

Oxygen consumption in the light by unicellular algae

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Introduction

Phytoplankton primary production forms the basis of many aquatic foodwebs. For ecosystem foodweb studies net primary productivity is the relevant parameter as this gives the amount of C-fixed available to higher trophic levels. In order to calculate net primary production (P_{net}) respiration (R) is subtracted from the measured gross primary production (P_{gross}): $P_{\text{net}} = P_{\text{gross}} - R$. As respiration cannot be measured with the ^{14}C -method it is often assumed that respiration is a fixed percentage of the maximum rate of photosynthesis measured during the P/E-curve (Smetacek & Passow, 1990; Cole et al., 1992). An assumption implicitly made when using this approach is that respiration does not vary with irradiance. The validity of this assumption and its effects on net primary productivity forms the basis of the paper.

There is mounting evidence that the rate of respiration is variable. Culture work demonstrated that respiration can be divided into a constant fraction related to maintenance processes and a variable fraction related to the growth (Geider & Osborne, 1986; Langdon, 1993). Weger et al (1989) studied the uptake of $^{18}\text{O}_2$ in a marine diatom and observed that the rate of oxygen consumption approximately doubled in the light. The increased rate of dark respiration persisted for several minutes after the light was turned off. Weger et al. (1989) argued that it is unlikely that the increased rate of mitochondrial respiration is due to catabolism because the capacity to produce ATP is greatly enlarged in the light, and that it is more likely that the requirement for TCA cycle intermediates is higher because of accelerated biosynthesis during photosynthesis. The results obtained by Weger et al. (1989) would imply that respiration is limited by the substrate supply. Thus, it seems fair to assume that respiration in the light is depending on the irradiance conditions. In this paper we investigate the extent of variable respiration in a number of eukaryotic micro-algae and cyanobacteria belonging to different taxonomic groups. Additionally we studied the contribution by the Mehler reaction to the total uptake of oxygen and discuss the effect of variable respiration on estimates of net primary productivity.

Material and Methods

Batch cultures of marine green alga *Chlorella autotrophica* (CCMP243), the marine diatoms *Thalassiosira pseudonana* (CCMP1013), *Phaeodactylum tricornutum*, *Skeletonema costatum* (CCMP1332) and *Cylindrotheca closterium*, the haptophyte *Isochrysis galbana* and the cyanobacteria *Nodularia spumigena* SN15a, *Synechococcus* BS4 (a green picocyanobacterium), *Synechococcus* BS5 (a phycoerythrin containing picocyanobacterium) and unknown red picocyanobacteria BS14 were all, with the exception *I. Galbana*, axenic. The cultures were grown at an incident irradiance of $35 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 20°C in 16h light: 8h dark cycle. Cultures were sampled in the 2nd half of the logarithmic phase for

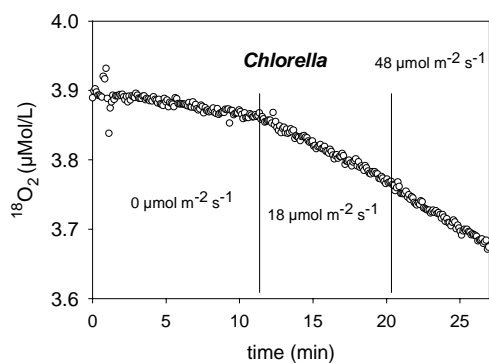


Fig. 1. Uptake of O_2 by the green algae *Chlorella autotrophicum* in the dark and two low, limiting irradiances.

sample was sparged with N_2 -gas to reduce the oxygen concentration after which $^{18}O_2$ gas (ARC-laboratories B.V.) was added as tracer. The O_2 enrichment never exceeded 10%. All calculations were done following Peltier & Thibault (1985). DCMU, 2.5 μM final (dissolved in water as ethanol disturbed the measurements) was added at the end of the incubation at the highest irradiance to inhibit the Mehler reaction (donation of electrons to O_2 by Photosystem I (PSI) (Kana, 1922, 1993).

Results

Fig. 1 shows an example of a trace of *C. autotrophica*. When the irradiance was increased from 0 to 18 $\mu mol photons m^{-2} s^{-1}$, the rate of oxygen uptake increased, showing a clear stimulation of respiration in the light. A further increase to 48 $\mu mol photons m^{-2} s^{-1}$ again caused a rise in the oxygen uptake. An example of a P/E-curve obtained with the green alga *C. autotrophica* is shown in Fig. 2a. It clearly shows that when the rate of photosynthesis increased, the rate of oxygen uptake increased as well. At saturating irradiances the total oxygen uptake seems to level off. As a result of the light stimulated “respiration”, the ratio of respiration to the maximum rate of photosynthesis (R/P_{max}) increased from 0.05 to 0.33, i.e. a more than 6-fold increase in the rate of respiration. When the measurement was repeated two weeks later with a fresh culture, similar results were obtained, although the ratio R/P_{max} in this case increased from 0.15 to 0.30. Similar results were obtained with the haptophyte *I. galbana* (Fig. 2B). R/P_{max} increased from 0.04 to 0.2. The diatoms showed a pattern similar to the green alga *Chlorella* (not shown). From these results it is therefore clear that P_{net} will be overestimated when the respiration measured at the start of a P/E-curve is assumed to be constant.

measurements of photosynthetic performance. The eukaryotic algae were grown in modified ASN-III and the cyanobacteria were grown a mixture of 1/3 ASN-III and 2/3 BG11 (Rippka et al. 1979).

Photosynthesis was measured as described by Dubinsky et al (1987). Rates of photosynthesis and respiration were measured at 8 light steps and each step lasted approx. 6-8 minutes. Gas exchange was measured on-line using a quadrupole membrane inlet mass spectrometer (MIMS, a Balzers Omnistar), fitted with a silicone membrane-containing probe, which was inserted into the cuvet.

Before the measurements were started the

The cyanobacteria tested showed a completely different pattern compared to the eukaryotic micro-algae. Both *Nodularia* (Fig. 3A) and *Synechococcus* (Fig. 3B) rapidly decreased their respiration rate when the light was turned on, and the minimal rate of respiration was reached

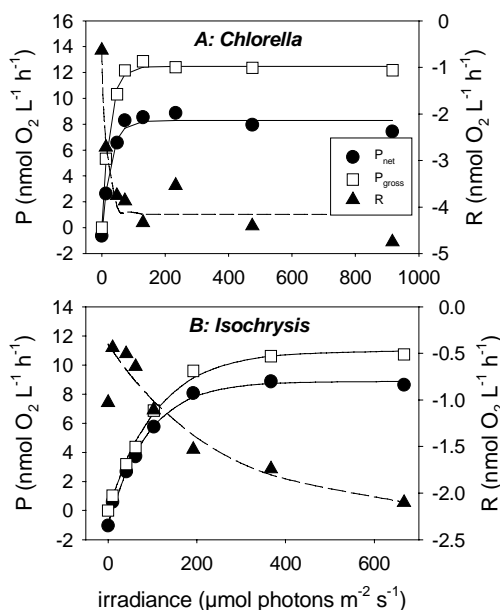


Fig. 2. Net (P_{net}), gross (P_{gross}) and respiration (R) as a function of irradiance for green algae *Chlorella* (A) and the haptophyte *Isochrysis* (B).

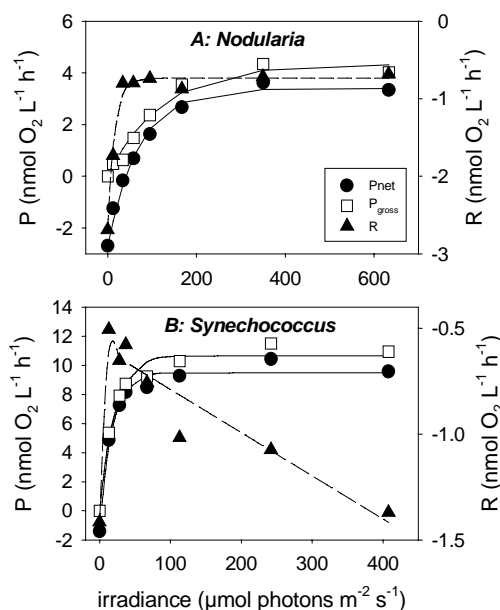


Fig. 3. Net (P_{net}), gross (P_{gross}) and respiration (R) as a function of irradiance for *Nodularia* (A) and *Synechococcus* (B).

before the rate of photosynthesis became maximal. Whereas the diazotrophic filamentous *Nodularia* maintained a minimal rate of respiration at saturating irradiances, *Synechococcus* BS4 showed an increase in the rate of oxygen uptake at saturating irradiances. Both cyanobacteria showed a rather small difference between P_{net} and P_{gross} compared to the eukaryotic algae. Oxygen uptake is not only mediated by respiratory electron transport, but can also be the result of oxygenase activity of RUBISCO (in photorespiration) or of reduction of oxygen by PSI in the Mehler reaction. The Mehler reaction donates electrons from PSII, via PSI to O_2 , producing both ATP and superoxide radicals, which are rendered harmless in a complex series of reactions (Asada 2000). The Mehler reaction plays an important role in dissipation of excess photons. Although photorespiration is depending on the O_2/CO_2 ratio, it also dissipates excess photons and electrons by consuming NADPH and ATP via the photorespiratory pathway, while at the same time it supplies CO_2 to the thylakoid membranes. These processes might thus play an important role at high light and we therefor studied the contribution of both processes by adding DCMU at the highest irradiance used in the P/E-curve). Fig. 4 shows an example of an experiment performed with the diatom *Thalassiosira pseudonana*. Adding DCMU to a sample exposed to an irradiance of $188 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, caused a 62% decrease in the rate of oxygen uptake, indicating a significant combined action of the Mehler reaction and photorespiration. At a higher irradiance of $388 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, the contribution of “dark” respiration was even less. The results obtained with other algae and data taken from other sources are summarized in table 1. It is clear that in all algal groups the Mehler reaction can contribute significantly to the overall oxygen uptake in the light. From the data in table 1 it can also be concluded that the different taxonomic groups do not differ in their Mehler reaction capacity: the range in the cyanobacteria was as high as

in the diatoms or in the green algae, despite the fact that respiration is regulated differently in cyanobacteria.

Discussion

Our results clearly demonstrate that light stimulates O_2 uptake in eukaryotic algae. That this stimulation takes part in the light-limited part of a P/E-curve corroborates the conclusion by Weger et al. (1989) who argued that respiration is substrate limited since the

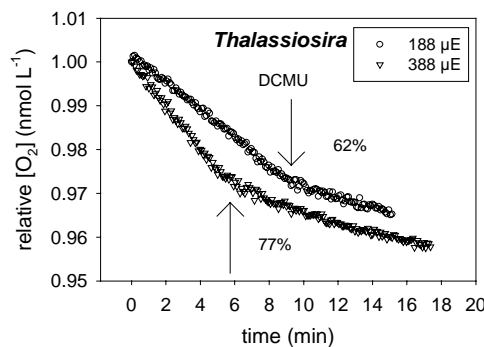


Fig. 4. The effect of addition of DCMU to block the Mehler reaction and photorespiration on a culture of the diatom *Thalassiosira pseudonana* at two different irradiances.

requirement for TCA cycle intermediates is likely to be higher in the light because of accelerated biosynthesis during photosynthesis. This also indicates that the degree of stimulation of respiration will be depending on the growth rate and nutrient conditions. The cyanobacteria investigated by us behaved quite differently. Upon an increase in irradiance there was a decrease in the rate of oxygen consumption, which became minimal before saturating irradiance were reached. In the 3 tested picoplankton species the rate of oxygen uptake increased again at saturating

irradiance. The filamentous *Nodularia* did not show an increase in oxygen uptake after the initial decrease (Fig. 3A). The decrease with increasing E can be explained by the fact that cyanobacteria share redox components on the thylakoid membrane for both photosynthesis and respiration. These processes thus interact in the light, causing an inhibition of respiration in the light (Schrerer 1990), although there is also good evidence of a spatial separation between photosynthesis and respiration in cyanobacteria (Peschek, 1996). Our results, however, clearly demonstrate an interaction between photosynthesis and respiration. The Mehler reaction might play an important role in preventing oxidative stress: in high light, oxygen may act as alternative electron acceptor to PSI, keeping the reduction state of Q_A in PSII constant. The concomitant ΔpH formed might activate the xanthophyll cycle and downregulate photosynthesis. The Mehler ascorbate reaction is thus an alternative electron sink for electrons produced by PSII, and might explain the discrepancy between C-fixation estimated from variable fluorescence measurements and measured rates of C-fixation in high light, as observed by Osmond & Grace (1995) and Flameling & Kromkamp (1998). But considering the fact that the electron flow associated to O_2 uptake seldom exceeded the electron flow associated with O_2 -production by more than 30-40%, the photosynthetic electron flow associated the Mehler reaction would be less than 30% of the total ETR. We believe that photorespiration only played a minor role in our experiments because: a), algae generally have very efficient carbon concentrating mechanisms (CCM, Raven et al., 2000); b) the oxygen-saturation at the end of incubations varied between 81-132%, and c), we noticed CO_2 production in the light or immediately after switching off the light, suggesting a very active CCM.

Table 1. Contribution of the Mehler reaction to total oxygen uptake in the light.

Species	Mehler (% R)	Source
<i>Trichodesmium</i>	48	Kana, 1993
<i>Synechococcus</i> <i>WH7803</i>	72	Kana, 1992
<i>Nodularia</i>	9	This study
<i>Synechococcus</i> <i>BS4</i>	26	This study
<i>Synechococcus</i> <i>BS5</i>	4	This study
<i>Pico-BS14</i>	76	This study
<i>S. costatum</i>	16	This study
<i>T. pseudonana</i>	60	This study
<i>T. weissflogii</i>	0	Weger et al., 1989
<i>P. tricornutum</i>	34-45	This study
<i>C. closterium</i>	23	This study
<i>Navicula</i>	9	This study
<i>Cryptomonas</i>	22	Lewitus & Kana, 1995
<i>I. galbana</i>	29-35	This study
<i>Pavlova</i>	25	Lewitus & Kana, 1995
<i>Closterium</i>	19	Lewitus & Kana, 1995
<i>Hemiselmis</i>	45	Lewitus & Kana, 1995
<i>C. autotrophica</i>	60-71	This study

We earlier suggested that an increased rate of substrate formation by photosynthesis would stimulate biosynthesis, concomitant with an increased rate of respiratory electron transport necessary to re-oxidize the reducing equivalents formed in the TCA-cycle. If the increase in light stimulated oxygen uptake is entirely due to Mehler activity, this hypothesis would not be valid. However, the observed stimulation of respiration in the eukaryotic algae was generally larger than can be explained by the Mehler reaction alone, supporting the hypothesis that part of the light stimulated oxygen uptake is indeed due to increased TCA-cycle activity supporting biosynthesis.

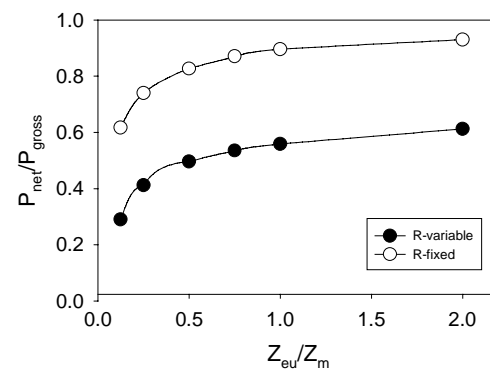


Fig. 5. The effect of the euphotic zone to mixing zone (Z_{eu}/Z_m) ratio on estimates on the the ratio of net (P_{net}) to gross (P_{gross}) photosynthesis.

Many primary productivity estimates made on marine phytoplankton are based on ^{14}C -based P/E-curves. Attempts to calculate net rates of primary productivity are based on assumption that respiration is a fixed percentage of P_{max} , which is species dependent (Langdon, 1993). The effect of light stimulated respiration on estimates of P_{net} will also be depending on the light climate experienced by the algae when circulating through the mixed layer. We calculated the effect using the P/E-curve shown in Fig. 2a for the green alga *C. autotrophica* at different ratio's of the euphotic zone (Z_{eu}) to the depth of the mixed layer (Z_m). Light stimulated respiration was calculated using the following equation:

$$R/P = (R/P)_{max} (1 - e^{-E \times \alpha_R / (R/P)_{max}}) + (R/P)_{min}$$

R/P is the ratio of respiration of photosynthesis, E the irradiance and α_R the slope of the R/E-curve. The results of the calculations can be seen in Fig. 5. In a transparent water column, the net primary production is approx. 40% of the gross primary production, and when the water column gets more turbid, respiratory losses increase slowly. When the Z_{eu}/Z_m -ratio drops below 0.5, the P_{net}/P_{gross} -ratio decreases rapidly. When it is assumed that respiration does not vary with irradiance and equals the amount measured at the start of a P/E-curve, a similarly shaped curve is found, but the respiratory losses are much smaller: thus, not accounting for the variability in respiration introduces an error as large as 33% in this example. This result clearly demonstrates that light stimulated rates of respiration can significantly affect true rates of net primary photosynthesis.

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