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INCUBATION UNDER FLUCTUATING LIGHT CONDITIONS PROVIDES VALUES MUCH CLOSER TO REAL IN SITU PRIMARY PRODUCTION

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ABSTRACT

Comparison of measured primary production, by radioactive carbonate incorporation or oxygen production, with diel variations of oxygen, inorganic carbon and particulate carbon, shows that the net in situ production often exceeds the measured activity, especially in deeper marine ecosystems. In vitro incubations under fluctuating light conditions provide higher values of primary production at low light intensities, both with cultures and with natural populations. The same results are obtained with in situ incubations, by varying the depth of incubation. This method allows reevaluation of primary productivity at low light intensities and provides results in agreement with the in situ variations of oxygen, inorganic carbon and particulate carbon. It can explain why primary productivity in the ocean is higher than previously calculated.

In the framework of a team approach to the ecological structure in the Southern North Sea (C cycling: Joiris et al., 1982), a contradiction became evident between the measured primary production and the determination of the activities consuming organic matter. This means that the primary production has been underestimated and/or the consumption rate over-estimated (Joiris, 1977). This is why the measured activities [primary production: radioactive bicarbonate method and Vollenweider (1965)-type of model; planktonic respiration: dark oxygen consumption rate] were compared with the in situ variations of oxygen and carbonates concentration during 24-h cycles (Joiris and Hecq, submitted[1]; Joiris and Bouquegneau, unpubl.). These results show that the respiration is correctly evaluated, but that the primary production is often underestimated by the methods used. Not only the gross, but even the net primary productions were underestimated.

Since these discrepancies are more important in deep marine systems than in shallow ones (Joiris, 1977), it seemed interesting to reevaluate the primary production methodology especially at low light intensities. One of the main differences between the natural conditions and the measurements even under in situ conditions seems to be the stability of the light conditions during the measurements, while in nature, turbulence causes the displacement of phytoplankton within the water column. This is the reason why we have compared estimates of integral photosynthesis in natural populations of phytoplankton between samples provided with vertical movement in a mixed layer and samples incubated at fixed light depths. Similar experiments have been performed by Marra (1978).

Conclusions reached from these field studies are supported by laboratory experiments on a *Dunaliella* and a *Skeletonema* culture. Comparison of the photosynthesis–light intensity relationship and primary production kinetics has been worked out under constant and fluctuating light conditions.

Materials and Methods

Field experiments were performed in the English Channel (50°N, 04°W) on 29 April 1983 at an euphotic depth of 24 m and a total depth of 50 m. Similar experiments were conducted in the Southern

[1] Joiris, C. and J. H. Hecq. Planktonic metabolism and diel oxygen curves during FLEX 76, Northern North Sea. Submitted.

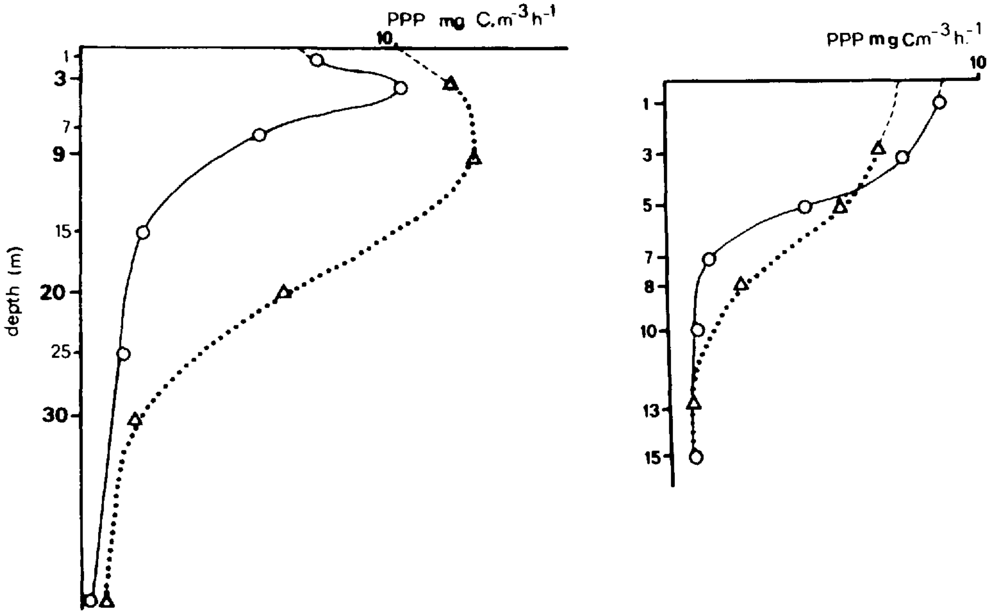

Figure 1. In situ primary production determination under constant and fluctuating light intensities (O——O constant light, △·····△ fluctuating light). A. (Left) English Channel; B. (Right) Southern North Sea.

Bight of the North Sea (Station Ostend 51°24′N, 02°48′E) on 4 May 1983 at an euphotic depth of 10 m and a total depth of 15 m.

A seawater sample taken at one single depth (−3 m) from the mixed layer with a polyethylene Nyskin bottle was used for all incubations. For each depth two bottles (Jena Glass), 50 ml each, were inoculated wtih 10 μCi $NaH^{14}CO_3$. The bottles were fixed in a transparent plastic rack, attached to a line and distributed at various depths in the mixed layer. One line had fixed depths of constant light at depths of −5, −10, −15 and −20 m for example. The second line was moved up or down 5 m every 15 min (fluctuating light). Each experiment lasted 4 to 5 h. The samples were incubated in the English Channel from 1200–1630, for Station Ostend from 1400–1700. After the incubation we filtered the samples on Whatman GF/C filters, using a gentle vacuum (<100 mm Hg). Filters were rinsed 3 times with 5 ml filtered seawater. The radioactivity retained on the filter was counted by liquid scintillation.

In the laboratory, similar experiments were performed with cultures of *Dunaliella tertiolecta* and *Skeletonema costatum*. Photosynthesis experiments were performed using a light incubator. Winkler bottles were inoculated with 400 μCi $NaH^{14}CO_3$ and incubated in light-bags under different light intensities. Samples remained in their light-bags to prevent a light shock during the whole incubation. During the fluctuating light incubation a second bag was added every 15 min.

Also photosynthesis in the laboratory culture experiments was determined by the classical oxygen method. Depending on the long respectively short altering of the fluctuating light, the light intensities were changed every 2 hours respectively every 30 min.

In all the laboratory experiments, filtrations (5-ml aliquots) and radioactive determinations took place as mentioned above.

RESULTS

Natural phytoplankton, in situ incubations.—In the English Channel an important difference in primary production is noted between the "constant- and fluctuating light" incubation measurements. The production measured under fluctuating light intensities is a factor 2.2 higher than those obtained under constant light conditions

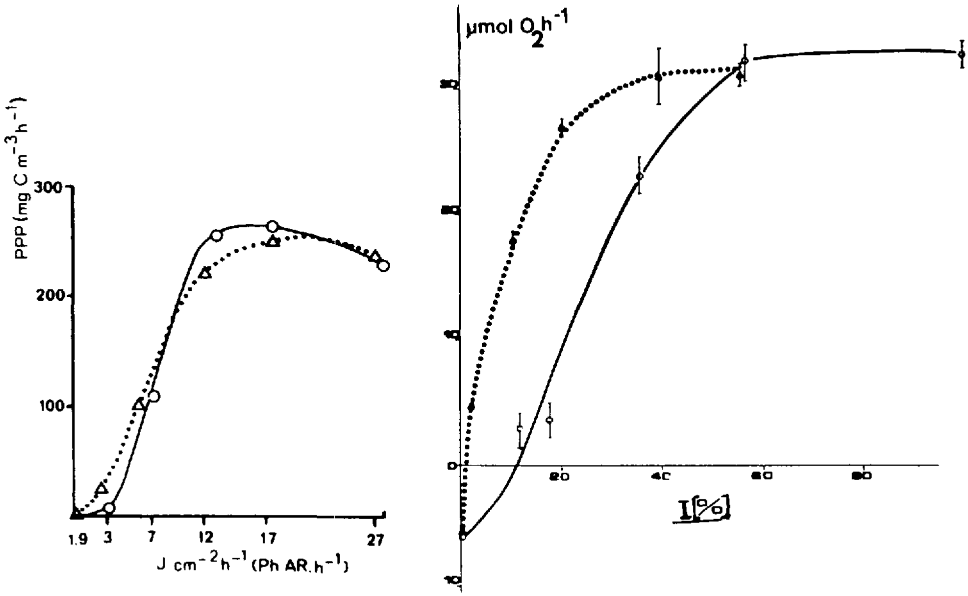

Figure 2. Primary production—light curves for a culture of *Dunaliella* under fluctuating and constant light conditions: typical examples A. (Left) 02.02.1983: 3 h incubation, ^{14}C bicarbonate method; B. (Right) 31.08.1983: 3 h incubation, oxygen method (O——O constant light intensities, △·····△ fluctuating light intensities).

(Fig. 1A, Table1). This difference is essentially to be attributed to the low light intensities corresponding with the deeper incubation depths. Note the slightly lower or comparable maximal primary production values. These experiments can be seen as representive for the primary production results obtained during the same period. In the Southern Bight of the North Sea the difference between both types pf incubations are very low, if any (factor 1.1). This factor results from two effects, fluctuating light being the cause of a slightly, lower maximal production, but higher values at low light intensities (Fig. 1B, Table 1).

Cultures, In vitro Incubation.—The effects of fluctuating light intensities on the photosynthesis-light curves on a *Dunaliella tertiolecta* culture was worked out using the radio-active bicarbonate method and the oxygen method. (Fig. 2A–B). The results of both methods reflect a clear tendency to provide comparable or slightly lower maximal production under fluctuating light, but higher values at the low light intensities. As an example, typical kinetics done with cultures in the laboratory are shown for *Dunaliella* and for *Skeletonema* (Fig. 3), both with long (A, C) and short (B, D) fluctuating periods. The variations of primary production under fluctuating light are as a rule clearly smaller than the expected values for kinetics realized under constant light of the same intensities. In the extreme situation (Fig. 3A, B, D), no difference can be detected between the two light intensities after an adaptation period, where more than a factor two is observed for comparable constant light intensities.

Discussion

Incubations under fluctuating light clearly show a tendency to provide comparable or slightly lower values of maximal primary production, but higher values

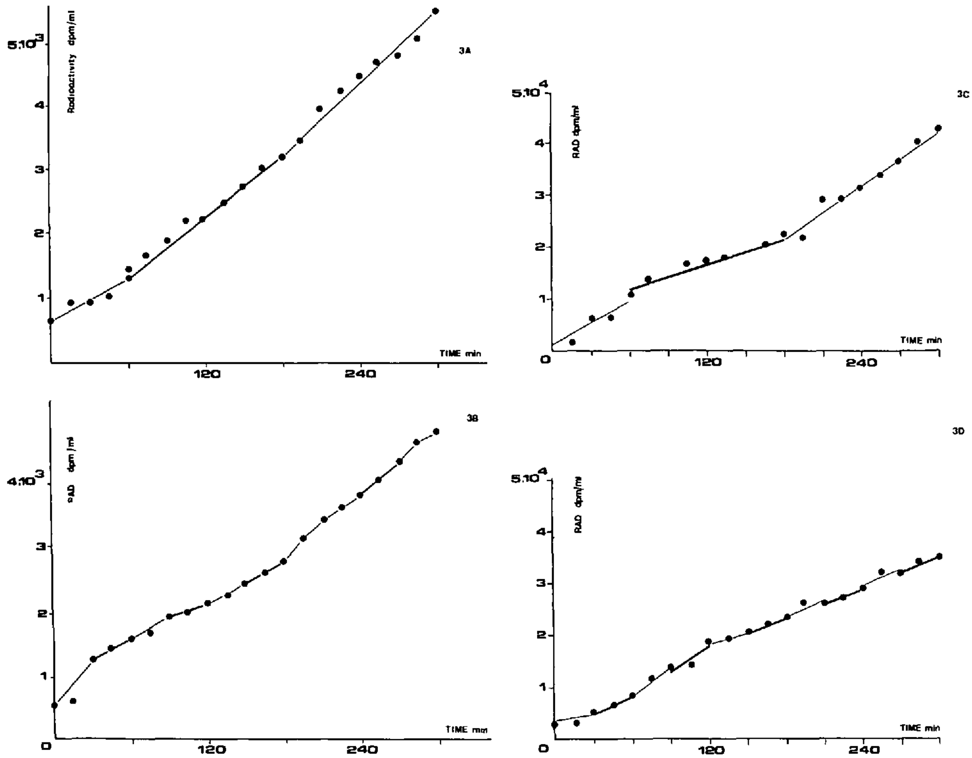

Figure 3. In vitro primary production kinetic of *Dunaliella* (A, B) and *Skeletonema* (C, D) under fluctuating light intensities. A, C: long fluctuations (on the 2 h) ——: 30% I, ——14% I. B, D: short fluctuations (on the 30 min) ——: 21% I, ——13% I. 100% I = 26,000 lux.

than constant light incubations at low light intensities. As a consequence, in deep systems, this provides higher integrated primary production values for the whole water column (per surface unit). Not only is this a qualitative difference in the expected direction, but the observed difference can even quantitatively explain the discrepancy between measured and natural primary production: during the diel cycle of 28–29 April 1983 in the English Channel, a net production of 2,500 mg $C \cdot m^{-2} \cdot d^{-1}$ is to be expected from the natural variations of O_2 and CO_2 concentrations (Joiris and Bouquegneau, unpubl.); constant light incubation provides 860 mg $C \cdot m^{-2} \cdot d^{-1}$ and fluctuating light incubations 1,940 mg $C \cdot m^{-2} \cdot d^{-1}$ (Table 1). During the diel cycle of 4 May 1983 at Station Ostend, a net production of 500 mg $C \cdot m^{-2} \cdot d^{-1}$ is to be expected from the variations of O_2 and CO_2 (ibidem); in situ constant light incubation provides 350 mg $C \cdot m^{-2} \cdot d^{-1}$ (Table 1). This fluctuating experiment provides the confirmation that no discrepancy between measured and real primary production is to be expected in shallow systems. In the same circumstances, no important difference between constant and fluctuating light incubations is detected.

For these reasons, we suggest that incubations under conditions of light intensities fluctuating at a rhythm of 15 min to two hours provide values of primary production much closer to the real in situ values than incubations under constant light intensity. The deeper the ecosystem, the bigger the discrepancy between constant light values of production, on the one hand, and fluctuating light values and real production, on the other hand.

Table 1. Comparison between the in situ primary productions of a shallow and a deeper biotope [η: extinction coefficient, I_k: saturation light intensity, P_{max}: maximum production, PPP: particulate primary production, calculated from the modified Vollenweider formula (Mommaerts, 1982)]

	Incubation time	η	I_k ($J \cdot cm^{-2}$ h^{-1})	P_{max} ($mg\ C \cdot m^{-3}$ h^{-1})	PPP ($mg\ C \cdot m^{-2}$ d^{-1})
English Channel (50°N, 04°W) 29.04.83					
Constant light incubation	1200–1630	0.19	34	10	856
Fluctuating light incubation			7	12	1,939
Southern North Sea (51°24′N, 02°48′E) 04.05.83					
Constant light incubation	1400–1700	0.50	24	8	317
Fluctuating light incubation			8	6	348

Within a more general discussion on the validity of the actual evaluations of primary production in the ocean, these results suggest that both the ^{14}C bicarbonate and the oxygen methods could be basically correct especially at high light intensities, both in the laboratory and in shallow systems (for the North Sea biotopes: see Joiris, 1977). The main problem seems to lie in the extrapolation of such results to the deeper systems where low light intensities play a very important role. This is why, for instance, important difficulties for determining a correct primary production are located in the Central Pacific Ocean (Shulenberger and Reid, 1981; Kerr, 1983). In this situation, incubation under fluctuating light conditions seems to provide a better picture of the dependence of primary production on the light intensity and thus a possible answer to the problem.

This approach to the effects of fluctuating light is very different from—but complementary to—the study of its physiological effects to be noticed on a much shorter time scale (Fréchette and Legendre, 1978; Legendre, 1981). It must indeed be noted that both types of experiments with fluctuating light concern entirely different time scales, and thus probably different phenomena: the very short fluctuations (sec) can correspond to the effect of surface waves. Such effects are however already integrated in our experiments at least for the in situ measurements. The time scale we used (fluctuation periods from 15 min to 2 h) concerns another phenomenon, in addition to the effects of short time variations. It corresponds probably much more to the vertical water movements (turbulence and/or internal waves, for instance) within the mixed layer. Other results concerning the same time scale can easily be incorporated in our interpretation (Harris and Lott, 1973; Jewson and Wood, 1975; Marra, 1980). A possible mechanism for explaining the effects of fluctuating light could be found by establishing a link with the light or shade adaptation of algae. Marine phytoplankton does indeed respond to decreasing light intensities by increasing its photosynthetic pigments content (Falkowski, 1980). On the other hand, the potential photosynthetic activity per chlorophyll unit is dependent on the previous light intensities (Demers and Legendre, 1981). Finally, a relation exists between photosynthetic capacity and the stability of the water column (Legendre, 1981). This allows us to formulate the following hypothesis: the differences between constant and fluctuating incubations could be that the phytoplankton cells are respectively light- and shade-adapted. The effect of fluctuating light on primary production is thus clearly dependent on the depth of the mixed layer: no important effect was detected in the Southern North Sea (15 m). This could explain why other authors came to the conclusion that vertical mixing has little or no effect on primary production (Falkowski and Wirrick, 1981; Gallegos and Platt, 1982).

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