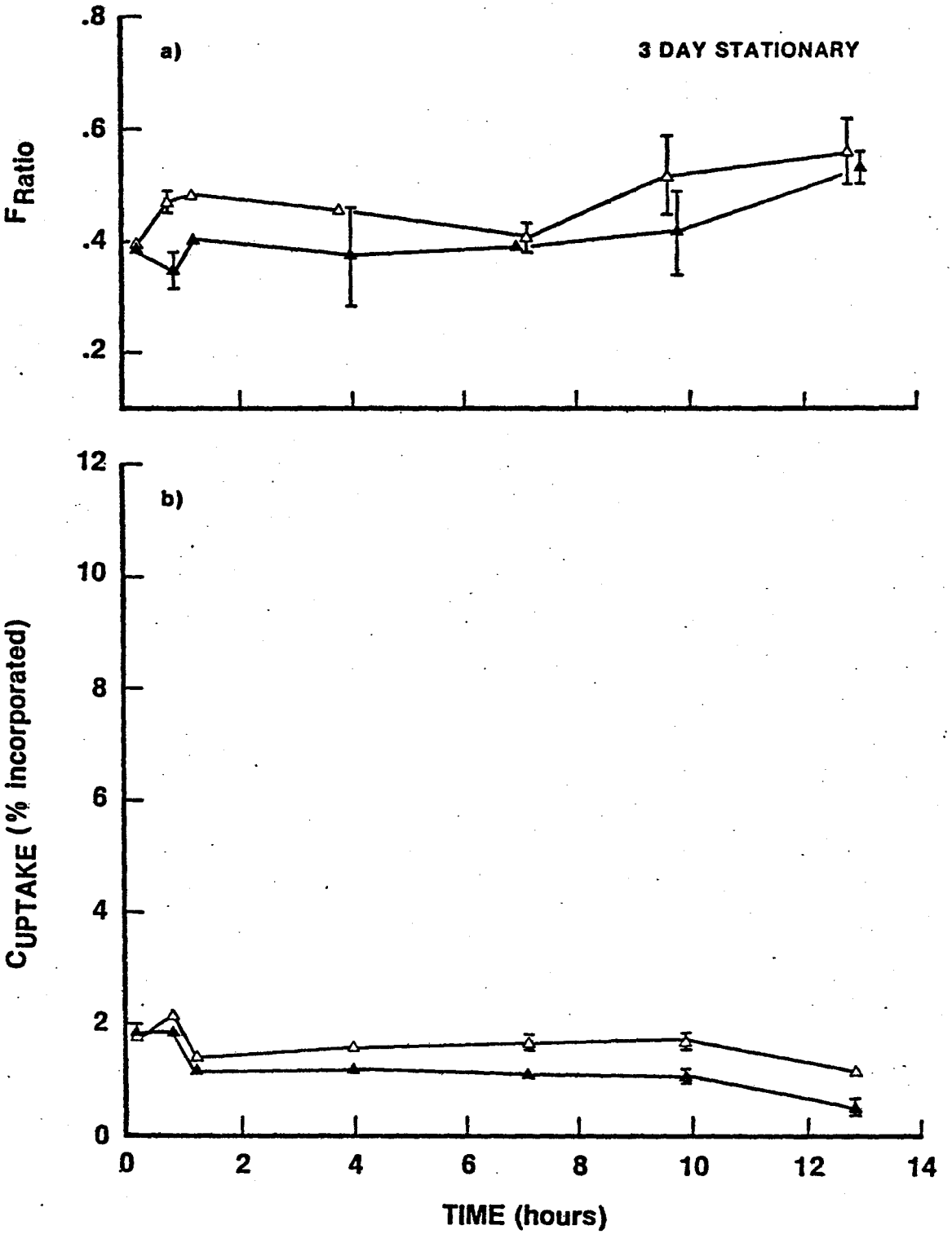
Appendix 2.1.5 The effect of NO_3^- enrichment on a) the F ratio and b) ^{14}C uptake for Chlamydomonas in 6 day stationary phase.

▲—▲ F ratio (in the control culture)

△—△ (in the culture following NH_4 addition)

▲—▲ ^{14}C uptake (% ^{14}C incorporated in the control culture)

△—△ (% ^{14}C incorporated in the culture following NH_4 addition)


a)
3 DAY STATIONARY
F Ratio
.8
.6
.4
.2
b)
C UPTAKE (% incorporated)
12
10
8
6
4
2
0
0
2
4
6
8
10
12
14
TIME (hours)

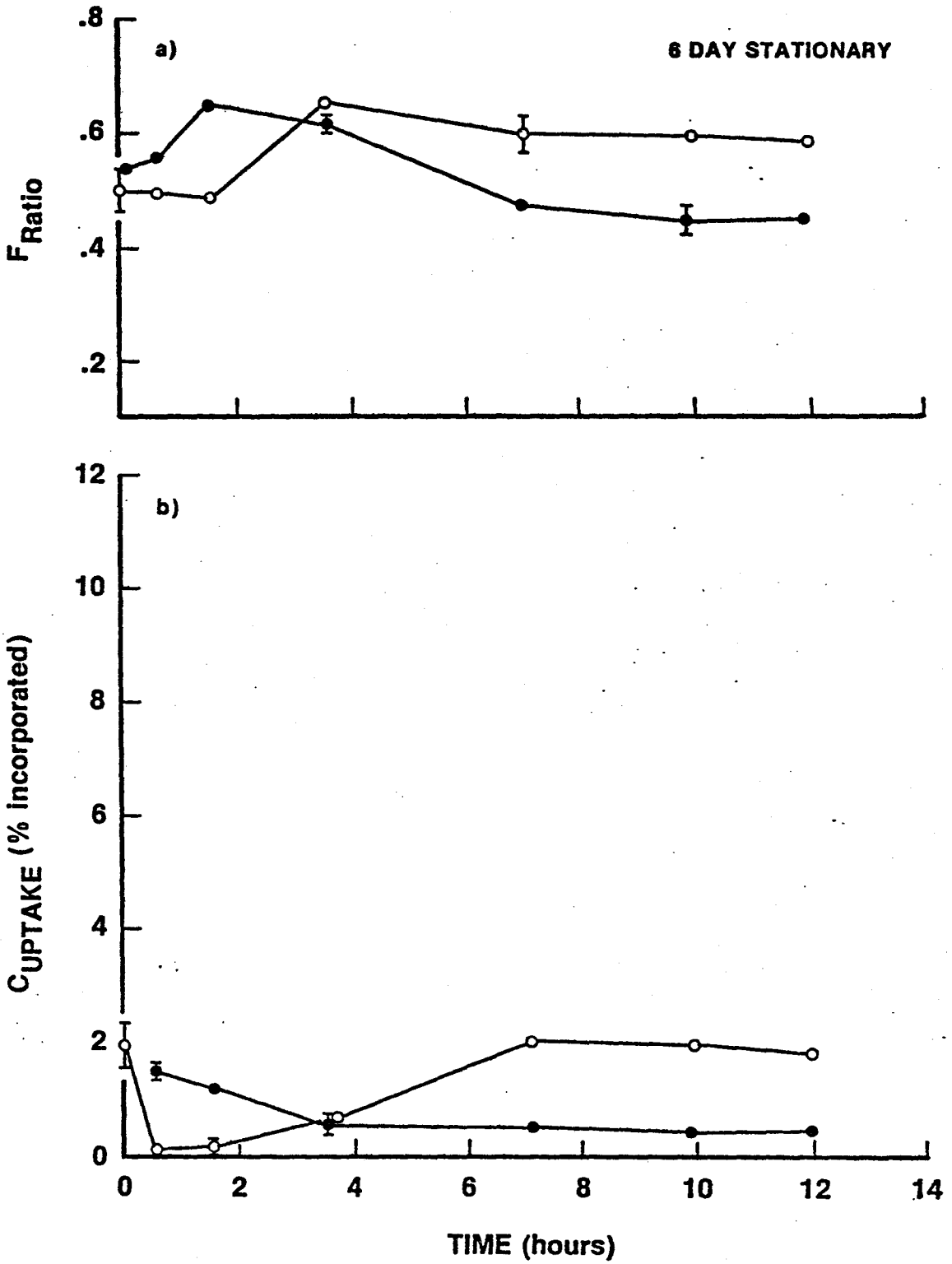
Appendix 2.1.6 The effect of NO_3^- enrichment on a) the F ratio and b) ^{14}C uptake for Chlamydomonas in 6 day stationary phase.

▲—▲ F ratio (in the control culture)

△—△ (in the culture following NH_4 addition)

▲—▲ ^{14}C uptake (% ^{14}C incorporated in the control culture)

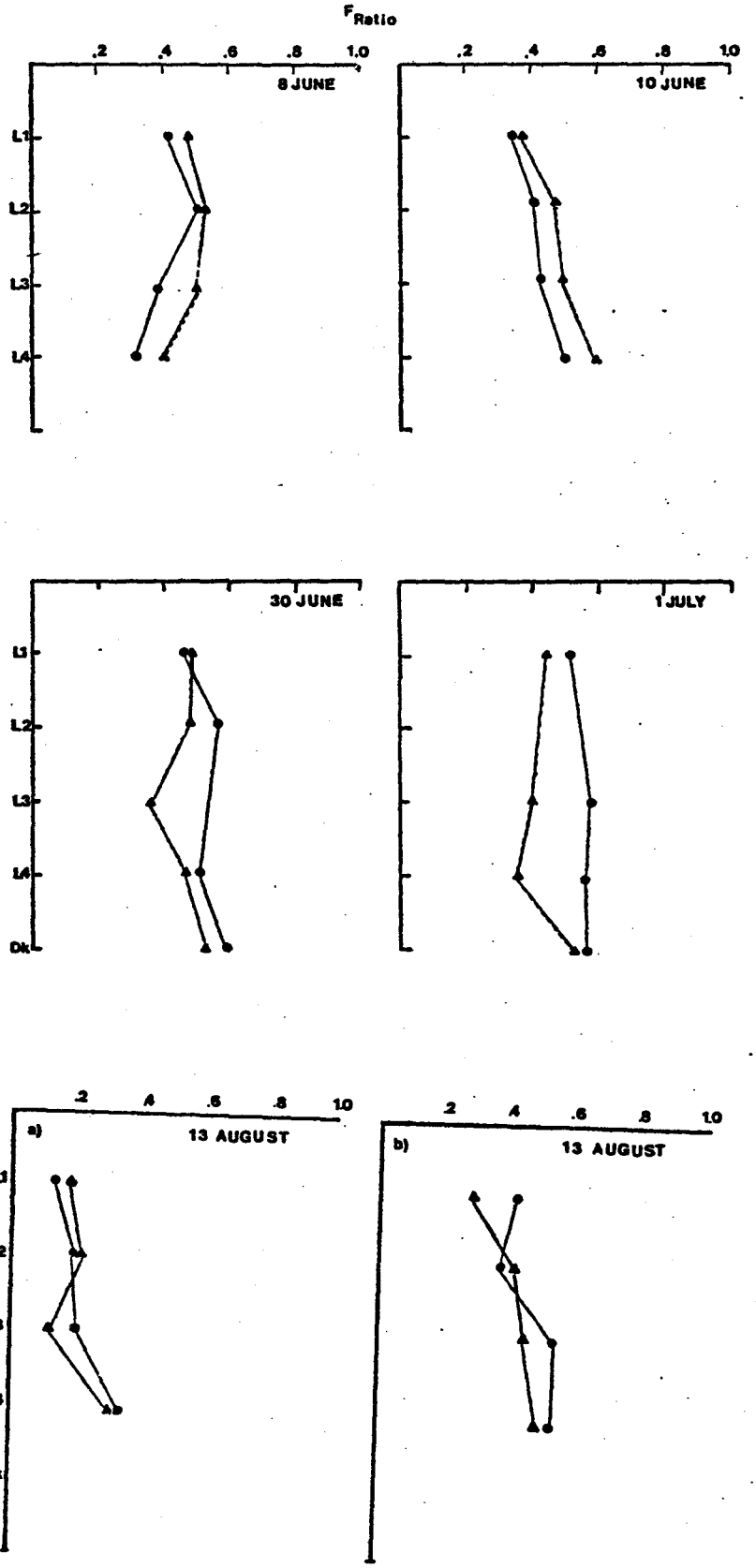
△—△ (% ^{14}C incorporated in the culture following NH_4 addition)


a)
6 DAY STATIONARY
.8
.6
.4
.2
F Ratio
b)
12
10
8
6
4
2
0
C UPTAKE (% incorporated)
0
2
4
6
8
10
12
14
TIME (hours)

Appendix 2.2.1 F ratio following addition of NH_4 and incubation at 4 light levels (Lake Ontario 1982). L1 is the brightest light level. For details of the incubations see Table 4.5. On 13 August a) 10m and b) 150 meter water were compared.

●—● F ratio in control water

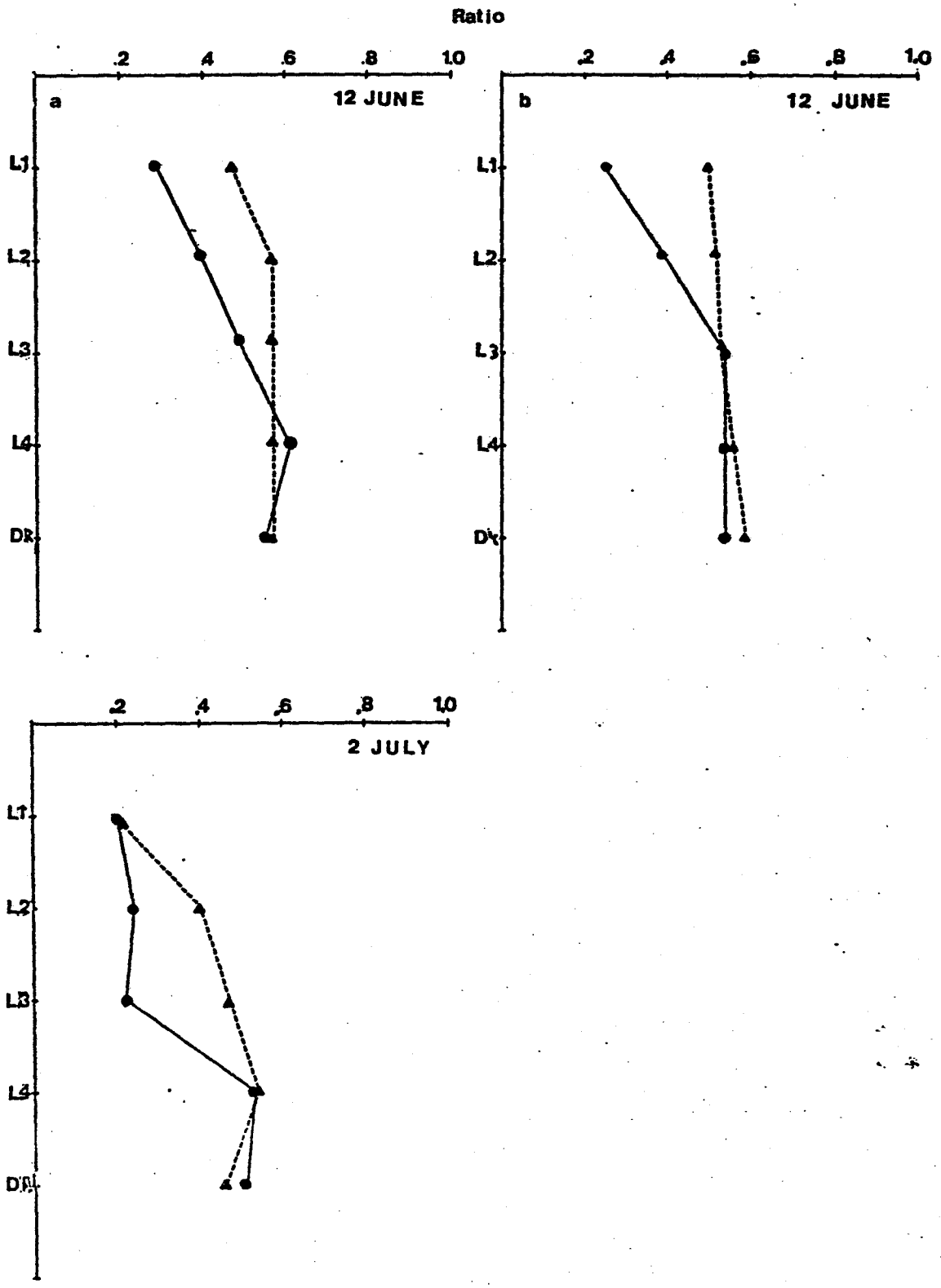
▲—▲ F ratio in treated water


F Ratio
.2
.4
.6
.8
1.0
8 JUNE
10 JUNE
30 JUNE
1 JULY
13 AUGUST
a)
b)
L1
L2
L3
L4
Dk

Appendix 2.2.2 F ratio following addition of NH_4 and incubation at 4 light levels (Lake Ontario, 1982). L1 is the brightest light level. For details of the incubations see Table 4.5. On 12 June a) 1m and b) 150m water were compared.

●—● F ratio in control water

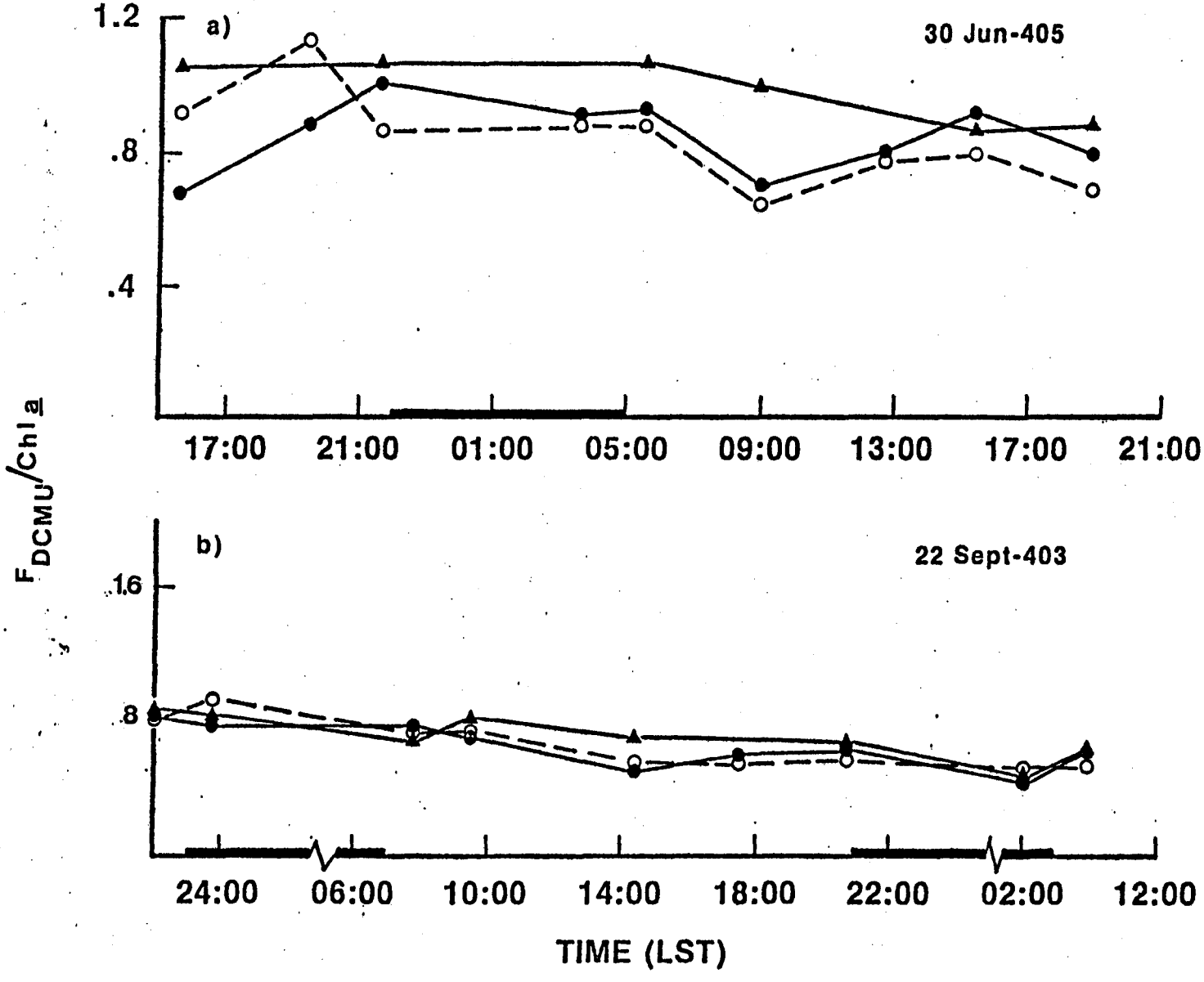
▲—▲ F ratio in treated water


Ratio
.2
.4
.6
.8
1.0
a
12 JUNE
L1
L2
L3
L4
DR
b
12 JUNE
2 JULY

Appendix 3.1.1 Diurnal patterns of F_{DCMU}/chl*a* in Lake Ontario water from (●—●) 1m, (○---○) 5m, and (▲—▲) 10m on:

a) 30 June,1982 (station 405)

b) 22 September,1982 (station 403)

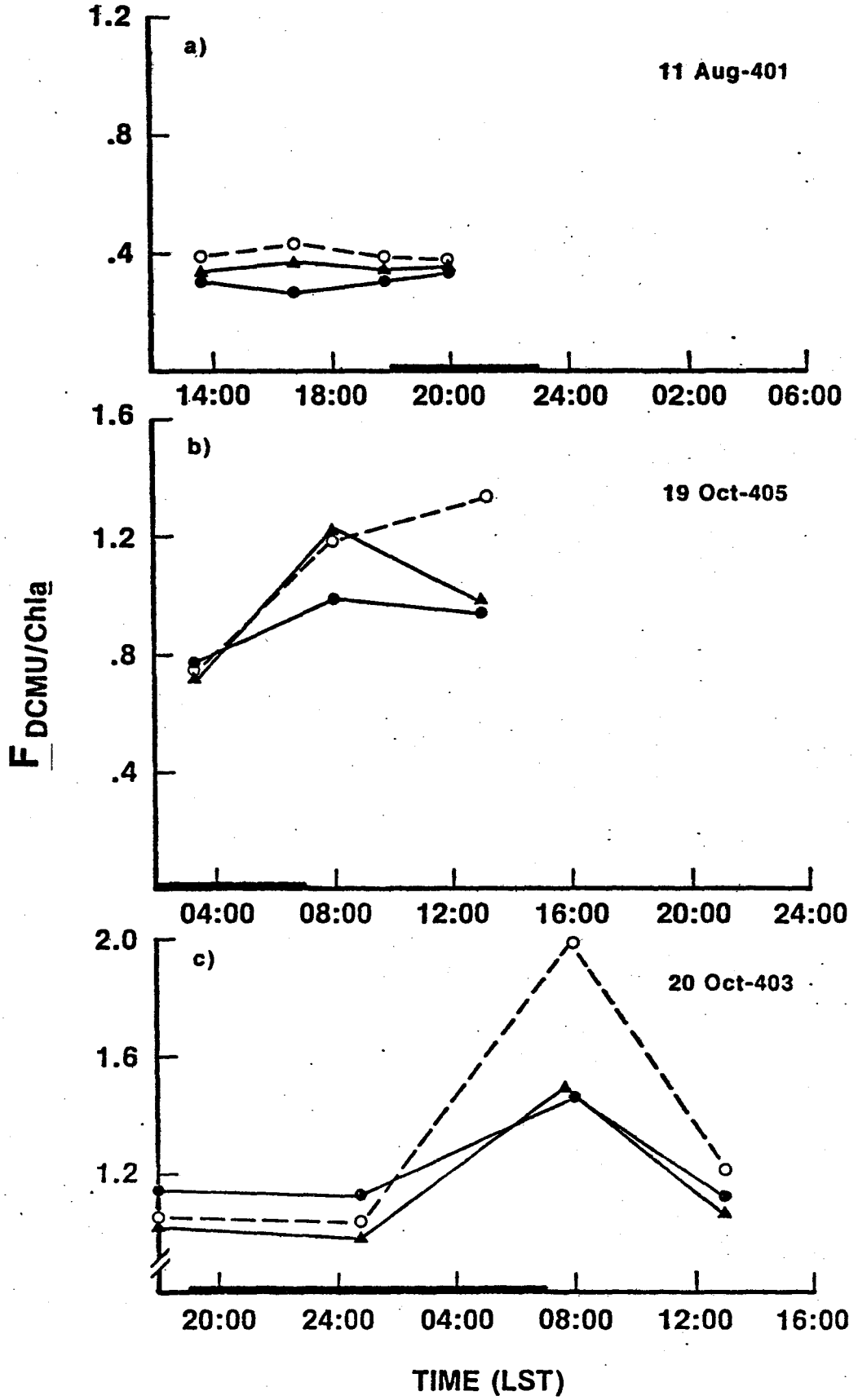

1.2
.8
.4
a)
30 Jun-405
17:00
21:00
01:00
05:00
09:00
13:00
17:00
21:00
$F_{DCMU}/Chl\,a$
1.6
.8
b)
22 Sept-403
24:00
06:00
10:00
14:00
18:00
22:00
02:00
12:00
TIME (LST)

Appendix 3.1.2 Diurnal patterns of F_{DCMU}/chl*a* in Lake Ontario water from (●—●) 1m, (▲—▲) 5m, and (○--○)10m on:

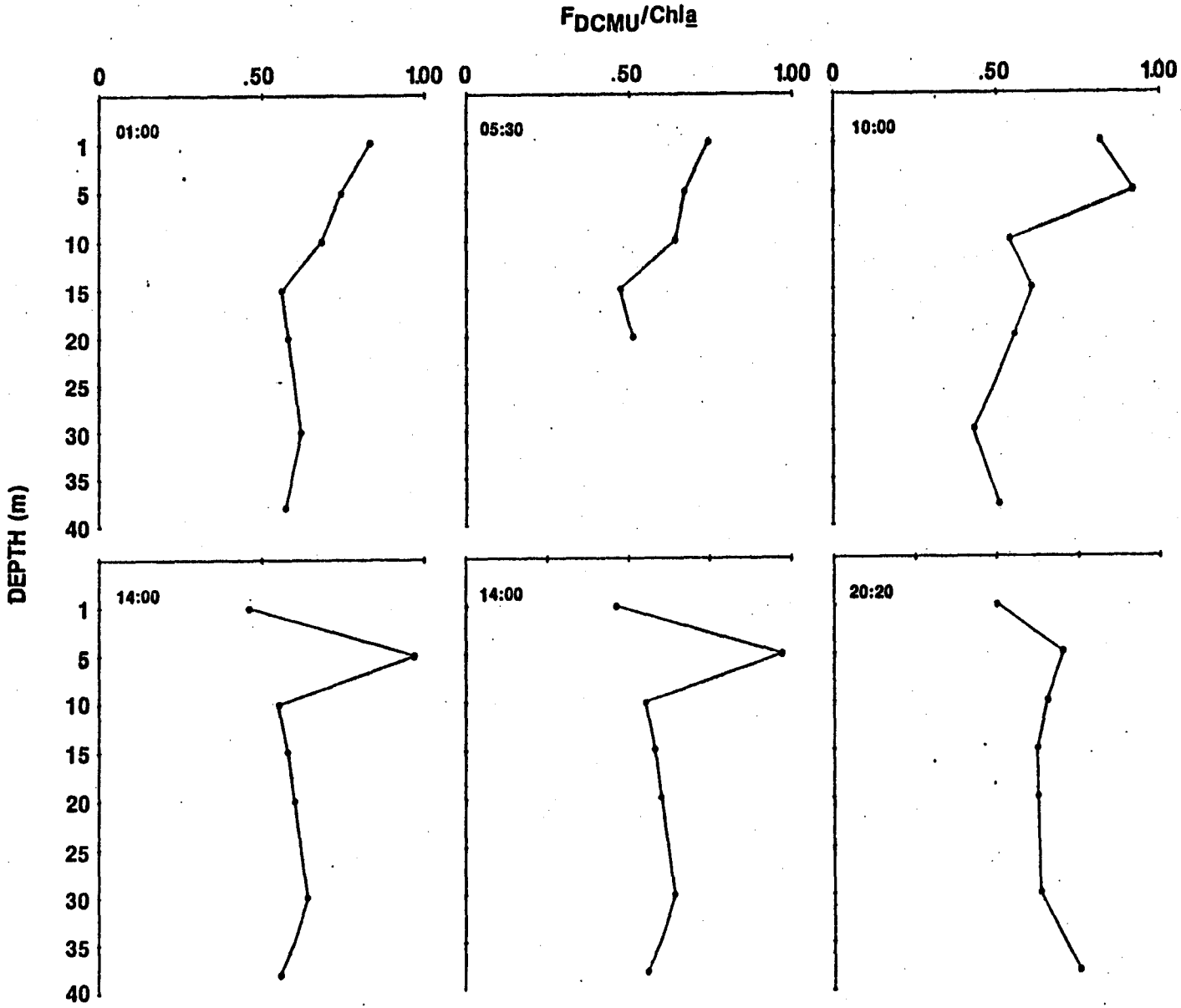
a) 11 August,1982 (station 401)

b) 19 October,1982 (station 405)

c) 20 October,1982 (station 403)


a)
11 Aug-401
1.2
.8
.4
14:00
18:00
20:00
24:00
02:00
06:00
b)
19 Oct-405
1.6
1.2
.8
.4
04:00
08:00
12:00
16:00
20:00
24:00
c)
20 Oct-403
2.0
1.6
1.2
20:00
24:00
04:00
08:00
12:00
16:00
F DCMU/Chla
TIME (LST)

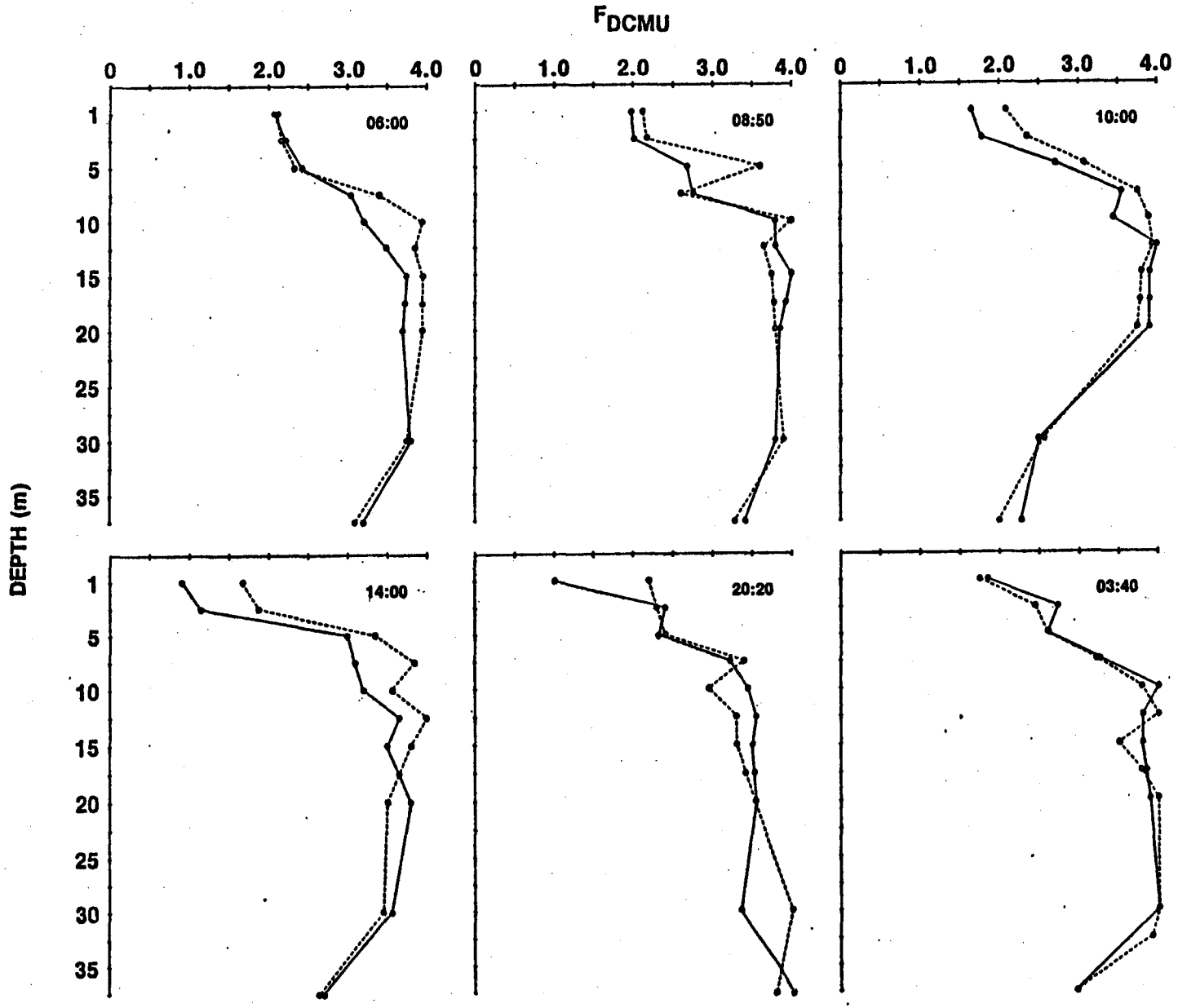
Appendix 3.2 Vertical profiles of F_{DCMU}/chl*a* on 8 June, 1982 at station 401 (Lake Ontario).


$F_{DCMU}/Chl\underline{a}$
0
.50
1.00
01:00
05:30
10:00
14:00
14:00
20:20
DEPTH (m)
1
5
10
15
20
25
30
35
40

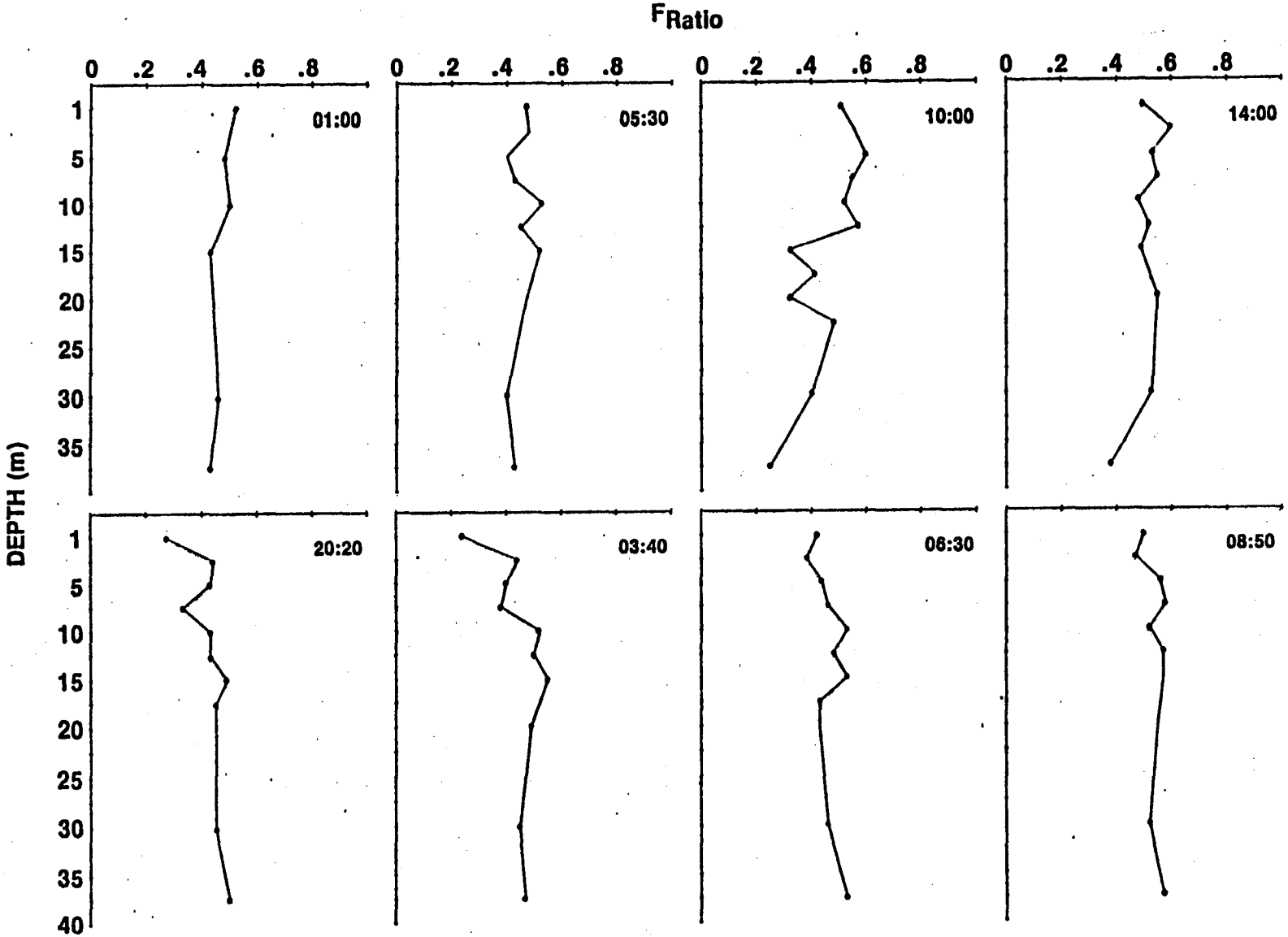
Appendix 3.3 Vertical profiles of F_{DCMU} prior to and after a 1 hour treatment in the dark on 8 June, 1982 at station 401 (Lake Ontario).

●—● F_{DCMU} prior to treatment

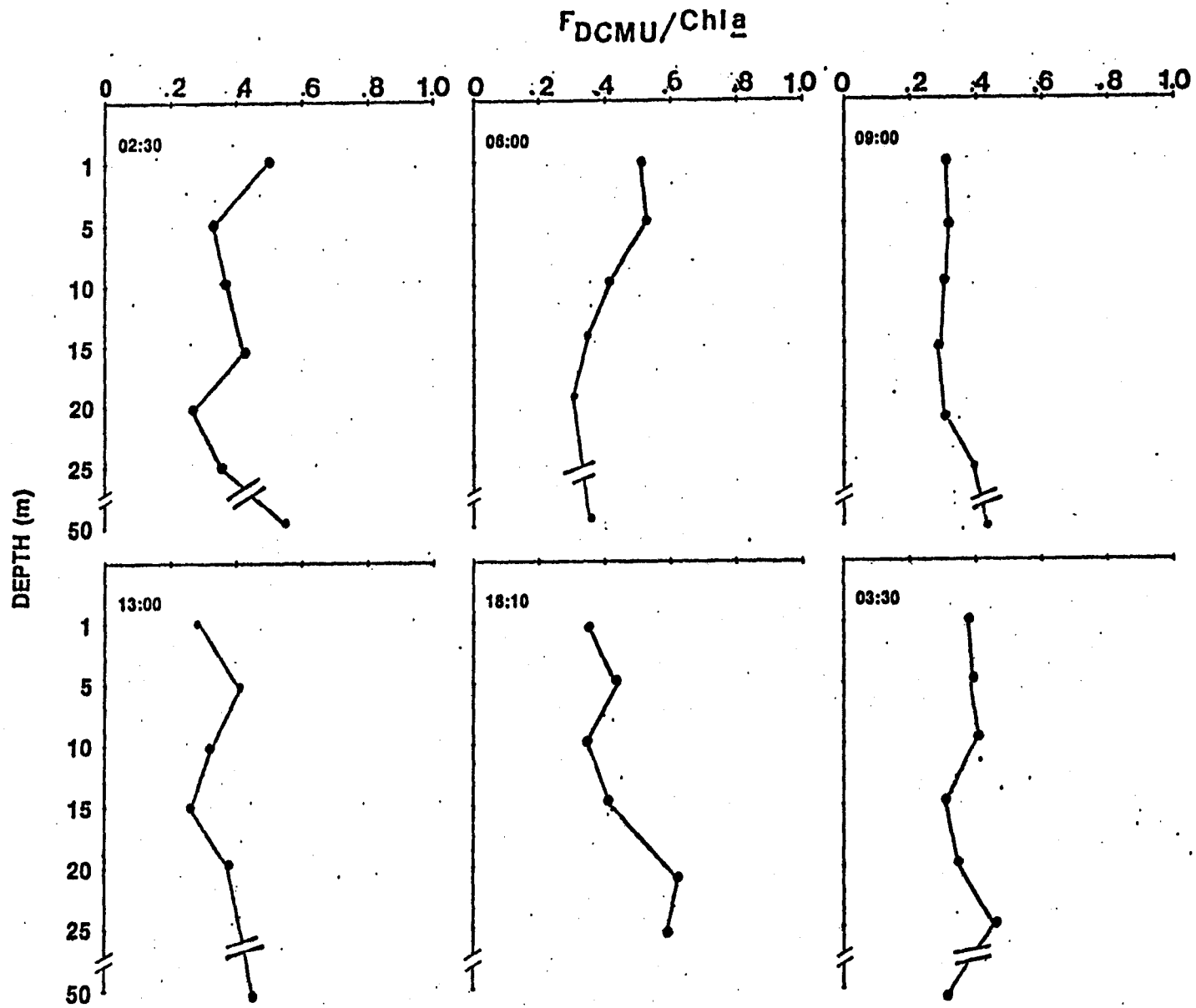
○-○ F_{DCMU} after dark treatment


F_{DCMU}
0
1.0
2.0
3.0
4.0
DEPTH (m)
1
5
10
15
20
25
30
35
06:00
08:50
10:00
14:00
20:20
03:40

Appendix 3.4 Vertical profiles of F ratio on 8 June, 1982 at station 401 (Lake Ontario).


F Ratio
0 .2 .4 .6 .8
DEPTH (m)
1 5 10 15 20 25 30 35
01:00
05:30
10:00
14:00
1 5 10 15 20 25 30 35 40
20:20
03:40
06:30
08:50

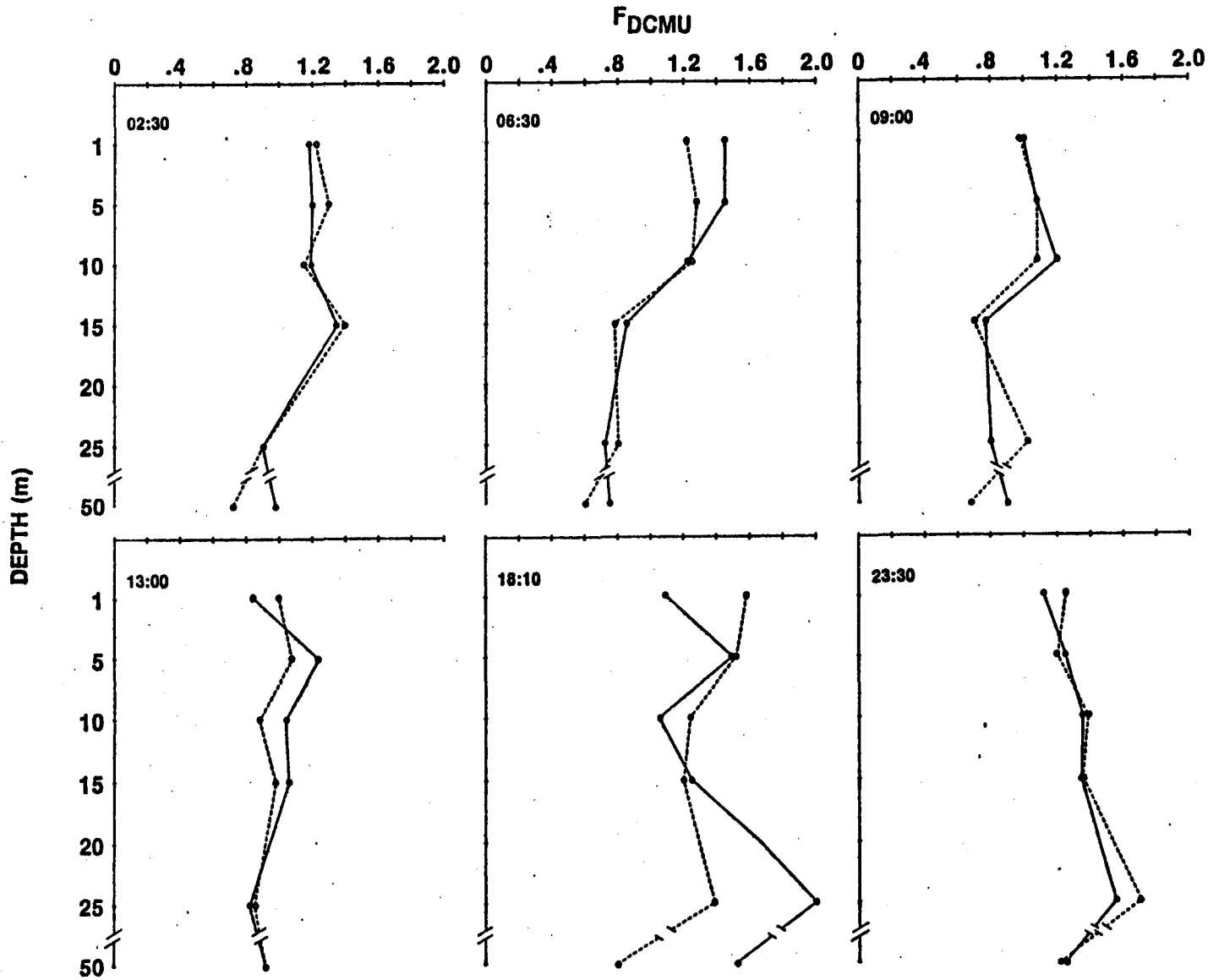
Appendix 3.5 Vertical profiles of F_{DCMU}/chl*a* on 12 August,1982 at station 403 (Lake Ontario).


F_{DCMU}/Chl_a
0 .2 .4 .6 .8 1.0
02:30
06:00
09:00
13:00
18:10
03:30
DEPTH (m)
1
5
10
15
20
25
50

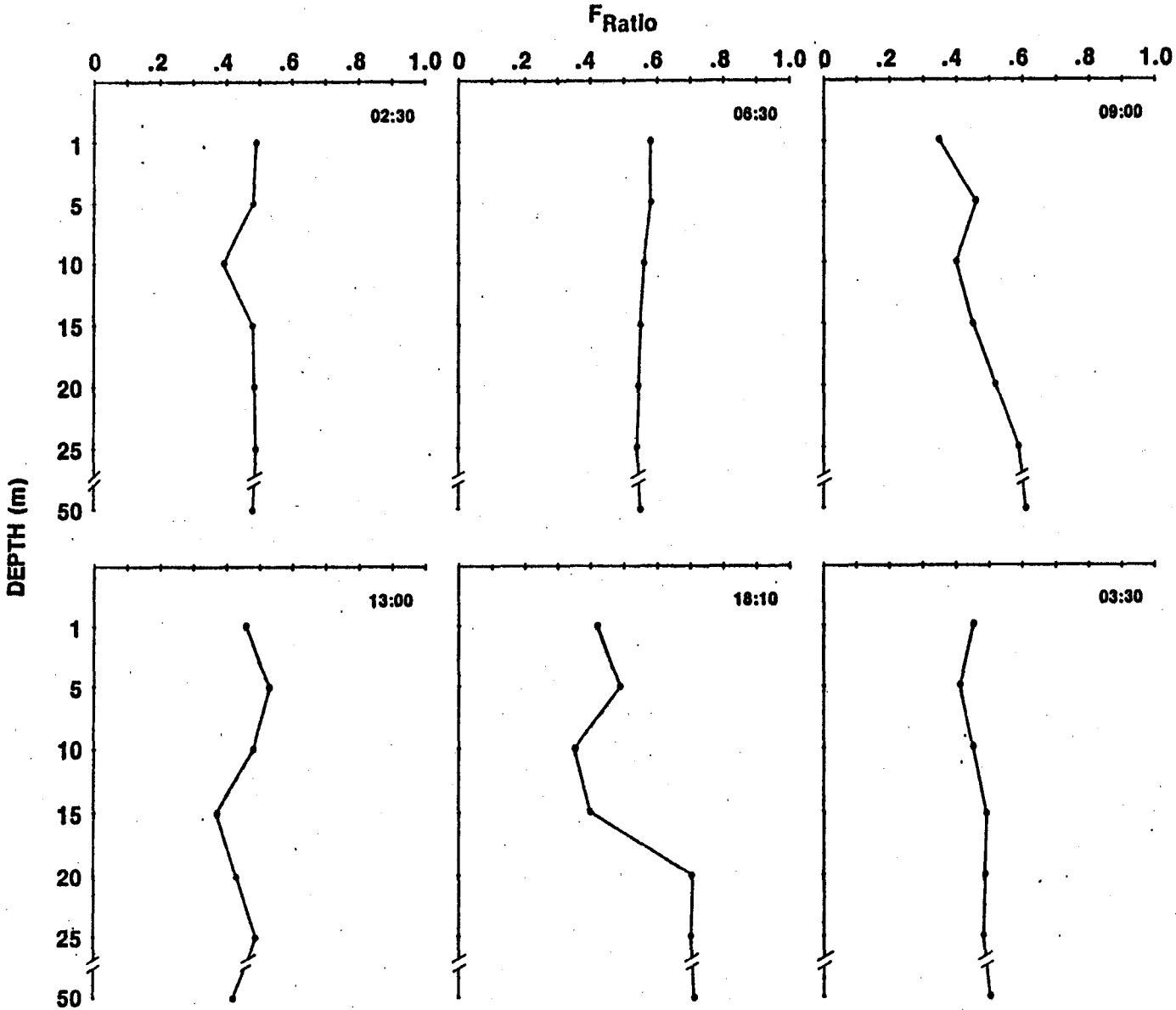
Appendix 3.6 Vertical profiles of F_{DCMU} prior to and after a 1 hour treatment in the dark on 12 August,1982 at station 403 (Lake Ontario).

●—● F_{DCMU} prior to treatment

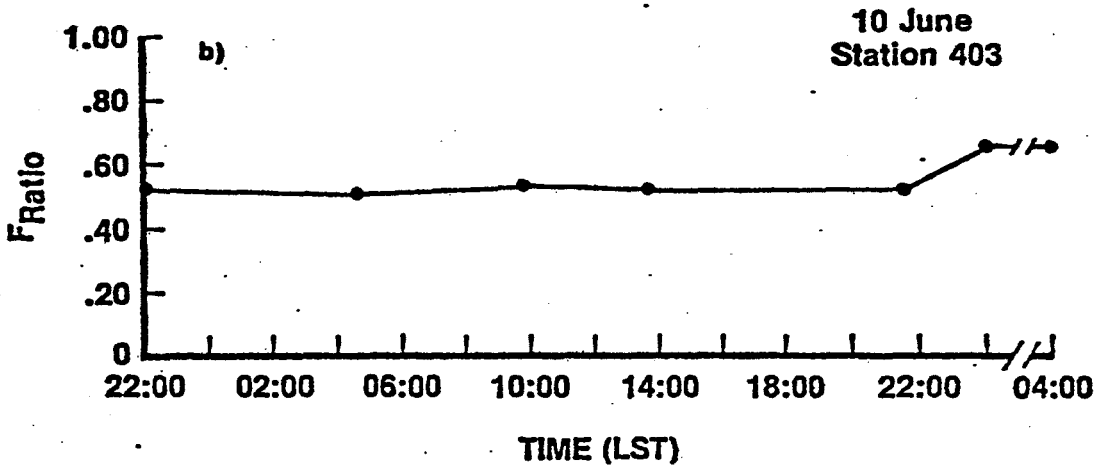
○--○ F_{DCMU} after dark treatment

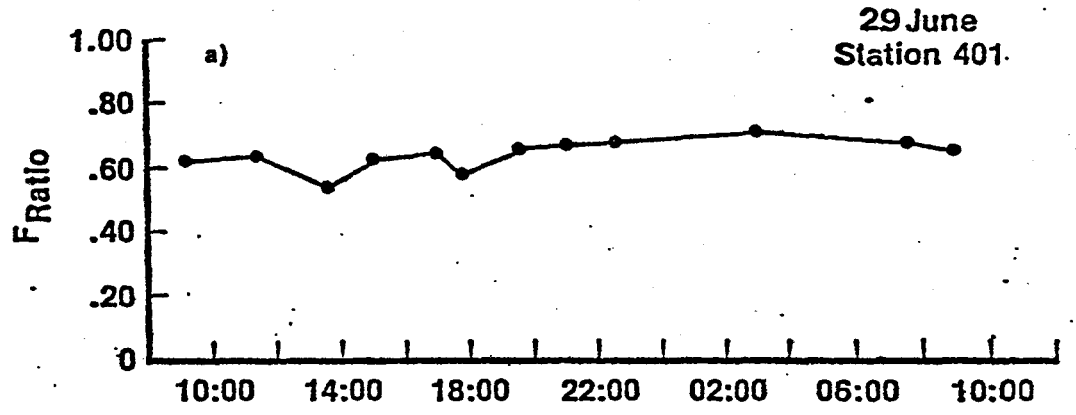

F_{DCMU}
0 .4 .8 1.2 1.6 2.0
02:30
06:30
09:00
13:00
18:10
23:30
DEPTH (m)
1
5
10
15
20
25
50

Appendix 3.7 Vertical profiles of F ratio on 12 August, 1982 at station 403 (Lake Ontario).

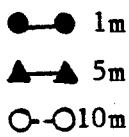

F Ratio
0
.2
.4
.6
.8
1.0
02:30
06:30
09:00
13:00
18:10
03:30
DEPTH (m)
1
5
10
15
20
25
50

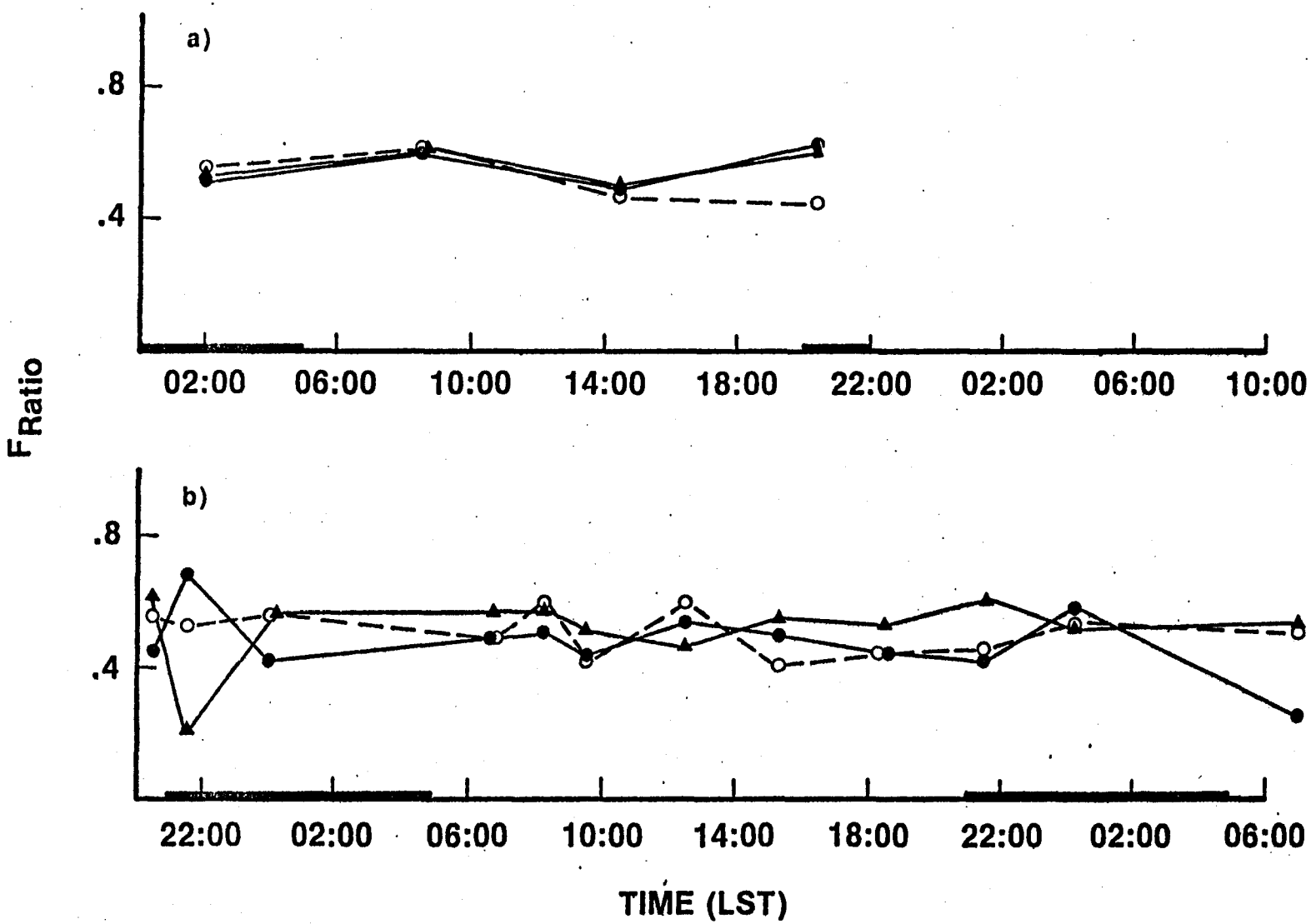
Appendix 4.1.1 F ratio of samples from 5m on a) 10 June, 1982 station 403 (Lake Ontario) and b) 29 June, 1982 station 401 (Lake Ontario) incubated under continuous low light (40 μE m^{-2} s^{-1}) for at least 24 hours.


b)
10 June
Station 403
1.00
.80
.60
.40
.20
0
F Ratio
22:00
02:00
06:00
10:00
14:00
18:00
22:00
04:00
TIME (LST)

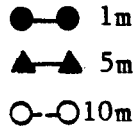

a)
29 June
Station 401
1.00
.80
.60
.40
.20
0
F Ratio
10:00
14:00
18:00
22:00
02:00
06:00
10:00

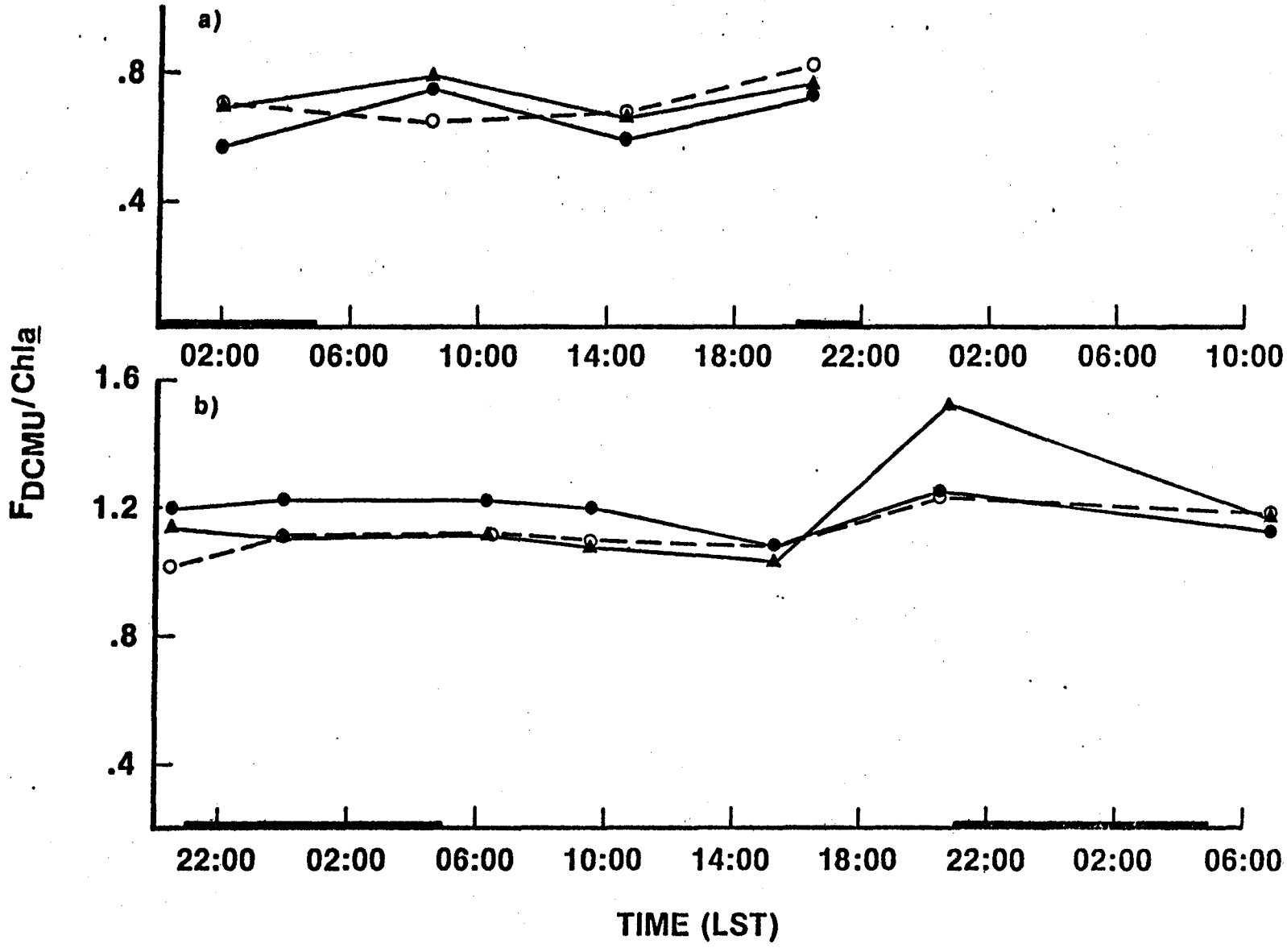
Appendix 4.1.2 Diurnal changes in the F ratio in the water column on a) 10 June, 1982 at station 403 (Lake Ontario) b) 29 June, 1982 station 401 (Lake Ontario).




a)
.8
.4
02:00
06:00
10:00
14:00
18:00
22:00
02:00
06:00
10:00
b)
.8
.4
22:00
02:00
06:00
10:00
14:00
18:00
22:00
02:00
06:00
F Ratio
TIME (LST)

Appendix 4.1.3 Diurnal changes in the F_{DCMU}/chl*a* in the water column on a) 10 June, 1982 at station 403 (Lake Ontario) b) 29 June, 1982 at station 401 (Lake Ontario).

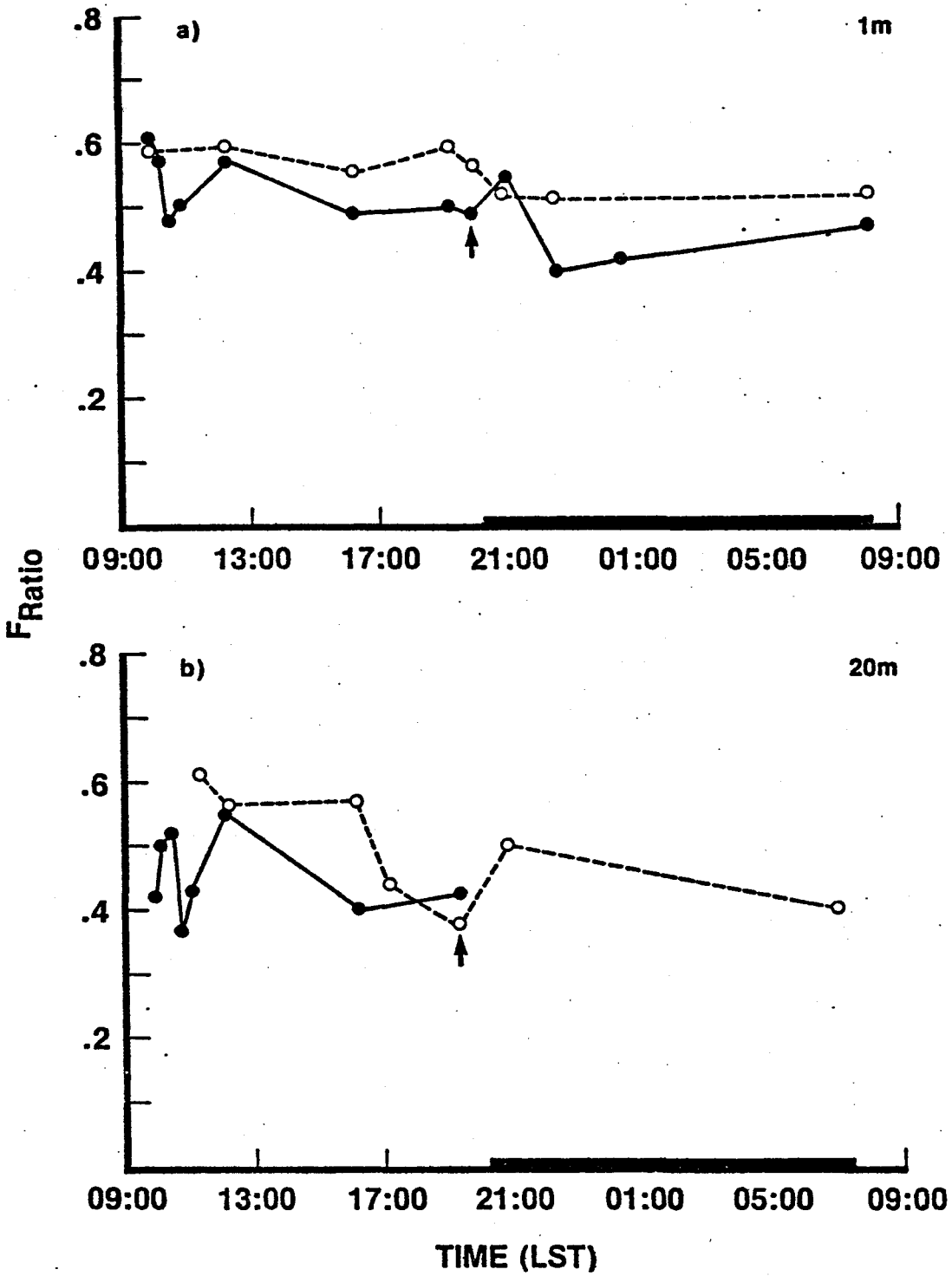



a)
.8
.4
02:00
06:00
10:00
14:00
18:00
22:00
02:00
06:00
10:00
b)
1.6
1.2
.8
.4
22:00
02:00
06:00
10:00
14:00
18:00
22:00
02:00
06:00
F_{DCMU}/Chl_a
TIME (LST)

Appendix 5.1.1 Comparison of the F ratio of water from a) 1m and b) 20m at station 405 (Lake Ontario, 1982) on 23 September during an 8 hour incubation at high light and in the dark:

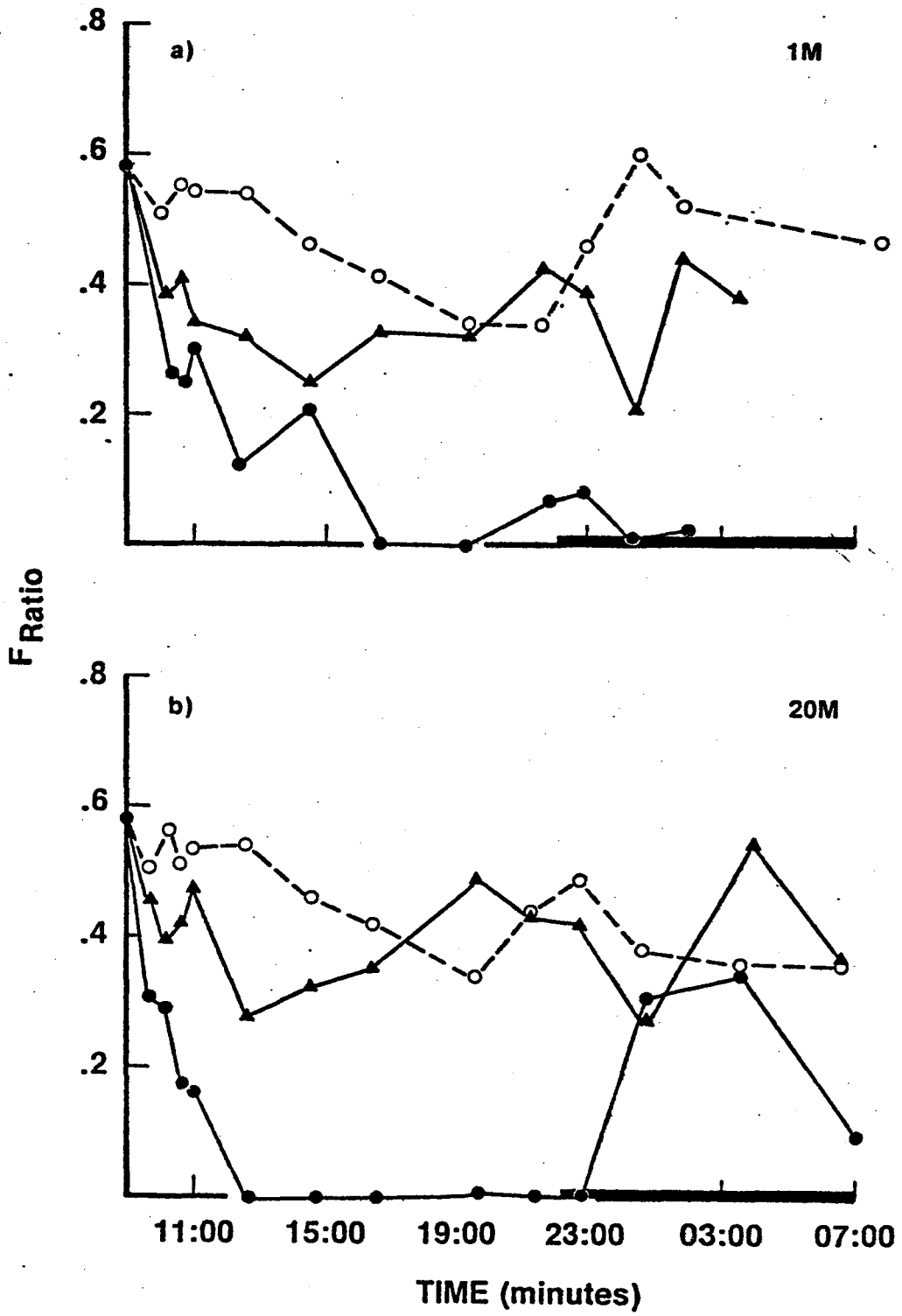
●—● (1300 $\mu E\ m^{-2}\ s^{-1}$)

○--○(Dark)


a)
1m
.8
.6
.4
.2
09:00
13:00
17:00
21:00
01:00
05:00
09:00
F_{Ratio}
b)
20m
.8
.6
.4
.2
09:00
13:00
17:00
21:00
01:00
05:00
09:00
TIME (LST)

Appendix 5.1.2 Comparison of the F ratio of water from a) 1m and b) 20m at station 405 (Lake Ontario) on 19 October,1982 during an 8 hour incubation at two light levels and in the dark:

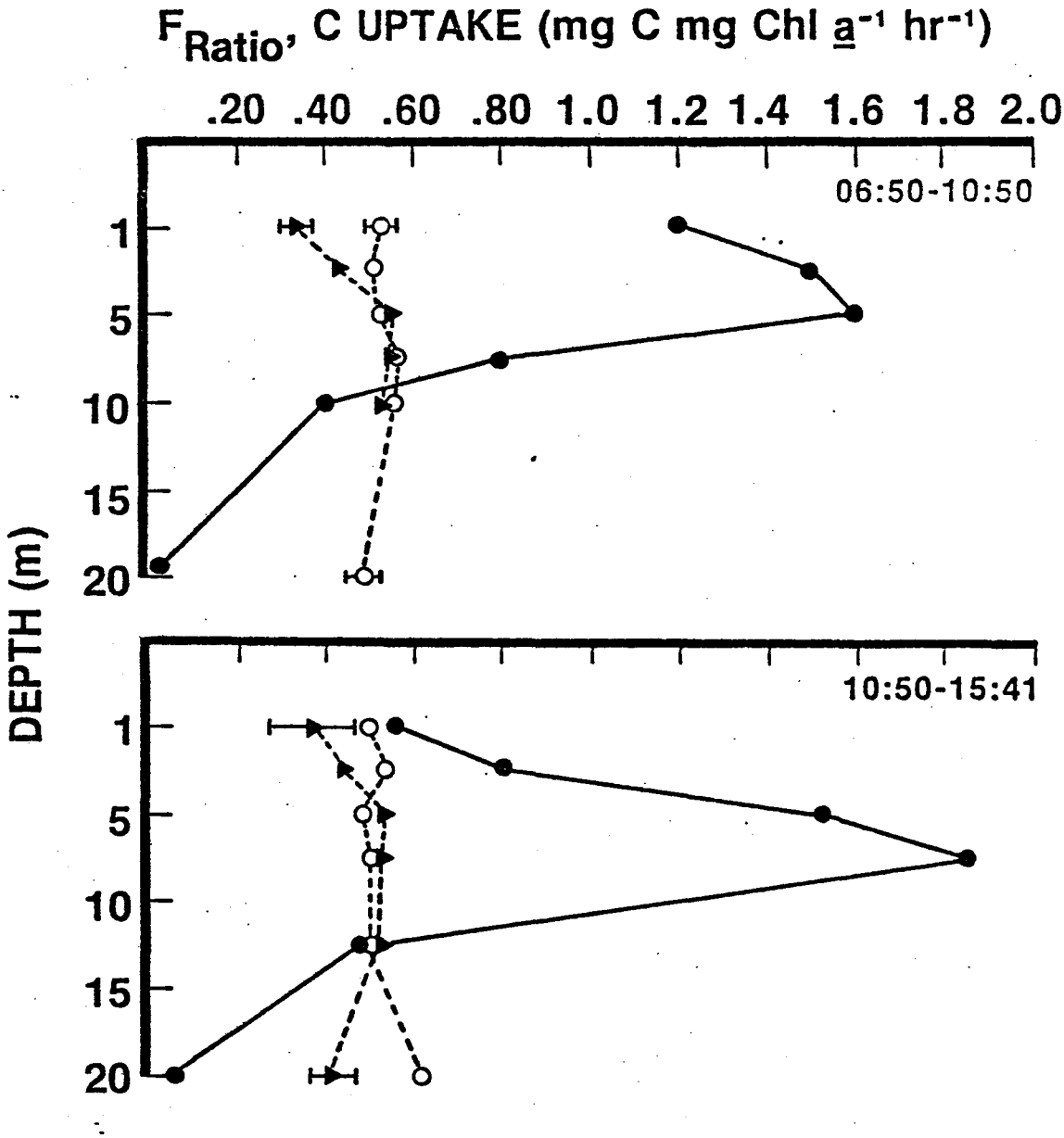
●—● (1300 μE m^{-2} s^{-1})
▲—▲ (250 μE m^{-2} s^{-1})
○--○ (Dark)


a)
1M
b)
20M
.8
.6
.4
.2
F Ratio
11:00
15:00
19:00
23:00
03:00
07:00
TIME (minutes)

Appendix 6.1 Comparison of the F ratio of water incubated in bottles (○—○), the F ratio in the water column (▶—▶), and ^{14}C uptake (●—●) on 26 August, 1982 at station 403 (Lake Ontario) for incubations between:

a) 06:50-10:50

b) 10:50-15:41

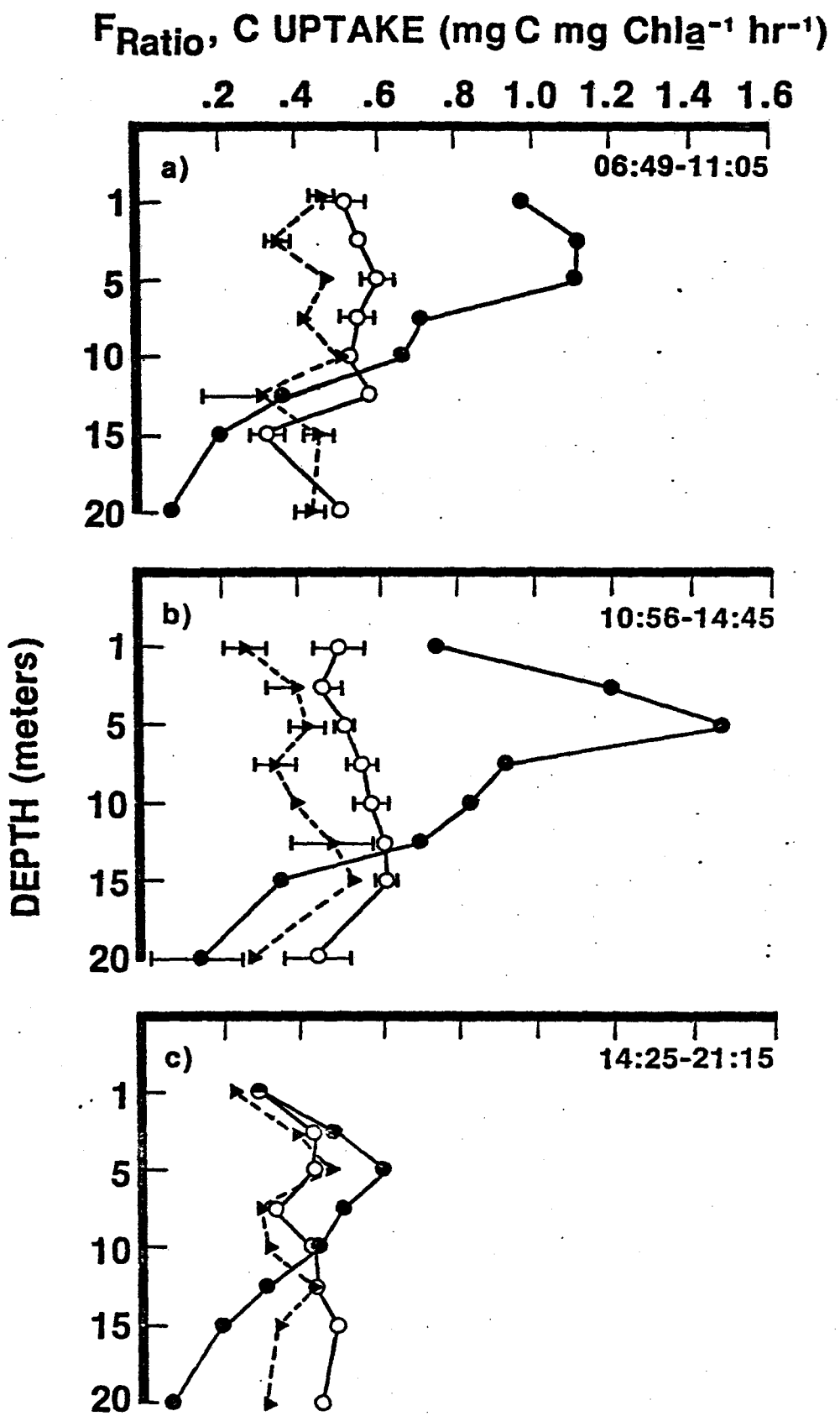

F Ratio, C UPTAKE (mg C mg Chl a⁻¹ hr⁻¹)
.20 .40 .60 .80 1.0 1.2 1.4 1.6 1.8 2.0
06:50-10:50
10:50-15:41
DEPTH (m)
1
5
10
15
20

Appendix 6.2 Comparison of the F ratio of water incubated in bottles (O—O), the F ratio in the water column (▶—▶), and ^{14}C uptake (●—●) on 8 June,1982 at station 401 (Lake Ontario) for incubations between:

a) 06:49-11:05

b) 10:56-14:45

c) 14:25-21:15


F Ratio, C UPTAKE (mg C mg Chla⁻¹ hr⁻¹)
.2 .4 .6 .8 1.0 1.2 1.4 1.6
a)
06:49-11:05
b)
10:56-14:45
c)
14:25-21:15
DEPTH (meters)
1
5
10
15
20