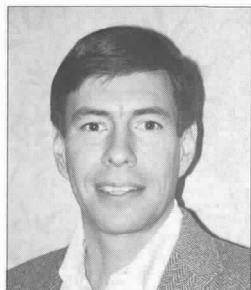


MEASUREMENT OF PHOTOSYNTHETIC RESPONSE TO EUPHOTIC ZONE PHYSICAL FORCING



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THE INFLUENCE of vertical water motion on the photosynthetic response of phytoplankton entrained in the upper mixed layer of the ocean is an aspect of biological oceanography that has received considerable interest for a number of years. Thorough reviews of phytoplankton response parameters, environmental variables, and the space and time scales of interest to phytoplankton ecology can be found in Harris (1980, 1984), Richardson *et al.* (1983), Denman and Gargett (1983), Falkowski (1984), Legendre and Demers (1984) and Harding *et al.* (1987). Light, temperature and nutrients are the principal environmental variables eliciting responses that ultimately are biochemically based but are manifested in changes to phytoplankton photosynthetic rates, behavior and growth. These biological responses, resulting from physical forcing, consist of nonlinear, multivariate relations on broad time and space scales involving complications such as time delays, hysteresis and filtering, to name a few. The discipline of establishing an understanding of this complex biophysical system was termed "dynamic biological oceanography" by Legendre and Demers (1984) to emphasize the non-steady-state nature of the problem. Due to the primary position that the phytoplankton occupy in the cycle of life in the ocean and the importance of the oceans to the rest of the earth, considerable effort has been and will continue to be dedicated to investigating this system.

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The approach to this task has included laboratory studies involving both steady-state and dynamic components, field experiments necessarily involving dynamic components and the integration of the resulting data into explanations of the characteristics of the phytoplankton response to mixed layer dynamics (Marra, 1978; Gallegos and Platt, 1982; Marra and Heinemann, 1982; Falkowski, 1983; Walsh and Legendre, 1983; Lewis *et al.*, 1984b; Yoder and Bishop, 1985; Kamykowski *et al.*, 1988; Cullen and Lewis, 1988; Savidge, 1988). Modelers have endeavored to assimilate what is known about physical, chemical and biological interactions into mathematical descriptions for use in predicting the future behavior of the system. Areas where models are developing include euphotic zone turbulence effects on phytoplankton distribution (Kamykowski, 1990), physiology and productivity (Falkowski and Wirick, 1981; Lewis *et al.*, 1984a; Gallegos and Platt, 1985; Geider and Platt, 1986) and internal wave effects on phytoplankton productivity (Lande and Yentsch, 1988; Holloway and Denman, 1989).

New Tools

Our project seeks to apply new technologies to the investigation of photosynthetic response to the natural variability of light and temperature experienced by phytoplankton entrained in the moving water of the upper mixed layer. Our approach involves an instrument to measure the vertical motion of the water, an instrument to measure multiple photosynthetic parameters of an enclosed, laboratory phytoplankton culture, and a mechanism to move the biological package in the upper mixed layer as directed by the real-time measurements of motion. This paper first briefly describes the physical and biological instrument packages. It then focuses on a sample of the data obtained by the biological package during a recent field trial in order to demonstrate the

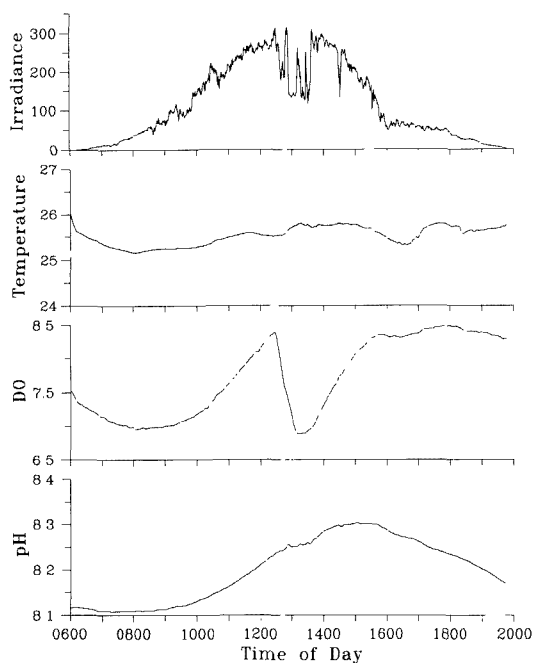


Fig. 2: Raw data from SUPA phytoplankton culture during field trial at 5 m depth. Sampling period was one minute. Irradiance in $\mu\text{E m}^{-2} \text{s}^{-1}$; temperature in $^{\circ}\text{C}$; DO (dissolved oxygen) in mg l^{-1} .

unique capabilities of this instrument for investigations of the dynamic response of phytoplankton.

The measurement of vertical water velocity is accomplished by what we termed the Physical Measurement Instrument (PMI) (Fig. 1, p. 20). This instrument is composed of four dual axis electromagnetic speed sensors (Sorrell *et al.*, 1986), a geomagnetic compass, a dual axis tilt sensor, and a Woods Hole Oceanographic Institute Instrument Bus Computer (IBC) for data acquisition, storage and communication. The electromagnetic speed sensors are designed with robustness and survivability in mind and incorporate features producing exceptional electrode stability, resistance to fouling and reduction of boundary layer flow interference. The sensors have a lower speed detection threshold of $< 0.1 \text{ cm s}^{-1}$ and a frequency response up to 1 Hz. The four sensors, located on one meter orthogonal spacing, make possible an averaged 3-dimensional description of the flow in a several cubic meter volume around the instrument. Future applications of the PMI in conjunction with the Self-contained Photosynthesis Apparatus (SUPA) (described below) will utilize the measured vertical flow component from the PMI to direct the movement of SUPA containing phytoplankton.

SUPA (Fig. 1) contains a laboratory phytoplankton culture within a transparent quartz dome at the top of the underwater assembly. Ambient irradiance, culture temperature, pH and dissolved oxygen are acquired and stored by an IBC on a one-minute sampling schedule. In addition, the IBC directs a dissolved gases control system which removes oxy-

gen from and/or adds carbon dioxide to the culture medium to keep those parameters within a predetermined range over the course of an unattended deployment. This system presently corrects only for the photosynthetic alteration of the dissolved gases concentrations, although it could be extended to correct for dark respiration also. The ambient irradiance is measured by a modified Biospherical Instruments, Inc. QSL-100 sensor fitted within the culture chamber. Culture temperature is measured by an immersed thermistor. Dissolved oxygen within the culture is measured with a pulsed oxygen electrode developed for this system using the techniques of Short and Shell (1985). The culture pH is measured with a specially constructed glass pH probe and reference. These electrodes were fabricated by Innovative Sensors, Inc. to operate in the inverted position. The pH electrode is shaded to reduce photoelectric effects and the signal is digitally processed to further reduce direct light effects and correct for temperature changes. The change in pH is related to the net photosynthesis of the phytoplankton through the carbonate buffer system of the seawater medium (Axelsson, 1988).

Field Trial

The field trial discussed here took place at the U.S. Army Corps of Engineers Field Research Facility at Duck, North Carolina, in August 1989. SUPA contained a culture of the marine diatom *Thalassiosira pseudonana* (clone 3H) at a density of 1.8×10^5 cells per milliliter. It was suspended 5 m below the surface (10-15% surface light level) for approximately fourteen hours. It experienced vertical excursions of approximately one meter due to the effect of changes in the direction and strength of longshore currents and the patterns of flow around the pier pilings. The sky on the day of deployment was clear except for a period of about eighty minutes in the middle of the day when a bank of clouds passed overhead. This cloud passage is expressed clearly in the irradiance record (Fig. 2). The temperature within the culture chamber showed an initial drop, while the culture temperature equilibrated with the ambient water temperature, and then varied less than 0.5°C through the remainder of the day. This temperature data tracked well with that recorded by an independent temperature probe located nearby at the same depth as SUPA. The initial decrease in dissolved oxygen was caused by the dominance of respiration over photosynthesis in the low light of early morning. The upward sloping dissolved oxygen signal between 0830 hrs and 1230 hrs shows net photosynthesis. At 1230 hrs the dissolved oxygen began a rapid decrease. This was caused by the programmed purging of oxygen from the media to prevent extreme supersaturation. At 1310 hrs this control system shut off and the photosynthesis signal is seen again as an increase of dissolved oxygen. At first glance, the photosynthetic evolution of oxygen appears very smooth when viewed on a daylong time scale. However, as will be illustrated in later figures there is significant variability on smaller time scales (tens

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Fig. 1: The Physical Measurement Instrument (PMI) (right) and the Self-contained Underwater Photosynthesis Apparatus (SUPA) (left). Both units are approximately 1.2 m tall. PMI has four dual-axis water velocity sensors mounted 1 m apart on the four arms at the top of the assembly. SUPA contains a laboratory phytoplankton culture in the transport quartz dome at the top of the housing.

of minutes). The initial decrease in pH represents the same dominance of respiration over photosynthesis in low light as seen in the dissolved oxygen signal. As light levels increase, the rate of photosynthesis increases and yields the positive slope that characterizes the data from 0830 hrs to 1230 hrs. The carbon dioxide infusion system, intended to return the pH of the media to a programmed level malfunctioned so that the pH data is unusable after 1230 hrs. Since this was only the second field trial some problems were expected; corrective measures have been implemented. The change in the pH signal due to respiration and photosynthesis in the phytoplankton culture was converted to net release or uptake of carbon dioxide by the culture as a direct measure of net photosynthesis that can be compared to net oxygen uptake or release. This conversion utilized the relationship between carbonate alkalinity, pH and total carbon dioxide published by Skirrow (1975).

During this deployment SUPA measurements of photosynthetic carbon uptake and oxygen evolution were compared with similarly derived rates from

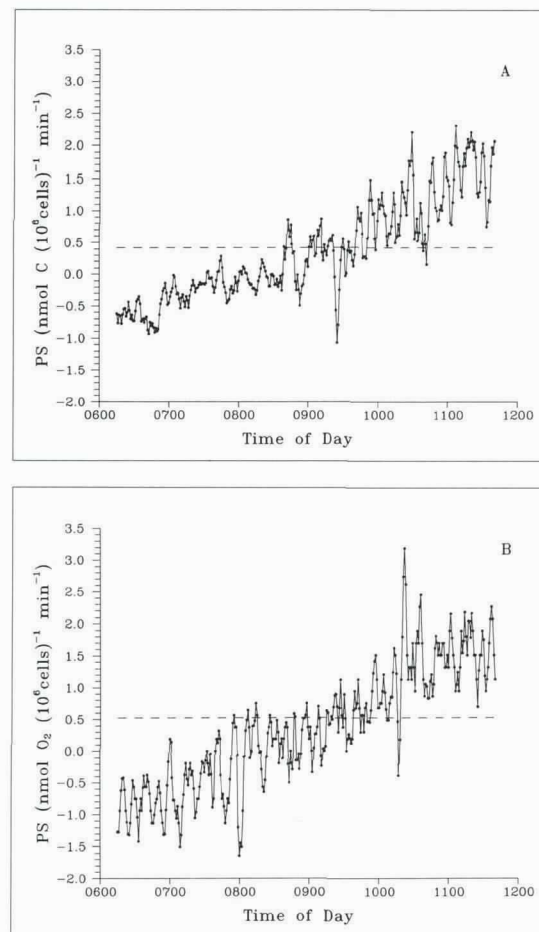


Fig. 3: Comparison of average photosynthetic rates from the BOD bottles (dashed lines) and the measured rates from SUPA (solid lines connecting data points). (a): carbon uptake rates. (b): oxygen evolution rates. Time scale covers the incubation period when the BOD bottles and SUPA were colocated.

cultures in biological oxygen demand bottles (BOD), standard field incubation containers. SUPA data, converted to photosynthetic rates, are overlaid on the average photosynthetic rates obtained from three BOD bottles incubated at the same location as SUPA (Fig. 3). The time period shown corresponds to the incubation period of the BOD bottles. The rates of photosynthesis obtained from the BOD bottles are shown as average (net) values over the time period of incubation to emphasize that this technique provides only a beginning and ending value of dissolved oxygen or total carbon dioxide from which only an average rate is obtained. When averaged over the same period SUPA's data differed from the BOD data by -18% for rate of oxygen change and -10% for rate of carbon dioxide change. Some of this difference is attributable to the omnidirectional light exposure of the BOD bottles compared to principally downwelling irradiance exposure of SUPA's culture chamber. This difference would be expected to enhance the BOD rates, especially in shallow water with a light-colored sandy bottom. The very detailed

time record of the rate changes recorded by SUPA also provide several additional parameters such as: 1) the base respiratory rate at zero light, 2) the initial slope of the photosynthesis versus irradiance curve for the culture at dawn, and 3) the gain function of photosynthetic response to light variability for this time of day.

Observed Photosynthetic Response

The standard photosynthesis versus irradiance (PI) analysis has multiple samples simultaneously incubated at different irradiance levels, producing a snapshot of the time integrated PI relationship over the time interval of incubation. The morning photosynthetic oxygen production and carbon uptake rates from SUPA are plotted against light in Fig. 4. These PI curves do not represent the traditional PI approach because time is a covariable in this data. SUPA, therefore, produces a time record of the PI relationship incorporating the light history of the culture. The data in Fig. 4 integrate the physiological adaptations the cells make in response to the measured light (and temperature) variability. Fig. 4 clearly illustrates the light saturation phenomenon of photosynthetic oxygen production. As irradiance exposure level in the morning increases past the level at which these cells were grown and adapted, the rate of increase in oxygen production follows a curve that levels off. The oxygen-producing photosynthetic apparatus of the low-light adapted cells saturated at the higher light levels encountered. After a few hours of high light exposure, however, the response efficiency recovered. In contrast, the carbon assimilation in Fig. 5 does not follow this same light saturation pattern in the morning. Photosynthetic carbon assimilation is not directly linked to oxygen evolution over short time periods, although this type of imbalance cannot exist for long term growth.

The photosynthetic quotient (PQ), the ratio of oxygen evolved to carbon taken up, is thought to be indicative of the physiological state of the cell (Bell, 1985; Langdon, 1988; Axelsson, 1988). The PQ computed from these data range from 1.4 at low to moderate irradiance values, to 0.9 at the highest irradiance values (which exceed the adapted irradiance level). Bell (1985) predicted a value of 0.75 for cells which are producing glycolate, an organic compound which is the principal organic compound released during light stress (Fogg, 1983). This type of biological response has protective benefits for phytoplankton which are transported into shallow water where the photosynthetic apparatus could be damaged by the high light levels. We do not have the measurements necessary to definitively point to extracellular release of organic compounds, but this dataset does illustrate SUPA's ability to resolve the time-course of this type of physiological change. Phytoplankton readjust their biochemical structure over longer periods in ways that accommodate the high light levels experienced in shallower water on clear days and thus contribute to efficient energy utilization.

Photoadaptation describes a number of changes

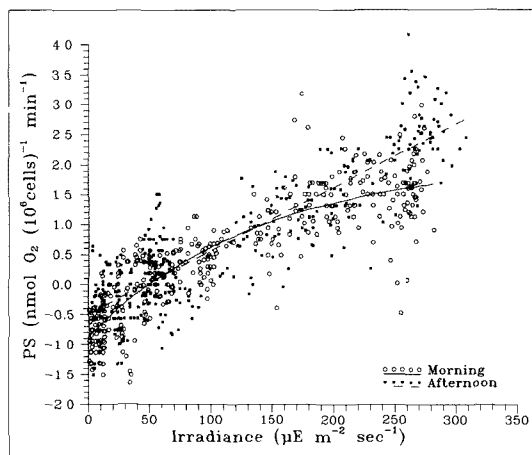


Fig. 4: Rates of photosynthetic oxygen evolution for the morning (0615 to 1223 hrs) and afternoon (1317 to 1946 hrs). The morning data shows light saturation, but the afternoon data does not. The above fitted lines are nonlinear least-squares regressions with $r^2 = 0.77$ for the morning and 0.78 for the afternoon.

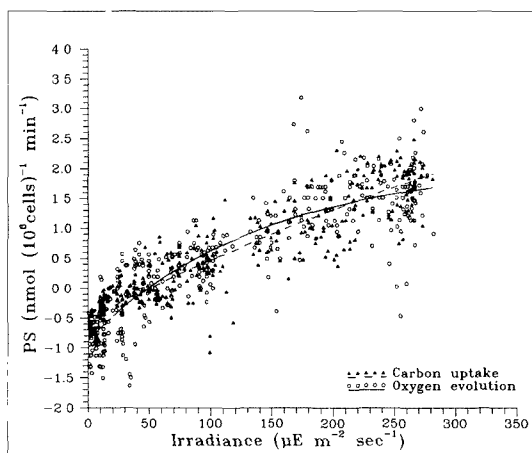


Fig. 5: Rates of photosynthetic carbon uptake and oxygen evolution for the morning (0615 to 1223 hrs). The course of change of the photosynthetic quotient can be estimated by the ratio of solid line to the dashed line over time. The oxygen data and fitted line are the same as the previous figure. The dashed line above is also a nonlinear least-square regression with $r^2 = 0.84$.

that take place in cells in response to increases and decreases of light over time scales of hours or more (Falkowski, 1984; Cullen and Lewis, 1988). In our trial, the high light levels which caused the saturation response persisted long enough for the cells to adapt and saturation to no longer be apparent (Fig. 4). The measurable result of the photoadaptation process, a higher light saturation level, was recorded over a period of approximately two hours from the time saturation was first observable to the time prior saturating light levels were reached again after the cloudy period. It should be stressed that we do not

know from this data record what degree of adaptation to the higher light level has occurred, because we do not know what the 'final' adapted saturation level would be. Direct comparison of this two hour adaptation time with those of Cullen and Lewis (1988) is not feasible. However, for the scale of change involved, this number is of a similar order of magnitude. Although much work has been done in the laboratory and field to elucidate these responses, SUPA provided a unique ability to obtain detailed resolution of this response under field conditions.

Summary

A field trial demonstrates the operational capability of SUPA and, due to fortuitous conditions, shows detailed evidence of the biological response to light variability. The present data verify our ability to measure two photosynthetic parameters using a short sampling period. Moving SUPA in the upper ocean in response to realistic vertical water motion will provide new views of the photosynthetic responses of phytoplankton to real-time turbulence, Langmuir cells and internal waves.

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References

Axelsson, L., 1988: Changes in pH as a measure of photosynthesis by marine macroalgae. *Mar. Biol.*, 97, 287-294.

Bell, L.N., 1985: Energetics of the photosynthesizing cell. In: *Soviet Scientific Reviews Supplement Series*, vol. 5. Harwood Academic Publishers, New York, 159-163.

Cullen, J.J. and M.R. Lewis, 1988: The kinetics of algal photoadaptation in the context of vertical mixing. *J. Plankton Res.*, 10(5), 1039-1063.

Denman, K.L. and A.E. Gargett, 1983: Time and space scales of vertical mixing and advection of phytoplankton in the upper ocean. *Limnol. Oceanogr.*, 28(5), 801-815.

Falkowski, P.G., 1983: Light-shade adaptation and vertical mixing of marine phytoplankton: A comparative field study. *J. Mar. Res.*, 41, 215-237.

_____, 1984: Physiological responses of phytoplankton to natural light regimes. *J. Plankt. Res.*, 6(2), 295-307.

_____, and C.D. Wirick, 1981: A simulation model of the effects of vertical mixing on primary productivity. *Mar. Biol.*, 65, 69-75.

Fogg, G.E., 1983: The ecological significance of extracellular products of phytoplankton photosynthesis. *Bot. Mar.*, 26, 3-14.

Gallegos, C.L. and T. Platt, 1982: Phytoplankton production and water motion in surface mixed layers. *Deep-Sea Res.*, 29, 65-76.

_____, and T. Platt, 1985: Vertical advection of phytoplankton and productivity estimates: A dimensional analysis. *Mar. Ecol. Prog. Ser.*, 26, 125-134.

Geider, R.J. and T. Platt, 1986: A mechanistic model of photoadaptation in microalgae. *Mar. Ecol. Prog. Ser.*, 30, 85-92.

Harding, L.W., Jr., T.R. Fisher, Jr., and M.A. Tyler, 1987: Adaptive responses of photosynthesis in phytoplankton: specificity to time-scale of change in light. *Biol. Oceanogr.*, 4, 403-437.

Harris, G.P., 1980: Temporal and spatial scales in phytoplankton ecology. Mechanisms, methods, models and management. *Can. J. Fish. Aquat. Sci.*, 37, 877-900.

_____, 1984: Phytoplankton productivity and growth measurement: Past, present and future. *J. Plankton Res.*, 6(2), 219-237.

Holloway, G. and K. Denman, 1989: Influence of internal waves on primary production. *J. Plankton Res.*, 11(2), 409-413.

Kamykowski, D., S.A. McCollum and G.J. Kirkpatrick, 1988: Observations and a model concerning the translational velocity of a photosynthetic marine dinoflagellate under variable environmental conditions. *Limnol. Oceanogr.*, 33(1), 66-78.

_____, 1990: A random walk model examining how phytoplankton distribute in the upper mixed layer. In: *Toxic Marine Phytoplankton*. E. Graneli, B. Sundstrom, L. Edler and D. M. Anderson, eds., Elsevier, New York, 183-188.

Lande, R. and C.S. Yentsch, 1988: Internal waves, primary production and the compensation depth of marine phytoplankton. *J. Plankton Res.*, 10(3), 565-571.

Langdon, C., 1988: On the causes of interspecific differences in the growth-irradiance relationship for phytoplankton. II. A general review. *J. Plankton Res.*, 10(6), 1291-1312.

Legendre, L. and S. Demers, 1984: Towards dynamic biological oceanography and limnology. *Can. J. Fish. Aquat. Sci.*, 41, 2-19.

Lewis, M.R., J.J. Cullen and T. Platt, 1984a: Relationship between vertical mixing and photoadaptation of phytoplankton: Similarity criteria. *Mar. Ecol. Prog. Ser.*, 15, 141-149.

_____, E.P.W. Horne, J.J. Cullen, N.S. Oakey and T. Platt, 1984b: Turbulent motions may control phytoplankton photosynthesis in the upper ocean. *Nature*, 311(6), 49-50.

Marra, J., 1978: Effect of short-term variations in light intensity on photosynthesis of a marine phytoplankton: a laboratory simulation study. *Mar. Bio.*, 46, 191-202.

_____, and K. Heinemann, 1982: Photosynthesis response by phytoplankton to sunlight variability. *Limnol. Oceanogr.*, 27(6), 1141-1153.

Richardson, K., J. Beardall and J.A. Raven, 1983: Adaptation of unicellular algae to irradiance: An analysis of strategies. *New Phytol.*, 93, 157-191.

Savidge, G., 1988: Influence of inter- and intra-daily light-field variability on photosynthesis of coastal phytoplankton. *Mar. Bio.*, 100, 127-133.

Short, D.L. and G.S.G. Shell, 1985: Pulsing amperometric oxygen sensors: earlier techniques evaluated and a technique implemented to cancel capacitive charge. *J. Phys. E: Sci. Instr.*, 18, 76-87.

Skirrow, G., 1975: The dissolved gases—carbon dioxide. In: *Chemical Oceanography*, vol. 2, 2nd edition. J.P. Riley and G. Skirrow, editors, Academic Press, New York, 1-192.

Sorrell, F.Y., T.B. Curtin and M.D. Feezor, 1986: An improved electromagnetic current meter for application in unsteady oceanic flows. In: *Current Practices and New Technology in Ocean Engineering*, T. McGuinness and H.H. Shih, eds., American Society of Mechanical Engineers, Ocean Engineering Division, OED-Vol. II, 395-403.

Walsh, P. and L. Legendre, 1983: Photosynthesis of natural phytoplankton under high frequency light fluctuations simulating those induced by sea surface waves. *Limnol. Oceanogr.*, 28(4), 688-697.

Yoder, J.A. and S.S. Bishop, 1985: Effects of mixing-induced irradiance fluctuations on photosynthesis of natural assemblages of coastal phytoplankton. *Mar. Biol.*, 90, 87-93. □