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New approaches to acquire photo-physiological information from natural freshwater phytoplankton communities.

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Introduction

Freshwater ecosystems are bio-optically and photo-physiologically different from marine systems. In turbid waters chemical gradients are nearly lacking, but light intensity can vary by several orders of magnitude due to high density of particulate matter, whereas in stratified lakes gradients can be very steep. These gradients change in space and time producing patchiness in phytoplankton distribution and activity (Becker et al. 2001). Traditional bottle techniques are not adequate to assess true primary production (PP) and become extremely expensive and time-consuming. However, water agencies monitoring eutrophication have to measure true PP as carbon fixed per area and time. This is the only parameter which allows the calculation of nutrient fluxes. Most of the data are still collected by sampling methods on the basis of oxygen production rates or radiocarbon isotope incorporation. The latter will become less usable in freshwater systems due to the increasingly restrictive permission practice. We have recently been working on the development of a new method that allows for direct and reliable PP measurements baced on PAM fluorescence.

An important issue here is whether the photosynthetic electron transport rate is a reliable measure of dry mass related PP. Numeral processes influence the relationship between photosynthesis and biomass: respiration, photorespiration, excretion of organic carbon, Mehler reaction, nitrogen assimilation. Several authors estimated the excretion rate under varying environmental conditions (Berman-Frank & Dubinsky, 1999). Under physiological conditions less than 10% of organic material are lost, even under high light which causes excretion rates to rise. It is still under discussion if excretion is a mechanism to keep the photosynthetic reaction chain running under rate limiting conditions (Berman-Frank & Dubinsky, 1999). Supporting this assumption Leboulanger et al. (1998) postulated an extracellular excretion of amino acids under high light along with a reincorporation in dark period. Active transport of CO_2 under C-limiting conditions leads to reduced ratios of photosynthetic electron transport per dry weight produced (Rotatore et al., 1995). Fluorescence based electron transport rates plotted against oxygen production have yielded linear relationships with varying slopes. Recently we have shown that, under identical geometric conditions for both fluorescence and oxygen measurement the slopes, of PI-curves are identical. At light saturation physiological regulation leads to non-linear relationships

(Gilbert et al. 2000). This non-linearity was shown to depend on the species and on the physiological state. In this study we addressed the following problems: (1) reliability of bio-optical modeling of oxygen production based on fluorescence data, (2) relationship between photosynthetic electron transport rates and true biomass, (3) new approaches to aquire species specific information of PM in natural systems.

Materials and methods

Cultures of diatom *Phaeodactylum tricornutum* (Bohlin) were kept at 20°C and 45 µmol m^{-2} s^{-1}. The algae were grown in the exponential growth phase in semicontinuos cultures in 16/8 h light/dark cycles in modified medium described by Provasoli et al. (1961). Chlorophyll determination was taken through according to Jeffrey & Humphrey (1975). In cultures cell absorption was measured with a ZEISS M 500 spectrophotometer. For single cells from natural phytoplankton probes a Zeiss Diode Array (MCS) spectrometer coupled with a ZEISS microscope "Axioskop" was used for absorption spectra measurement. The same microscope was coupled with a MICROSCOPY PAM (Walz, Effeltrich, Germany) equipped with a blue LED for actinic light.

Oxygen measurement was carried out simultaneously with a Clark type electrode and micro-optode (Microx 1, Presens Regensburg, FRG). The set-up consisted of a temperature-controlled ED-101 US cuvette compartment (Walz, Effeltrich, Germany) and a Light Pipette (Illuminova (Uppsala, Sweden) as the source for continuos actinic light. This set-up allowed for simultaneous determination of the variable Chl a-fluorescence with a Xe-PAM (Gilbert et al., 2000b) as well as for two independent oxygen monitors. The absorbed photosynthetic radiation (Q_{phar}) and the bio-optically based model for oxygen production were calculated as described by Gilbert et al. (2000).

For oxygen and biomass determination *Phaeodactylum tricornutum* were subjected to a permanent light period of 8-12 h by 20°C under 50, 200 and 1000 µmol m^{-2} s^{-1} in 1 L Erlenmeyer flasks. The increase in biomass was determined by dry weight analysis. For biomass composition, 5 mg of freeze-dried *Phaeodactylum tricornutum* were analysed by element analysis before and after the light period. In the flasks, the oxygen production was continuously measured via microoptodes. The samples were bubbled with N_2 to keep oxygen concentration below supersaturation. For the direct comparison between oxygen release and biomass production we assumed the consumption of 4 electrons per molecule carbon, 10 electrons per molecule N in the amino acids (Falkowski & Raven, 1997) and one electron per reduced H in the biomass. Sulphate reduction accounts for only about 1% of the total photosynthetic electron flow and was neglected.

Results and Discussion

The optode technique is advantageous for long-term measurements, as it does not consume oxygen itself. Therefore, we decided to use this technique to quantify photosynthetic oxygen evolution during biomass accumulation experiments. In order to test the reliability of optode measurement in illuminated cell suspensions at high oxygen pressure we introduced a micro-optode in addition to a clark electrode in the setup for PI-measurements. Fig. 1 shows the results for two physiologically different cultures of *Phaeodactylum tricornutum*. The photosynthetic rates measured via optodes turn out to be practically identical to those measured with the Clark-type electrode.

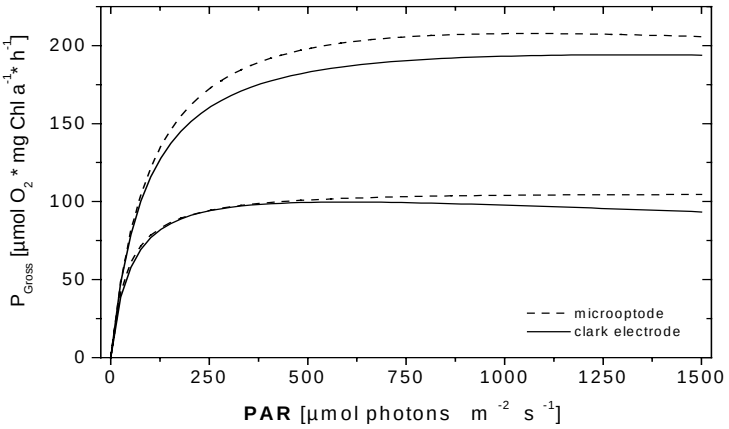

Fig 1. PI-curve from HL and LL-grown *Phaeodactylum* cells measured via Clark-type electrode (solid line) and via micro-optode (broken line)

Fluorescence quantum yields were multiplied with the incident light intensity, and the photosynthetic activity is expressed as relative electron transport rates in the resulting PI-curves. However, these relative rates are of limited importance, as the ratio of incident to absorbed light in algae is highly variable due to differences in pigmentation and adaptation. Absolute electron transport rates are much more informative and allow for realistic quantitative PP estimates. Given the spectrum of the light source as well as the absorption, one can calculate Q_{phar}, and the absolute electron transport rates. Dividing these rates by the quantum yield for oxygen (8), the results can be directly compared to Clark-type measured photosynthetic rates. The outcome of this experiment is shown in Fig. 2.

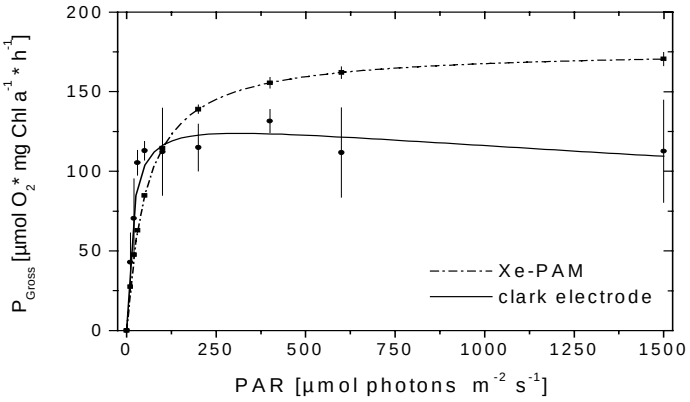

Fig 2. PI-curve from *Phaeodactylum* measured via a Clark-type electrode (solid line) and modeled oxygen evolution on the basis of fluorescence data obtained with a Xenon-PAM (broken line)

The photosynthetic rates calculated from bio-optically measured electron transport rates and Q_{phar} fit the direct measurement very well, especially in the linear part of the curve. Under light saturation certain discrepancy occurs, which can be attributed to cyclic electron flow (around PSI as well as PSII) or by the Mehler reaction (Geel, 1999, Gilbert et al. 2000). In follow-up studies we are going to show that differences between net oxygen production and corresponding PSII electron transport rates can be used as a tool to study the regulation of electron distribution under different physiological conditions. This may support important ecophysiological information e.g. abuot changes in the ratio of C/N assimilation or P-limitation.

In the third experiment, the relation between absolute amount of oxygen produced in a 12 h measurement and the absolute values of carbon, nitrogen and hydrogen assimilation during this period was examined (Fig. 3). The light intensity used for this experiment was 50µmol

photons m^{-2} s^{-1} at which oxygen production and PSII electron transport rates yield identical results (see Fig. 2).

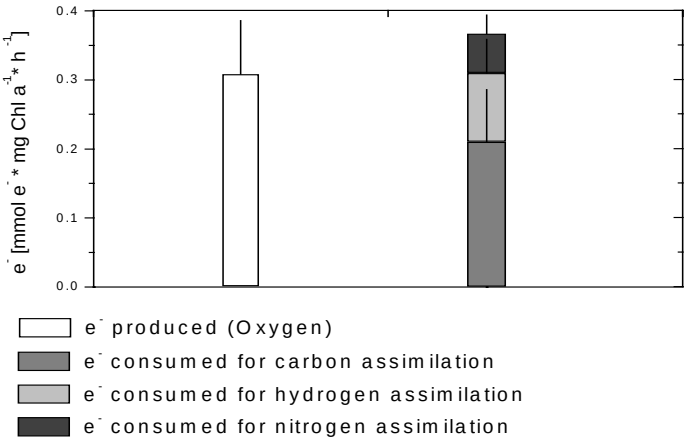

Fig. 3 The Photosynthetic electrons stemming from water oxidation (white bar) in comparison to the electrons stored in the assimilated biomass during permanent illumination of limited intensity (12 h with 50 µmol $m^{-2}s^{-1}$)

Summing up the electrons needed for the assimilation of these three elements the outcome is equivalent to the electrons determined from oxygen production. To examine the possible influence of changes in light conditions an the relationship shown above, the experiment was carried out under saturating light intensities as well (Fig. 4).

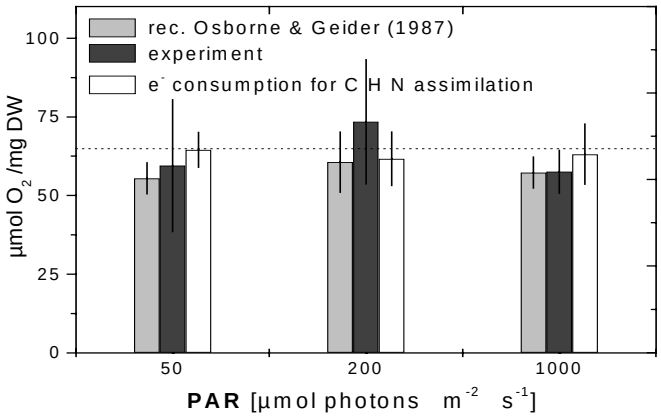

Fig. 4. Comparison of electrons stemming from water oxidation (black bar) and electrons stored in the biomass related to dry weight (DW). The horizontal dotted line indicates a 1:1 stochiometry of stored and photosynthetically measured electrons.

The results bear clear evidence of total electron flow is being a reasonable measure of absolute PP under permanent light, even under light saturation. It can be concluded that, given the photosynthetic quantum yields and the ratio of incident to absorbed light, the bio-optical modeling delivers reliable absolute values for PP in freshwater systems.

However, this approach does not yield taxon-specific information. Dealing with freshwater, the challenge in measurement of the PP or growth rates of cyanobacteria in relation to other phototrophs is an important possibility to assess the risk of developing toxic blooms. In a recent paper Becker et al. (2001) have shown that by means of flow cytometry the chlorophyll content per cell and/or per biovolume can be measured. The possibility of sorting cells of interest will enable us to measure their photosynthetic characteristics. However, preparative cell sorting is expensive and time consuming and thus not appropriate for routine analysis. Therefore, we developed a single cell measuring setup. To aquire values of PP single cell measurements should provide for a $_{PHY}$ (for Q_{phar} calculation) as well as photosynthetic quantum yields via variable Chl a fluorescence. The latter one is commercially available as

so-called MICROSCOPY-PAM whereas the other was developed on the basis of a diode array spectrophotometer from ZEISS coupled to a Microscope.

In fig. 5 are shown a comparision of a $_{PHY}$ measurements from conventional spectrometers with those carried out by single cell spectrophotometry.

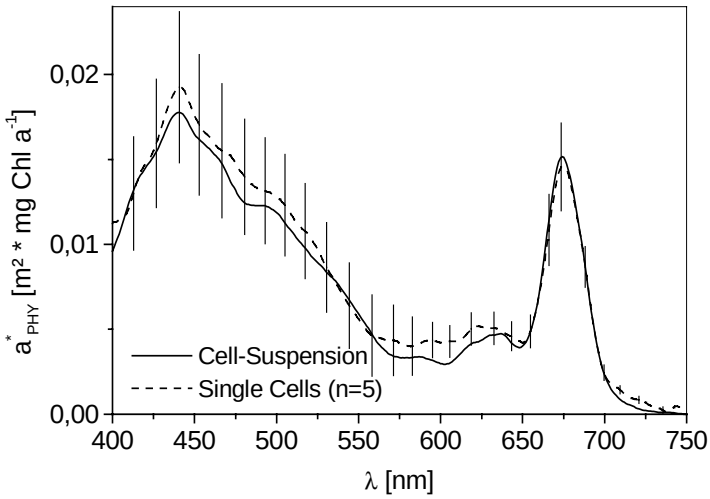

Fig. 5. Determination of the normalised in-vivo absorption coefficient a $_{PHY}$ of *Phaeodactylum* culture by conventional methods (solid line) in comparison to single cell absorption spectroscopy (dotted line)

In fig. 6 absorption spectra of single cells numeral taxa in natural phytoplankton from lake Auensee (Leipzig, Germany) were diagrammed. After the determination of the chlorophyll a content per cell via flow cytometry, each taxon´s a $_{PHY}$ can be computed.

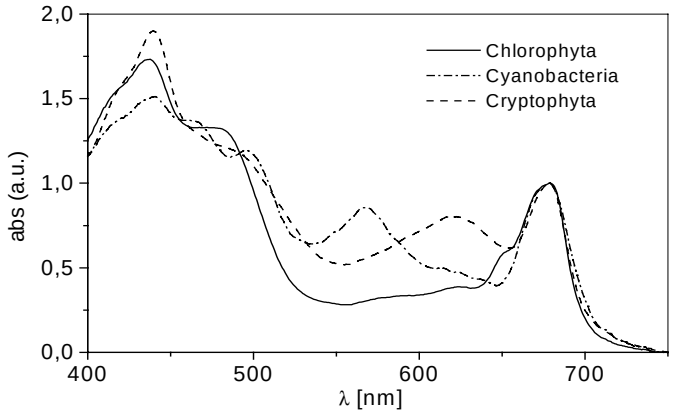

Fig. 6. Absorption spectra of single cells from *Chlorophyta*, *Cyanobacteria* and *Cryptophyta* from phytoplankton samples taken from lake Auensee**.**

For each taxon a cell specific PI-curve can be determined as shown in fig. 7. Again, in combination with the incident light the fluorescence measure yields have to be converted relative electron transport rates into absolute values through single cell Q_{phar}**.**

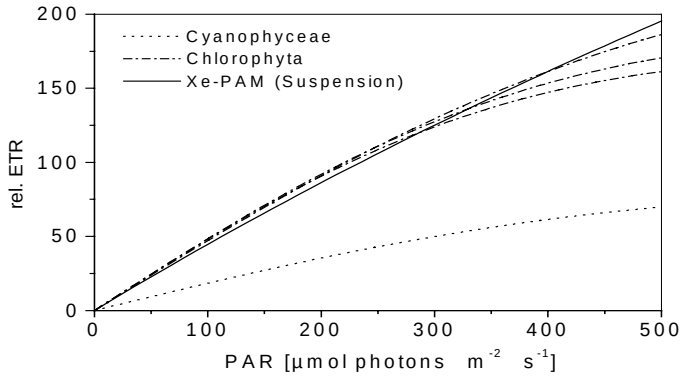

Fig. 7. PI-curve measured with natural phytoplank-ton population via a Xenon-PAM (solid) in comparison to single cell measurements via a MICROSCOPY-PAM for cyanobacteria (dotted) and *Chlorophyta* (broken line)

The latter are shown exemplarity for the model

organism *Phaeodactylum* in Fig 8. The comparison of a PI-curve from a cell suspension with a single cell measurement shows considerable differences. These concern the initial slope, as well as the P_{max} values. We do not know yet, if these differences are due to the different actinic light source or due to other effects e.g. the temperature control .

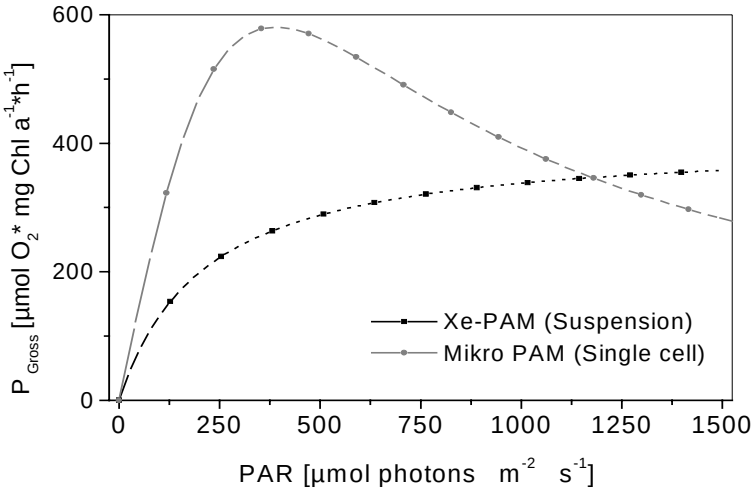

Fig. 8. Bio-optically modeled oxygen evolution on the basis of fluores-cence data from cell suspension by means of Xenon-PAM and by MICROSCOPY-PAM from single cells.

However, our data reliable show that this approach will not only be able to differentiate between spectrally different groups, but also between cells of different size or other bio-optical relevant features. Further work has to be done to optimize the single cell system in a way that also absolute values of PP per cell become measurable.

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