



The influence of irradiance on the apparent photosynthetic quotient in the unicellular alga *Pycnococcus provasolii*

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Abstract: The influence of irradiance on the apparent photosynthetic quotient (PQ) was studied for cultures of the unicellular alga *Pycnococcus provasolii*. The apparent PQ was in the range 0.7 to 4.2. and two trends of variation with irradiance were apparent: a) PQ's decreased with increasing incubation irradiance above saturation levels; b) At any given incubation irradiance PQ's were generally higher for algae grown at higher irradiances. Possible explanations to these patterns are discussed. It is suggested that variations of PQ in phytoplankton can partly be due to mechanisms related to photoacclimatization.

Résumé : L'influence de l'éclairement sur le quotient photosynthétique apparent de l'algue unicellulaire *Pycnococcus provasolii*.

L'influence de l'éclairement sur le quotient photosynthétique (PQ) apparent pour des cultures de l'algue unicellulaire *Pycnococcus provasolii* a été étudiée. Le PQ apparent a oscillé entre 0.7 et 4.2 et nous avons mis en évidence deux types de variation par rapport à l'éclairement : a) les PQ apparents ont diminué avec l'éclairement d'incubation pour des valeurs situées au dessus du niveau lumineux de saturation de la photosynthèse ; b) pour un éclairement d'incubation donné, en général, plus l'éclairement auquel l'algue a été acclimatée était élevé, plus le PQ apparent était grand. Les explications possibles pour ces types de variation sont discutées. Il apparaît que les variations du PQ dans le phytoplancton peuvent être dues, en partie, à des mécanismes en relation avec la photoacclimatation..

Keywords : photosynthetic quotient, phytoplankton, irradiance, *Pycnococcus provasolii*.

Introduction

The photosynthetic quotient (PQ) is defined as the molar ratio of O₂ produced to CO₂ fixed in the process of photosynthesis. Based on the stoichiometry of the photosynthetic reactions and the works of Ryther (1956),

the PQ had conventionally been assumed to range between 1.1 and 1.35 with an average of 1.25. Later on Williams et al. (1979) proposed to separate the PQ into two components, the 'carbon PQ', which is dependent on the ratio of glucids, lipids, proteins and nucleic acids produced and is considered to range between 1.1 and 1.35, and the 'nitrogen PQ' which is determined by the state of reduction of the inorganic nitrogen source assimilated by the algae. Davies and Williams (1984) thus suggested that for phytoplankton

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growing on nitrate, total values of PQ in the range 1.8 to 2 could be expected. However, Laws (1991), after a detailed examination of the biochemical composition of phytoplankton and the chemical equations for the production of the phytoplankton cell constituents has more recently suggested that for growth on nitrate the PQ lies around 1.4 and for growth on ammonium the PQ takes the value of 1.1.

Most of the empirical values of PQ have been obtained by comparison of oxygen production measurements using either the Winkler assay or electrochemical sensors and the ^{14}C derived CO_2 fixation measurements. The disagreements between the O_2 and the ^{14}C approaches have rarely been found to be explainable in terms of the expected theoretical PQ (e.g. Williams & Robertson, 1991) and therefore the term "apparent PQ" has been used for PQs estimated as the ratio of gross oxygen production to $^{14}\text{CO}_2$ assimilation (see for example Williams, 1993). These discrepancies prompted speculation on what is measured exactly by each of the techniques used to estimate primary production. There is still great uncertainty as to whether the ^{14}C -technique (Steemann Nielsen, 1952) measures gross or net primary production or something in between. With the light and dark oxygen technique there are basically three difficulties: a) the uncertainty of whether the rates of dark respiration measured in the dark are comparable to the rates of dark respiration in the light; b) the failure to account for the rates of photorespiration, leading to an underestimation of the rates of gross primary production; and c) the failure to distinguish between the photosynthetically produced oxygen and the oxygen liberated from photochemical reactions unrelated to carbon synthesis.

The measurement of water column primary production is an essential step for determining the trophic structure of the plankton, as well as for establishing the role of the ocean in the regulation of atmospheric CO_2 (i.e. the biological pump). Therefore the determination of what is measured by each of the techniques used to measure primary production and whether this varies as a function of environmental and/or biological factors is an unresolved question to which attention should be paid. The investigation of the factors that may have an influence on the apparent PQ can provide useful information in this field of research.

In the present work the influence of irradiance on the apparent PQ in laboratory cultures of the picoplanktonic alga *Pycnococcus provasolii* Guillard, 1991 has been investigated.

Material and Methods

Pycnococcus provasolii (clone $\Omega 48-23$) (division Chlorophyta, class Micromonadophyceae) is a pico-

ultraplankton sized alga that is very common in oceanic waters (Guillard et al., 1991). The algae were grown in natural seawater (salinity ≥ 33 PSU) enriched with Guillard's f/2 nutrient recipe without added silicate (Guillard, 1980) at various irradiances (25, 140, 260 and $370 \mu\text{E m}^{-2}\text{s}^{-1}$) under continuous light and 20°C .

Algae from each of the cultures grown at different irradiances (acclimatization period of several weeks) were incubated for 3 hours in a light gradient box at 20°C in order to measure the rates of photosynthesis over a range of photon flux densities (10 to $2500 \mu\text{E m}^{-2}\text{s}^{-1}$). Details about the incubations for these measurements are given in Iriarte & Purdie (1993).

The rates of photosynthesis were measured using two methods, the ^{14}C technique to determine the rates of CO_2 fixation (Steemann Nielsen, 1952) and the light and dark oxygen technique to measure the gross oxygen production. The concentration of dissolved oxygen was measured with the Winkler titration technique, using an automated and microprocessor controlled titration system with a photometric end point detector (Williams & Jenkinson, 1982).

The rate of gross primary production versus irradiance was fitted to the equation of Platt et al. (1980) (including the photoinhibition parameter) by nonlinear least square regression analysis, using a modified Marquardt procedure for the minimization of sum of squares (Nash, 1979) (P vs. I curve).

In the light gradient box, the rates of carbon fixation and the rates of oxygen production cannot be measured at exactly the same irradiance. Therefore apparent PQs were estimated and expressed as a function of irradiance, by dividing the equation of the oxygen based P vs. I fitted curves by the equation of the carbon based ones.

The biomass of the algae was estimated in terms of carbon content. Particulate organic carbon was determined by combustion in oxygen of samples retained in precombusted GF/F filters and measurement of the CO_2 produced in an infra-red gas analyser.

Results and Discussion

The apparent PQs obtained in the present work for cells of *Pycnococcus provasolii* are presented in Fig. 1. These ranged between 0.7 and 4.2 and two trends of variation with irradiance were apparent: a) PQs decreased with increasing incubation irradiance above saturation levels, i.e. relatively less oxygen production was detected at high photon flux densities; b) at all incubation irradiances, PQs were markedly lower for algae acclimatized at lower irradiances, decreasing from values around 4 for algae grown at $370 \mu\text{E m}^{-2}\text{s}^{-1}$ to around 2 for algae grown at $25 \mu\text{E m}^{-2}\text{s}^{-1}$. Thus,

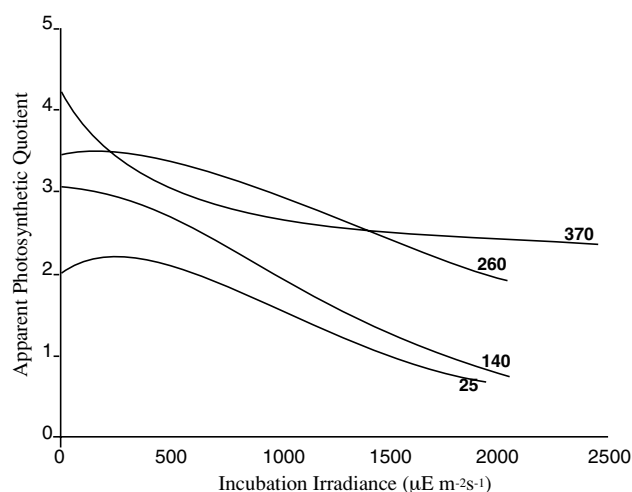


Figure 1. Apparent photosynthetic quotient estimated over a range of incubation irradiances for cultures of *Pycnococcus provasolii* grown at various irradiances (25, 140, 260 and 370 $\mu\text{E m}^{-2}\text{s}^{-1}$).

Figure 1. Variation du quotient photosynthétique apparent en fonction de l'éclairement pour des cultures de *Pycnococcus provasolii* préalablement acclimatées à différents éclaircements (25, 140, 260 et 370 $\mu\text{E m}^{-2}\text{s}^{-1}$).

growth at high irradiances resulted in comparatively less carbon fixation per unit of O_2 produced than growth at low irradiances.

The algae are assumed to have grown on nitrate, given that the culture medium was prepared with nitrate (900 μM). According to Davies and Williams (1984) PQs in the range of ca. 2 would have been expected on grounds of the nitrogen PQ, whereas following Laws (1991) the PQs should be 1.4. However, the apparent PQs estimated in the present work showed great variability and values obtained encompassed widely the expected ones. PQs markedly different from the theoretical PQs have been measured in a number of works, both in field measurements with mixed plankton populations as well as in laboratory measurements with monoalgal cultures (see Table 1).

There is still great uncertainty as to whether the ^{14}C technique measures gross or net primary production or something in between. Some workers support the hypothesis that rates of $^{14}\text{CO}_2$ uptake represent gross primary production rates only with experimental incubation periods of less than 1 hour and under conditions of maximal growth rates (Harris & Piccinin, 1983). Others, however, have suggested that with incubation periods such as those used in the present study (i.e. 3 hours) $^{14}\text{CO}_2$ uptake rates are much closer to gross primary production rates than to net primary production rates (e.g. Williams, 1993).

Results obtained in the present work are in agreement with those obtained in other studies with monoalgal cultures

Table 1. Summary of apparent PQs in natural plankton assemblages and monoalgal laboratory cultures.

Tableau 1. Résumé des valeurs du quotient photosynthétique apparent (PQ) publiées pour des communautés planctoniques naturelles et des cultures monoalgales.

Mixed plankton populations

Location	Apparent PQ	Reference
<i>Marine</i>		
Coastal waters off Hawaii	0.92-1.66	Williams et al. (1983)
Coast of Finland	0.60-2.40	Kuparinen (1985)
English Channel	1.60-4.50	Holligan et al. (1984)
English Channel	1.20-3.70	Garcia (1989)
Canadian Arctic	1.30-1.80	Platt et al. (1987)
<i>Freshwater</i>		
Lake Erken (Sweden)	1.47-1.75	Bell & Kuparinen (1984)
Lake Conzanz (Switzerland)	0.60-1.98	Sakamoto et al. (1984)
Lakes Huron and Michigan (Canada)	1.58-4.90	Fahnenstiel & Carrick (1988)

Monoalgal cultures

Species	Apparent PQ	Reference
A range of species	0.90-1.90	Eppley & Sloan (1965)
<i>Dunaliella</i> sp.	1.10-2.25	Williams et al. (1979)
<i>Selenastrum capricornutum</i>	1.14-8.00	Andersen & Sand Jensen (1980)
<i>Pavlova lutheri</i> and <i>Glennodinium</i> sp.	1.00-1.80	Burris (1981)
<i>Pycnococcus provasolii</i>	0.70-4.20	Present work

and with mixed natural plankton populations in which the apparent PQ also showed a tendency to increase at low incubation irradiances (Andersen and Sand Jensen, 1980; Fahnenstiel & Carrick, 1988). It has been suggested that at low irradiances algae incorporate $^{12}\text{CO}_2$ produced in respiration in preference to dissolved ^{14}C -bicarbonate (Andersen & Sand Jensen, 1980). Furthermore, Harris & Piccinin (1983) have suggested that at low growth rates ^{14}C is more readily transferred from photosynthetic to respiratory pathways. Therefore at these low irradiances the rate of $^{14}\text{CO}_2$ uptake underestimates the rate of gross CO_2 fixation. On the other hand, Megard et al. (1985) have pointed out that much of the reductant generated during oxygenic photosynthesis is used for nitrate reduction at low irradiances, because "... the enzymes (nitrate reductase and nitrite reductase) for reduction of nitrate to ammonium have higher affinities for reductant at low irradiances than the enzymes for reduction of carbon dioxide have".

The marked depression of the photosynthetic rates observed at high incubation irradiances (above saturation level) in the oxygen P vs. I curves was not apparent in the

carbon based P vs. I curves. This suggests that the decrease in the photosynthetic rates was not essentially due to photochemical damage to the photosynthetic apparatus, unless these damages had affected more severely the oxygenic photosynthesis. Thus the decrease of the apparent PQ at irradiances above saturation was most likely due to an underestimation of the gross photosynthetic rate by the oxygen method resulting from the failure to account for the losses due to photorespiration. A similar trend of variation has been observed in measurements undertaken with laboratory cultures of *Gyrodinium aureolum* Hulburt, 1957 (Garcia, 1989).

For *Pycnococcus provasolii* the values of apparent PQ measured over a range of irradiances during short incubation periods varied as a function of the photon flux density at which the algae had been growing prior to the photosynthesis measurement. The effect of the incubation irradiance and that of the growth irradiance, however, were opposite. A similar trend of variation of reduced PQs for cells grown at low growth irradiances has also been observed for the dinoflagellate *Gyrodinium aureolum*, but the opposite trend was noted for the green algae *Brachiomonas* sp. (Garcia, 1989). The results obtained for *Pycnococcus provasolii* and *Gyrodinium aureolum* are also somewhat contrasting with some observations of increased apparent PQs with depth in field measurements with natural plankton communities (Megard et al., 1985; Fahnenstiel & Carrick, 1988). Some workers have argued that for some algal species PQs will tend to increase with decreasing growth irradiance because at these low growth irradiances these species tend to have lower C:N assimilation ratios (Falkowski et al., 1985). However, the magnitude of the apparent PQs has not always been explainable in terms of the C:N assimilation ratios (Megard et al., 1985).

Given the different trends of variation of PQ with growth irradiance observed for different phytoplankton species it can be hypothesized that the light-induced effect is species-specific and therefore it probably reflects the different strategies of photoadaptation/acclimatization. With the data obtained in the present study it is not possible to determine the exact mechanisms involved, but they show that growth at low light not only enables cells of *Pycnococcus provasolii* to achieve higher rates of photosynthesis when exposed to an array of irradiances, but it also results in relatively more photoreductant channeled to carbon reduction. It should be remembered that the ¹⁴C-method measures carbon fluxes, whereas the oxygen technique gives results more associated with energy fluxes (Williams et al., 1979).

Results obtained in the present study thus suggest that when analysing data on phytoplankton primary production rates, measured with the ¹⁴C technique and with an oxygen technique, it would be advisable to know, not only the irradiance at which these rates have been measured, but also

the light conditions at which the algae have been growing prior to undertake the photosynthesis measurements and the strategies of photoacclimatization that they display.

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