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Studies on enhanced post-illumination respiration in microalgae

John Beardall[1], Tineke Burger-Wiersma[2,8], Machteld Rijkeboer[3], Assaf Sukenik[4], Jacques Lemoalle[5], Zvy Dubinsky[6] and Daniel Fontvielle[7]

[1]*Department of Ecology and Evolutionary Biology, Monash University, Clayton, Victoria, 3168, Australia,* [2]*Laboratory for Microbiology, University of Amsterdam, Nieuwe Achtergracht 127 1018 WS, Amsterdam,* [3]*Netherlands Institute of Ecology, Centre for Limnology, Rijksstraatweg 6, 3631 AC Nieuwersluis, The Netherlands,* [4]*Israel Oceanographic & Limnological Research Institute, PO Box 8030, Haifa 31080, Israel,* [5]*Laboratoire d'Hydrobiologie, ORSTOM BP 5045, Montpellier Cedex, France,* [6]*Department of Life Sciences, Bar-Ilan University, 52100 Ramat-Gan, Israel and* [7]*Laboratoire d'Ecologie, Universite de Savoie, BP 1104, 73011 Chambery, France*

[8]*Present address: RIVM-LWD, PO Box 1, 3720 BA Bilthoven, The Netherlands*

Abstract. The extent of enhanced post-illumination respiration (EPIR) has been investigated in a number of microalgae. Respiration rates, as determined by O_2 consumption, were enhanced (in all but one case) by 50–140% following pre-exposure to high photon flux compared to rates obtained for steady-state dark respiration. The extent of EPIR was dependent more on photon flux than on duration of exposure, although the latter did have some effect. In *Isochrysis galbana* and *Chaetoceros calcitrens*, EPIR effects were also demonstrated using $[^{14}C]CO_2$ evolution. In *I.galbana*, release of CO_2 from cells pre-exposed to a period of high photon flux was most rapid from carbohydrate and low-molecular-weight metabolites. Data obtained from *Thalassiosira weisflogii* indicate that cells grown at low photon flux are more susceptible to EPIR than those grown under high photon flux. These results are discussed in the context of various hypotheses that have been proposed regarding the mechanism of EPIR effects.

Introduction

A large proportion of the primary productivity of aquatic environments depends on photosynthesis by microalgae. This is particularly true of open ocean, in which a large fraction of total global primary production is brought about by the phytoplankton, but is also true of many other aquatic ecosystems. Whilst the initial process in primary productivity is the light-driven assimilation of inorganic carbon into organic matter, there are a number of processes that take place in microalgae that run counter to this reductive assimilation of carbon. These are generally grouped together as 'respiration' (Beardall and Raven, 1990). Although not as obvious as photosynthesis, respiratory processes are essential to the functioning of plant cells and can have important consequences for overall rates of primary production (Beardall and Raven, 1990).

Dark respiration rates are generally of the order of 10% of gross photosynthesis at light saturation (Beardall and Raven, 1990). However, there are a number of reports in the literature that suggest that the respiratory rate of plants is dependent on the preceding 'photosynthetic history'. An enhancement of dark respiration rates following a period of photosynthesis was originally reported for higher plants by Weigl *et al.* (1951), Brown and Van Norman (1952) and Heichel

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(1970). Later studies on microalgae have established that dark respiration rates, in these organisms also, are stimulated after exposure of cells to light (Jassby, 1978; Stone and Ganf, 1981; Falkowski *et al.*, 1985). Such effects could explain the well-documented 'hysteresis' effects in microalgal photosynthesis when cells are moved through an increasing and then decreasing light field (Harris and Lott, 1973; Falkowski and Owens, 1978). This hysteresis has important consequences for the overall primary productivity of phytoplankton that are exposed to a fluctuating light regime as they circulate through a water column.

The experiments reported here were designed to investigate the extent of enhanced post-illumination respiration (EPIR) in a range of microalgae grown at different photon fluxes. In selected organisms, further studies were carried out on the effects of photon flux and duration of illumination on EPIR, and on changes in metabolite pools during a period of EPIR.

Method

Organisms and growth conditions

Cultures of *Isochrysis galbana*, *Microcystis* sp., *Thalassiosira pseudonana*, *Tetraselmis suecica*, *Chaetoceros calcitrens* and *Phaeodactylum tricornutum* were grown in 2 l batches using the medium of Conway *et al.* (1976). Cultures were maintained in a constant-temperature room under high (100 μmol photons m^{-2} s^{-1}) or low (10 μmol photons m^{-2} s^{-1}) photon flux. Under these conditions, temperatures in the high and low photon flux cultures were 24 and 19°C, respectively. NO_3^- was used as the nitrogen source.

Experimental

Studies with O_2 electrodes. Samples were taken from late exponential phase cultures and introduced into an O_2 electrode constructed as described by Dubinsky *et al.* (1987). The temperature in this chamber was maintained at 21 ± 0.5°C by means of a thermoregulator and dissolved O_2 measured using a YSI 5331 O_2 electrode. The O_2 electrode was calibrated using sodium sulphite or by purging with O_2-free N_2 to achieve 0% O_2 and by purging with air to give equilibration with 100% O_2. Light was supplied by a quartz–halogen lamp in a Kodak SA 2050 projector. The output signal from the O_2 electrode was recorded on either a Linseis or a Kipp & Zonen 8041 chart recorder. Unless otherwise stated, EPIR was determined following a standard incubation of 10 min at 1000 μmol photons m^{-2} s^{-1}.

In a separate series of experiments, *Thalassiosira weisflogii* was grown in 3.2 l light-limited turbidostat cultures at 19°C. The cultures were kept optically thin, to minimize self-shading, by an optical sensor–dilution pump feedback loop operated by computer. Photon flux was adjusted to the desired level by changing the distance of two banks of three cool-white fluorescent lamps and by switching on an appropriate number of these lamps. These cultures were harvested and the magnitude of any EPIR determined following a standard incubation of 10 min at 1000 μmol photons m^{-2} s^{-1}.

Studies using [^{14}C]HCO_3^-. Cultures of *I.galbana* and *Chaetoceros gracilis* were grown in f/2 medium (Guillard and Ryther, 1962) containing $NaH^{14}CO_3^-$ at an activity of 0.15 μCi ml^{-1} (1 mCi $mmol^{-1}$). Cultures were grown for 3 days at 70 μmol photons m^{-2} s^{-1} to allow isotopic equilibration of the internal carbon pools. At various time intervals thereafter, samples of cell suspension were withdrawn for measurement of acid-stable ^{14}C content by liquid scintillation spectrometry. Samples were taken before and during dark periods following exposure of cells to 70 or 700 μmol quanta m^{-2} s^{-1}.

For *I.galbana*, 20 ml samples were collected on GF/C filters and the cells fractionated for the four major cell components, i.e. lipids, proteins, carbohydrates and low-molecular-weight metabolites, according to the scheme of Taguchi and Laws (1985).

Results

In all organisms examined, there was a marked stimulation of respiration following a period of exposure to high proton flux (Figure 1). In all but one case, this stimulation represented an increase in oxygen uptake of 50–140% above steady-state rates of dark respiration, i.e. EPIR values of 1.5–2.4 (Table I). Enhancement of respiration following illumination was evident not only from changes in O_2 concentration but also, in the case of *I.galbana* and *C.gracilis*, from changes in the ^{14}C content of cells (Figures 2 and 3). In these experiments, the extent of EPIR was 420 and 240% greater than steady-state dark respiration, respectively.

The characteristics of EPIR in *I.galbana* were examined in more detail by varying either the photon flux or the duration of exposure. The magnitude of the EPIR was markedly stimulated by increasing the photon flux prior to the dark period (Figure 4). Although increasing the duration of exposure to a photon flux of 1000 μmol photons m^{-2} s^{-1} also resulted in an increase in EPIR (Figure 5), such an effect was not so large as when the photon flux was varied. The latter feature is illustrated in Figure 6, in which the EPIR is plotted as a function of 'photon dose' (the number of photons supplied to the electrode chamber during the incubation).

The fate of the organic carbon pools during EPIR in *I.galbana* is shown in Figure 7. The decrease in the total ^{14}C content of cells (Figure 3) was accompanied by a similar decay in the amount of label associated with the carbohydrate and low-molecular-weight metabolite fractions. In contrast, the amount of label in the lipid and protein fractions did not decrease during the period of EPIR.

In *T.weisflogii*, there was an inverse relationship between the extent of the EPIR and growth rate in light-limited cultures (Figure 8). The lower the photon flux for growth, the greater was the magnitude of the EPIR after a 10 min exposure to 1000 μmol photons m^{-2} s^{-1}. That this trend was not exhibited by a number of the microalgae in the experiments reported in Table I probably reflects variations in the physiological state of these batch cultures.

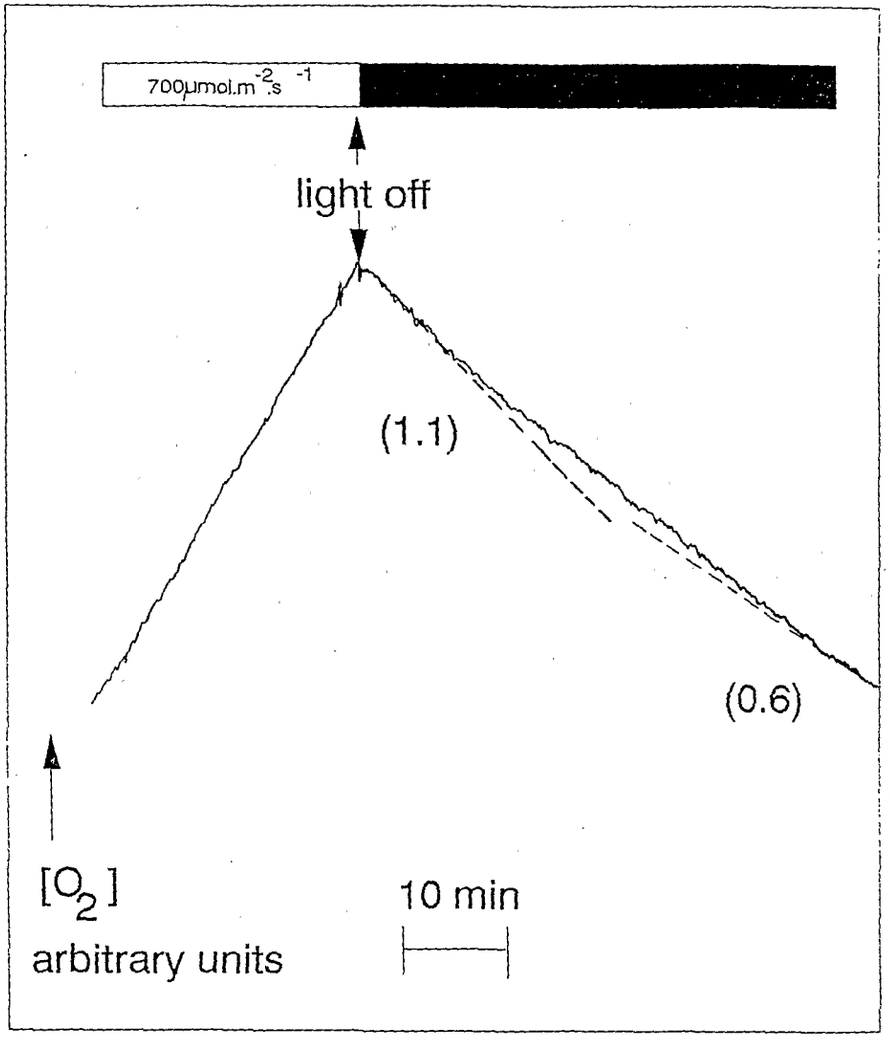

Fig. 1. O_2 uptake by *Microcystis* sp. following 10 min illumination at a photon flux of 1000 μmol m^{-2} s^{-1}. Numbers in parentheses indicate measured rates of O_2 uptake (arbitrary units min^{-1})

Discussion

The data obtained in this study suggest that the phenomenon of EPIR is widespread in microalgae. Respiration rates were enhanced by 10–140% following exposure of the microalgae to high photon flux. This is in agreement with previous observations with a number of microalgae (Jassby, 1978; Stone and Ganf, 1981; Falkowski *et al.*, 1985). Indeed, it appears that enhancement of respiration following illumination is widespread and also occurs in systems other than phytoplankton (e.g. the coral *Porites porites*; Edmunds and Spencer

Table I. Stimulation of dark respiration of a range of microalgae following a 10 min illumination at 1000 μmol m^{-2} s^{-1}. Values are given relative to a value of one for steady-state dark respiration (n/m = not measured)

Organism	Photon flux for growth (μmol m^{-2} s^{-1})	
	10	100
Isochrysis galbana	2.4	1.5
Microcystis sp.	1.5	1.9
Thalassiosira pseudonana	1.7	1.6
Tetraselmis suecica	2.3	1.7
Chaetoceros calcitrens	1.1	1.8
Phaeodactylum tricornutum	2.0	n/m

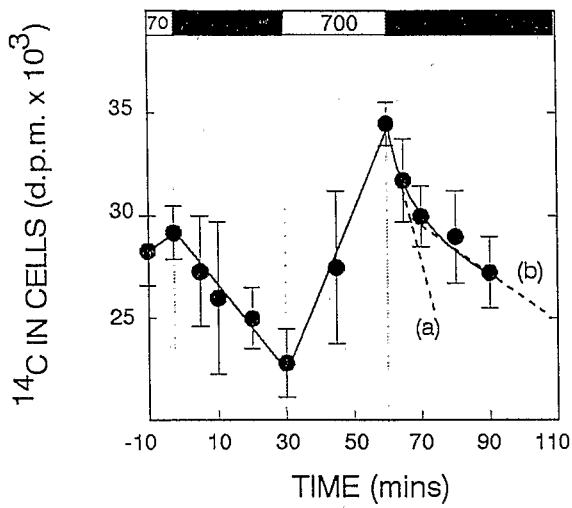

Fig. 2. Effects of illumination on loss of ^{14}C, in a subsequent dark period, from cultures of *C.calcitrens* exposed to photon fluxes of 70 and 700 μmol m^{-2} s^{-1}. The ratio of slope (a) to slope (b) is 5.2.

Davies, 1988). From the data reported here, it is evident that the extent of enhancement is dependent on both the photon flux and the duration of exposure, although the effect of photon flux is more marked.

The oxygen electrodes used to obtain data on EPIR effects are notoriously sensitive to small changes in temperature. It is imperative, therefore, to differentiate between true enhancement of respiration and temperature effects on the electrode. Falkowski *et al.* (1985) showed that EPIR in *T.weisflogii* was sensitive to metabolic inhibitors such as cyanide. In our present experiments, we have been able to demonstrate EPIR effects not only with the oxygen electrode, but also by changes in cellular ^{14}C. The latter are not so affected by small variations in temperature, thereby suggesting a metabolic rather than a physical basis for our O_2 electrode observations. Furthermore, Weger *et al.* (1989) have used mass spectroscopy to show EPIR effects in a marine diatom. It is unlikely,

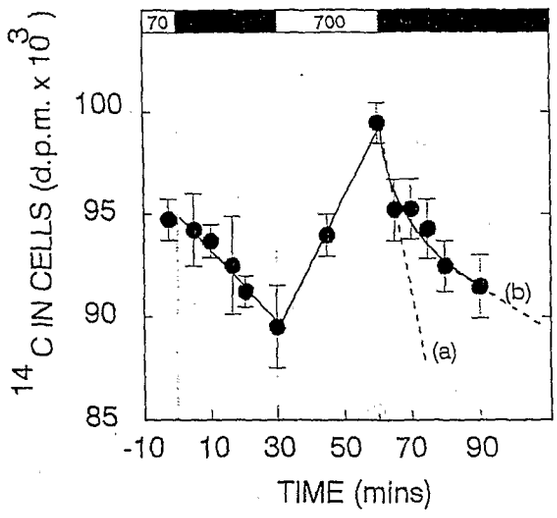

Fig. 3. Effects of illumination on loss of ^{14}C, in a subsequent dark period, from cultures of *I.galbana* exposed to photon fluxes of 70 and 700 μmol m^{-2} s^{-1}. The ratio of slope (a) to slope (b) is 3.4.

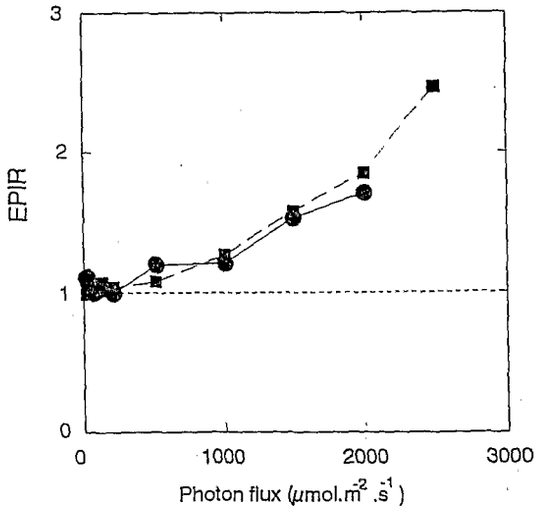

Fig. 4. Effect of photon flux on EPIR in *I.galbana*. Cell suspensions were exposed to increasing photon fluxes for 5 min intervals at each photon flux. Data are shown for cells given 5 min (●) or 10 min (■) dark periods between exposure to the appropriate photon flux.

therefore, that our experimental data reflect temperature variations within the oxygen electrode chamber.

It is also feasible that the stimulation of respiration following a period of high illumination could be a consequence of repair of photodamage. The photon flux used for EPIR experiments was considerably larger than that used for growth

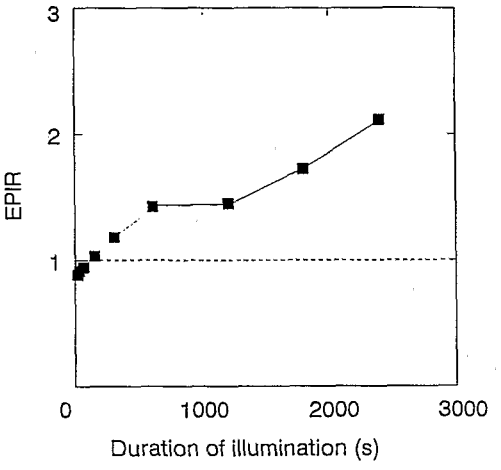

Fig. 5. Effect of duration of exposure of cells to a fixed photon flux of 1000 μmol m^{-2} s^{-1} on EPIR in *I.galbana*.

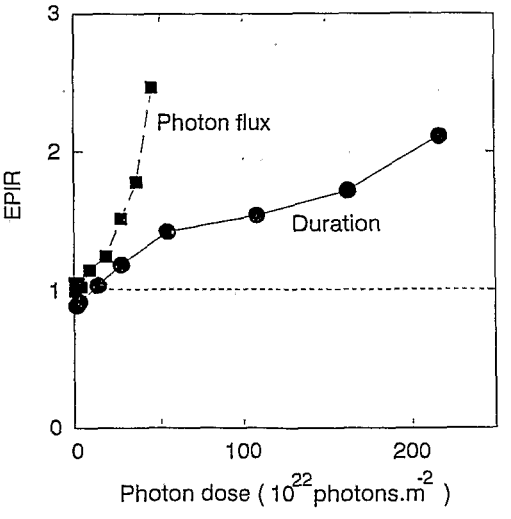

Fig. 6. Effect of photon dose (photon flux × duration of exposure) on EPIR in *I.galbana*.

and EPIR effects are greater the lower the photon flux for growth (conditions favourable to photoinhibition by high-intensity light). There is little evidence in the data reported here for a gradual decline, with time, in rates of $[^{14}C]CO_2$ incorporation or of O_2 evolution, as might be expected if photoinhibition and its repair were occurring. However, it must be recognized that a decrease in quantum yield due to photoinhibition would not be picked up by our experiments. Nonetheless, we believe that stimulation of repair of photodamage is not the source of the EPIR effect as EPIR is frequently observed after exposure to photon fluxes much lower than those considered to cause

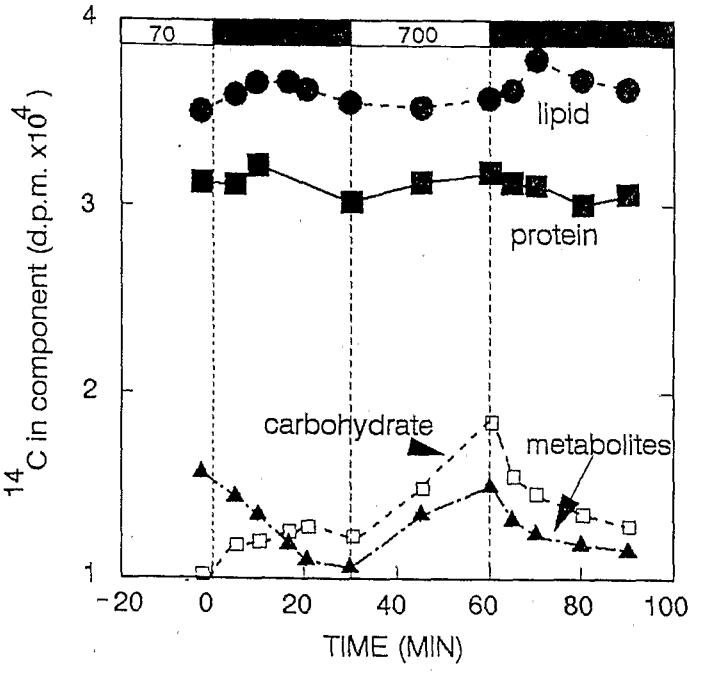

Fig. 7. Compartmentation of ^{14}C in *I.galbana* during transitions between high and low photon flux and darkness. The distribution of ^{14}C in lipid (●), protein (■), carbohydrate (□) and low-molecular-weight metabolites (▲) is as indicated.

photoinhibition. For example, *T.weisflogii* will show a significant EPIR effect even after exposure to as little as 6 min light at a photon flux of 20 μmol photons m^{-2} s^{-1} (Z.Dubinsky, unpublished data). Such a photon flux is less than that necessary to saturate growth and is thus unlikely to cause photoinhibition.

Falkowski *et al.* (1985) postulated that EPIR effects were due to an increase in intracellular pools of photosynthate following a period of intense illumination. The increased availability of respiratory substrates thus provided is believed to stimulate glycolysis and the TCA cycle, which in turn increases the rate of oxidative phosphorylation and O_2 consumption. The data obtained in this study are consistent with this hypothesis in that the cellular components from which ^{14}C label is lost after a period of illumination are carbohydrate and low-molecular-weight metabolites. It must be pointed out, however, that stimulation of oxygen consumption via the electron transport chain is normally tightly coupled to ADP/ATP levels. Thus, a sudden increase in the ATP demand of cells will, under the normal processes of respiratory control, lead to increased rates of electron transport and oxygen consumption. That cells of *T.weisflogii* grown under low photon flux show more marked EPIR effects than cells from high photon flux conditions could reflect the extent of operation of cellular biosynthetic machinery. Under high photon flux, cells are growing rapidly and metabolism is geared to biosynthesis. Under low photon flux conditions, however, biosynthesis and cell growth are decreased. In this situation, a sudden

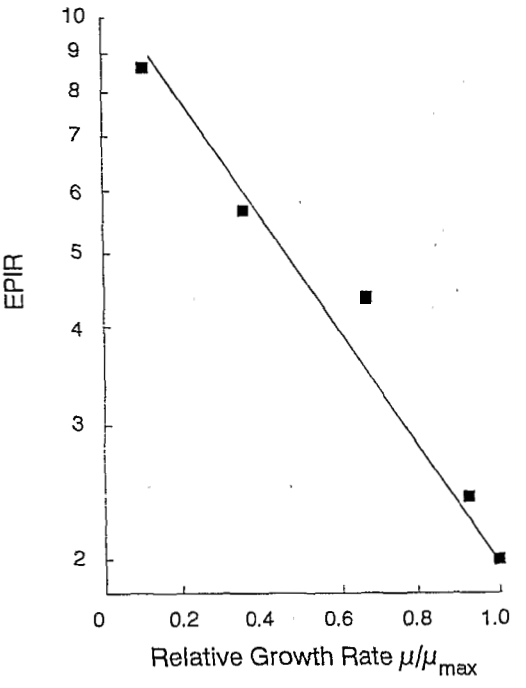

Fig. 8. The effects of photon flux for growth on EPIR in *T.weisflogii*. Algae were grown in light-limited turbidostat culture and EPIR determined after a 10 min exposure to 1000 μmol photons m^{-2} s^{-1}. Growth data are expressed relative to a maximum specific growth rate of 1.0.

increase in photon flux and subsequent photosynthate levels overloads the capacity of cells for biosynthesis and the photosynthate is rapidly oxidized. Explanations of EPIR in terms of exercise of respiratory control are, however, complicated by observations that not only is oxygen consumption stimulated by short-term exposure to high photon flux, but also that the capacity for electron transport is stimulated (Martinez, 1992). Clearly, further studies are required to clarify the phenomenon and to differentiate between the alternative hypotheses.

These observations are consistent with the hysteresis effect observed by Falkowski and Owens (1978) and by Harris and Lott (1973). Presumably, exposure to the increasing photon fluxes stimulates respiratory rates so net oxygen evolution at a given photon flux is lower after prior exposure to a higher photon flux. In the natural environment, this is critical in water bodies where vertical movement will mean that suspended cells are sequentially exposed to low and high proton fluxes (Ferris and Christian, 1991).

Observations of EPIR effects in a wide range of microalgae suggest that respiration rates could be higher than previously thought and are dependent on the 'light history' of the cells. This has important repercussions for our understanding of and attempts to model and predict net primary productivity in aquatic ecosystems (Ferris and Christian, 1991). Ignoring EPIR effects will lead to an underestimation of respiratory losses and consequent errors in estimations

of net productivity in natural systems. Experiments designed to measure primary production rates must, therefore, also take EPIR effects and fluctuating light regimes following vertical movements of phytoplankton cells into account if we are to gain meaningful insights into the initial steps in aquatic food chains.

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