

Photosynthetic characteristics of five high light and low light exposed microalgae as measured with ^{14}C -uptake and oxygen electrode techniques

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Abstract

Tetraselmis suecica, *Thalassiosira pseudonana*, *Chaetoceros calcitrans*, *Isochrysis galbana* and a species of *Microcystis* were used in ^{14}C -uptake and O_2 -liberation experiments to determine light adaptation capabilities and differences between the two methods used. The algal cultures were exposed to high light (HL: $100 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) and low light (LL: $10 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) intensities for two days, prior to the measurements. The efficiency of light energy conversion (α^{P} =the initial slope of P/I-curves) and the maximum chlorophyll specific photosynthetic rates (P_m^{B}) were generally higher in the LL cultures. As to the α^{P} -values, the results are much more pronounced using the photosynthetic rates related to a per cell basis. The minimum quantum requirements (ϕ^{-1}) were generally higher in the HL-exposed cultures. The various photosynthetic parameters as measured with the oxygen chamber method

differed from those obtained with the ^{14}C -uptake measurements. On average higher P_m^B rates were measured using the O_2 -liberation method, whereas higher ϕ^{-1} were determined from the ^{14}C -uptake measurements. Care should be taken when oxygen evolution is converted into carbon uptake rates, because considerable differences were found in PQ (photosynthetic quotient)-values (O_2/CO_2) of HL and LL cultures of the same species and also between the species tested.

Key words: Microalgae, P/I-curves, Light adaptation, Photosynthesis measurement, ^{14}C -uptake, O_2 -production.

Résumé

Caractéristiques photosynthétiques, mesurées par assimilation de ^{14}C et électrode à oxygène, de cinq espèces de micro-algues adaptées à des éclairagements fort et faible

Tetraselmis suecica, *Thalassiosira pseudonana*, *Chaetoceros calcitrans*, *Isochrysis galbana* et *Microcystis* sp. ont été utilisées pour déterminer leur capacité d'adaptation et pour tester les différences entre les deux méthodes de mesure. Les cultures avaient été exposées à un éclairage fort (HL : 100 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) ou à un éclairage faible (LL : 10 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) pendant deux jours avant l'expérience.

L'efficacité de la conversion de la lumière (α^B = pente initiale des courbes photosynthèse-lumière) et l'activité photosynthétique spécifique maximale par unité de chlorophylle (P_m^B) ont été généralement plus élevées pour les cultures LL. En ce qui concerne α^B , les résultats sont plus contrastés si l'on compare l'activité photosynthétique par cellule algale. Le nombre minimal de quanta par carbone synthétisé (ϕ^{-1}) a été généralement plus élevé pour les cultures HL.

Les divers paramètres photosynthétiques mesurés par la méthode de l'électrode à oxygène ont été différents de ceux obtenus par celle du ^{14}C . Dans l'ensemble, les valeurs de P_m^B étaient plus élevées avec la méthode de l'électrode à oxygène, tandis que celles de ϕ^{-1} l'étaient pour les mesures avec le ^{14}C . Les importantes différences de quotient photosynthétique (O_2/CO_2) observées, aussi bien entre cultures HL et LL d'une même espèce qu'entre espèces différentes, soulignent la difficulté de convertir les productions d'oxygène en assimilation de carbone.

Introduction

The quantity of assimilated carbon fixed per unit area and time (Thienemann, 1931) through primary production generally determines the trophic level of aquatic ecosystems. A variety of methods are available to quantify the primary productivity in aquatic ecosystems, such as short-term (4 to 8 hours) measurements of oxygen release (Gaarder and Gran, 1927), ^{14}C -uptake (Stemann Nielsen, 1952), both by means of small flasks suspended over the water-column. Furthermore, primary productivity can be estimated from carbon dioxide accumulation (Ohle, 1956), or the rate of oxygen depletion in the hypolimnion during the stratified season (Hutchinson, 1983). Although the light quality and quantity differs over the photic zone, it is generally assumed that phytoplankton populations that circulate in the

epilimnion are more or less in a homogeneous physiological condition (Talling, 1957 a).

The vertical profiles of *in situ*-primary production rates have been used to determine the relationships between photosynthesis and irradiance (so-called P/I-curves, Talling, 1957 b). This, together with variations in the underwater light field and the phytoplankton biomass, allows for the estimation of primary productivity depth profiles during periods spanning days to years (Gächter, 1972; Bangerter, 1988). Several mathematical procedures have been proposed to calculate the areal primary productivity from such depth profiles (Vollenweider, 1965; Jassby and Platt, 1976). From this the trophic level of lakes could be judged (Friedli and Tschumi, 1981; Tilzer and Beese, 1988). Moreover, comparisons of data of several years of measurement can help to determine the effects of both eutrophication and lake restoration measures (Tschumi *et al.*, 1982).

In a review by Schanz and Wälti (1982) high variations were shown between the areal annual primary productivities of lakes with similar trophy. Sakamoto *et al.* (1984) noted that these variations may not be due to the different methods used for measuring primary production. It is also becoming more and more evident, that assumptions made in the past, have introduced errors in the calculations. The most important ones are:

1. The epilimnion is not a continuously mixed layer. The mixing depth is determined by the heat budget and the wind energy (Reynolds, 1989). Whenever the heat budget is positive and the weather calm, the epilimnion stratifies, thus stimulating the growth of many phytoplankton species (Cushing, 1989).

2. According to their growth regime (*e. g.* light; temperature and nutrients) phytoplankton species can adapt physiologically to a wide range of conditions (Kohl and Nicklisch, 1988). Algal adaptation depends on several factors such as changes in cellular pigment contents, differing pigment ratios, and/or enzymatic activity. Therefore, the extrapolation of short-term primary production measurements to longer periods renders doubtful results (Falkowski and Owens, 1980).

From the above it is apparent that the influence, of micro-temperature stratification in the euphotic zone and the movement therein on phytoplankton productivity, should be studied. Besides further research is needed on the adaptation capabilities of algae. In order to determine the effect of light-shade adaptation and the influence on the P/I-curves, cultures of five algal species were used in experiments to determine their photosynthetic responses. High (HL) and low light (LL) exposed algal cultures were used in ^{14}C -uptake and oxygen liberation experiments. HL and LL should be understood in relation to each other, where algae grown under the light intensities of 100 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (HL cultures) may still be light-limited. Results obtained with the above mentioned two methods were compared and discussed in respect to the determination of primary productivity.

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Materials and methods

Symbols, definitions and units

The following symbols, definitions and units were used:

α^B = slope of the linear part of the P/I-curve (at light limited photosynthesis) or the maximum efficiency of light energy conversion, chlorophyll *a* related, in mmol C or O₂ h⁻¹ mg⁻¹ Chl *a* (mmol quanta m⁻² h⁻¹)⁻¹.

α^C = the same as α^B , but related to a per cell basis as 10⁻¹⁵ mol C or O₂ h⁻¹ cell⁻¹ (mmol quanta m⁻² h⁻¹)⁻¹.

C_{ass} = assimilated carbon, in mmol C h⁻¹ l⁻¹.

Chl *a* = concentration of chlorophyll *a* in mg l⁻¹.

ϕ^{-1} = minimum quantum requirement at limiting light intensities, in mol quanta mol⁻¹ C or O₂.

I_k = light intensity at the onset of light saturation, in μmol quanta m⁻² s⁻¹.

P_{net} = net oxygen evolution measured in mmol O₂ h⁻¹ l⁻¹.

P^B = chlorophyll specific photosynthetic rate, in mmol C or O₂ h⁻¹ mg⁻¹ Chl *a*.

k_c = chlorophyll specific light attenuation coefficient of algal suspensions, in m² mg⁻¹ Chl *a*.

P_m^B = maximum chlorophyll specific photosynthetic rate, in mmol C or O₂ h⁻¹ mg⁻¹ Chl *a*.

P_{gross}^B = gross chlorophyll specific photosynthetic rate, in mmol C or O₂ h⁻¹ mg⁻¹ Chl *a*.

P_{net}^B = net chlorophyll specific photosynthetic rate, in mmol C or O₂ h⁻¹ mg⁻¹ Chl *a*.

R^B = chlorophyll specific respiration rate, in mmol C or O₂ h⁻¹ Chl *a*.

Algal material, culture conditions and chlorophyll determinations

The following algal species from CREMA-L' Houmeau were used in the experiments (originally axenic and cultivated under sterile conditions): *Tetraselmis suecica* (Chlorophyta, Volvocales), *Thalassiosira pseudonana* (Bacillariophyceae, Centrales), *Chaetoceros calcitrans* (Bacillariophyceae, Centrales), *Isochrysis galbana* (Chryso-phyceae, Chrysomonadales), and an undetermined *Microcystis* species (Cyanophyceae, Chroococcales).

The culture medium was that of Conway *et al.* (1976). The cultures flasks were either 2 or 5 liter Erlenmeyer-flasks and the cultures were continuously aerated with bubbling air and handshaken from time to time to keep the cells in suspension. The HL intensity was about 100 (5 Sylvania Gro-Lux F85W/GRO tubes at one-side and a distance of 10 cm) and the LL approximately 10 μmol quanta m⁻² s⁻¹ (2 tubes of the type Sylvania GRO-LUX F36W/GRO at the bottom in a distance of 30 cm from the culture vessel). The light path in the culturing flasks differed because of their different volumes. Duplicate cultures of each species were exposed to the

mentioned light intensities and all measurements were done 48 hours later (see also Fontvieille *et al.*, submitted).

Chlorophyll *a* was measured by fluorometry using a TURNER model 112 fluorometer and the method of Holm-Hansen *et al.* (1965).

P/I-curves

1. ¹⁴C-uptake

To about 170 ml of the various algal cultures, 2.78 × 10⁵ Bq NaH¹⁴CO₃ solution was added, whereafter 12.5 ml aliquots were dispensed in each scintillation vial. Twelve of these vials were placed into a photosynthesetron similar to that of Lewis and Smith (1983). An irradiance range of 0.7 to 440 μmol quanta m⁻² s⁻¹ was achieved in the photosynthesetron and incubations lasted for 1 hour (light samples). One sample was darkened by aluminium foil and incubated at the same temperature and for the same period as those in the photosynthesetron (dark sample). After the incubation period, the samples were acidified with 0.2 ml of 2 N HCl to a final pH of 2.0 to 2.3. Then air was passed through the samples for 30 minutes to remove all the extracellular H¹⁴CO₃⁻ in solution. After having added 10 ml Aquasol 2 (Du Pont) scintillation fluid, the radioactivity was determined as disintegrations per minute (dpm) using a Packard 1500 Tri-Carb Scintillation Counter, with external standardization.

The dissolved inorganic carbon contents (DIC, in mmol C l⁻¹) of the culture media were measured after acidification and passing the gas through an Infrared-Gas-Analyser (calibrated with a standard gas mixture). The assimilated carbon (C_{ass}, in mmol C l⁻¹ h⁻¹) was calculated as follows:

$$C_{\text{ass}} = \frac{(\text{Light-Dark}) \times 1.06 \times \text{DIC}}{^{14}\text{C}_{\text{add}} \times \text{time}} \quad (1)$$

where Light = incorporated ¹⁴C in the illuminated sample (dpm); Dark = incorporated ¹⁴C in the dark sample (dpm); ¹⁴C_{add} = ¹⁴C added at the beginning of the experiment (dpm); time = incubation period (h); 1.06 = discrimination factor to compensate for differences in the uptake of ¹²C and ¹⁴C.

2. Oxygen electrode technique

The equipment used was described in detail by Dubinsky *et al.* (1987), who also gives information on the characteristics, sensitivity and reproducibility of the electrode system. The measurements were done according to Schanz and Dubinsky (1988) allowing us to determine directly the net assimilation rate P_{net}. The mean light intensity in the oxygen chamber (I, in μmol quanta m⁻² s⁻¹) was calculated as follows:

$$I = (I_0 - I_2) (\ln I_0 - \ln I_2)^{-1} \quad (2)$$

where I_0 = light intensity through distilled water, measured behind the oxygen chamber with a calibrated 2π air quantum sensor (LI 190SB) and I_z = light intensity with the chamber filled with the algal sample.

3. Further determinations and calculations

The chlorophyll specific photosynthetic rate P^B was calculated as follows:

(i) in case of ^{14}C -uptake measurements:

$$P^B = C_{\text{ass}} (\text{Chl } a)^{-1} \quad (3)$$

and (ii) in case of oxygen electrode technique:

$$P_{\text{net}}^B = P_{\text{net}} (\text{Chl } a)^{-1} \quad (4)$$

The gross chlorophyll specific photosynthetic rate, P_{gross}^B , was calculated using:

$$P_{\text{gross}}^B = P_{\text{net}}^B + R^B \quad (5)$$

The maximum chlorophyll specific photosynthetic rate, P_m^B , is the constant value of P^B in the light-saturated part of the P/I-curve, which was usually calculated as the mean of four or five measurements. For the oxygen chamber technique P_{gross}^B values were used in the calculations.

The slope of the linear part of the P/I-curve, α^B , was determined by linear regression analysis (least square fit) using the production values in the light intensity range from 0 to $120 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$. The light intensity at the onset of light saturation, I_k , was the ratio of P_m^B to α^B . Nineteen of the twenty calculations were significant at the 1% level, whilst it was 2.4% significant for the LL-exposed *Tetraselmis*.

The mean chlorophyll specific light attenuation coefficient for chlorophyll *a*, k_c , was calculated using the light measurements behind the oxygen chamber (see Dubinsky et al., 1987) and the following equation:

$$k_c = \text{Ln } I_z (d \times \text{Chl } a \times \text{Ln } I_0)^{-1} \quad (6)$$

where d = thickness of the oxygen chamber cell (in m); $\text{Chl } a$ = chlorophyll concentration (in $\text{mg Chl } a \text{ m}^{-3}$) and for I_z and I_0 , see equation (2).

The minimum quantum requirement, ϕ^{-1} , was calculated from the equation given by Kirk (1983):

$$\phi^{-1} = k_c / \alpha^B \quad (7)$$

Results

The chlorophyll *a*-related maximum efficiencies of light energy conversion (α^B), for the HL and LL-exposed algae, for both the ^{14}C -uptake results and the oxygen

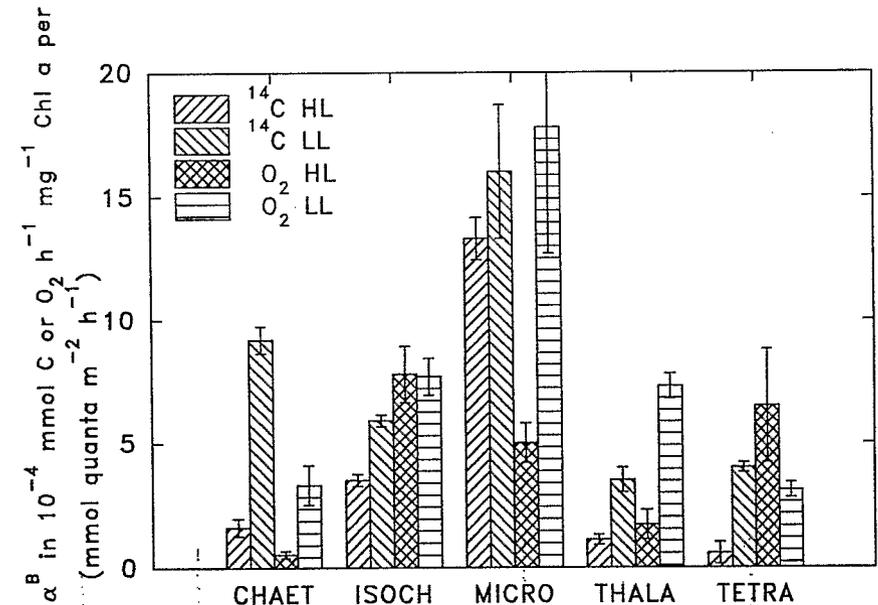


Fig. 1. The slopes of P/I-curves at limiting light intensities, α^B , of HL and LL algal cultures related to chlorophyll *a* with the standard deviations. CHAET = *Chaetoceros calcitrans*, ISOCH = *Isochrysis galbana*, MICRO = *Microcystis* sp., THALA = *Thalassiosira pseudonana*, and TETRA = *Tetraselmis suecica*.

chamber measurements are shown in Figure 1. In most of the measurements (eight out of ten) the LL algae showed a higher light energy conversion efficiency than the HL algae. The only two exceptions were found for *Isochrysis* and *Tetraselmis* and then only for the oxygen liberation measurements. The difference was not significant for *Isochrysis*, whereas it was for *Tetraselmis*. When the results of ^{14}C -uptake and the oxygen chamber measurements were compared, statistically comparable α^B -values were only found for the LL culture of *Microcystis* and the HL culture of *Thalassiosira*.

Shown in Table I is a comparison between α^B as measured with the two methods (O_2 and ^{14}C) and treatments (HL and LL) related to the species investigated. From this and the results shown in Figure 1, it is clear that not only do the α^B differ between the methods used, but also between the species and depending on whether they were subjected to HL or LL. The largest different in α^B between the two methods used, was for the HL *Tetraselmis*, where it came second of the algae used with the O_2 measurements and last for the ^{14}C assimilations (Table I).

TABLE I. — Comparison of the species dominance order as measured for α^B between the methods and treatments. Chaet = *Chaetoceros calcitrans*; Isoch = *Isochrysis galbana*; Micro = *Microcystis* sp., Thala = *Thalassiosira pseudonana*; Tetra = *Tetraselmis suecica*; HL = High Light-exposed cultures and LL = Low Light-exposed cultures.

Method	Treatment	Species dominance order
^{14}C	HL	Micro > Isoch > Chaet > Thala > Tetra
O_2	HL	Isoch > Tetra > Micro > Thala > Chaet
^{14}C	LL	Micro > Chaet > Isoch > Tetra > Thala
O_2	LL	Micro > Isoch > Thala > Chaet > Tetra

The data of the cell specific maximum efficiencies of light energy conversion (α^C), for the HL and LL-exposed algae, for both the ^{14}C -uptake results and the oxygen chamber measurements are given in Table II. The LL algae always showed higher values than the HL ones. In most of the species and treatments, the results of oxygen measurements were higher than those of ^{14}C . The exceptions were *Chaetoceros* HL and LL and also *Tetraselmis* LL.

TABLE II. — The slopes of P/I-curves at limiting light intensities, α^C , related to a per cell basis of four algal species exposed to high (HL) or low light (LL) intensities for two days. O_2 = oxygen liberation experiments; ^{14}C = ^{14}C -uptake experiments; α^C in 10^{-15} mol C or O_2 h^{-1} cell $^{-1}$ (mmol quanta m^{-2} h^{-1}) $^{-1}$; relative confidence interval of α^C (Rel. Conf. α^C) = $(\alpha_{95\%o, \text{upper}}^C - \alpha_{95\%o, \text{lower}}^C) / \alpha^C$ (in %), where $\alpha_{95\%o, \text{upper}}^C$ = upper 95% confidence limit of α^C .

	Treatment	O_2		^{14}C	
		α^C	Rel. Conf. α^C	α^C	Rel. Conf. α^C
<i>Chaetoceros calcitrans</i>	HL	0.006	49	0.019	38
	LL	0.039	38	0.119	12
<i>Isochrysis galbana</i>	HL	0.059	32	0.025	98
	LL	0.200	18	0.155	7
<i>Thalassiosira pseudonana</i>	HL	0.086	88	0.062	35
	LL	0.650	12	0.322	29
<i>Tetraselmis suecica</i>	HL	0.244	104	0.078	134
	LL	0.786	19	1.375	10

The minimum quantum requirements (Eq. 7) were calculated from the following k_c -values; *Tetraselmis suecica*, HL, $k_c = 0.015$ m^2 mg^{-1} Chl *a*, LL = 0.005; *Isochrysis galbana*, HL = 0.012, LL = 0.005; *Thalassiosira pseudonana*, HL = 0.005, LL = 0.007; *Chaetoceros calcitrans*, HL = 0.015, LL = 0.011; *Microcystis* sp., HL = 0.014, LL = 0.030. In all ten experiments except one, the minimum quantum requirements

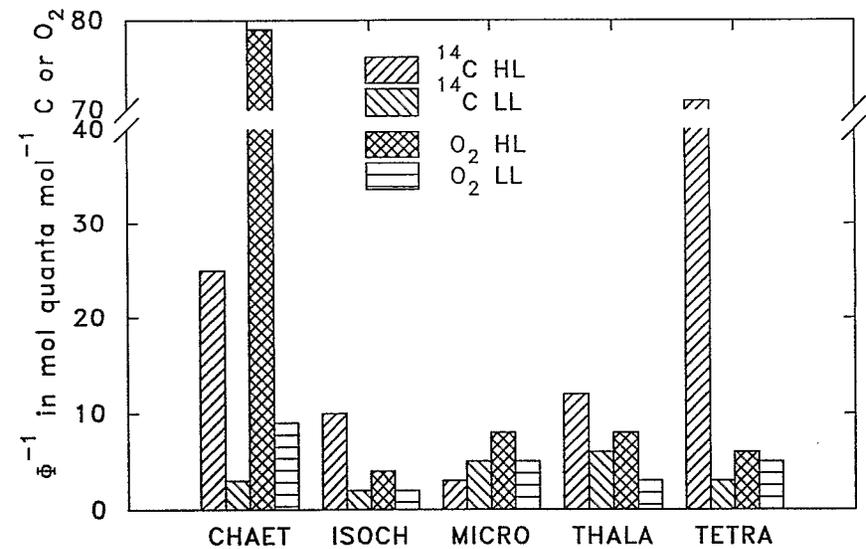


Fig. 2. The minimum quantum requirements, ϕ^{-1} , as measured by ^{14}C -uptake or by O_2 -release using HL and LL-exposed cultures. Abbreviations of the algae are the same as in Figure 1.

(ϕ^{-1}) showed that the HL cultures had higher quantum requirements than the LL cultures (Fig. 2). The only exception was for the HL *Microcystis* as measured with ^{14}C -uptake. The quantum requirements were low and sixteen of the 20 values were equal to, or below 10 mol quanta mol^{-1}C or O_2 .

The maximum chlorophyll specific photosynthetic rate (P_m^B), for the five algal species are shown in Figure 3. In all, but one of the measurements, the HL cultures had lower P_m^B -values than the LL adapted algae. The exception was for *Microcystis* as measured with ^{14}C -uptake. In general the oxygen chamber measurements lead to higher rates than the ^{14}C -uptake (Fig. 3 and the PQ-values given in Table III: the three exceptions from ten experiments were *Chaetoceros* LL, *Microcystis* HL and *Tetraselmis* LL). It should, however, be remembered that more O_2 is liberated than the C assimilated and that such a comparison is therefore not valid. Some of the differences between the two types of measurements shown in Figure 3 are statistically not significantly different from each other as indicated the high relative confidence intervals (*Isochrysis* HL, *Thalassiosira* HL, *Tetraselmis* HL).

The light intensities at the onset of light saturation (I_k), as well as their relative 95% confidence intervals are given in Table IV. For both ^{14}C -uptake and oxygen

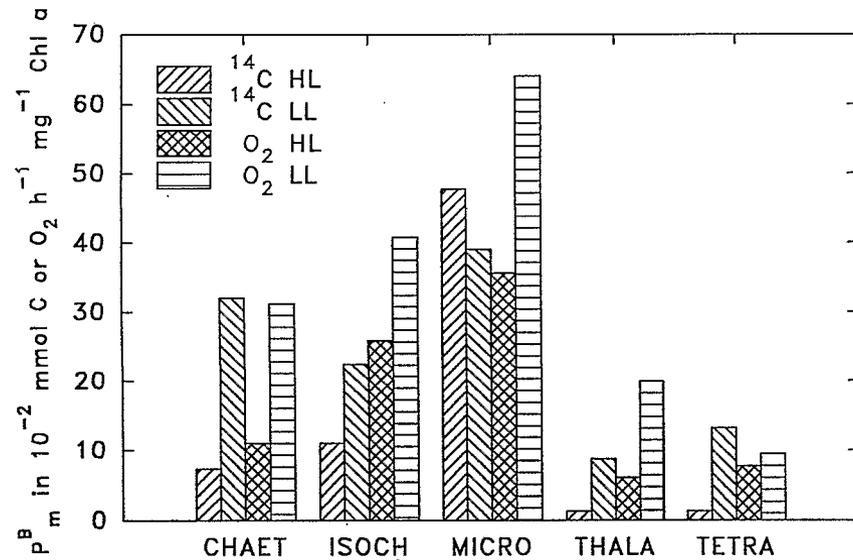


Fig. 3. Maximum chlorophyll specific photosynthetic rate, P_m^B , as measured by ^{14}C -uptake or by O_2 -release using HL and LL-exposed cultures. Abbreviations of the algae are the same as in Figure 1.

TABLE III. — The photosynthetic quotient PQ ($=\text{O}_2/\text{CO}_2$, dimensionless) at saturated light intensities of five algal cultures exposed to high (HL) and low light (LL) intensities. Avg = average of measurements, and sd the standard deviation ($n=4$).

Test Organism	PQ			
	HL		LL	
	Avg	sd	Avg	sd
<i>Chaetoceros calcitrans</i>	1.04	0.15	0.52	0.14
<i>Isochrysis galbana</i>	2.55	0.76	1.50	0.23
<i>Microcystis</i> sp.	0.57	0.10	1.46	0.23
<i>Thalassiosira pseudonana</i>	3.23	0.37	1.82	0.49
<i>Tetraselmis suecica</i>	5.35	0.35	0.94	0.33

chamber measurements the HL cultures of *Chaetoceros* and *Microcystis* showed higher I_k -values than the LL cultures. The high light exposed cultures of the other three species, however, had lower values than the low light exposed ones, with the exception for the O_2 measurements of *Thalassiosira*. The relative confidence intervals mostly are smaller with the ^{14}C -uptake method than with the oxygen chamber

TABLE IV. — Chlorophyll specific respiration rates, R^B , and light intensities at the onset of light saturation, I_k , of five HL and LL grown algal cultures. The measurements were from either ^{14}C -uptake or O_2 -liberation experiments. R^B , in 10^{-2} mmol C or $\text{O}_2\text{h}^{-1}\text{mg}^{-1}$ Chl a , calculated by means of the regression line where $I=0$ ($P^B = \alpha^B I + R^B$; P^B = chlorophyll specific photosynthetic rate, α^B = slope of P/I-curve and I = light intensity). The relative respiration rates (Rel. Resp.) = $-R^B/P_m^B$ are given as a percentage. I_k was calculated as the ratio P_m^B/α^B and the relative confidence interval of I_k (Rel. Conf. I_k) = $(I_{k95\%upper} - I_{k95\%lower})/I_k$ (in %), where $I_{k95\%upper}$ is the confidence limit of I_k calculated by the regression line using the upper 95% confidence limit of α .

Test organism	Treatment	Method	R^B	Rel. Resp.	I_k	Rel. Conf. I_k
<i>Chaetoceros</i>	HL	^{14}C	-0.16	2	129	39
		O_2	-2.06	19	578	55
	LL	^{14}C	-0.52	1.6	99	12
		O_2	-3.63	12	262	53
<i>Isochrysis</i>	HL	^{14}C	-0.16	1.4	89	16
		O_2	-6.6	25	91	31
	LL	^{14}C	-0.31	1.4	107	7
		O_2	-2.2	5.3	146	18
<i>Microcystis</i>	HL	^{14}C	-0.90	1.9	102	12
		O_2	-21.4	23	196	33
	LL	^{14}C	0.67	-1.7	69	33
		O_2	-21.4	33	100	63
<i>Thalassiosira</i>	HL	^{14}C	0.07	-5.0	34	35
		O_2	-3.0	49	97	91
	LL	^{14}C	0.13	-1.5	68	29
		O_2	-3.2	16	75	15
<i>Tetraselmis</i>	HL	^{14}C	0.13	-10	57	339
		O_2	-4.8	62	32	84
	LL	^{14}C	-0.04	-0.3	91	10
		O_2	-1.5	16	86	17

technique. The exceptions are for LL *Thalassiosira* and HL *Tetraselmis*. The I_k -values of the HL and the LL cultures were significantly different from each other (at the 5% confidence level; Sachs, 1984).

The chlorophyll specific respiration rates (R^B) as determined from the y-intercept of the linear regression lines of the P/I-curves are also given in Table IV. The method used (based on Jassby and Platt, 1976) implies that photosynthetic rates are net rates. Whereas this is true for O_2 -values, it is extremely doubtful for ^{14}C -values, especially in short incubation experiments (Harris, 1978). All of the y-intercepts calculated for O_2 experiments were <0 , yet this was not so for the ^{14}C -rates (exceptions being *Chaetoceros* and *Isochrysis*). For the ^{14}C -uptake experiments the rates varied between -0.90 to $+0.67 \times 10^{-2}$ mmol C $\text{h}^{-1}\text{mg}^{-1}$ Chl a and for the oxygen between -21.4 to -1.5 . If we compare the respiration rates to the maximum photosynthetic rates (Table IV: Rel. Resp.), the ^{14}C -method gave values between 0.3 and 10%, whereas with the O_2 -procedure it ranged between 5.3 and 62% (8 of 10 values $>15\%$). The highest relative respiration rates were measured for *Thalassiosira* and *Tetraselmis*.

Discussion

For many years the dependence of photosynthetic rates on light intensities (P versus I), has been used to characterize the potential activity of the photosynthetic apparatus (Blackman, 1905). Several mathematical models have been proposed to describe P/I-curves (summarized by Kohl and Nicklisch, 1988). In most of these, α^B and P_m^B are included as constants. Iteration procedures are available to estimate these constants by computer (e.g. Ben-Zion and Dubinsky, 1988). In general a simple plot of the photosynthetic rates against the corresponding light intensities shows that many models do not accurately predict the P/I-curve (some examples are given in Tilzer, 1984). We, therefore, decided to calculate α^B by linear regression of all points showing linearity in P/I-plots (we looked for minimum probability values when calculating the regression lines). P_m^B was determined as the mean values of more or less constant P^B -values at light saturation intensities. We consider these procedures to provide the most realistic estimates of α^B and P_m^B .

Dubinsky (1980) has shown that considerable variation were found in the P/I-curves of the same alga, when grown under different light intensities. Morel *et al.* (1987) have shown that this adaptation period could be less than one generation time. However, in general it is assumed that adaptation requires one generation time. In a review, Reynolds (1984) reported generation times for laboratory algal cultures of between 2 hours and 6 days. Cullen and Lewis (1988) showed that the time needed for chemical or physiological changes in algal cells after a light shift depends on several factors investigated and that there is a hysteresis of adaptation depending on exposure from low to high or high to low light. The algal experiments done by Post *et al.* (1985) showed faster adaptation when LL cultures were exposed to HL than when HL cultures were exposed to LL. It seems, therefore, that adaptation kinetics differ in the two directions of adaptation. Under natural conditions minimal generation times reported are around 20 hours (early summer stratification: *Cryptomonas ovata*; Sommer, 1981), but for most of the species generation times, even during phytoplankton spring bloom, are about 30 hours (Bleiker and Schanz, 1989). Our cultures were exposed for 48 hours to the two light regimes, which should be long enough for the algae to have adapted.

Light adaptation can have an effect on both α^B and P_m^B , depending on the strategy of photoadaptation (Richardson *et al.*, 1983). These authors found that HL cultures in general showed low values of α^B and high values of P_m^B , whereas LL cultures have high α^B and low P_m^B . In our experiments, as expected, the α^B -values of the LL-exposed algal cultures mostly were considerably higher than those of the HL-exposed ones. This difference has been ascribed to changes in the transfer efficiency of antennae pigments (Beardall, 1976). P_m^B was mostly lower for cells grown at high light intensities than for those grown at low light, which is in contrast to the above mentioned expectation. This could be the result of photoinhibitory damage at or near the reaction center of PSII by high energy bands of the fluorescence lamps used (Samuelsson and Richardson, 1982). Gerath and Christholm (1989) have shown that P_m^B decreased during illumination and that this continued into the first

hours of a dark period, for the dinoflagellate *Amphidinium carteri*. They also found decreasing slopes of P/I-curves (i.e. α^B) at limiting light intensities. Changes in the photosynthetic performance also occurred over the cell cycle when a diurnal light/dark cycle was imposed, but this did not occur with constant light. Dujardyn and Foyer (1989) found evidence of photoinhibition in the leaves of *Hordeum vulgare*, when they moved from low light (280 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) to high light intensities (1,400 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$). Initially they observed a marked increase in photosynthetic activities and subsequently a slow decrease by limiting amounts of ribulose-1,5-biphosphate, which they related to a restriction of the supply of reducing equivalents. These results are in some contrast to those of Sukenik *et al.* (1987), who pointed out that the absolute rate of light-saturated photosynthesis is limited by carbon fixation rather than electron transport.

In our experiments, the HL cultures were illuminated continuously at 100 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ which is normally not high enough to inhibit photosynthesis. It can be supposed that in fact both treatments really result in more or less low light adapted cultures. In most experiments done to study light adaptation of algae, P_m^B increased with increasing light intensities (e.g. Tilzer and Goldman, 1978; Post *et al.*, 1985; Meffert, 1989). The unexpectedly low P_m^B rates of the HL cultures are ascribed to:

1. the relatively low light intensities used for the HL-cultures,
2. the possible damaging effects of the high energy bands emitted in the spectrum of the fluorescence tubes,
3. a possible high sensitivity (genetic) to light of some of the species used in our experiments,
4. and a greater sensitivity to photoinhibition, because of the relatively low initial growth light intensity.

This conclusion is partly supported by the extremely high quantum requirements of the HL cultures of *Chaetoceros*, *Thalassiosira* and *Tetraselmis*. Falkowski *et al.* (1985) found that the quantum requirements increased, when algal populations were adapted to high light intensities. However, they did not find quantum requirements higher than 70 mol quanta mol⁻¹ C or O₂ for either *Isochrysis galbana* or *Thalassiosira weissflogii* (see also Morel *et al.*, 1987). We measured a quantum requirement of 79 mol quanta mol⁻¹ O₂ for *Chaetoceros* and 71 mol quanta mol⁻¹ C for *Tetraselmis* (Fig. 2). However, the quantum requirements were generally low and may reflect the low adaptation light intensities to which the algae were subjected to.

Evidently adaptation occurred in our cultures as we observed differences in α^B . From the results it is also clear that great differences existed between species and methodology, being either in terms of O₂-liberation or ¹⁴C-uptake. We found no influence of light intensity on the photosynthetic quotient (PQ). The calculated values were in the range 0.6 to 5.4 and only the latter value is outside the data published by Williams and Robertson (1991). These authors explained the wide PQ-range from 0.5 to 3.5 as an effect of the nitrogen sources used and the metabolites formed. However, they found good evidence that PQ-values of natural

algal cells ranged between 1.0 and 1.36. High PQ-values may also be explained by photo-oxidative processes, but it is doubtful whether these played a role in our experiments.

The respiration rates varied considerably, where relative respiration rates between 5.3 and 62 % were measured with the oxygen method. Although a rate of 10 % is commonly used, Grobbelaar and Soeder (1985) measured rates of 4 to 20 % in outdoor mass algal cultures and Grobbelaar et al. (1990) used a rate of 3 to 41 % in a predictive production model. It is also known that the light history greatly affects the respiration rates of algae (Grobbelaar and Soeder, 1985) and Falkowski and Owens (1978) measured an average respiration of 25 ± 16 % for algae grown at light saturation. The fact that negative relative respiration rates were measured with the ^{14}C -method, confirms the doubt of Harris (1978) concerning this method for such measurements. Also, it supports the many conclusions that the ^{14}C -method measures a rate between net and gross primary production.

The time course of any specific primary production rate changes mainly, because of differences in photoinhibition and the fact that turbulent mixing neutralises this loss factor (Vermij et al., 1985). Because of the time required for light-shade adaptation to take place, this process according to Falkowski and Wirick (1981), plays a secondary role and may not affect integral column productivity at all. This is because a single planktonic cell is normally not able to adapt within the time span of the wind induced mixing of the uppermost water layers of the water column. The time-scale of such mixing modes range from minutes to a few hours (Spigel and Imberger, 1980). Consequently all phytoplanktonic organisms in the turbulent epilimnetic water layers have more or less the same physiological state. However, during windless periods temporary thermal stratification can occur for hours to days, which might be long enough to induce photoadaptation (Cullen and Lewis, 1988). Besides adaptation which could influence the photosynthetic characteristics of the entire phytoplankton population, we have shown that different results are obtained for the same alga using either ^{14}C -uptake or O_2 -liberation measurements. Caution should, therefore, be exercised when such results are interpreted.

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