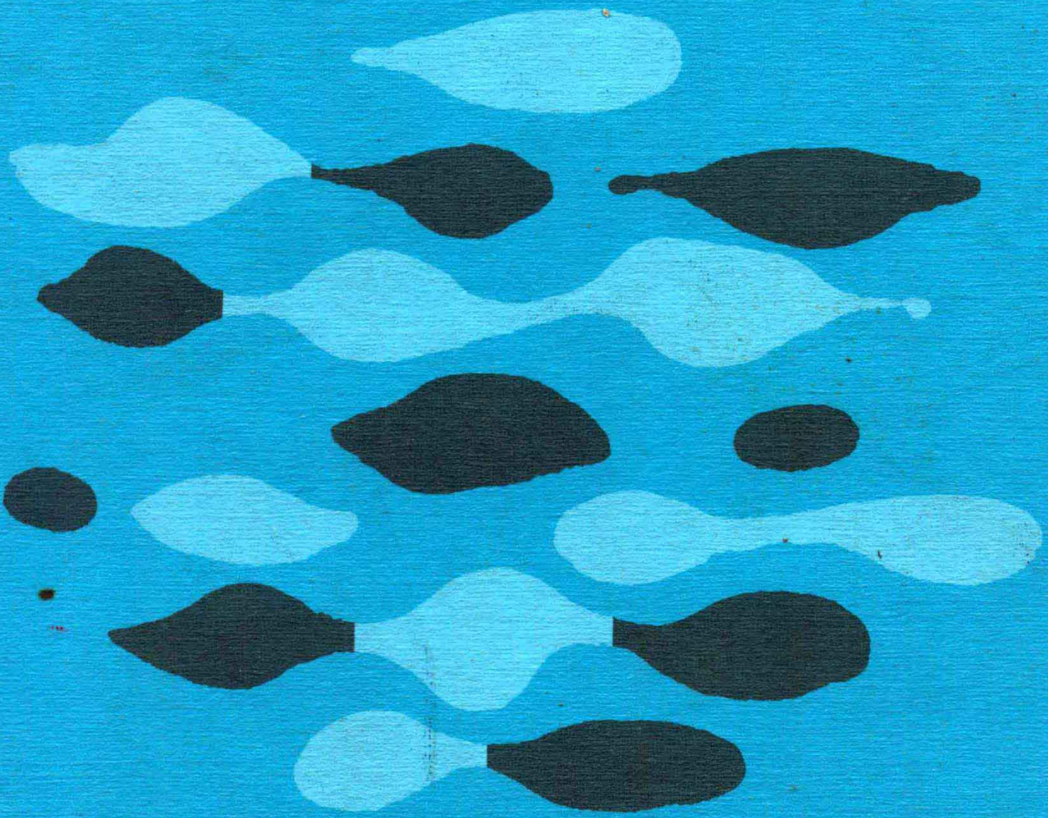
DEVELOPMENTS IN HYDROBIOLOGY

The Daily Growth Cycle of Phytoplankton

edited by
T. Berman, H. J. Gons and L. R. Mur



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Proceedings of the Fifth International Workshop of the Group for Aquatic Primary Productivity (GAP), held at Breukelen, The Netherlands, 20–28 April 1990

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The Daily Growth Cycle of Phytoplankton

Developments in Hydrobiology 76

Series editor
H. J. Dumont

Foreword

'...This Chequer-board of Night and Days...'
Omar Khayam/Fitzgerald (1859)

'Tomorrow and Tomorrow and Tomorrow
Creeps in this petty pace
From day to day'
Shakespeare (1605)

This year marks the tenth anniversary of the Group for Aquatic Primary Productivity (GAP) and this special Volume of *Hydrobiologia* presents material from the Fifth GAP International Workshop which was held in April 1990 at Breukelen, The Netherlands. The theme of the Workshop was 'The Daily Growth Cycle of Phototrophic Microorganisms'. Most of the papers in this volume, which include the two Keynote Lectures, papers based on experimental work carried out during the Workshop and upon contributed posters, have some bearing on this important topic (see key references above). At this meeting, the experiments were done at the Limnological Institute, Nieuwersluis, at two nearby lakes (Loosdrecht and Maarsseveen) and at the laboratories of the Departments of Aquatic Ecology and Microbiology, University of Amsterdam.

The basic aims of GAP have remained unchanged over the decade of its existence. GAP International Workshops (and 'Mini-GAP' projects such as the Eastern Mediterranean Cruises held in September 1989) provide a common meeting ground for scientists interested in freshwater and marine primary production to exchange state-of-the-art information and techniques during the course of a 'hands on' working meeting. This has given participants the possibility to compare and test methods, instrumentation and experimental approaches in a variety of aquatic settings. At each Workshop, several Working Groups have carried out joint experiments, the results of which have been subsequently published. (J. Plankton Research 6(2): 1984; J. Plankton Research 7(5): 1985 and Mar. Microbial Food Webs 4(1): 1990).

The Sixth International GAP Workshop will be held in June 1993 at the National Hydrology Research Institute, Saskatoon, Canada. The theme for this Workshop will be 'Effects of physical forcing on primary production process in inland and marine environments'.

TOM BERMAN
GAP Chairman

Preface

The present volume contains the keynote lectures, reports on the experimental work, and papers contributed to posters at the Fifth International Workshop of the Group for Aquatic Primary Productivity (GAP) held at Breukelen, The Netherlands (20–28 April 1990). GAP Workshops bring together a diverse group of scientists interested in many aspects of aquatic primary productivity and the programs are designed to promote the sharing of expertise through joint experimental work. Therefore, we emphasize the practical, experimental phases of these Workshops.

The Fifth Workshop was hosted by the Limnological Institute (the recent Netherlands Institute of Ecology – Centre for Limnology) of the Royal Netherlands Academy of Arts and Sciences, and the Departments of Microbiology and Aquatic Ecology of the University of Amsterdam. Forty-two visiting participants, representing sixteen countries, were welcomed in Breukelen.

The Workshop theme was the Daily Growth Cycle of Phototrophic Microorganisms. Participants were able to exploit continuous cultures of selected algal species and facilities for studies of phytoplankton communities in two 'natural' systems: the very shallow, eutrophic Lake Loosdrecht and the relatively deep, oligo-mesotrophic Lake Maarsseveen. Special facilities included a custom-made flow cytometer, labelled silicon uptake and laboratory scale enclosures of lakewater.

The experiments were organized in two work groups, chaired by B. B. Prézelin and W. F. Vincent with the assistance of M. Rijkeboer and L. R. Mur. Before the meeting the participants exchanged research ideas and technical information with the chairpersons and local organizers. Equipment brought by participants included instrumentation for measurements of photosynthesis and respiration, UV-radiation, temperature microprofiles and a flow cytometer.

The Workshop theme incorporated different aspects of growth: increase in algal biomass, specific cell components, and in cell numbers. The Keynote Lectures, 'Time course of photosynthesis and respiration' and 'Interaction of physical environment and daily growth cycle', were given by B. B. Prézelin and W. F. Vincent, and served to set the overall background for the proceedings. These contributions constitute Part One of this volume. The research facilities and field sites were then introduced by T. Burger-Wiersma (continuous cultures), H. J. Gons (Lake Loosdrecht) and J. Ringelberg (Lake Maarsseveen).

After the introductions, the chairpersons co-ordinated the practical work. Research plans were formed on a basis of pre-Workshop communications and adapted to the facilities at the three laboratory and two field sites. Teams were set up to work on (1) continuous cultures, (2) flow cytometry of culture and natural populations, and (3) the two lakes, including laboratory scale enclosures. Each team made a detailed initial work plan, which was presented for plenary discussion. Finally, sampling schedules and responsibilities for analyses were assigned. Logistical and instrumental bottlenecks were minimized thanks to much tact and hard work on the part of the staffs of the host Institutes. Special thanks are due to B. J. G. Flik (Lake Maarsseveen) and L. van Liere (Lake Loosdrecht), who organized the field work, and to the Workshop Secretary, H. de Haan, who took care of the transportation to the study sites, general liaison and many unexpected chores.

The unpleasant weather of the preceeding weeks gave way to calm, sunny days. On the Sunday morning, the participants were shown the way to instruments, chemicals and glassware at the laboratories. Equipment which had been brought was installed and tested. Next came an all-out search for accessories varying from ropes and binders to thermocryostats and dataloggers. Chemicals and apparatus were moved among the laboratories and field sites. Frantic appeals were sent home for technical references and to speed up deliveries of missing or forgotten items. Meanwhile, radio-isotope lab protocols (apparently rather strict) and operational details of instruments were checked. By noon Sunday the first samples were collected. Dinner was cold for several participants, but soon all had firmly settled into their experimental mode 'niches'.

Like its precursors, the Fifth Workshop became a stimulating and even exciting event for the participants and 'invaded' Institutes. The cooperative research efforts – persistently inspired by the chairpersons, day and night – turned out to be a rewarding experience for all involved. Long shifts of intensive experimental work followed until Thursday, to be interrupted only by the poster session and excursion. Friday was used for data analysis and evaluation within the work groups. At the plenary discussion on Saturday, seven reports were presented on algal growth patterns as observed with the cultures, in the laboratory scale enclosures, in the two lakes and by flow cytometry. The meeting was concluded by reviewing the inventory of potential articles and a schedule for publication.

Experience from previous Workshops has shown that it is no easy task to transform experimental results obtained in this manner to scientifically acceptable text. Therefore, the efforts of the twenty-six participants who produced the nine experimental articles in Part Two of this volume, based on work done within only a few days, mostly with unfamiliar equipment and with 'strange' systems, are particularly commendable.

Fifteen posters dealing with aspects of cell cycle and primary productivity were displayed at the Workshop. Nine of these appear as contributed papers in Part Three of this volume.

All manuscripts have been peer-reviewed. Special thanks are due to the referees, whose comments greatly helped to improve both the scientific and linguistic level of these contributions.

Nieuwersluis/Tiberias/Amsterdam
HERMAN J. GONS
TOM BERMAN
LUUC R. MUR

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PART THREE: CELL CYCLE AND PRODUCTIVITY

Papers contributed to Posters

Hydrobiologia **238**: 1–35, 1992.
T. Berman, H. J. Gons & L. R. Mur (eds), The Daily Growth Cycle of Phytoplankton.

Diel periodicity in phytoplankton productivity

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Key words: periodicity, diel, circadian, clocks, photosynthesis, phytoplankton

Abstract

Daily variation in phytoplankton productivity influences the dynamics and linkages between several large scale processes in aquatic ecosystems. As part of an opening address to the 5th International workshop for the Group for Aquatic Productivity (GAP), the daily patterns of variability in photosynthesis for different algal classes was introduced and accompanied by a discussion of the sources of environmental and endogenous regulation of repeating biological oscillations that occur in phytoplankton on timescales of one day. It is suggested that one way to develop a database that serves to sort and predict phytoplankton variability over the day may be to encourage the creation of a 'temporal library'. Such a library would be comprised of temporally fixed maps of circadian clock-controlled rhythms for individual species, as well as temporally variable maps of diel periodicities that only can be defined for a selected set of environmental conditions.

Introduction

Phytoplankton productivity occupies a central position in several large scale aquatic processes, including food web dynamics, biogeochemical cycling, particle flux, bioluminescence, and water column optics (Fig. 1). Temporal variability in phytoplankton productivity influences the dynamics of and linkages between components of these large scale processes. Mastering small-scale temporal changes is crucial to current biophysical attempts to model primary production at larger scales from environmentally monitored and remotely sensed optical data (*cf.* Smith *et al.*, 1987, 1989; Prézelin *et al.*, 1991; Bidigare *et al.*, 1992). Organizers of the 5th international GAP (Group for Aquatic Primary Productivity) conference recognized that significant and often repeatable variations in phytoplankton biology are often induced and commonly observed on time scales less than a day. Thus the workshop chose to focus on assessing and defining predictive relationships between the daily variability in phytoplankton growth and productivity and their possible driving forces.

Over the course of a day, adjacent water masses can display significantly different patterns of photosynthetic variability which can be partially attributable to differences in hydrography as well as phytoplankton size, group and/or physiological state (Fig. 2). These daily (= everyday) variations in phytoplankton photophysiology have a direct effect on bio-optical parameters such as photosynthetic potential (Fig. 2) and quantum yield (Fig. 3A) and lead to small scale temporal and spatial variability in rate of primary production (Fig. 3B). An increasing number of studies have documented that attempts to predict *in situ* rates

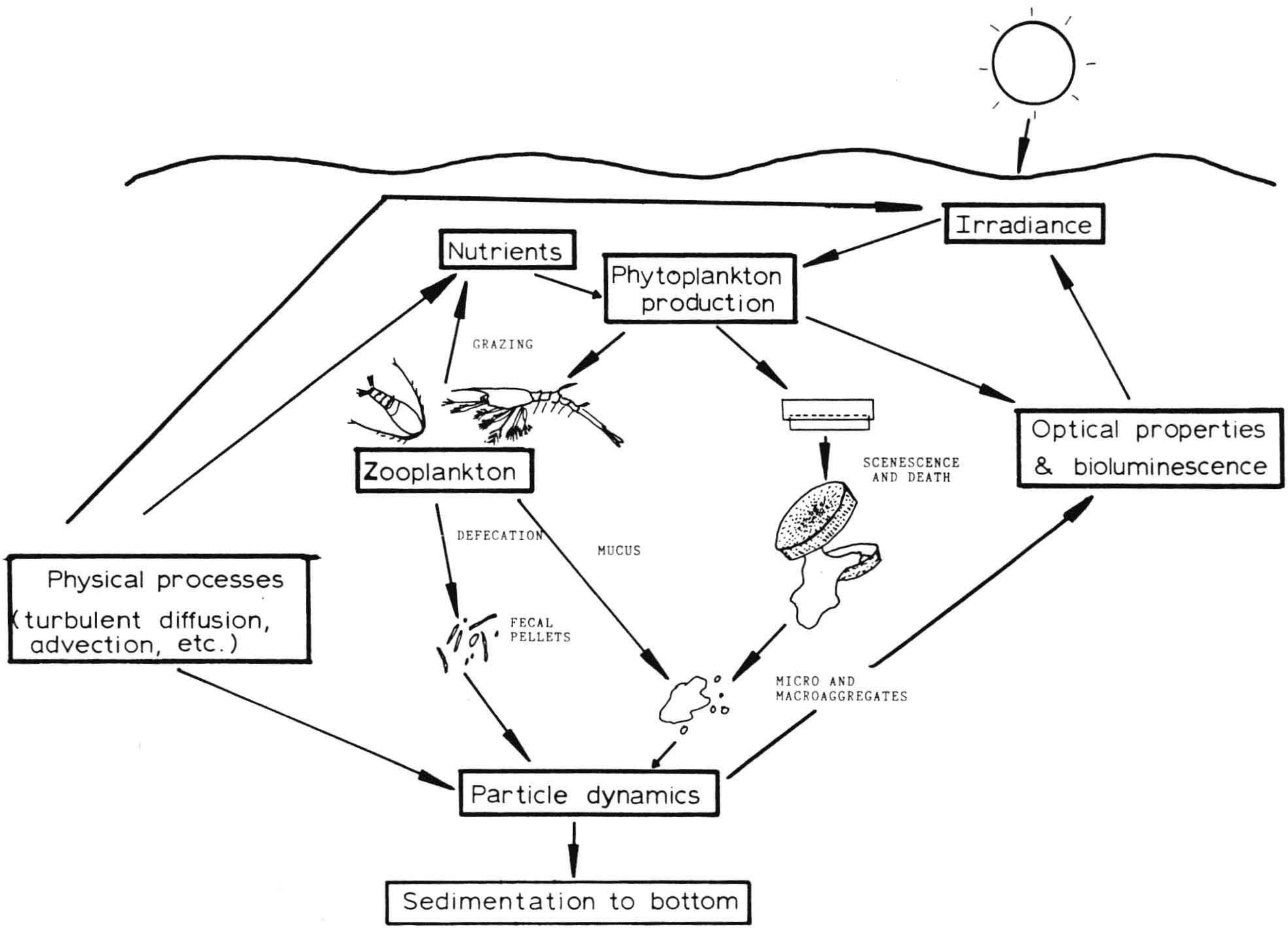

Fig. 1. Schematic cartoon of linkages between phytoplankton production and other large scale processes in aquatic ecosystems (Alldredge, unpubl.).

of primary productivity would err significantly if the diel variations in photosynthesis were not taken into account (*cf.* Fee, 1975; Harding *et al.*, 1982; Brown & Field, 1985; Prézelin *et al.*, 1987a & b, 1989; Smith *et al.*, 1987, 1989; Prézelin & Glover, 1991). What follows is an introduction to daily patterns of variability in photosynthesis for different algal classes and a discussion of the sources of environmental and endogenous regulation of biological oscillations in phytoplankton that generally repeat on the timescale of one day.

Proxy measures of daily variability in algal biology

Table 1 provides a partial list of proxy measures of phytoplankton biology commonly used as indicators to quantify changes in metabolic rate processes, which are also amenable to field studies, and which vary on timescales of less than one day. They are provided here to clarify terminology used in the text and to illustrate the following points. While there are numerous biological parameters that can be chosen to characterize some aspect of algal biology, the choice will directly affect the design, data interpretation and networking of field monitoring programs. Knowledge of the biology underlying a proxy measure can be especially helpful when logistic restraints require that alternative approaches to a field question be considered or when interpretations based on one proxy measure require validation. Readers interested in detailed characteristics of proxy measures of phytoplankton biology are referred

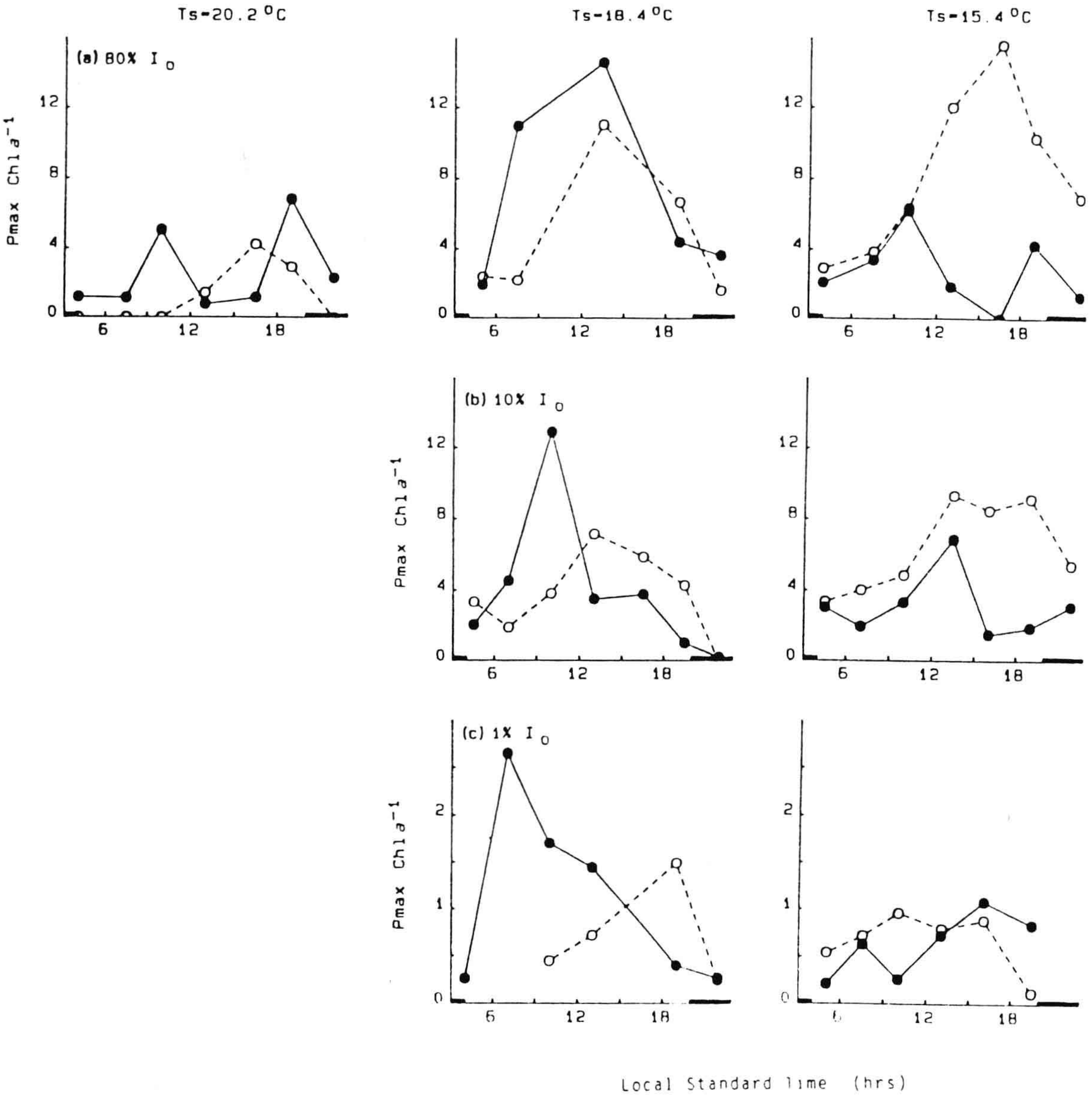

Fig. 2. Daytime variations in Chl-specific photosynthetic potential for 0.4–0.5 μ (closed circles) and $> 5.0\ \mu$ (open circles) phytoplankton collected from the surface (top row), the depth of the Chl maximum (middle row) and the bottom of the euphotic zone (bottom row) in the Southern California Counter Current (left column), the Southern California Current (middle column) and localized upwelling (right column) off Santa Barbara during a July 1985 cruise (Fronts '85) (adapted from Prézelin *et al.*, 1987a).

to a wide selection of texts on the subject (*cf.* Falkowski, 1980; Morris, 1980; Platt, 1981; Kirk, 1983; Reynolds, 1984; Platt & Li, 1986; Harris, 1986; Taylor, 1987).

Rarely do comparable proxy measures monitor phytoplankton variability in precisely the same way. For instance, estimates of plant biomass based on extracted chlorophyll (Chl) *a* will depend largely upon light and nutrient regulation of cellular concentrations of Chl. Biomass estimates based on *in vivo* F_{680} intensities will depend upon the photochemical state of the photosynthetic membranes (thylakoids) as well as Chl synthesis rates. And biomass estimates based on the beam

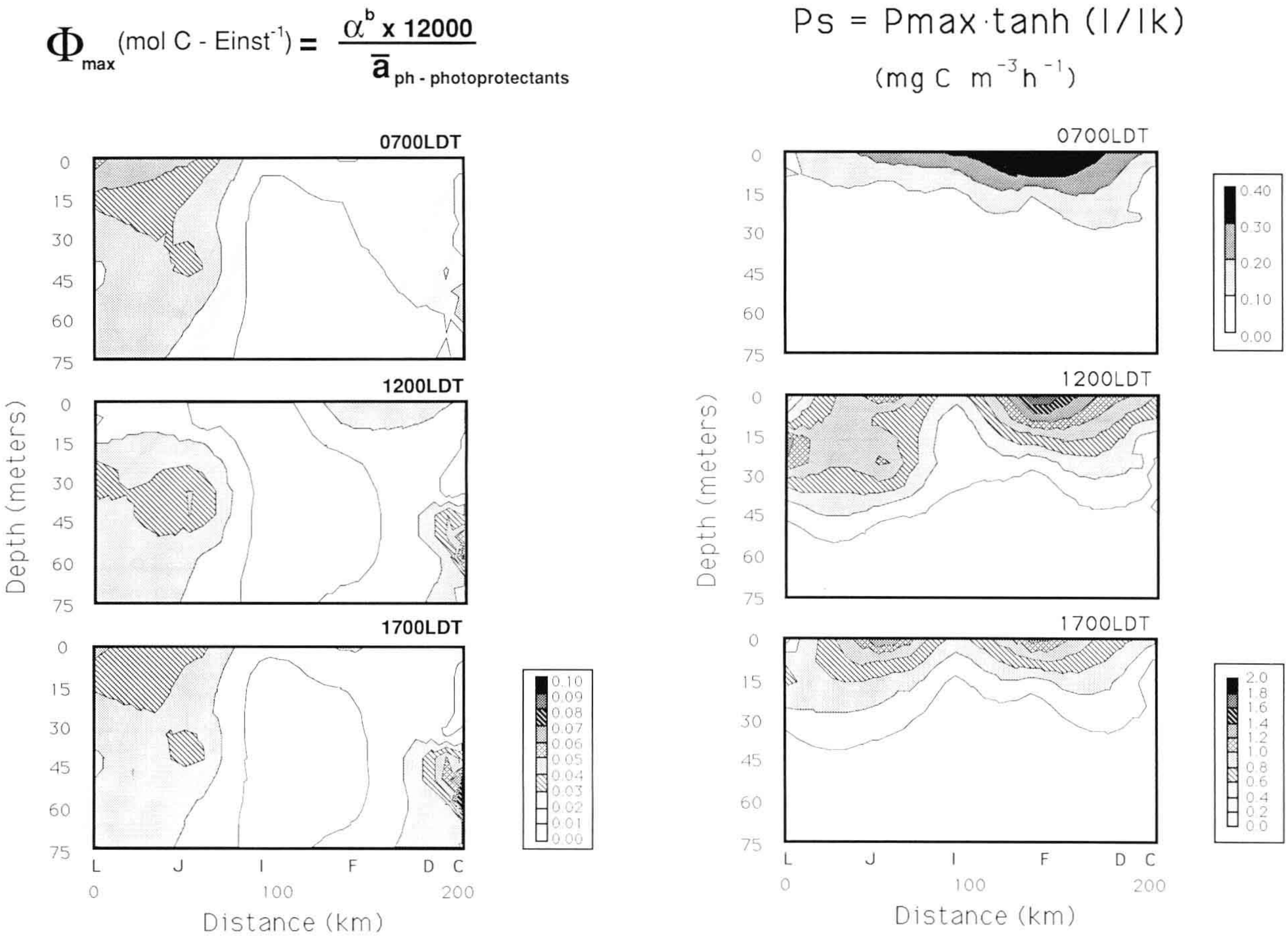

Fig. 3. Daytime variations in measured rates of A) maximum quantum yield of photosynthesis (ϕ_{max}) and B) *in situ* photosynthetic performance (Ps or P_i) for a west-east (station L-C) transect of the Southern California Bight during Watercolors '88 cruise in July 1988 (Prézelin, unpubl.). LDT, local daylight time.

attenuation coefficient for Chl-containing particles (C_{660}) will depend upon Chl synthesis rates, attenuation by other red light absorbing substances, and cell scattering parameters like cell size and surface morphology (Kirk, 1983). Since these different means of estimating plant biomass can differ in sensitivity, convenience and utility, each may provide different patterns of the apparent temporal/spatial variability in phytoplankton distribution and activity (Smith *et al.*, 1987; Siegel *et al.*, 1989).

A single environmental perturbation can induce changes in several cell properties at once, with the individual response times varying by several orders of magnitude (Fig. 4; Harris, 1986). For instance, a sustained change in irradiance can induce changes in Chl fluorescence on time scales of seconds to minutes while changes in Chl concentrations will be evident on timescales of hours to a few days. For those interested in documenting how variability in a single environmental factor may drive variability in algal biology, it is advisable to choose a biological parameter which responds on a similar timescale and with sufficient sensitivity to track the environmental change. It is best if the proxy measure chosen is one least affected by other environmental or biological variability that might be occurring on overlapping timescales. It is also worth noting that physical forcing of phytoplankton biology is dynamic and exhibits a parallell spectrum of timescales (*cf.* Harris, 1986; Prézelin *et al.*, 1991).

Table 1. Examples of proxy measurements that are amenable to field studies.*

Cell density:
- Flow cytometry
- Direct microscopic counts

Biomass:
- extracted Chlorophyll *a*
- IVF (*in vivo* fluorescence F680)
- beam attenuation coefficient for Chl-containing particles (C660)

Species composition:
- Microscopy (species specific)
- HPLC accessory pigmentation (class specific)

Cell composition and doubling times:
- particulate C, N, P, etc. and their ratios
- pigment: particulate C or N ratios
- organic constituents (amino acids, nucleic acids, lipids, etc)
- DNA content

Metabolic state and enzymatic activity:
- RUBPcase, PEPKcase, carbonic anhydrase: patterns of carboxylation
- Nitrate Reductase activity as an index of rates of NO_3uptake
- Phosphatase activity as an index of rates of PO_4 uptake
- Organic excretion rates and chemical composition of DOM
- Turnover rates of cell constituents (i.e. pigments, amino acids, sugars, and lipids)

Parameters of primary production:
- Cell absorption (A)
- P–I parameters including photysynthetic potential, P_{max}; the light-limited slope of P–I curves, alpha; the minimum irradiance required for the onset of light-saturated rates of photosynthes, I_k; the bright light-inhibited slope of P-I curves, beta: and the minimum irradiance required to photoinhibit photosynthesis, I^b
- Ps, the *in situ* rate of photosynthesis
- Fluorescence intensity (F_{680}) and fluorescence emission/excitation spectra ($F\lambda$), as probes of thylakoid membrane state and quantum efficiency of PSII
- Qpar (photosynthetically available radiation)
- AQphar (photosynthetically absorbed radiation)
- Radiation absorption efficiency: AQphar/Qphar
- Maximum quantum yield, ϕ_{max} = alpha/AQphar
- Photosynthetic quantum eficiency *in situ*, ϕ = Ps/AQphar
- Radiation utilization effciency: Ps/Qpar $\cdot$ k_d where kd is the downwelling attenuation coeficient

* Response time scales and sensitivity of proxy measures differ within and between categories.

It is characteristic of phytoplankton biology to be adaptable and cued to ongoing environmental and endogenous change. The response time for many proxy measures can vary with the nature of the environmental perturbation and the physiological state of the cell. For instance, the response times for both low light and high light photoadaptation are nutrient-dependent in dinoflagellates (Prézelin & Matlick, 1983; Prézelin *et al.*, 1986). For low light adaptation, there is a slowing and desynchronization of rates of pigment synthesis, quantum yield and photosynthetic performance as cells become increasingly nutrient limited (Figs 5 & 6). Furthermore, the instantaneous measurements of phytoplankton properties and rate constants are rarely constant or stay in phase with each other for long (Figs 7 & 8), unless growth conditions enable the cells to become syn-

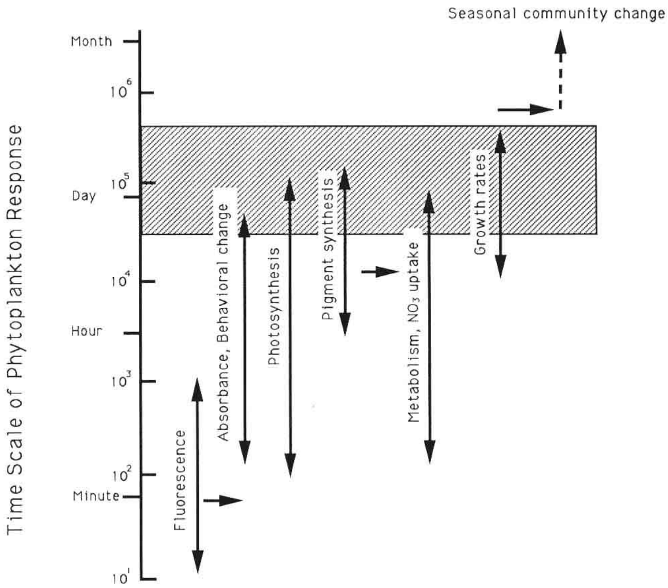

Fig. 4. The response time scale for phytoplankton physiology (adapted from Harris, 1986).

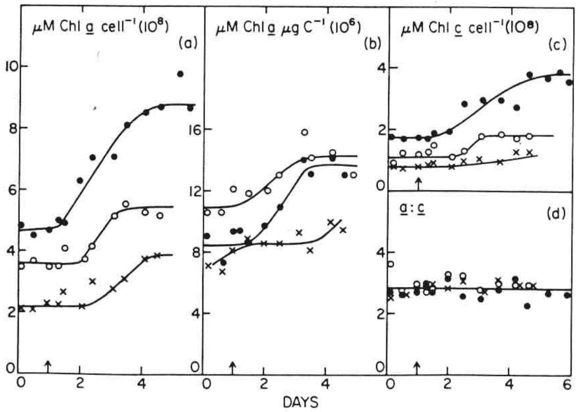

Fig. 5. Gonyaulax polyedra. Comparison of rates of pigmentation change when early log (filled circles), late log (open circles) and stationary (crosses) phase cells were transferred from bright light (330 μE m^2 s^{-1}) to low light (80 μE m^2 s^{-1}) conditions. Semi-continuous cultures were maintained in log phase on 12:12 h LD cycle at 18 °C. Time of transfer occurred at the beginning of the light period and is indicated by vertical arrows (Prézelin & Matlick, 1983).

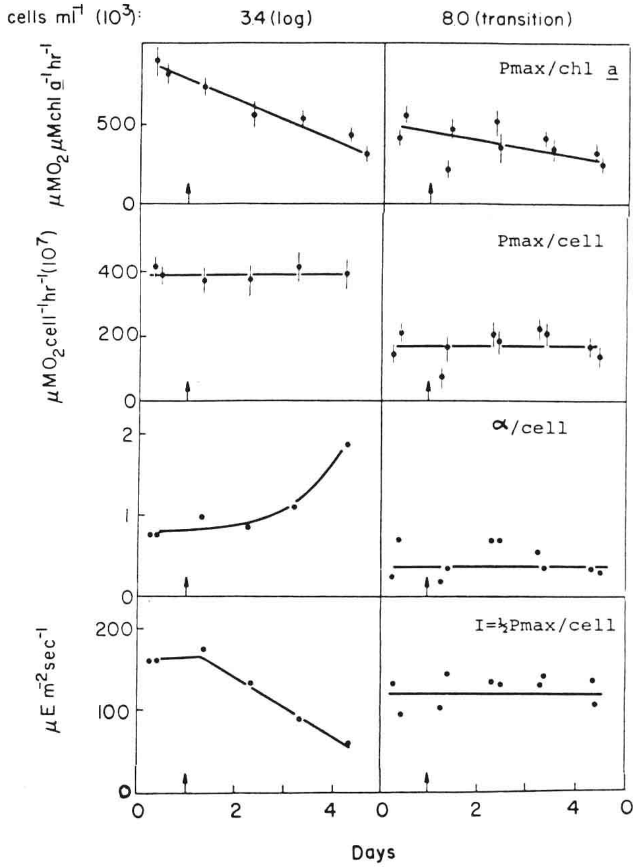

Fig. 6. Gonyaulax polyedra. Comparison of photoadaptation rates for early and late log populations. Conditions were identical to those in Fig. 5 (Prézelin & Matlick, 1983).

chronized to a steady state (*cf.* Eppley, 1980). And while a biological parameter like pigmentation may respond to a perturbation within hours, it can take several days for steady state physiology to be reestablished (Fig. 9). High and low light adaptation are not reverse processes. With increasing nutrient limitation of low light adapted cells, subsequent high light photoadaptation is more likely to be accompanied by photoinhibition where rates of photobleaching of pigments and the susceptibility of the quantum efficiency of photosystem II electron transport rates to photoinactivation are increased (Prézelin *et al.*, 1986).

While the variability of some biological rate constants may make it more difficult to develop models that successfully predict the temporal variability of phytoplankton, they do enable field biologists to establish the physiological state of natural populations. For instance, when natural populations of red tide dinoflagellates (Boczar *et al.*, 1990) or oligotrophic marine cyanobacteria (Prézelin *et al.*, 1987b) are placed under simulated *in situ* conditions and then only the irradiance is lowered or nutrient availability increased,

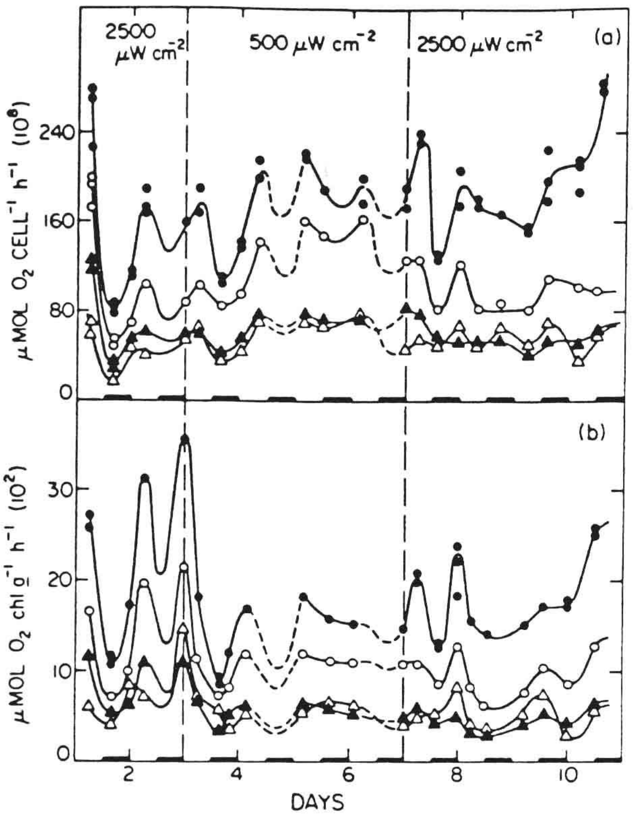

Fig. 7. Glenodinium sp. (aka *Heterocapsa pygmaea*). Variability in the diel periodicity of photosynthesis and respiration as steady-state populations are light-shifted from light-saturating (2500 μW cm^{-2}; *ca* 190 μE m^2 s^{-1}) to light-limiting (500 μW cm^{-2}; *ca* 38 μE m^2 s^{-1}) growth conditions. Semi-continuous cultures were maintained in log phase on 12:12 h LD cycle at 18 °C and light transfers were made at the beginning of the light period. Photosynthesis rates were measured at 6000 (closed circles), 2300 (open circles) and 1100 (closed triangles) μW cm^{-2}. Diel variability in absolute rates of dark respiration (open triangles) are also shown (Prézelin & Matlick, 1980).

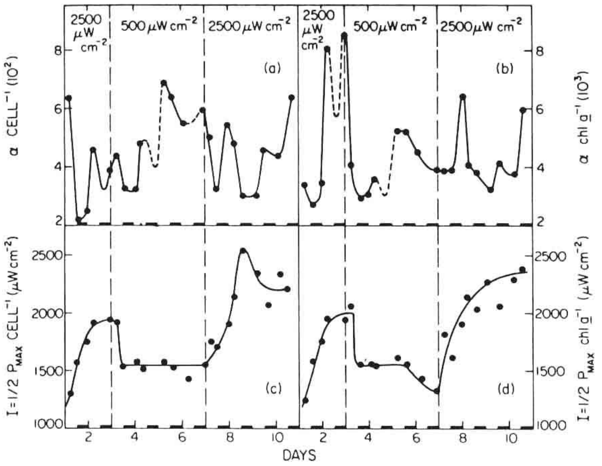

Fig. 8. Glenodinium sp. (aka *Heterocapsa pygmaea*). Comparison of the temporal variability in a) Cell-specific and b) Chl-specific relative quantum yield; and c) Cell-specific and d) Chl-specific half-saturation constants for photosynthesis as populations are light-shifted between light-saturating and light-limiting growth conditions. Conditions were the same as those in Fig. 7 (Prézelin & Matlick, 1980).

the rate of adaptation of photosynthesis and growth can be used as indices of the physiological state of the cell at the time of collection. In one field study of oligotrophic picoplanktonic cyanobacteria (Fig. 10), the photosynthetic potential of high light cells increased immediately after transfer to low light conditions and argued that *in situ* cells were experiencing some photoinhibitory effects (Prézelin *et al.*, 1987b, 1989). While a nutrient pulse at dawn did shift the timing of peak cell division (Fig. 10c), it had no effect on daily division rates and thus argued that cyanobacterial growth was not limited by inorganic nutrients (NO_3 and PO_4). Figure 10 also illustrates how the timing and pattern of cell division (expressed as the frequency of dividing cells, FDC) and photosynthesis in *Synechococcus* spp. can be differentially altered by manipulating environmental conditions.

Finally, daily fluctuations in algal biology can underlie and influence patterns of biological variability on longer timescales (*cf.* Reynolds, 1984; Harris, 1986). One example is the linkage between daily variability in rates of photosynthesis or nutrient uptake and rates of cell division (Fig. 4). Cellular linkages controlling growth and reproduction of phytoplankton are differentially regulated in different algal groups and contribute to observed weekly to seasonal changes in phytoplankton population and community dynamics. Timescales longer than a day are not considered here and interested readers are referred to reviews addressing topics of biological timekeeping (Brady, 1982; Sweeney, 1987; Winfree, 1987; Edmunds, 1988) and timescales of phytoplankton ecology (Sournia, 1974; Chisholm, 1981; Reynolds, 1984; Harris, 1986; Prézelin *et al.*, 1991).