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Poster

Photosynthetic parameters of planktonic microalgae by fluorometric and ossimetric measurements, for the estimate of pelagic primary production.

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Abstract

Phytoplankton is the main pelagic primary producer. Models evaluating primary production, integrated along the water column, are based on the correct estimate of photosynthetic rates, traditionally measured by gas exchange (e.g. moles of evolved oxygen). A quick, sensitive, not invasive method for photosynthesis estimate is modulated variable fluorescence. Values of photosynthetic parameters depend on the measuring protocol: experiments performed on *Skeletonema costatum* and *Phaeodactylum tricornutum* show that photosynthetic rates depend both on the colour of the actinic light (ETR_{max}-blue>ETR_{max}-white, E_c-blue<E_c-white) and on the saturation pulse intensity (ETR_{max} higher at a higher saturation intensity). Photosynthesis is influenced also by the physiological state of the sample: in nutrients limiting conditions, alpha, ETR_{max} and Fv/Fm are reduced. In light-limiting condition, there is linear correlation (R^2 >0.87) between photosynthetic rates by variable fluorescence (ETR) and by evolved oxygen (O_2P^B). From this correlation it is possible to calculate the photosynthetic speed and thus the moles of transferred electron / moles of evolved oxygen ratio. These results show that the analysis of variations in the photosynthetic parameters enables the interpretation of results as physiological state of cells population. However the measuring protocol which has been chosen has to be carefully taken in account.

Keywords: phytoplankton; primary productivity; photosynthetic rates; variable fluorescence; oxygen evolution; physiological state

1. Introduction

Mathematical models for the estimate of pelagic primary production are based on the correct estimate of photosynthetic rates. Traditionally, photosynthetic rates are measured by gas exchange, such as fixed radioactive carbon or evolved oxygen. Nevertheless gas exchange measurements are not specific for photoautotrophics, because they measure net production rates, as the difference between gross photoautotrophic production and autotrophic and heterotrophic respiration rates, unavoidable *in situ*.

Variable fluorescence measurements are an alternative approach for primary production estimate, specific for photoautotrophics: at room temperature, only photosystem II (PSII) chlorophyll *a* molecule,

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which is present in every photoautotrophic capable of oxygenic photosynthesis, emits red fluorescence. Fluorescence measurements are quick, non invasive and very sensitive. They allow the estimate of photosynthetic rates as electron transport rate ETR = PAR*Y, where PAR is the photosynthetically available radiation (μE m^{-2} s^{-1}, 400-700 nm) and Y is the fluorescence efficiency (Genty *et al.* 1989). Environmental factors as light intensity, temperature, O_2, CO_2 and nutrients concentrations influence the *in vivo* fluorescence emission.

The purpose of this research is to analyse and interpret the variation of the photosynthetic rate measured by modulated variable fluorescence on phytoplanktonic cultures. We first studied the variations of photosynthetic parameters with different measurement protocols (OXYPAM experiment). Since the photosynthetic yield depends also on the physiological state of the cells, we also studied the variation of fluorescence parameters in a batch culture of *Skeletonema costatum* (ETA' experiment). P-E curves parameters alpha, P_{max} and Ek obtained by variable fluorescence on *Skeletonema costatum* and *Phaeodactylum tricornutum* were compared with those by evolved oxygen, to investigate correlation between the different measurement methods and to compare production data by fluorescence and by oxygen exchange (OXYPAM experiment).

The more ecological purpose of comparing these different methods is obtaining indirect estimates of production by variable fluorescence measurements only, for a more complete knowledge of spatial-temporal variations of pelagic primary production.

2. Materials and methods

The experiments were conducted on monospecific cultures of diatoms (*Skeletonema costatum* and *Phaeodactylum tricornutum*) from the algae collection of the Laboratory of Marine Ecology. Coltures were cultivated in "f/2" medium (Guillard & Ryther 1962) at a temperature of 18-19°C, in white light, within a PAR range of 50-100 μE m^{-2} s^{-1} (Biospherical QSL-100).

During the ETA' experiment, 90 ml of culture were diluited in 900 ml of "f/2" medium (t0 time) and kept in a thermostat (18-19°C) in white light at a mean PAR of 130 μE m^{-2} s^{-1} for two weeks, without renewing the medium.

2.1. Variable fluorescence measurements

Variable fluorescence was measured by a modulated pulse fluorometer PAM (Walz), equipped with a PAM-101 unit, a PM-101 detector (with RG9 filters, Schott), the L450 diode as exiting source, two light sources for the saturating pulse (length: 0.8 s) and the actinic light: the led lamp HPL-C (Walz) which emits blue light, or the KL-1500 lamp (Schott) which emits white light. The time of acclimation at each PAR was 90 s.

2.2. Absorption coefficient a_λ

Absorption coefficients of the cells suspension a_λ *(m^{-1})* at nine wavelenghts (412, 440, 488, 510, 555, 630, 650, 676 and 715 nm) were measured by the spectrophotometer AC-9 (Wetlabs). The values were corrected with the absorption of the filtered (Millipack 0.22 μm) culture and the value a_{715} was subtracted to remove the turbidity and temperature effects. Finally, spectra of the chlorophyll-specific absorption coefficients a^* (m^2 mg chl a^{-1}) were calculated.

2.3. Dissolved oxygen

Oxygen measurements were made by a liquid-phase polarographic electrod (Oxygen Electrod Disc, Hansatech). The measuring chamber was not thermo stated, since the temperature increase between the beginning and the end of the measurements was less than 1°C. The stirrer speed was 60 r/min. Each sample was preacclimated for 15 minutes in the dark to reach a stable signal. We added the saturation pulse to the actinic light, so as to make P-E curves by the two methods, fluorometric and ossimetric, as much comparable as possible. The same measuring protocol was used both in white and in blue light.

3. Results and discussion

3.1. Measuring protocol of P-E curves

Measuring protocol of P-E curves: P-E curves were made by PAM fluorometric measurements on *S. costatum* and *P. tricornutum* in actinic and saturating blue and white light, at three and two different saturation pulse intensities (Fig. 1). In *S. costatum*, the values of ETR_{max}-blue are two to fourfold lower than the corresponding ETR_{max}-white. *P. tricornutum* shows similarly ETR_{max}-blue values 2-2.5 times lower than the corresponding ETR_{max}-white. Furthermore, as saturation intensity increases both the two species show an increase in ETR_{max} which is more evident in white light than in blue light.

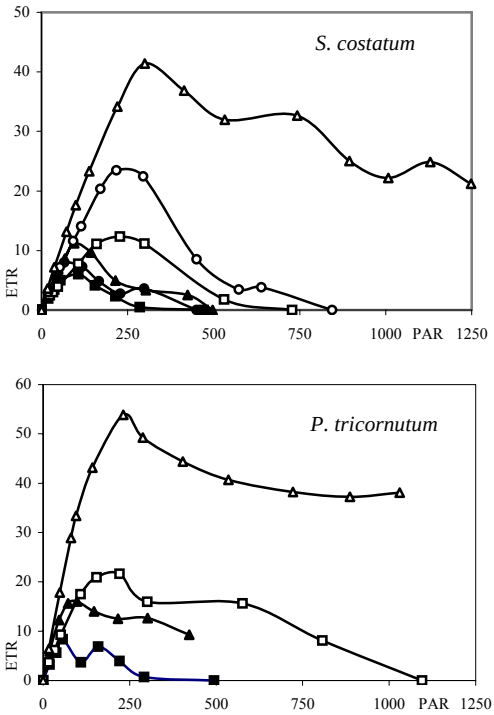

Fig. 1: PAM P-E curves (OXYPAM experiment) in the two species, in blue (filled symbols) or white (empty symbols) actinic light at different saturation pulse intensities (square: 900 μE m^{-2} s^{-1}; circle: 1600 μE m^{-2} s^{-1}; triangle: 2000 μE m^{-2} s^{-1}).

In *S. costatum*, oxygen P-E curves were measured at two different saturation pulse intensity: P_{max}-blue is lower than P_{max}-white. The only couple of P-E curves for *P. tricornutum* shows quite similar values of P_{max}-blue and P_{max}-white (Fig. 2). In both the two species, the compensation point E_c is lower in blue light than in white light.

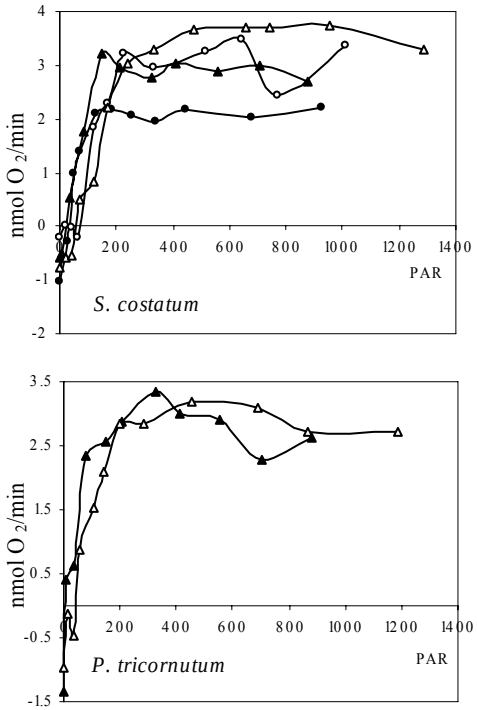

Fig. 2: oxygen P-E curves (OXYPAM experiment) in the two species, in blue (filled symbols) or white (empty symbols) actinic light. In *S. costatum* measurements were made with two different saturation pulse intensities (circle: 1600 μE m^{-2} s^{-1}; triangle: 2000 μE m^{-2} s^{-1}).

The pulse modulated fluorometer PAM (Walz) makes possible to measure photosynthetic rates indipendently from the actinic light. Nevertheless, these measurements show that the light source colour causes significant variations in the shape of the P-E curves. A partial explanation can be found in the *in vivo* absorption spectrum (*a**) of the two algal species. The comparison between the values of *a** and the emission spectra of the two light sources, the blue and the white ones (Fig. 3), shows that at a given value of incident PAR, i. e. number of photons sent to the sample, the fraction of blue photons actually absorbed is greater than for the white ones. A consequence is the quicker saturation of photosystems in blue light at a lower PAR, which means an earlier saturation. The lower E_c-blue than E_c-white obtained with evolved oxygen supports this hypothesis. A further explanation can be found in an unbalance between photosystems, caused by an

uncomplete overlapping of absorption and action spectra of photosystems. This occurrence is frequent in chlorophytes and cyanobacteria (Falkowski & Raven 1997). Nevertheless, values of P_{max} by evolved oxygen don't show marked variations as the colour of the light source changes (less than 50%).

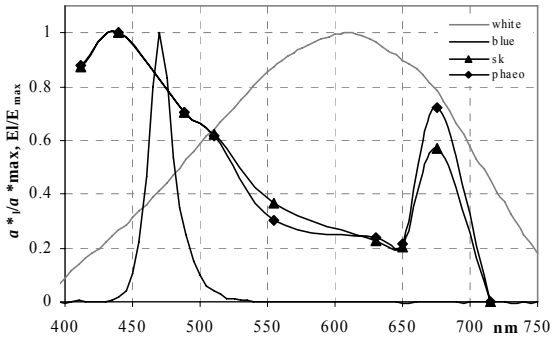

Fig. 3: comparison between the emission spectra of the two light sources (blue and white) and the absorption spectra of the two species.

The electron transport rate ETR increases with the saturation pulse intensity, from 900 to 1600 to 2000 µE m^{-2} s^{-1}. It can be assumed that 900 µE m^{-2} s^{-1} is a fluence rate which is not enough to close all the photosynthetic units. Since the large variability of saturation pulse intensities found in literature, it's not possible to fix a general value of intensity, which is indipendent from the phytoplanktonic species examined. The right pulse should rather be chosen for each experiment through some preliminary tests (Mouget and Tremblin 2002; Ting & Owens 1992).

3.2. Effects of the physiological state of the sample on P-E curves

During the "ETA' " experiment on a batch culture of *S. costatum*, PAM fluorometric parameters Fv/Fm, alpha, ETR_{max} and the growth rate µ (div/day) were studied. Fv/Fm decreases from t4 to t9, then it becomes stable while alpha decreases as far as t12. Both ETR_{max} and µ decrease progressively from t9 to t14 (Fig. 4).

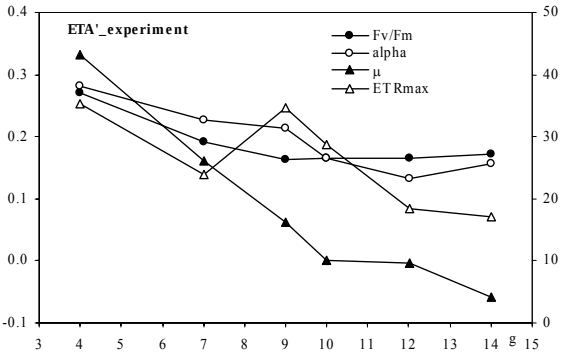

Fig.4: PAM fluorescence parameters (Fv/Fm, alpha, ETR_{max}) and growth rate (µ) with time in *S. costatum* (ETA' experiment).

Pigments ratios did also reveal the aging of the culture, as the total carotenoids to chlorophyll *a* ratio changes from 0.6 to 0.9 and to 1.12 passing from t2 to t9 and t12.

Stress generally caused by environmental factors can determine the decrease of variable fluorescence parameters Fv/Fm, alpha and ETR_{max}. Thus, through the analysis of these parameters it is possible to assess the physiological state of the sample. During the experiment, the batch culture of *S. costatum* consumed nutrients and accumulated catabolits, with a consequent decrease of the growth rate µ. The increase of carotenoids content against chlorophyll *a* content and the decrease in the P-E parameters could mean an increase of stress starting from t9. In particular, Fv/Fm, which can reach values of 0.8-0.7 in optimal growth conditions, decreases to very low values, suggesting a small efficiency in light harvesting (Krause & Weis 1991).Thus the analysis of fluorometric P-E curves' shape supports the hypothesis that the culture entered a phase of stasis, followed by aging, since the 9th day from the inocule.

3.3. Correlation between alternative measurements of photosynthetic rates

The comparison of photosynthetic rates by evolved oxygen $P^B(O_2)$ and by variable fluorescence (ETR) for both the two species of diatoms, both in blue and in white light (Fig. 5), shows linear relationships for the limiting and saturating light conditions, while for the photoinibiting part of the curve ETR values are

lower than the corresponding $P^B(O_2)$. In fact the large photoinibition at high PAR shown by variable fluorescence PAM P-E curves is nearly absent in the oxygen curves. Usually, many comparisons between photosynthetic yields, measured on different photoautotrophic species, show at high PAR photosynthetic rates by gas exchange lower than rates by variable fluorescence, owing to NPQ (non photochemical quenching) mechanisms. These are an alternative sink for the electrons, instead of the non cyclic transport chain, and reduce the potential damage due to excess light absorption (Geel *et al*. 1997; Flameling & Kromkamp 1998).

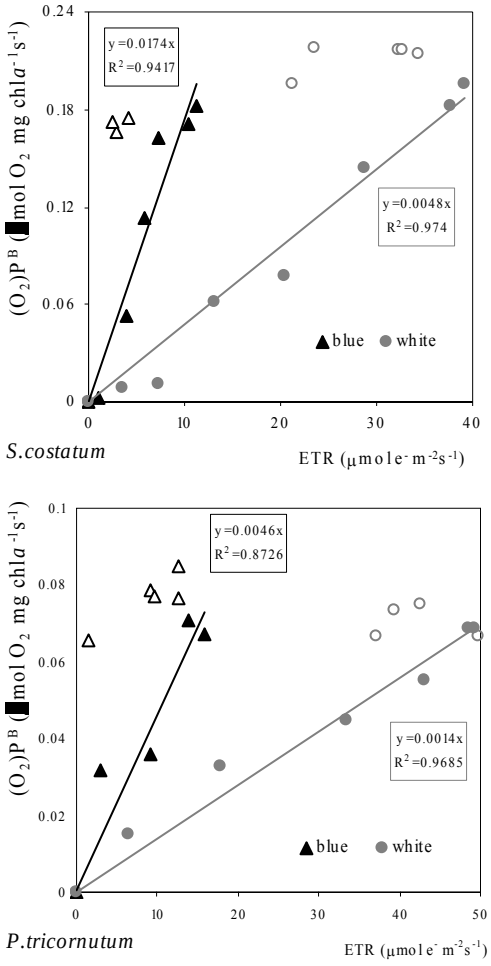

Fig. 5: covariation between variable fluorescence (ETR) and by evolved oxygen (O_2) P^B rates, in blue and white light, for the two species. In the squares, the parameters of the linear regressions relative to the limiting and saturating light intensities are shown.

Nevertheless, at high PAR we measured ETR< $P^B(O_2)$. Also Carr & Björk (2003) and Beer & Axelsson (2004) have similar findings and they propose as explanation the underestimate of fluorescence yield Y due to the chosen acclimation time at each PAR: not long enough to enable the complete relaxation of the fluorescence kinetics from *Fm'* to *Ft*. Thus the underestimate of Y should be tightly dependent on the modalities of PAM usage and maybe independent on the examined species.

4. Conclusions

To apply most of the models of aquatic primary productivity it's essential to correctly measure photosynthetic parameters by P-E curve. These parameters are subject to variations, according to the measuring protocol (correct estimates of *Fo*, *Fm*, *Ft*, *Fm'*), which depends on both the light source quality and the saturation pulse intensity. The collected datas thus show the importance of the choice of the measuring protocol.

Photosynthetic parameters variability depends also on physiological factors: in *S. costatum* nutrient limitation and cellular aging caused the decrease not only of the growth rate, and also of the photosynthetic efficiency and rates. Thus informations about the cells physiological state are easily achievable from the shape of variable fluorescence P-E curves.

The analysis of the PAR ranges of linear relationship between photosynthetic rates measured by the two different methods (fluorometric and ossimetric) enables to emphasize the limits of each method and to make hypothesis about the causes at biophysical and cellular scales (i.e. electron sinks, alternative to non cyclic transport). Once the ranges of linear correlation are fixed, it's possible to quantify the ratio between the photosynthetic rates by the two methods. Thus, it would be more simple, efficient or less time-consuming to make measurements by the quickest and less invasive method (the fluorometric one) and hence deduce the evolved oxygen rate.

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