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Do light/dark cycles of medium frequency enhance phytoplankton productivity?

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Abstract

It has been suggested that turbulence with the resultant light/dark cycle and light gradient through which phytoplankton move, enhances their productivity. The stationary bottle incubation technique for estimating rates of primary productivity has mainly been criticized because of bottle effects, the elimination of natural turbulence and the presence of photo-inhibition. In a series of experiments where productivity was measured over static profiles and compared to the productivity in a mixed system, no definite conclusion could be reached regarding the effect of varying light/dark cycles of medium frequency (seconds to minutes). It appeared as though the ratio of the euphotic depth to mixing depth (Z_{eu}/Z_m) influenced productivity more than the duration of the light/dark cycle. The static bottle incubation method gave higher integral productivities than the mixed samples at low ratio's of Z_{eu}/Z_m. It is suggested that mixing has two separate, but synergistic effects i.e. it not only moves the phytoplankton cells through a light/dark cycle, but also decreases the boundary layer, which increases the rate of exchange through the cell wall of nutrients and metabolites. In doing so more nutrients are available and light could be utilized more efficiently and therefore, productivity is increased.

Introduction

Algal growth can be influenced by three ranges of intermittent illumination (light/dark cycles):

(1) high frequency fluctuations of 100 ms (10 Hz) and less,
(2) medium frequency fluctuations of seconds to minutes, and
(3) low frequency cycles of hours to days and years.

High frequency fluctuations (> 10 Hz) give rise to the 'flashing light effect' (Kok, 1953; Friedrickson & Tsuchiya, 1970; Terry, 1986), whereby the rate and efficiency of photosynthesis are increased under specific conditions of illumination. Legendre *et al.* (1986) included frequencies of between 0.1 and 10 Hz in their definition of high frequency light fluctuations, because Dera (1970) observed this range in the euphotic zone of the sea. Because these frequencies include both the 'flashing light' range as well as those being to long too enhance photosynthesis due to this phenomena, in this paper high frequency fluctuations are only those > 10 Hz.

Low frequency fluctuations influence the periodicity of cell division, where synchronous populations would establish under light/dark cycles similar to that of day/night cycles (9–15 h either way to a total of 24 h) (e.g. Sorokin, 1957). The

growth rate of a synchronous algal culture tends to be greater during the light period, than the growth rate of an asynchronous culture illuminated continuously at the same intensity (e.g. Sorokin & Krauss, 1959). Low frequency fluctuations would also influence the circadian rhythm of photosynthetic activity as observed by Doty & Oguri (1957) and others. In this paper only intermittent illumination of medium frequency would be considered, as the influence of both high and low frequency cycles have to a large extent been elucidated.

Light fluctuations of medium frequency could be brought about by waves at the water surface (Dera, 1970) or because of turbulence, especially in shallow systems. Conflicting results are reported in the literature, where on the one hand it is suggested that turbulence, which is typical of natural systems, enhances productivity, whereas on the other hand no evidence of such stimulatory effect could be found. Westlake (1967) found that the metabolism of aquatic macrophytes were stimulated by turbulence. Mann *et al.* (1972) found that by rotating their incubation bottles in the water, with a device similar to a paddle wheel, the overall productivity increased by 30%. Marra (1978), using $^{14}CO_2$ with 4-h incubations, found that if some of the bottles were vertically cycles on days when surface photo-inhibition would occur in stationary bottles, estimates of areal productivity from the cycled bottles were 19–87% (average 46%) higher than those from the fixed bottles. However, there was a good agreement between the two incubation techniques on days when no surface photo-inhibition occurred and it was, intimated that vertical mixing may increase column productivity (Marra, 1980). Walsh & Legendre (1983) reported 30% higher photosynthetic efficiencies under fluctuating light, as experienced in surface waters of the ocean, when compared to constant illumination. Legendre *et al.* (1986) showed that microalgae can adapt to the dominant frequencies of their environment. They measured higher photosynthetic efficiencies when the algae were subjected to fluctuation light regimes, as compared to steady light, when they were taken from an environment of fluctuation light and lower photosynthetic efficiencies than when the algae were taken from an environment of relatively constant light.

On the other hand, Jewson & Wood (1975) found that there was a good agreement (not more than 10% difference) between estimates of areal productivity obtained with stationary bottles and water circulating in a tube through depths corresponding roughly to the euphotic zone. Falkowski & Wirick (1981) found from a mathematical simulation that vertical mixing had little effect on the integral water column primary productivity. Little difference in the accumulation of carbohydrates were found between captive and freely circulating phytoplankton (Gibson, 1984) and Barlow (1984) found that although photosynthesis appeared to be greater in circulating water, protein synthesis and hence growth was faster in a stable environment.

The influence of fluctuating light regimes has also been a topic of discussion in the massive cultivation of algae, where some form of turbulence is usually provided, which ensures that the algae move continuously through a light gradient. Richmond & Vonshak (1978) reported that the growth rate of *Spirulina* increased significantly, when the stirring speed of their cultures were doubled and attributed these findings to a more favorable dark/light cycle, in that the time period of each interval becomes smaller with increasing turbulence. Laws *et al.* (1983) introduced wing-shaped foils in an algal production system, which induced vortex circulations of about 0.5 to 1 Hz. This resulted in an almost doubling of the productivity, which they attributed to a flashing light effect.

It is obvious that time intervals, which would be short enough to produce the 'flashing light effect' (Kok, 1953), would only be found near water surfaces due to the focusing effect of waves or in highly turbulent massive microalgal cultures. In nature, phytoplankton are therefore, subjected to light/dark cycles ranging from fractions of seconds to seasons. Short light/dark cycles are also common in turbid waters (Grobbelaar, 1985), or in rivers and streams (Mann *et al.*, 1972). Richmond *et al.* (1980) suggested that

cycles ranging from a few seconds to minutes will enhance the output rate from mass algal cultures.

The estimation of phytoplankton primary productivity has received wide attention since the introduction of the ^{14}C-method by Steemann-Nielsen (1952) and numerous methods have been employed for its measurement (e.g. Vollenweider, 1969; Hall & Moll, 1975). A major criticism of most of the techniques used to measure phytoplankton productivity, is that a discrete isolated sample is incubated for a time interval, where errors due to bottle effects and decreased turbulence may be large (Hall & Moll, 1975). It has, furthermore, been suggested that photo-inhibition, as measured with static bottle methods, may simply be an artifact and that it does not occur under natural turbulent conditions (Harris & Piccinin, 1977), which could partly explain the higher productivity rates of mixed systems.

Should light/dark cycles of medium frequency (seconds to minutes) have an influence on the rates of phytoplankton photosynthesis, this would be an important factor to consider when such rates are measured. In this paper a series of experiments are described which were designed to determine the effect of varying light/dark cycles on phytoplankton productivity. The duration of the light/dark cycles were not only altered (i.e. the fluctuations), but also the length of the dark period in relation to the light period (thus intermittent light). The latter would be found in nature when the mixing depth is greater than the euphotic depth, which is common for highly turbid systems (Grobbelaar, 1985) or in dense mass algal cultures.

Materials and methods

Chlorella sp. (culture collection, University of the OFS, Bloemfontein) was used as test organism and was cultivated in a 1 l capacity continuous culture, using PAAP nutrient solution (Porcello *et al.*, 1970). Light was provided by four Philips TL50 fluorescent tubes and was the rate limiting factor in the continuous culture operation. Nutrient solution was supplied at a rate of 10 ml h^{-1} with a Watson-Marlow 101U/R peristaltic pump and the culture volume was maintained at 525 ml with a continuous overflow. 200 ml of the culture suspension, diluted to 1000 ml with PAAP nutrient solution, was used in each experiment. The chlorophyll *a* content of the diluted culture suspension was determined before each experiment, using the method of Sartory & Grobbelaar (1984) and a Beckman Model 26 spectrophotometer.

Algal productivity was measured from the uptake of ^{14}C, where 500 ml of the suspension was enriched with 20 μ Ci $NaH^{14}CO_3$ solution (New England Nuclear). Duplicate 2 ml samples of this enriched solution were placed in 10 ml scintillation liquid (Insta-Gel II, Packard Instruments) in order to determine the amount of added radioactivity. Scintillation vials were used as incubation bottles, where each received 28 ml of the enriched suspension, whereupon two were immediately darkened, first with Al-foil and then with black cloth. Incubations lasted 30 minutes whereafter the contents of the vials were filtered through 25-mm diameter Whatman GF/C filters, rinsed with 5 ml of a 0.01 N HCL solution and 10 ml PAAP nutrient solution to remove any extra-cellular ^{14}C. The filters with algae were placed in scintillation vails containing 10 ml Insta-Gel II and the radioactivity of all the samples was measured as disintegrations per minute (dpm) after quench corrections in a LKB Rackbeta scintillation counter. Measurement of alkalinity and calculations of productivity were done according to Grobbelaar (1984; 1985).

Incubations were done in a waterbath with the following dimensions, 430 mm (l) × 430 mm (w) × 260 mm (h). Natural clay, collected from the Modder River (South Africa) was suspended in the water and depending on the concentration, different turbidities could be achieved (i.e. different underwater light regimes could be experimented with). Two stirrers ensured that the clay particles remained in suspension. Two black anodized metal disc-like incubation devices were used to incubate the ^{14}C-enriched algal samples. Each disc held eight flasks. The holes were evenly spaced on the disc which could rotate, whilst they

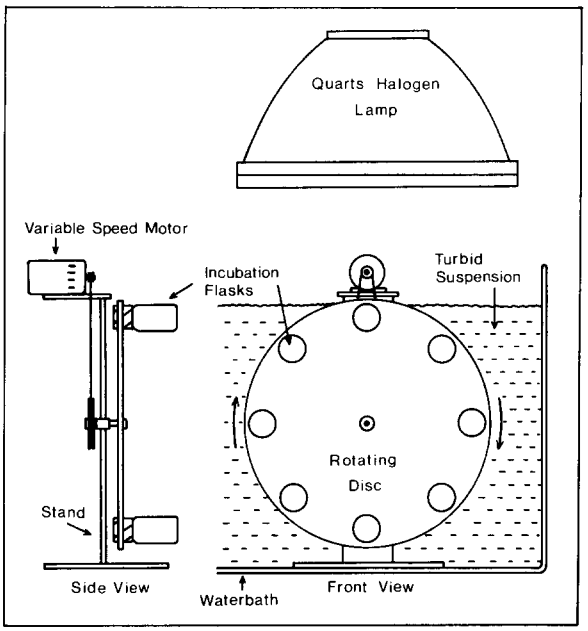

Fig. 1. Diagrammatic presentation of the experimental device, showing the disc with flasks, which could rotate. The flasks were spaced equidistantly from the surface downward on the stationary disc.

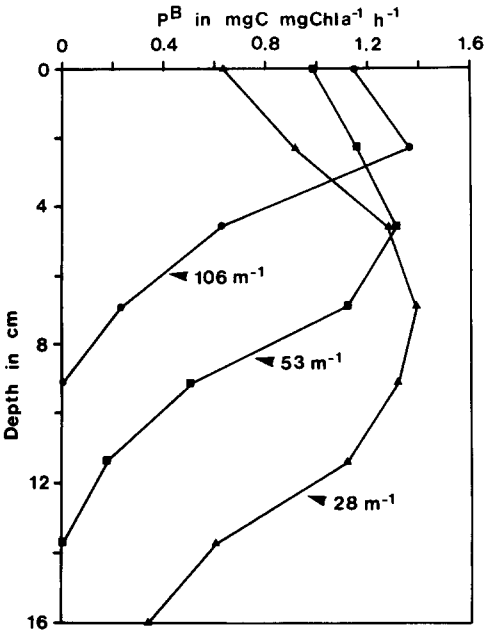

Fig. 2. Examples of three chlorophyll specific productivity profiles as measured with the stationary disc at three attenuation coefficients; 106, 57 and 28 m^{-1}.

were spaced such that a depth profile with equidistances between the flasks existed on the stationary disc (Fig. 1). The rotating disc, therefore had eight replicates, whilst single incubations were done at each depth on the stationary disc. A depth profile of 160 mm was covered by the discs and a variable speed motor could facilitate rotating speeds of 2 to 60 s per one rotation of the rotating disc (0.5 to 0.0167 Hz).

An OHD 1000 W quartz halogen lamp provided light and approximately 1700 μ Einst. $m^{-2}\ s^{-1}$ was measured at the water surface. The light intensity at the surface and over the depth profile were measured with a Li-Cor Model LI-185B Quantum/Radiometer/Photometer equipped LI-190 Quantum sensor. The attenuation coefficient for downward illumination (k_d in m^{-1}) was calculated from the following relation:

$$k_d = \frac{\ln I_o - \ln I_z}{z},$$

where I_z is the light intensity at depth z in meter and I_o the light intensity at the surface. The cultures were darkened with a black cloth before being exposed to the light and covered again after the incubation period, prior to being processed.

Results

'Typical' productivity depth profiles were recorded for the stationary incubation bottles, where photo-inhibition occurred at the surface, maximum productivity (P_{max}) at light saturation and a decrease in productivity with depth due to the attenuation of light. Increased turbidities not only influenced the production depth profiles, but also P_{max} where it decreased with increased turbidity. Turbidity did not influence the maximum chlorophyll specific productivity (P^B_{max}) and the marked influence, which different attenuations of the light had on the chlorophyll specific production (P^B) profiles, is shown in Fig. 2. The areas under the productivity curves were integrated to give areal productivities.

The eight flasks of the rotating disc always gave similar results and examples of three measure-

Table 1. Examples of differences among the eight flasks incubated on the rotating disc.

	Experimental conditions		
Rotation time (s)	9.5	2.2	32
k_d (m^{-1})	106	57	28
Productivity (mg C m^{-3} h^{-1})	35.0	48.8	59.2
	34.5	53.0	61.4
	37.7	45.5	61.9
	35.8	47.6	60.3
	33.4	45.7	58.9
	38.5	47.4	60.4
	37.2	49.3	63.0
	38.4	52.8	61.5
Standard deviation	1.79	2.69	1.30

ments are shown in Table 1. The standard deviation between the individual flasks never exceeded more than 5% and the average were, therefore, used in the comparison with the integrals of the static incubations. As the incubations lasted for only 30 min, differences due to the excretion of fixed organics or bottle effects would be at a minimum and consequently the results obtained with the two incubation techniques were used in a direct comparison to each other.

In order to compare the various experiments with each other, the productivities as measured with the static bottles were taken to be 100% and the results from the rotating disc were expressed as a percentage of the stationary flasks. The outcome is shown in Fig. 3 where five ranges of rotation times (speeds) and four conditions of different light attenuating conditions are presented. These four conditions of turbidity could also be expressed as different Z_{eu}/Z_m ratios, where Z_{eu} was taken as the depth to which 1% of the surface illumination penetrated and Z_m as the depth of mixing, which is 16 cm in these experiments. Conflicting results were obtained, where productivity increased with increased mixing at a light attenuation of 28 m^{-1} (ratio of Z_{eu}/Z_m = 1.025). The opposite was seen at a light attenuation of 57 m^{-1} (Z_{eu}/Z_m = 0.504), where productivity decreased with increased mixing. No clear trend could be seen at an attenuation coefficient of 75 m^{-1} (Z_{eu}/Z_m = 0.384), whilst

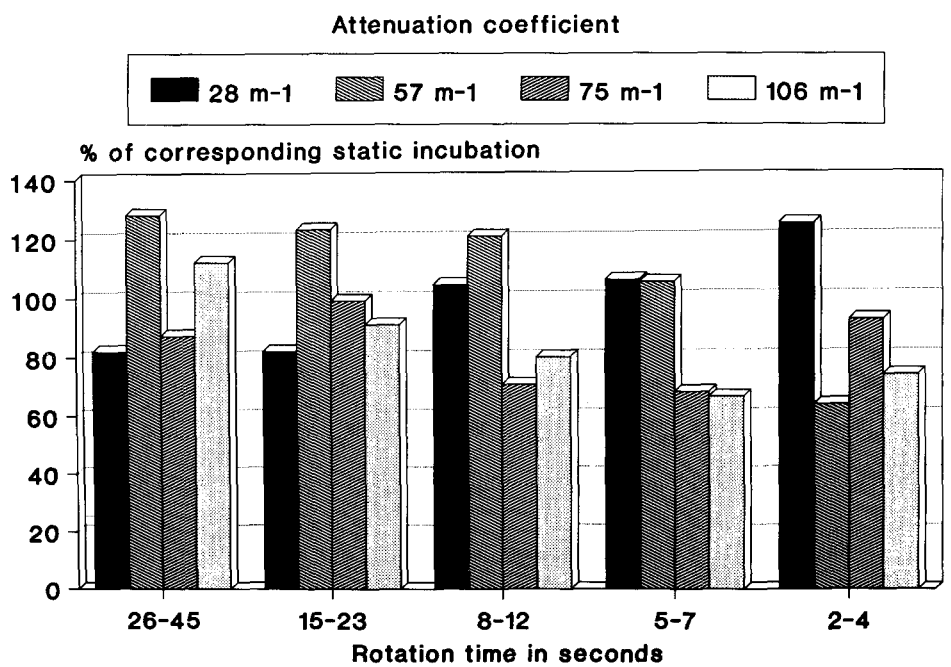

Fig. 3. Productivity at various mixing rates and at different turbidities. The values are as percentages of the static bottle incubations, where these were taken to be 100%.

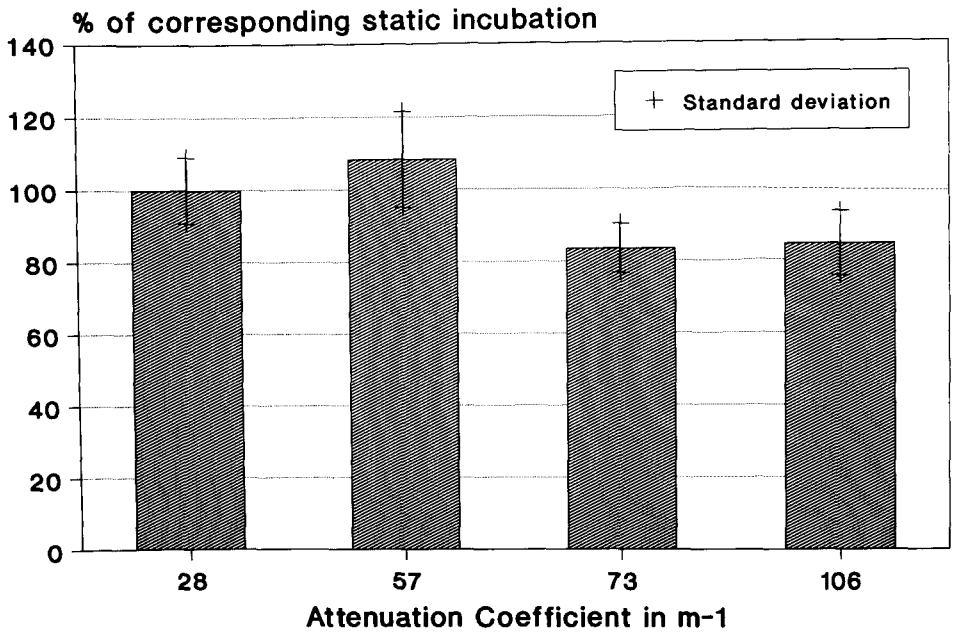

Fig. 4. Average productivities of the rotating flasks at different conditions of turbidity, together with the standard deviations for each treatment.

productivity decreased with increased mixing at the highest attenuation coefficient of 106 m^{-1} (Z_{eu}/Z_m = 0.271) up to the second fastest mixing rate, whereafter it increased slightly.

In comparison to the static bottle incubations, the productivities as measured in the rotating flasks were generally lower (Fig. 4). At an attenuation coefficient of 28 m^{-1} the average of the four rotating experiments were the same as the stationary measurements, whereas it was slightly higher for the experiments done at an attenuation coefficient of 57 m^{-1}. At both the higher attenuation coefficients the rotating disc incubations gave on an average lower production rates than the static bottle incubations.

Discussion

Although it has been suggested that light/dark cycles in the order of seconds to minutes could have a stimulatory effect on the rates of algal production (Richmond & Vonshak, 1978), this could not be shown by our results. In fact, our results showed that productivity as measured in a rotating system were on an average lower than that which was obtained in static bottle incubation over similar depth profiles and light fields (Figs 3, 4). As the incubations lasted for only 30 min, bottle effects and losses due to excreted organic compounds, would be at a minimum and the results obtained should represent what actually happened in the system (approaching gross photosynthesis). It should, furthermore, be noted that turbulence around the algal cells as such was at a minimum in the rotating disc system and could be compared to the static incubations. The reason for this was that the flasks were fixed and the disc merely rotated without any stirring device employed in the reaction vessel as such. Contrary to this, the influence of modulated light has usually been studied in well mixed (i.e. the reaction vessel) systems such as polarigraphic O_2 electrode chambers (e.g. Kok, 1953; Terry, 1986) or mass algal cultures (e.g. Laws *et al.*, 1983). It is suggested that a distinction should be made between the effect of light on the one hand and mixing on the other, as will be discussed at the end of this section.

The findings shown in Figs 3 and 4, also relate

to criticism leveled against the static bottle incubation method for measuring *in situ* primary productivity (Hall & Moll, 1975), where the 'unnatural' situation in the bottles and static nature of the incubations are seen as a major problem (e.g. Hall & Moll, 1975; Harris & Piccinin, 1977). Our results suggests that productivity may be overestimated at low ratios of Z_{eu}/Z_m with the static bottle method (Fig. 4), because the circulation flasks gave lower rates than those measured in the static flasks. This does not refer to the 'critical mixing depth' (Vollenweider, 1970; Grobbelaar, 1985) where the mixing depth determines the overall productivity of a system (Grobbelaar, 1989).

The actual productivity of a system decreases as the critical mixing depth is approached (Grobbelaar, 1985). This is not shown in the results presented in Fig. 4, because these were expressed as a percentage of the static incubations. The raw data, however, clearly show how a decreased euphotic/aphotic zone ratio limits overall productivity. For the rotating discs productivities decreased from an average of 60.8 mg C m^{-3} h^{-1} at a Z_{eu}/Z_m ratio of 1.025 to 36.3 mg C m^{-3} h^{-1} at a Z_{eu}/Z_m ratio of 0.271 (see Table 1).

Turbulence or free water movement would not only influence the light climate which phytoplankton are subjected to, but would also influence the nutritional and gaseous gradients which are formed around the cells during their metabolic activity. Increased turbulence would decrease this boundary layer (diffusion gradient) and *vice versa*, and would be especially important in oligotrophic waters or systems with high biomass concentrations, such as mass algal cultures. Märkl (1977) showed that the growth rate of algae could be increased by supplying CO_2, up to a certain critical concentration near the algae, whereafter no further influence of CO_2 on the growth rate could be seen. The thesis that turbulence would enhance exchanges between the cell and the environment and, therefore, would be able to utilize light more efficiently is suggested here. It is, furthermore, suggested that this influence is synergistic and that overall productivity would increase exponentially within a certain range of turbulences and available light energy.

The above thesis leaves many questions unanswered and also does not offer specific explanations for previously published results where higher productivities were attributed to variations in light/dark cycles (other than to remark that these were usually done in systems where the culture as such were thoroughly mixed). Cognisance should also be taken on the findings of Legendre *et al.* (1986), where they suggest that microalgae can adapt to the specific light regime under which they are grown, be it fluctuating or constant. In our experiments, the algae were simply moved through various light fields, so that the influence of turbulence which would effect the boundary layer and hence exchange rates between the cell and its environment, was at a minimum. A series of experiments have been designed to test the effects of turbulence and varying light fields and with these we hope to elucidate the question of turbulence, as the cell would experience it under natural conditions and perhaps establish the synergistic effect of mixing. Should such a synergistic effect exist, it would be important in the design and operation of mass algal cultures.

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