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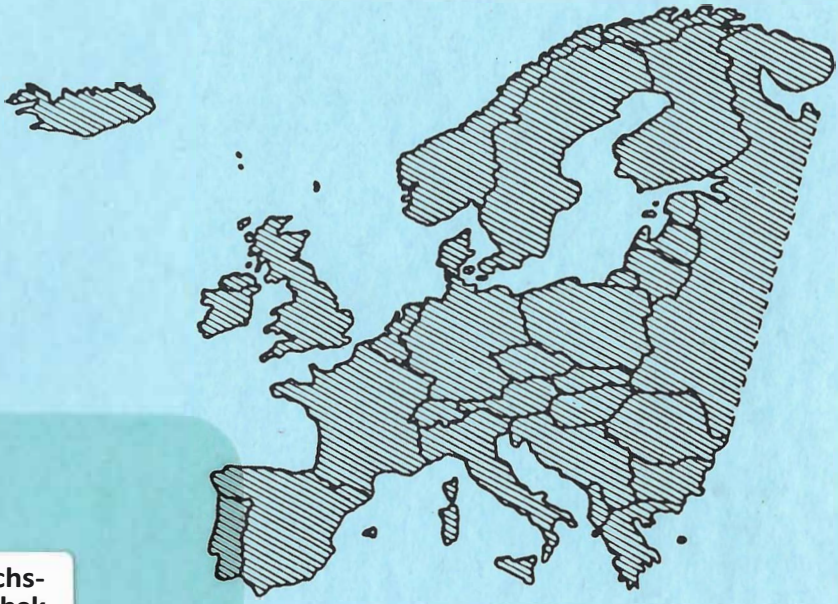

UNIVERSITÄT ROSTOCK

Rostocker Meeresbiologische Beiträge

Primärproduktionsbestimmungen in aquatischen Systemen

- Vergleich und Bewertung des Instrumentoriums -

Heft 6

Universität Rostock
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Vorwort

Kurz soll es sein, ein „gutes" Vorwort, und dieser Forderung will ich freudig genügen.

Der Workshop, dessen Vorträge und Ergebnisse auf den folgenden Seiten vorgestellt werden, war nicht unbedingt das Ergebnis einer Idee der Veranstalter selbst, sondern die Folge einer Tagung in Bad Saarow, initiiert von Prof. Nixdorf. Zu den Details der auslösenden Momente äussert sich Frau Prof. Nixdorf im ersten Beitrag selbst, daher hier nur Folgendes: Wenn man eine Idee äußert, und sei es noch so zaghaft, muß man in bestimmten Umgebungen immer damit rechnen selbst für die Umsetzung der Idee in die Praxis verantwortlich gemacht zu werden. Daß *diese* Idee umgesetzt werden konnte verdanken wir zu einem großen Teil der gesamten Crew der Laborstation Zingst des FB Biologie der Universität Rostock, für deren Unterstützung ich mich hier ganz herzlich bedanken möchte.

Die vorliegende Publikation kam durch die freundliche Vermittlung von Prof. Schlungbaum (Rostock) zustande, der uns ein ganzes Heft der durch ihn betreuten Schriftenreihe zur Verfügung stellte, ihm sei ausdrücklich gedankt dafür.

Ursprünglich wollte ich meine Kollegen weitgehend verschonen mit organisatorischen Vor- und Nachbereitungen des Workshops. Bei der Vorbereitung ist das noch gelungen, für exzessive Hilfeleistungen während und nach dem Workshop möchte ich mich jedoch an dieser Stelle bei Sigrid Sagert und Peter Feuerpfeil ganz herzlich bedanken. Rodney Forster muß einen eigenen Satz bekommen, er hat nicht nur währen und unmittelbar nach dem Workshop geholfen, ihm ist auch in großem Maße das termingerechte Erscheinen dieses Heftes zu verdanken. Vielleicht ist es ja wirklich gesetzmäßig, daß man mit zunehmendem Alter (Erfahrung? Dienstgrad?) mehr und mehr Dinge, die man eigentlich selber verantworten müsste „delegieren" muß. Gewöhnungsbedürftig ist das allemal und noch tun mir die „Opfer" leid.

Der Workshop selbst gliederte sich in die Untersuchungen zweier Forschungsvorhaben unseres Bereiche ein, zum Einen des BMBF-Projektes UV-MAOR (FKZ 03F0120B) und zum Anderen des MAST3-Programmes „BASYS" (contract no. MAS3-CT96-0058 DG12-DTEE) für deren finanzielle Unterstützung gedankt sei.

Rostock, Mai 1998 H. Schubert

Inhalt

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Brigitte NIXDORF

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Measuring primary production in aquatic systems - new attempts to solve old problems

Why this workshop and why this journal? We all know that primary production is one of the most important process, not only in aquatic systems, but globally. There are many scientists (not only in Germany) who are interested in the development of and comparison of methods for measuring primary production in aquatic ecosystems. A platform for intensive discussions was provided by the two yearly meetings of primary production in the GDR prior to 1990 and the experimental orientated GAP workshops (last two in Breukelen/The Netherlands 1990 and Saskatoon/Canada 1993, next in Zürich/Switzerland in 1999). The idea for our little national GAP-workshop in Zingst was born after seminar discussions of current problems concerning primary production methods in Bad Saarow, a limnological research station of Technical University Cottbus in 1996 and 1997. Here we discussed how to measure photosynthesis, primary production and phytoplankton biomass by different methods, especially the modern fluorometric devices. Further current aspects of photosynthetic research were measurements of underwater light, modelling photosynthesis-light relations and laboratory scale simulation of natural conditions of shallow waters.

Although the general definition of primary production as the photo- and chemoautotrophic inorganic carbon fixation into biomass is widely accepted, there are a number of misunderstandings or misinterpretations between scientists when measuring this process by different methods. Measurements of chlorophyll fluorescence are an attractive alternative or supplement to conventional methods of photosynthesis. After initial use of

different fluorescence detectors to detect photosynthetic pigments, we wanted to know: Are the new advances in these methods really useful as alternatives for measuring primary production? Are we able to measure the same process by relatively different approaches like bottle methods, dilution techniques, fluorescence techniques; either *in situ* or in combination with incubation in an artificial light gradient as in a light pipette or photosynthetron? As a result of the Bad Saarow-seminars, the participants decided to meet at an experimental workshop. The staff of Hendrik Schubert organised this workshop at the Rostock University field station in Zingst from 6-10 October 1997, where we met to present, discuss and test our approaches and devices for measuring primary production in a mesocosm experiment. This workshop was very well organised and took place in a very constructive atmosphere. The organizer are thanked again by all participants!

The theme of the workshop was 'Estimation of primary production in aquatic systems - comparison and evaluation of instruments'. The main objectives were:

1. To assess the state of knowledge on the workshop theme, and to discuss new methods such as fluorescence and photoacoustics.
2. To perform joint field experiments using different techniques (e.g. conventional light and dark bottle techniques, in-situ and laboratory fluorescence methods) to test their comparability and reliability.
3. To define gaps and urgent research needs.

The main questions relating to our project concerned the wide diversity of approaches used to describe and measure primary production. The spectrum of methods applied at the workshop were as follows: 'Traditional' static fixed-depth *in situ* measurement of ^{14}C fixation in different bottle volumes to quantify bottle effects, oxygen concentration measurements in an open mesocosm; dynamic incubation of ^{14}C bottles to simulate different frequencies of mixing to indicate the importance of mixing especially in shallow eutrophic waters (brackish and shallow lakes); ^{14}C-fixation and O_2-evolution under controlled irradiance conditions in a 'photosynthetron' and in a 'light pipette' respectively; fluorometric measurements by different types of modulated chlorophyll fluorometers. The 'true' nature of primary production is not only a question of a general accepted definition and further development of measuring devices. It also requires the careful analysis, integration and interpretation of results and application and testing of models.

Assuming that the energy source for primary production is light (phototroph) or chemical (chemotroph) energy, the electron donators are inorganic (lithotroph) compounds and that the carbon source for cell synthesis is carbon dioxide, we must be careful in interpretation and comparison of results obtained by the diverse methods tested at the workshop. We have to consider a complex of problems concerning the "old story" of what does each particular method really measure: net or gross production? Are we able to quantify losses like light and dark respiration or exudation? What about refixation of respired exudates, dark fixation or the kinetics of assimilation ? How did the conditions and duration of incubation affect the result, and, of course, what was the production rate itself?

The most important aspect of the workshop was to compare the photosynthetic parameters resulting from different types of fluorometric measurements with conventional and modified conventional methods, to model a chlorophyll-specific production rate and to validate the models. I (as a representative of the more "matter" orientated scientists) wished to compare our primary production results in terms of [gC m^{-2} d^{-1}]. This meant that our energy orientated partners had to convert their fluorometric signals across a number of models into production values.

Despite the fact that some questions (as expected) remained open, I am certain that the workshop itself was helpful for all participants. And finally, the output of results of the individual experiments was greater than to be expected from such a short period as can be seen in chapter 3.

Because of this, we have decided to continue with this type of workshop in autumn 1998 in Büsum and we are all looking forward to meeting again there.

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Measurement of photosynthetic carbon fixation in aquatic systems

1. Principle of measurement

The photosynthetic carbon fixation of autotrophic plants and bacteria is called primary production, since this process produces first organic compounds formed out of H_2O and carbon dioxide. The actual primary production rate depends on many factors such as temperature, incident light, availability of nutrients and the biomass and metabolic status of the primary producers. In the aquatic environment, it can be measured as the uptake of inorganic radiocarbon (^{14}C) by aquatic autotrophic organisms. The radiocarbon method was introduced into limnology by Steemann Nielsen (1952). Several reviews on possibilities and restrictions of the method are given in the literature (e.g. Coljin 1983, Harris 1984, Maestrini et al. 1993, Peterson 1980). Up to today, it is one of the most sensitive methods for the determination of primary production.

For the measurement of primary production, trace amounts of $NaH^{14}CO_3$ are added to the sample. The incorporation into biomass is determined either by filtration of particulate matter and measurement of its radioactivity or by the acidification and bubbling technique which measures both particulate and dissolved production. Under the assumption that ^{14}C is fixed with a comparable rate as ^{12}C, the carbon fixation rate can be calculated from the ^{14}C incorporation and the concentration of dissolved inorganic ^{12}C.

Use of the radiocarbon methods need an approved radionuclide laboratory. In Germany, use, storage, transport and disposal of radioactive isotopes are controlled by official federal regulations. Investigations using radiocarbon need permission if they exceed a rather low quantity.

Primary production measurements with the radiocarbon method are carried out in enclosed samples of pelagic as well as of benthic communities, incubation can occur in shipboard or laboratory incubators or *in-situ*. The radiocarbon method is suitable for primary production measurement in oligo- to hypertrophic water bodies. Coloured samples require an effective quench correction for the liquid scintillation counter. In acidic samples with low DIC-concentration (< 1 mg l^{-1}), a sensitive DIC-determination is necessary and the samples have to be handled carefully to avoid the loss of added $^{14}CO_2$.

2. Measured processes

Fixation of inorganic carbon takes place in the Calvin-Benson cycle and is catalysed by the enzyme ribulose biphosphate carboxylase. In eucaryotic algae, these reactions occur in the chloroplast stroma (Falkowski & Raven 1997). As well as photoautotrophic eucaryotes and procaryotes, chemolithoautrophic organisms are capable to fix inorganic carbon by anoxygenic photosynthesis.

Interpretation of primary production measurements can be made more difficult by the metabolic fate of the fixed ^{14}C. A part of the previously assimilated ^{14}C can be respired, depending on incubation time and metabolic activity of the investigated community. Another part of the fixed ^{14}C can be exuded as dissolved organic carbon or lost by breakage or lysis of cells. By removal of inorganic carbon from the sample by the acidification and bubbling method (Gächter et al. 1984, Riemann & Møller Jensen 1991, Schindler et al. 1972) exudation can be measured.

The amount of ^{14}C, which is respired or exuded, determines, whether net or gross primary production is measured by the radiocarbon method (Dring & Jewson 1982, Harris et al. 1989). Equilibrium between ^{12}C and ^{14}C inside the metabolic pools of phytoplankton can be reached after a period as short as two hours, but it may even take some days, depending on the metabolic activity of the phytoplankton.

Dark fixation of inorganic carbon usually occurs at a slow rate by organic and inorganic processes. The dark fixation rate is subtracted from the light fixation rates. As an alternative to dark incubated bottles, some authors prefer the use of DCMU (Yallop 1982, Legendre et al. 1983).

The slower uptake of ^{14}C compared to ^{12}C is usually corrected by the isotopic discrimination factor 1.06.

3. Different incubation methods

Three methods for estimation of integral primary production are in common use:

a) static incubation of several samples along a vertical profile in the euphotic zone *in-situ*,
b) dynamic incubation with vertical movement of samples through the euphotic zone *in-situ*, and
c) incubation in a light incubator with defined light intensities.

The traditional method of incubating several samples along a vertical depth profile to calculate the integral primary production has some disadvantages, which are independent of the used method (O_2 or ^{14}C). The measurement is laborious and time consuming (Nixdorf et al. 1990). The static exposition of samples in fixed depths does not meet the natural conditions in well mixed eutrophic water bodies. Algal movement induced by turbulence or active migration is suppressed. The algae are forced to stay under constant light conditions for several hours.

Dynamic incubation methods were developed to overcome these shortcomings. They are designed to imitate turbulent mixing to measure a more realistic primary production by consideration of vertical movement of algae in a mixed layer (e.g., Gervais et al. 1997). The measured value of production is an integral, describing the average of integral primary production within the zone, where the samples were moved.

There is a good agreement in many cases between the results of static and dynamic incubation. In some cases an enhancement or reduction of primary production by dynamic incubation could be observed. Dynamic incubation of just one sample suffices to measure areal production. In that way, radioactive material and filters as well as time can be saved.

The third incubation method is used to measure phytoplankton primary production under controlled light conditions in the laboratory (e.g., Tilzer et al. 1993). The so-called photosynthetron, a laboratory incubator, can be used to measure primary production by both ^{14}C-uptake and chlorophyll fluorescence. The relationship between light and photosynthesis can be used to calculate *in-situ* primary production. Important parameters for the calculation are the intensity and spectral

distribution of light, temperature and phytoplankton distribution in relation to depth. A model for calculation is given by Walsby (1997).

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Measurement of photosynthetic oxygen production

1 Introduction

All material methods for measuring primary production are based on the stoichometry of photosynthesis. They measure rates of CO_2 utilization, changes in the concentration of organic products or, in the case of oxygen techniques, rates of oxygen evolution during photosynthesis. The observed changes in oxygen concentration of the water result from photosynthetic release as well as respiratory consumption of oxygen. Changes in the light are defined as net oxygen production, oxygen decline in the dark is named community dark respiration.

Basically the oxygen method does not require expensive equipment or hazardous chemicals. It was used by GAARDER & GRAN (1927) to carry out the earliest measurements of in situ productivity and is still the most common method for monitoring metabolism of aquatic ecosystems. The development of oxygen-sensitive electrodes enabled continuous and short-time measurements in enclosures as well as in surface waters. Production and respiration of oxygen can be measured in enclosed samples of pelagic (GAARDER & GRAN 1927) or benthic communities (POMEROY 1959) or estimated from the oxygen budget of whole river stretches (ODUM 1956) or of epilimnion or hypolimnion of stratified lakes or sea basins (e.g., WELCH 1968). The latter calculations have to take atmospheric exchange into account.

2 Measured processes

The observed change in concentration of dissolved oxygen results from a variety of physiological processes (see, e.g., HARRIS 1978, FALKOWSKI & RAVEN 1997). During illumination oxygen is photosynthetically produced but also consumed by mitochondrial

respiration and perhaps by light respiration and the Mehler reaction. Mitochondrial respiration occurs by algae as well as by heterotrophic organisms (bacteria, fungi, animals) which (except for axenic cultures) jointly act in the water sample.

Photosynthesis: Cyanobacteria, prochlorophytes, eukaryotic algae and higher plants (but not anaerobic photosynthetic bacteria) use light energy to oxidize water. They yield molecular oxygen during this so-called light reaction of photosynthesis:

$$2\ H_2O + \text{light} \text{----->} 4\ H^+ + 4\ e^- + O_2$$

Light reaction occurs in photosystem II which is located in thylakoid membranes.

Dark respiration: „Dark" or mitochondrial respiration occurs in the light as well as in darkness. Its pathways include glycolysis, the oxidative pentose phosphate pathway, the tricarboxylic or Krebs cycle, and the mitochondrial electron transport chain. Dark respiration serves to form ATP and reductants and to produce carbon skeletons from complex organic compounds. Respiration is therefore essential for maintenance as well as growth of cells. Maintenance respiration rates are variable between species but fairly constant for a given population. The component of respiration associated with cell synthesis is proportional to the growth rate (see GEIDER 1992). Its rate increases with temperature (Q_{10} ca. 1.7-2.0). Mitochondrial respiration might be inhibited as well as stimulated in the light. GRANDE et al. (1989), e.g., found ratios between mitochondrial respiration in the light and in the dark ranging from 0.2 - 12.5.

Photorespiration: The light-dependent oxidation instead of carboxylation of ribulose bisphosphate is named photorespiration. It produces glycolate which is subsequently exudated, oxidised or used for synthesis of amino acids. Intensity of photorespiration increases with increasing ratios of oxygen to carbon dioxide. Photorespiration is an energy-dissipating process which reduces both the initial slope and the light-saturated rate of photosynthesis. It was reviewed, e.g., in a special issue of Aquatic Botany (1989, volume 34).

Mehler reaction: This is a light-dependent redox reaction, in which photosynthetically produced oxygen is reduced to H_2O_2 in photosystem I. There is no net oxygen consumption.

3 Common applications

Bottle method: Water samples from the various depths are enclosed in both duplicate transparent (light) and in dark bottles. Bottles must be filled immediately and carefully (to avoid exchange of oxygen or trapping of gas bubbles). The bottles are either incubated at the sampling depth or are moved vertically through the euphotic layer. The lenght of incubation must allow measurable changes in oxygen concentration but avoid supersaturated conditions in the light bottles. It ranges from less than one hour under conditions of very high algal biomass to about 6 hours in less productive waters. Simultaneous incubation of light and dark bottles enables the measurement of both net oxygen production and dark respiration. If the rate of respiratory oxygen consumption is the same in the dark as in the light then the difference between the oxygen values of the light and the dark bottle is a measure of gross photosynthesis. The method was described in more detail by, e.g., WETZEL & LIKENS (1991).

Laboratory incubations: A variety of incubators was used for laboratory or shipboard measurement of oxygen production. Samples are usually exposed to an artificial light gradient. Light sources should resemble the spectral composition of sunlight as close as possible but the vertical changes of the light spectrum in the water column were rarely simulated. An assemblage of microelectrode and light source of variable intensity but constant spectral composition („light pipette") enables measurements of oxygen evolution of the same concentrated algal sample at a range of light intensities within an hour or so. Laboratory measurements should take the temperature-dependence of the involved processes into account.

4 Priniciples of oxygen measurement

The Winkler method: This method is based on the oxidation of manganous hydroxide by the oxygen dissolved in the sample. The product is transformed to manganic sulfate which liberates iodine from previously added potassium iodide. The quantity of free iodine is

equivalent to the amount of dissolved oxygen. It is determined by titration with a standard solution of sodium thiosulfate. Errors may occur in waters containing suspended organic solids, dissolved organic compounds, nitrite, or iron salts. The precision of the Winkler method is improvable by microtitration with automatic detection of the endpoint. The method has been described in full detail in APHA 1989, and WETZEL & LIKENS (1991).

***Oxygen electrodes*:** Oxygen electrodes consist of a cathode, usually made of platinum or gold, and a silver / silver chloride anode. A constant potential is maintained between both poles to electrochemically reduce oxygen at the cathode to water and hydroxide ions. At the same time, silver of the anode is oxidized. The silver is regenerated by the surrounding hydrous solution of KCl. The electric signal of this polarographic method is proportional to the oxygen flux. It depends on the surface area of the cathode. The mostly used Clarke-electrodes separate sample and cathode by a thin (10-30 µm) membrane of Teflon or polyethylene. The diffusion of oxygen through membrane and electrolyte is often speeded up by stirring. Rate of diffusion and thus signal of the electrode depends on temperature and exchange rate of the sample. The output also depends on salinity and air pressure. Macroelectrodes require a rapid flow of sample across the membrane. Their output is usually compensated for temperature. Most of them are unreliable at oxygen concentrations below 1 mg l^{-1} and they are poisoned by hydrogen sulfide. Microelectrodes provide faster response and are less sensitive for flow of water but are more fragile. Their output is usually not corrected for temperature and is less constant compared with macroelectrodes. Oxygen electrodes require repeated calibration by chemical methods.

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Chlorophyll fluorescence measurements for assessment of primary production in aquatic ecosystems - the basics

Abstract

For many investigations on aquatic ecosystems it is desirable to assess the primary production with high resolution in time and space. Chlorophyll (Chl) fluorescence measurements have the potential to become the method of choice for such investigations. The biophysical and physiological background, the measuring techniques and the limitations of Chl fluorescence measurements as a tool for investigations on primary production in aquatic ecosystems are reviewed. The following aspects are discussed: contribution of Photosystem I fluorescence, variability of the Photosystem II fluorescence, photochemical quenching of Chl fluorescence (q_P), non-photochemical quenching (energy quenching, q_E; photoinhibitory quenching, q_I; state transitions, q_T), measuring technique, modulated fluorescence, special light programs (saturation pulse techniques using s or µs-pulses; 1 Hz-method); multi-color excitation and species-differentiation; basics of the use of fluorescence techniques for assesment of primary production.

Abbreviations

Chl, chlorophyll; ETC, electron transport chain; F_0, fluorescence yield after dark-adaptation, oxidized Q_A in all PS II; F_M, fluorescence yield after dark-adaptation and sudden application of saturating light, reduced Q_A in all PS II; F_0', fluorescence yield of light-adapted organisms for oxidized Q_A in all PS II ; F_M', fluorescence yield of light-adapted organisms for reduced Q_A in all PS II; $F_V = F_M - F_0$; $F_V' = F_M' - F_0'$; LED, light-emitting diode; LHC, light-harvesting chlorophyll-protein complex with molecular weights of 20-30 kDa; PAM, special fluorometer based on Pulse Amplitude Modulation; PS, photosystem; Q_A, primary quinone acceptor of PS II; REE, rapid exciton equilibration.

1 Introduction

Today, the chlorophyll fluorescence of algae and cyanobacteria can be accurately measured even on ultra-dilute suspensions of algae (Chl content below 100 ng/L). Precise *in-situ* measurements (e.g., sample collection and fluorescence measurement on a research vessel) or even *in-vivo* investigations (e.g., handy instruments controlled by divers or fully automated diving probes) are feasible using commercially available instruments. By means of special illumination programs reasonable estimates are obtainable on: (i) the amount of primary producers (i.e. algae and cyanobacteria), (ii) the actual rate of primary production, (iii) the 'fitness' of the primary producers (light stress effects, influence of toxic substances, etc.). However, it is rarely possible to obtain all this information simultaneously. Furthermore, heterogeneous samples (mixture of dissimilar species) may endanger the straightforward interpretation of fluorescence data and may require the use of instruments using a multi-color excitation technique. Thus, before starting a research program involving fluorescence measurements its is advisable to consider the strengths and limitations of the technique, to clarify the objectives and, then, to select the most appropriate instrumentation and experimental approach. The easiness of obtaining large heaps of fluorescence data and the knowledge on potential relations to various aspects of photosynthesis is seductive; not infrequently conclusions have been drawn on basis of misunderstood fluorescence data. Background knowledge on the involved biophysical basics and on the measuring technique is required to avoid experimental artefacts and to prevent misleading interpretations which might discredit this fascinating and powerful technique.

2 The variable chlorophyll fluorescence

In intact organisms the yield of Chl fluorescence is variable. In case the photosynthetic electron transport chain (ETC) is in a fully oxidized state (e.g. in dark-adapted organisms), the fluorescence yield is minimal (F_0-fluorescence); in case the ETC is in a fully reduced state (e.g. due to illumination with saturating light), the fluorescence yield is maximal (F_M-fluorescence). It is mainly a variability in the fluorescence yield; the shape of the emission spectrum changes only slightly (Fig.1).

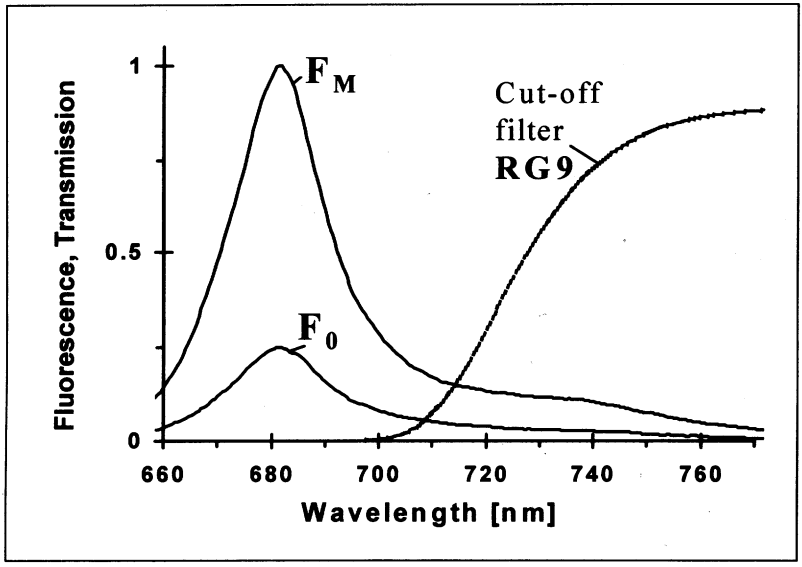

Fig. 1 Fluorescence emission spectra of a cell suspension of the green alga *Scenedesmus obliquus* in der F_M-state and in the F_0-state (collected at 17 °C by Dr. I. Heinze, Marburg). Additionally, the transmission characteristics of a long-pass filter (Schott glas RG9) are shown. The RG9 is often used as a cut-off filter for fluorescence detection in fluorometers which employ a red measuring-light LED. The F_0-to-F_M increase is due to the variability of the PS II fluorescence. In the F_0-state, roughly 5% of the emission at 683 nm stems from PS I; for wavelengths greater than 700 nm the PS I contribution may be as high as 30% (in green algae) or even higher (in cyanobacteria).

The variable fluorescence of plants and photosynthetic bacteria has been subject of research for decades. Instead of attempting an exhaustive review on this subject, a highly simplified extract of the results essential to the use of fluorescence measurements for assessment of primary production is presented; for a mechanistically more accurate discussion, see DAU 1994a. Fluorescence emission by anoxygenic photosynthetic bacteria is not considered. To avoid an overwhelming reference list, only a small selection of the relevant original works and review articles is cited. Numerous references on earlier works can be found in various chapters of GOVINDJEE et al. (1986); for more recent references see review articles of KRAUSE & WEIS (1991), DAU (1994a, 1994b), SCHREIBER et al. (1994) and GOVINDJEE (1995).

PS I fluorescence

In oxygenic photosynthesis fluorescence photons are emitted by the antenna pigments of PS II and PS I. The yield of the PS II fluorescence varies between about 2% (F_0-level) and 10% (F_M-level) as discussed further below in more detail. For reasons which are not fully understood, the PS I fluorescence yield is significantly lower (at least at temperatures above 0°C, see legend of Fig. 1) and not variable. Therefore, often the contribution of PS I-chlorophylls to the fluorescence emission of intact organisms is assumed to be negligible. However, in particular if fluorescence emission is detected at wavelengths greater than 700 nm, neglect of PS I contributions may not be a reasonably good approximation.

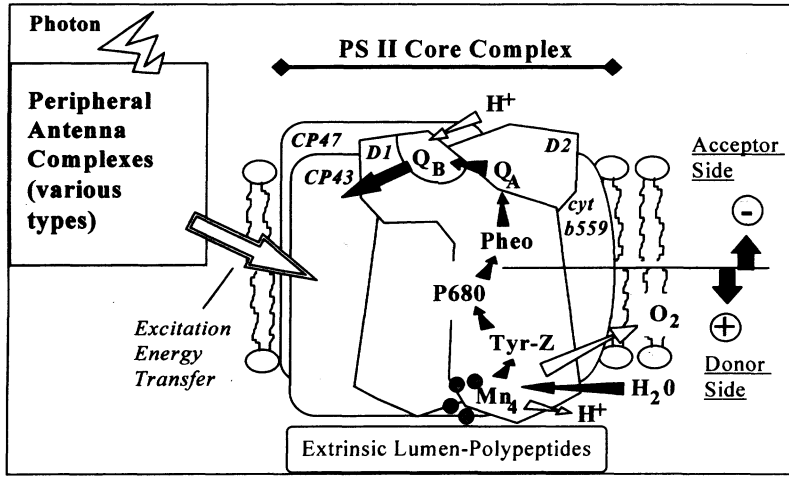

Fig. 2 The Photosystem II.
The type of the peripheral antenna is species-dependent. The core complex consists of a core antenna (CP 43 and CP 47) containing 35-80 Chl *a* (the precise number of Chl *a* per CP 43 and CP 47 in intact organisms is unknown) and the D1/D2/Cyt_{b559} reaction-center, where the light-induced electron transfer reactions proceed.

PS II light-harvesting and photochemistry.

The PS II consists of 'peripheral' antenna complexes and the PS II core complex (Fig. 2). The biochemical and spectroscopic characteristics of the peripheral antenna are species-dependent. For example, in most cyanobacteria the membrane-attached phycobilisomes serve as

peripheral PS II antenna, whereas in green algae membrane intrinsic chlorphyll *a/b*-protein complexes, the so-called LHC's, fulfil the same function. The species dependent differences in the peripheral PS II antenna system affect the color of the organisms which traditionally serves as a taxonomic criterium for algae. Thus, there are various types of peripheral PS II antenna which are each characteristic for a distinct group of organisms (*Chlorophyceae, Cyanophyceae, etc.*). In contrast to the peripheral PS II antenna, all relevant characteristics of the PS II core complex are evolutionary fully conserved (see Fig. 2). For this reason, various molecular mechanisms related to the variability of the PS II fluorescence are a common trait of all organisms of oxygenic photosynthesis. This holds in particular for the most prominent source of fluorescence variability, the so-called photochemical quenching of the PS II fluorescence.

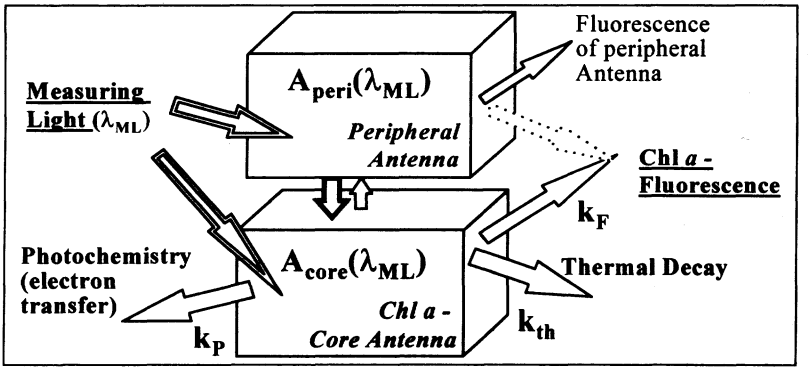

Fig. 3 Creation and decay of excited states in the PS II. An excited state is created by absorption of a photon; $A_{peri}(l_{ML})$ and $A_{core}(l_{ML})$ are the absorption coefficients (precisely spoken: the absorption cross-section at l_{ML}) of the peripheral and the core antenna, respectively. Due to rapid hopping between antenna pigments a so-called 'exciton equilibrium' is established. Then the excited state decays *via* four major pathways: (1) initiation of charge separation (electron transfer), (2) thermal dissipation (non-radiative decay), (3) emission of a fluorescence photon (radiative decay), (4) formation of triplet states (intersystem crossing). Triplet-formation is of particular relevance with respect to initiation of photodamage, but due to its extremely low yield this decay process is, in the present context, negligible. Due to REE occurence the rate constants for excited state decay (k_P, k_{th}, k_F) are independent of the excitation wavelength (i.e. l_{ML}, the wavelength of the measuring light). In case the primary quinone acceptor is singly reduced (F_M-state), photochemistry is inhibited ($k_P = 0$) and the fluorescence yield is maximal. For non-reduced Q_A (F_0-state), the fluorescence yield is minimal.

PS II fluorescence

The photon absorption event in the peripheral antenna or the PS II core complex is typically followed by 'rapid exciton equilibration' (REE, see DAU, 1994a; DAU & SAUER, 1996). This means that the excited state (or the exciton) is moving energetically down-hill to Chl *a* and then its is rapidly hopping back and forth between these chlorophylls until eventually (and clearly later) the excited state decays (see Fig. 3). Due to the random hopping the exciton 'forgets' where it has been created by an absorption event. Consequently, the chlorophyll fluorescence yield and emission spectrum are essentially independent of the excitation wavelength. Due to REE occurence it is usually sufficient to measure the PS II fluorescence at a single emission wavelength (or wavelength range); based on REE relatively uncomplicated models can be used for interpretation of fluorescence data (DAU, 1994a).

For the model depicted in Fig. 3, the fluorescence signals detectable in the F_0- and F_M-state are reasonably well described by (N_{PSII}, number of PS II in the sample volume; c_{instr}, a fluorometer-dependent constant; I_{ML}, intensity of the measuring light):

$$F_{0/M} = N_{PSII}\ c_{instr}\ I_{ML}\ \{A_{peri}(I_{ML}) + A_{core}(I_{ML})\}\ F^F_{0/M} \quad (1)$$

with $$F^F_0 = k_F / \{k_{th} + k_F + k_P\} \quad (2)$$

and $$F^F_M = k_F / \{k_{th} + k_F\}\ . \quad (3)$$

For the quantum yield of the photochemical reaction (here Q_A reduction) we obtain:

$$F^P_0 = k_P / \{k_{th} + k_F + k_P\} = F_V' / F_M' \quad (4)$$

with $$F_V' = F_M' - F_0' \quad (5)$$

Following the nomenclature of VAN KOOTEN & SNELLE (1990), F_M' and F_0' are used for organisms which are not dark-adapted, whereas F_M and F_0 are used exclusively in the case of complete dark-adaptation. The light-values, F_M'- and F_0', may differ from the dark-values, F_M- and F_0, due the non-photochemical quenching discussed below. Often the experimental protocol does not allow for a clear-cut distinction between light- and 'true' dark-values. Equations 1-5 hold for light- and dark-values.

The parameter F_V/F_M is rarely greater than 80%. There is, however, considerable evidence that the 'real' quantum yield for Q_A reduction can exceed 90%. In the model of Fig. 3 the reversibility of the primary charge separation is neglected. One consequence of this approximative treatment is that by using Eq. 4 the value for the Q_A-reduction quantum yield is underestimated (DAU 1994a).

Photochemical quenching (q_P)

Because the PS II fluorescence yield is significantly diminished in comparison to (isolated) chlorophyll in organic solvents, the variability of the PS II fluorescence yield is usually discussed as a fluorescence quenching of variable strength. 'Photochemical quenching' denotes a decrease in the fluorescence yield which results from excited state decay by initiation of a photochemical reaction. In PS II the initiated photochemical reaction is the transfer of an electron from the primary chlorophyll donor, P680, *via* the primary pheophytin acceptor, Pheo, to the quinone acceptor, Q_A, which becomes singly reduced by this process. If Q_A is already reduced, this process cannot take place and the photochemical quenching is assumed to be absent. Thus, for reduced Q_A the fluorescence yield is maximal (F_M) whereas for oxidized Q_A it is minimal (F_0). In intact organisms illuminated with light of sub-saturating intensity typically Q_A is reduced in a fraction of all PS II. Consequently, the measurable fluorescence yield, F_S, is determined by the percentage of PS II with reduced Q_A, and the extent of photochemical quenching is described by a so-called quenching coefficient:

$$q_P = \{F_M'-F_S'\} / \{F_M'-F_0'\} \quad (6)$$

Per definitionem, the photochemical quenching coefficient (q_P) is zero in case all PS II are in the F_M-state (all Q_A reduced). For a more accurate discussion, however, it needs to be considered that the excited state decay by initiation of pheophytin reduction can proceed even in the presence of reduced Q_A; for details see DAU 1994a.

Based on the model sketched in Fig. 3, we obtain for the rate of PS II electron flux (which is, more or less closely, related to the rate of CO_2-fixation):

$$R_{ET} = q_P \, F_P^{0\prime} \, I_{abs} = F_P I_{abs} \quad (7)$$

with $$F_P^{0\prime} = \{F_M'-F_0'\} / F_M' \quad (8)$$

and $$F_P = \{F_M'-F_S'\} / F_M' \quad (9)$$

where I_{abs} denotes the rate of photon absorption by all the PS II in the sample volume (thus, I_{abs} is proportional to the light intensity).

Non-photochemical quenching (q_N)

Various quenching mechanisms which are not directly related to changes in the Q_A redox state affect the PS II fluorescence. The three most prominent ones are: energy quenching (q_E), photoinhibitory quenching (q_I) and state transition quenching (q_T) (see KRAUSE & WEIS 1991; DAU 1994b). Minor effects include quenching by oxidized plastoquinone (of the PQ-pool), fluorescence increase due to an effect of the thylakoid membrane voltage on charge separation reactions, quenching by oxidized P680 and quenching of excited Chl-singlet states by carotenoid triplets (see DAU 1994a, GOVINDJEE 1995). Resolution of various quenching effects by fluorescence measurements on intact organisms is often possible by utilization of differences in the rates for formation or decay.

Thylakoid energization (q_E)

The q_E-formation parallels the acidification of the thylakoid lumen (therefore also denoted as 'pH-dependent quenching'). Upon illumination q_E is built up within a few seconds (3-30 s); q_E relaxes in the dark in about 30-120 s (the 'rapidly-reversible non-photochemical quenching'). The F_M value is strongly quenched (by 10-60%); the F_0-quenching is clearly smaller (2-25%). Even though this phenomenon has been intensively studied for higher plants (but rarely for algae and cyanobacteria), its biophysical basis and physiological role is only inadequately understood (see RUBAN & HORTON 1995). There is, however, considerable evidence that the q_E-quenching is caused by an increased rate of thermal deactivation of excited chlorophylls (increase of k_{th} in Eqs. 1-5 as demonstrated for green algae by HEINZE & DAU 1996); a role in protection against photoinhibitory damage is often assumed.

Photoinhibition (q_I)

Prolonged exposure (e.g., 30 min) to high-intensity light causes PS II-inactivation (photoinhibition, photodamage, ARO et al. 1993, BAKER & BOWYER 1994) and an accompanying effect on the PS II-fluorescence which is irreversible in the minute range. This q_I-effect is typically characterized by a clear increase of F_0' and decrease of F_M' and, consequently, in a pronounced, not rapidly-reversible decrease of F_V'/F_M'. Several stress factors (e.g. aging of algae cultures) result in a fluorescence phenotype which resembles photoinhibitory quenching.

State-transitions (q_T)

In higher plants and green algae the q_T-formation is related to LHC-phosphorylation and the resulting decrease in the PS II-antenna size (change of $A_{peri}(I_{ML})$ in Eqs. 1-5), a phenomenon denoted as State 1-State 2 transition (BONAVENTURA & MYERS, 1969; ALLEN 1992); there seem to be analogous state transitions in cyanobacteria (and perhaps red algae) involving the phycobilisome system (see, e.g., CANAANI 1986). State tranistions are involved in adaptation not only to changes in spectral quality, but also in intensity of light (see DAU 1994b). State transitions proceed within 5-20 min; their effect on F_M' and F_0' is usually relatively small (5-25%).

3 Measuring technique

Already the basic experimental set-up shown in Fig. 4A allows Chl fluorescence measurements, but it is neither suited for *in-situ* (and *in-vivo*) measurements on dilute suspensions, nor for an experimental approach involving special light programs.

The type of instrument sketched in Fig. 4B is superior due to the following features: (1) By using a modulated measuring light and a correlation amplifier solely the magnitude of the fluorescence stemming directly from the modulated measuring light is detected; ambient light artefacts are supressed and a signal is obtained which is a (relative) measure of the fluorescence yield. (2) The perpendicular arrangement of light sources and fluorescence detector minimizes stray-light artefacts. (3) Color filters are never fully non-fluorescent; the use of interference cut-off filters minimizes artefacts due to filter fluorescence. Instruments for measurement of modulated fluorescence are commercially available (various companies).

The employment of a modulated measuring light does not completely solve all technical problems related to measurements on ultra-dilute suspensions. In particular, there seems to exist no perfect combination of optical filters which fully prevents artefactual contributions of scattered measuring light to the fluorescence signal. Furthermore, fluorescent humic substances in the sample suspension inevitably give rise to an undesired contribution to the fluorescence signal. These problems can be overcome, at least partially, by using special light programs (see 4 and 6).

Considerable inaccuracies which result from quantitatively significant absorption or scattering of the measuring light and the emitted

fluorescence by turbid suspensions is another potential problem. This problem can, at least partially, be solved by a correction on basis of the simultaneously-measured attenuation of the measuring light (MOLDAENKE & al. 1995). Presently, only a few commercially available instruments are equipped with an attenuation correction.

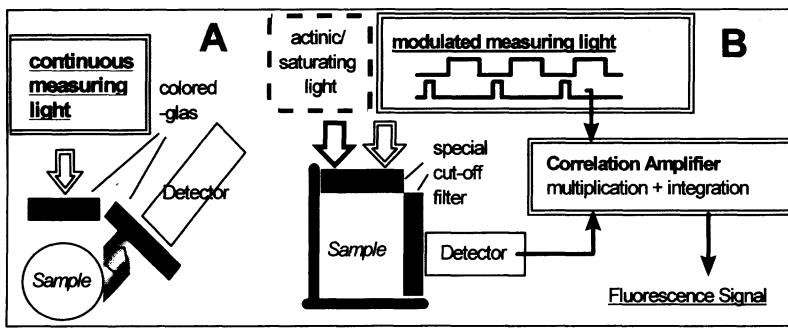

Fig. 4 Set-up for fluorescence measurements using a continuous (**A**) or a modulated (**B**) measuring light. The modulated light may be either symetrically modulated (equal length of on- and off-period, approach in use for decades) or asymetrically modulated (e.g., short light-on period, PAM-approach, SCHREIBER 1986). Today the source of the modulated light typically is either a LED, or Laserdiode, or a Xenon-Flash lamp. The Correlation Amplifier (correlator, Lock-in amplifier, phase-controlled rectifier or demodulator) provides an output signal proportional to the amplitude of the modulated fluorescence which originates from the modulated measuring light. The correlation facilitates, first, the discrimination between modulated fluorescence and the non-modulated fluorescence resulting from additional non-modulated light-sources (ambient light, actinic light, saturation light) and, second, a significant noise reduction. An increased integration time-constant of the correlation amplifier results in reduced noise, but also in a lengthened response time. In some commercially available instruments the correlation is done by a micro-processor unit.

4 Special light programs

Due to the variability of the PS II fluorescence special light programs can be utilized for (1) suppression of light-scattering contributions and of fluorescence of 'dead' substances (to obtain an estimate of the amount of chlorophyll in the sample which is not affected by artefactual contributions), (2) obtaining estimates on the actual rate of

photosynthesis, (3) gathering information on PS II quantum yield, 'fitness', stress effects and influences of toxic substances (biotest).

More or less complex light-programs which involve determination of the fluorescence yield in the presence of reduced Q_A^- (F_M or F_M') by application of high-intensity light-pulses (see Figure 5) are frequently used. The duration of the light-pulse employed for reduction of Q_A mostly is either greater than 300 ms or less than 20 µs. In the former case, the frequently used 'saturation pulse method' , Q_A and the total ETC are reduced (BRADBURY & BAKER 1981; QUICK & HORTON 1984; SCHREIBER et al. 1986; SCHREIBER et al. 1995). In the latter case, Q_A is reduced by a µs 'pump pulse' and the fluorescence yield is determined roughly 100 µs later by a µs 'probe pulse' (MAUZERALL 1972; FALKOWSKI et al. 1986; KOLBER & FALKOWSKI 1993).

An alternative light-program involves determination of the variability in the fluorescence yield induced by an actinic light modulated with 1 Hz (SCHROETER et al. 1991; MOLDAENKE et al. 1995, see Fig.6), a method derived from analysis of the variable PS II fluorescence in the frequency domain (DAU & HANSEN 1989). The 1-Hz fluorometer is particularly useful for continuous environmental monitoring.

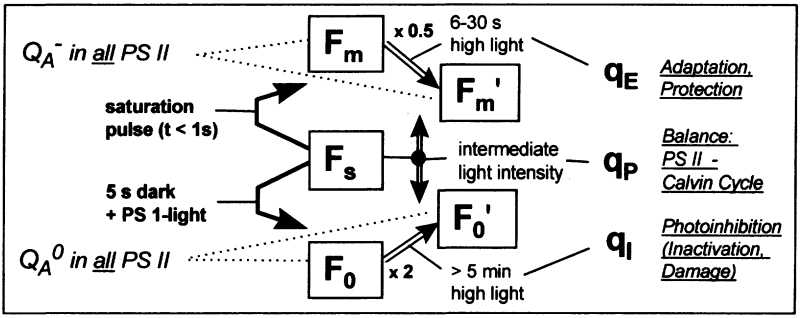

Fig. 5 Fluorescence yield and its response to special light programs (e.g. by the saturation pulse method). The corresponding experimental set-up involves a fluorometer for measurement of modulated fluorescence as sketched in Fig. 4B (e.g., the PAM fluorometer, Walz, Effeltrich). It should be noted that photoinhibition is, typically, related to a F_0-increase (as indicated in the above scheme) and a significant F_M-decrease (not indicated in the above scheme). Determination of F_0' is often troublesome because full reoxidation of reduced Q_A is not readily achieved. In particular, in many cyanobacteria the plastoquinone pool redox-state is affected by the respirational activity of the organism; therefore, accurate determination of F_0 and F_0' by special light programs is not possible.

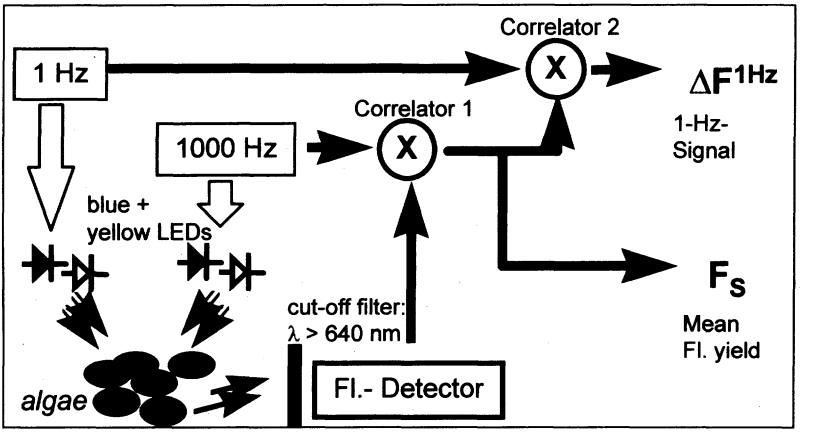

Fig. 6 Scheme of the 1-Hz-fluorometer. The 1-Hz-signal represents the magnitude of the variations in the fluorescence yield which are induced by the actinic light LED modulated with 1 Hz (MOLDAENKE et al. 1995). The F_S-determination involves measurement of modulated fluorescence as depicted in Fig. 4B. Due to the double-correlation technique exceptional sensitivity can be achieved. In contrast to the saturation-pulse techniqe, the light-program (i.e. the actinic light modulated with 1 Hz) is running all the time and the so-called 1-Hz-signal is continuously provided. The value of the ratio DF^{1Hz} / F_S is related, but not identical to the the ratio F_V'/F_M' as obtained by the saturation pulse method.

5 Current development: species-differentiation by multi-color excitation

For a homogenous algae population (single species, identical developmental and adaptational state) the fluorescence parameters $F_M^{(\prime)}$, $F_0^{(\prime)}$, F_S and DF^{1Hz} are proportional to the Chl *a* concentration; measurements of Chl fluorescence (as shown in Fig. 4b) can, e.g., be used to obtain a relative measure of the Chl *a* content. Frequently, however, the population is heterogeneous. For a mixture of various green algae species, the resulting inaccuracies may be tolerable. However, the errors are likely to be unacceptable for, e.g., a mixture of green algae and cyanobacteria because excitation spectra, $A_{peri}(\lambda_{ML})$ in Eq. 1, and fluorescence yields, $F^F_{0/M}$ in Eq. 1, of these organisms are pronouncedly different.

On basis of Eq. 1 (see also Fig. 3) we obtain for the fluorescence signal, F_x, of a heterogeneous organism population (i.e. a mixture of various species):

$$F_x(I_{ML}) = \sum_{i=1,2...} C_{Chla}^{\,i}\, f_x^{\,i}(I_{ML}) \qquad (10)$$

with $$f_x^{\,i}(I_{ML}) = c_{instr}\; I_{ML}(I_{ML})\; (N_{PSII}^{\,i} / C_{Chla}^{\,i})\; \{A_{peri}^{\,i}(I_{ML}) + A_{core}(I_{ML})\}\; F_x^{F,i} \qquad (11)$$

where the superscript 'i' indicates the reference to the i'th species and $C_{Chla}^{\,i}$ the concentration of Chl *a*-molecules (e.g., in µg/L) associated with the i'th species.

Using modern optoelectronics and special correlation schemes, it is technically feasible to detect, at a single emission wavelength, separately the fluorescence induced by measuring light sources (LEDs) of different wavelengths ($I_{ML} = I_1, I_2, ..I_j, ..I_N$). By collecting 'norm spectra' on samples with known Chl concentration which contain exclusively the i'th species, the $f_x^{\,i}(I_j)$ are obtainable. In case the number of excitation wavelengths is equal to or greater than the number of discernible species, determination of the $C_{Chla}^{\,i}$, the species-resolved chlorophyll content, is achievable by using appropriate fit algorithms.

Major limitations of this multi-color excitation approach are: (i) differentiation between species with similar PS II antenna systems is usually not feasible; (ii) the number of resolvable species or organism groups can never exceed the number of the number of excitation wavelengths; (iii) variations in the developmental or adaptational state of one species (or organism group) may cause significant variations in the norm-spectra and, consequently, misleading results (a potential problem particularly for cyanobacteria). Some 'resistance' against the third problem is achievable by intelligent fit algorithms, but only in case the number of excitation wavelengths exceeds the number of organism groups.

The multi-color excitation approach for 'algae differentiation' is perhaps the most promising recent development with respect to *in-situ* and *in-vivo* Chl-fluorescence measurements on algae suspensions. Potentially, this approach enables the fast and convenient, spatially-resolved and differentiated assessment of the amount primary producers and the rate of primary production in aquatic ecosystems by means of diving probes which employ on-line data transfer to a personal computer on board of a research vessel.

Recently, the first five-color diving-probe system became commercially available (l_{ML} = 450, 525, 570, 590 and 610 nm; detection at l > 670 nm; turbidity correction; measurement of water pressure for depth determination; C. Moldaenke, bbe Moldaenke, Schauenburgerstr. 116, D-24118 Kiel, Germany). A four-color table-top instrument (l_{ML} = 450, 590, 620 and 650 nm; detection at l > 710 nm; possibility to employ complex, computer-controlled light-programs; see KOLBOWSKI & SCHREIBER, 1995; J. Kolbowski, Büro für Umweltmeßtechnik, W.-v.-d.-Vogelweide-Str. 56, D-97422 Schweinfurt, Germany) has been presented at the workshop on determination of primary production in Zingst (October 97) and has been announced to become commercially available soon.

6 Assessment of primary productivity by Chl-fluorescence measurements

In the following it is assumed that the rate of oxygen evolution by PS II is a suitable measure for the gross rate of primary production. The O_2-evolution rate (e.g. in Mol evolved $O_2/(m^3\ h)$) is determined by:

(I) the amount and species composition of primary producers (the total number of PS II, $N_{PS\,II}$, in the sample volume per species),
(II) for each species the 'effective' quantum yield for O_2-evolution of the illuminated sample (F_{ox}), and
(III) the integrated product between photon-fluence rate, I(l), and the species-dependent absorption cross-section of PS II ($A(l) = A_{peri}(l) + A_{core}(l)$). Thus,

$$R_{ox} = \sum_i N_{PSII}^{\ i}\, F_{ox}^{\ i}\, L^i \qquad (12)$$

with

$$L^i = \int I(l)\, A^i(l)\, dl \quad . \qquad (13)$$

(I) Amount of primary producers.

Convenient *in-vivo* or *in-situ* determination of $N_{PSII}^{\ i}$ (which corresponds to the organism concentration) by fluorescence measurements is, in principle, possible. For a homogeneous population the values of F_0 or F_M are proportional to N_{PSII} (Eq. 1). However, due to non-photochemical quenching (see 2) there is a potential error in the fluorescence-based N_{PSII}-determination of, roughly, a factor 2. In case pronounced q_E- and q_I-formation can be excluded, the fluorescence-

based N_{PSII}-determination should be relatively precise. For concentrated or turbid samples, however, an attenuation correction is mandatory (MOLDAENKE et al. 1995). For heterogeneous populations with varying species compositions, N_{PSII}-determination for each organism group is required (see 5).

(II) Quantum yield for photochemistry. It is often possible to obtain a reasonable estimate for F_{ox} by fluorescence measurements. For a homogeneous population to a first approximation, 4 times F_{ox} (4 PS II-turnover are required per O_2-molecule) equals to the fluorescence paramter F_P of Eq. 9 (first experimental proof: GENTY et al. 1989, 'corrected' theory: DAU 1994a; application to algae, e.g., HEINZE et al. 1996, GEEL et al. 1997). Also the (alternative) fluorescence parameter DF^{1Hz} / F_S is closely related to F_{ox} (VANSELOW et al. 1997).
For ultra-dillute organism suspensions, a contribution to the fluorescence signal due to scattered measuring light and fluorescence of humic substances is unavoidable (see 3). In this context, it could be profitable to employ directly the fluorescence parameters F_V' or DF^{1Hz} for obtaining an estimate on the product 'N_{PSII} F_{ox}'. Furthermore, using this approach the errors due to q_E- or q_I-formation are, presumably, relatively small. Presently, however, this approach is merely an untested suggestion.

(III) Number of incident and absorbed photons. Determination of the L^i in Eq. 12 is, unfortunately, troublesome. Using a homogenous 'norm-sample', the wavelength dependence of the PS II absorption cross-section, $A_{PSII}(l)$, can be accurately determined by measurement of the F_V-fluorescence excitation spectrum (using a wavelength-scanning laboratory fluorometer). These measurements are relatively difficult (various lurking artefacts), but 'affordable' because frequent repetition is not required. However, spectrally resolved under-water measurements of the photon-fluence rate (for determination of $I(l)$ in Eq. 13) are required which are costly in terms of financial resources and manpower because spacial and temporal resolution is needed.

Approximative approaches

Occassionally a highly approximative, semi-empirical approach might be applicable involving:

(i) determination of the Chl-content of the sample (or N_{PSII}),
(ii) measurement of the photosynthetically active radiation (PAR) with a simple instrument and

(iii) calculation of R_{ox} for *in-situ* or *in-vivo* measurements of fluorescence yield ratios (F_V'/F_M' or $\Delta F^{1Hz} / F_S$) on basis of calibration factors. For example, the rate of oxygen evolution of the green algae *Scenedesmus obliquus* has been found to be :

$$R_{ox} = I_{PAR} \cdot F_V'/F_M' \cdot (0.9 \pm 0.16)\ (\mu mol\ mg^{-1}\ h^{-1}) / (\mu E\ s^{-1}\ m^{-2}) \qquad (14)$$

with R_{ox} in μmol O_2 per mg Chl and h, I_{PAR} in μE per s and m^2 (for lab cultures grown at various conditions, but in the absence of significant photoinhibition; see HEINZE et al. 1996).

For a more intricate, but perhaps more general approach see GEEL et al., 1997. New approximative approaches are needed for handling multi-color excitation data obtained on species mixtures (see 5).

Stress and toxicity

Often it is the objective to search for an influence of 'stress factors' (high light intensities, unfavorable O_2-concentration, pollutants, toxic substances, etc.) on photosynthetic organisms. The molecular basis of the involved phenomena is complex and can not be derived from fluorescence measurements. However, fluorescence parameters may be used as convenient and sensitive 'indicators' for the presence of a stress effect. The parameter F_V/F_M is sensitive to light stress, but it is usually insensitive to substances which inhibit reactions of the ETC or the Calvin cycle (e.g. various herbicides); the parameter $\Delta F^{1Hz} / F_S$ responds to both types of 'stress'. Thus, the former fluorescence parameter is more specific in its response, whereas the latter is senses a clearly broader spectrum of stress or toxicity effects.

7 Conclusion

For many investigations on aquatic ecosystems, it is desirable to assess the primary production with high resolution in time and space. However, additional work needs to be spend for development of instrumentation and the (approximative) methods which are indispensable for relating fluorescence data to primary production. Presently, a strict standard for instrumentation and data evaluation is neither existent, nor desirable because it would impede future developments. The choice of method and instrumentation depends on character and objectives of the intended investigation.

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Dilution-Methode - Möglichkeit der Bestimmung von Wachstum und Sterblichkeit durch Grazing in Planktongemeinschaften

Das Prinzip der Untersuchungsmethode besteht in der Veränderung der Nettowachstumsrate in den gegebenen Verdünnungsstufen durch reduzierten Grazingdruck. Die Auswertung der gemessenen Unterschiede erfolgt innerhalb einer definierten Inkubationsdauer (vorwiegend 24 h). Das Prinzip basiert auf dem Modell des exponentiellen Wachstums in Planktongemeinschaften, wobei die beobachtete Nettowachstumsrate r für das Zeitintervall t die Differenz zwischen momentaner Bruttowachstumsrate μ und der Sterblichkeitsrate m ist,

$$r = \ln (P_t/P_0)/t = \mu - m \qquad (1)$$

(P_0, P_t Abundanz der zu untersuchenden Planktonkomponente zu Beginn und Ende der Inkubationsdauer)

Die Sterblichkeitsrate m errechnet sich aus der Beziehung

$$m = F * (D) \qquad (2)$$

wobei F die Clearancerate (Wasservolumen, welches pro Grazer und Zeiteinheit beutefrei filtriert wird) ist und (D) die zeitabhängige Grazerdichte (Anzahl der Grazer pro Wasservolumen) bedeutet.

$$(D) = (D_t - D_0)/\ln D_t/D_0 \qquad (3)$$

Bei konstanter Bruttowachstums- und Clearancerate variiert die Nettowachstumsrate des Phytoplanktons linear als Funktion der Grazerdichte mit dem Schnittpunkt μ und dem Anstieg -F unter der

Annahme, dass sich die Sterblichkeitsrate durch Grazing entsprechend der Grazerdichte zu Beginn der Inkubation verhält.

$$r = \mu - m_n * (D)/D_{n,0} \quad (4)$$

Ein Berechnungsfehler entsteht, wenn anstatt der auf die natürliche Grazerdichte normalisierte Grazerabundanz $(D)/D_{n,0}$ der Verdünnungsfaktor $D_0/D_{n,0}$ verwendet wird.

Die Untersuchungsmethode kann unter Berücksichtigung der Annahmen

(i) Wachstum und Sterblichkeit können innerhalb kurzer Zeitintervalle variieren,
(ii) die mittlere spezifische Wachstumsrate des Phytoplanktons ist unabhängig von der Abundanz, d.h. unbeeinflußt durch die Verdünnung und
(iii) die durchschnittliche Clearancerate der Grazer ist in allen Verdünnungen konstant,

angewendet werden.

Erweiterungen der Methode wurden von Gallegos (1989) in Bezug auf die funktionelle Beziehung zwischen Grazingrate und Phytoplanktonkonzentration vorgenommen. Dabei erfolgte die Bestimmung der Wachstumsrate des Phytoplanktons in sehr hohen Verdünnungsstufen. Andersen et al. (1991) und Elser & Frees (1995) führten spezielle Untersuchungen zu P- und N-Limitierung durch, wobei sowohl interne und externe Pools als auch Recyclingprozesse berücksichtigt wurden. Evans & Paranjape (1992) führten Untersuchungen in Bezug auf nichtlineares Grazingverhalten durch.

Zum Anwendungsbereich der Methode kann gesagt werden, daß keine einheitlichen und optimalen Vorgaben für die Durchführung von Dilutionexperimenten genannt werden können. Die Verfahrensschritte und verwendete Ausrüstung müssen an die spezielle Problematik angepaßt sein.

Für die Ermittlung der Abundanz bzw. Konzentration des Phytoplanktons können verschiedene Methoden verwendet werden. Die Bestimmung von Chl*a* gilt als Idealparameter, um die Kontrolle der Verdünnungsverhältnisse vorzunehmen und bietet die Möglichkeit des schnellen Abcheckens der experimentellen Resultate. Die Anwendung von Mikroskopie/Flowcytometrie liefert die zahlenmäßige Unterteilung der Planktongemeinschaft anhand verschiedener Taxa, der Größe, des

morphologischen Charakters und Autofluorenszenzmerkmalen der Organismen.

Vorteile	Nachteile
Möglichkeit der Ratenabschätzung für Wachstum und Sterblichkeit durch Grazing des Phytoplanktons in einem Experiment	beträchtlicher Aufwand für die erfolgreiche Durchführung der Experimente und die Probenauswertung
geringe Beeinflussung der Organismen bzw. minimales „handling“	3 Annahmen der Methode sind routinemäßig schwierig zu testen
simultane Analyse der Dynamik der unterschiedlichen Komponenten der Planktongemeinschaft möglich	Beeinflussung der rel. Wachstumsraten in den Verdünnungsstufen durch Kontamination und NS-Limitation bzw. -Anreicherung
	„threshold“-feeding kann Grazingraten in höheren Verdünnungsstufen verringern
	rel. lange Inkubationszeiten führen ggf. zu unklaren bzw. ungenauen Raten-abschätzungen
	geforderte Abschätzung der mittleren Grazerdichte wird erschwert bzw. unmöglich, wenn sich die unterschiedlichen Kategorien der potentiellen Konsumenten während der Inkubationsdauer ändern

Für die Versuchsdurchführung wird Verdünnungswasser benötigt, welches frei von den zu untersuchenden Planktonkomponenten und frei von Kontaminationen, welche die Wachstums- und Grazingraten positiv (Nährstoffe) bzw. negativ (Inhibitoren) beeinflussen können, sein sollte. Als Filtermaterial werden möglichst große (∅ 142 mm) aus Methylcellulose (MC) oder Polycarbonat (PC) mit der Porenweite 0,2 µm verwendet. Vorteile der MC-Membranen bestehen in der schnelleren Flußrate als PC-Membranen und der geringeren Gefahr der Zellzerstörung bzw. des Herauslösens von Kontaminationen aus dem Filter. Wenn sehr große Volumina des Verdünnungswasser benötigt werden, können Filterkapsulen eingesetzt werden. Sie gewährleisten

einen effizienten und sterilen Filtrationsprozeß, sind aber sehr teuer. Das Filtrationssystem sollte aus nichttoxischem Kunststoff bzw. teflonbeschichteten Materialien bestehen. Als Verbindungelemente sollten Schläuche aus Silikon eingesetzt werden. Die Reinigung aller während des Filtrationsprozesses verwendeten Ausrüstungen sollte mit 10% HCl und sorgfältigem Spülen mit dest. Wasser durchgeführt werden.

Beim Herstellen der Verdünnungsstufen wird das unfiltrierte und filtrierte Wasser schonend je nach geforderter Verdünnungsstufe gemischt. Dabei kann das Anmischen der Verdünnungsstufen in großen Vorgefäßen und das Überführen in kleinere Experimentalgefäße erfolgen. Dieser Ablauf liefert eine bessere Reproduzierbarkeit und einfache Anfangsprobenahme und ist notwendig für die Arbeit mit Dialyseschläuchen. Sie bietet allerdings die Gefahr der negativen Beeinflussung fragiler Organismen. Eine zweite Möglichkeit besteht im Einfüllen des Verdünnungswassers in die Experimentalgefäße in Volumeneinheiten entsprechend des Verdünnungslevels. Anschließend erfolgt die Zugabe von frisch geschöpftem Wasser, wobei Blasenbildung zu vermeiden ist. Bei diesem Verfahren ist allerdings die Probenahme zu Beginn des Versuches erschwert.

Zur Berechnung der erforderlichen Parameter ist mindestens eine Probenahme zu Beginn und zum Ende der Inkubationsdauer notwendig. Beprobungen während der Inkubationsdauer sind wünschenswert, wenn hohe Wachstums- und Grazingraten zu erwarten sind bzw. zur Kennzeichnung von Tag-Nacht-Unterschieden. Die Charakterisierung der Anfangssituation (Chl*a* oder Mikroskopie) sollte mindestens 2fach, besser 3fach aus der unverdünnten Probe erfolgen. Generell ist die Präzision (< 5% SD) der Bestimmungsverfahren von großer Bedeutung für die spätere Auswertung und sichere Interpretation der Ergebnisse. Als Anfangssituation wird die Abundanz in den einzelnen Verdünnungsansätzen bestimmt. Am Ende der Inkubationszeit erfolgt die Beprobung jedes Experimentalgefäßes. Als Kontrolle sollte das Verdünnungswasser mit in die Versuchsdurchführung aufgenommen werden, um evtl. den Filter passierte Organismen in die Abschätzungen miteinbeziehen zu können.

Die Inkubationsgefäße sollten unter annähernd natürlichen Bedingungen inkubiert werden. Optimal ist *in situ* - Inkubation, um sicherzustellen, daß die Versuchsdurchführung unter natürlichen Temperatur- und Lichtverhältnissen in geeigneter Tiefe abläuft. Bei der Verwendung von Dialyseschläuchen bzw. Diffusionskammern sollten diese mit weichen Nylonnetzen geschützt werden.

Bei der Durchführung von Versuchen im Labormaßstab erfolgt die Inkubation im Wasserbad. Dabei ist auf die Simulation der gegebenen Lichtqualitäten und -quantitäten sowie auf die Kontrolle der Wassertemperatur (± 1°C) zu achten, um eine Erhöhung oder Verminderung der Wachstums- bzw. Grazingraten zu verhindern.
Unter Verwendung der linearen Standardabweichung werden bei der Auswertung die aus den Zählergebnissen der einzelnen Verdünnungsstufen errechneten Wachstumsraten in Diagramme eingetragen. Die daraus ableitbare Bruttowachstumsrate ergibt sich als Schnittpunkt mit der y-Achse. In Tab. 2 sind Situationen geschildert, die zur Beeinflussung der eindeutigen Interpretierbarkeit der Ergebnisse führen.

Störungen	**Ergebnis**	**Gegenmaßnahme**
1. Nährstoff-Zugabe	spezif. Wachstumsrate des Phytoplanktons verändert	Inkubation von unverdünnten Proben ohne Zugabe von Nährstoffen
2. unterschiedliche NS-Verfügbarkeiten in hohen Verdünnungsstufen	Anstieg der Regressionslinie erhöht, Grazingrate überschatzt	direkte Versuchsdurchführung in hohen Verdünnungsstufen
3. Kontamination des Verdünnungswassers	Anstieg der Regressionslinie positiv, Grazingrate unterschätzt	Kontrolle des Verdünnungswassers
4. Grazerdichte geht ohne konkrete Bestimmung als unabhängige Variable ein	Unter- bzw. Überschätzung der Grazingraten, wenn sich Grazer während der Inkubation verändern	Grazerdichte bestimmen
5. Sättigung der Ingestionsraten der Konsumenten in Fällen hoher Phytoplanktonabundanz	Grazingrate unterschätzt	direkte Versuchsdurchführung in hohen Verdünnungsstufen

Zusammenfassend ist zu sagen, daß die Dilutionmethode trotz der vorausgesetzten Annahmen und des vorhandenen Potentials von Fehlinterpretationen Informationen über die Dynamik der gesamten

Phytoplanktongemeinschaft liefern kann. Selektiv metabolische Inhibitoren können die Wachstums- und Grazingraten von prokaryotischen Populationen trennen, haben aber eine begrenzte Aussagekraft für die Auflösung der Beziehung zwischen eukaryotischem Phytoplankton und deren Konsumenten.

Die Dilutionmethode erweist sich als weniger sensitiv, wenn geringe Zelldichten vorliegen.

Die Untersuchung des Grazingverhaltens einzelner Taxa oder die Abschätzung der Raten in kleinen Zeitintervallen ist nicht möglich. Hierbei sollte die FLA/FLB-Technik zur Anwendung kommen, die ihrerseits wiederum unpraktikabel ist für routinemäßige Messungen auf dem „community level".

Im Bezug auf Primärproduktionsmessungen stellt die Dilutionmethode keine Alternative dar. Sie ist als Ergänzung zu anderen Methoden mit dem Ziel einer möglichen Verifizierung von Ergebnissen bzw. erhöhtem Erkenntnisgewinn zu sehen.

Die Methodendarstellung wurde in Anlehnung an Landry (1993) erstellt.

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Photoacoustic measurements as a potential tool for assessment of primary production in aquatic ecosystems - the basics

Abstract

The light-energy absorbed by photosynthetic antenna pigments is

(i) chemically stored in form of energy-rich intermediates,
(ii) dissipated as heat and
(iii) re-emitted as fluorescence light.

The photoacoustic (PA) technique bears the potential for direct monitoring of photochemical energy storage and thermal dissipation. Analysis of the PA signals could provide the means for determination of the following parameters:

(1) absorbance of light-energy by photosynthetic organisms and all other substances in the sample;
(2) straightforward determination of the total and relative extent of photosynthetic light-energy usage (even for samples with a highly heterogeneous population of photosynthetic organisms);
(3) for excitation at various wavelengths, energy usage by the individual groups of photosynthetic organisms in the sample volume (green algae, cyanobacteria, ..).

Intrinsic and ambient acoustic noise are presently a significant obstacle to profitable *in-situ* measurements.

Measuring Principle

Absorption of the modulated light by a photosynthetically active sample (see Fig. 1) leads to two distinct contributions to the detectable

photoacoustic (PA) signal: the (i) thermal and the (ii) photobaric signal (BULTS et al. 1982; for reviews see BUSCHMANN 1990, FORK & HERBERT 1993, MALKIN & CANAANI 1994).

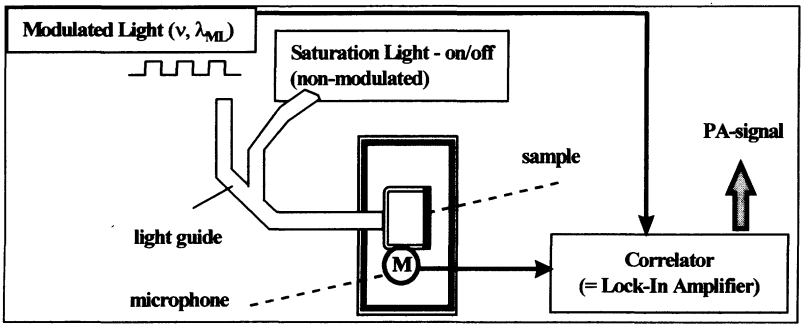

Fig. 1 Typical PA set-up. The modulated light (n = 1 Hz .. 10 kHz) is absorbed by an intact leaf (or macroalga), photosynthetic microorganisms on filter paper or by photosynthetic organisms in solution.

(i) Thermal signal.

The major part of the absorbed light-energy is converted into heat within a few milliseconds. The heat is transferred to a gas layer at the sample surface; the gas layer expands (according to the gas law: $P\ V = n\ R\ T$) and acts like a piston which compresses the air inside the closed measuring cell. This compression results in a pressure increase. Because the heat production is modulated with ν (frequency of the modulated light), the pressure changes with the same frequency. Consequently, these pressure changes are acoustically detectable by a microphone. The amplitude of this thermal photoacoustic signal, A_{th}, is proportional to the light-energy absorbed by the sample, but decreased due to reemission of fluorescence light and photochemical energy storage. Thus,

$$A_{th}(\nu) = \{1 - \psi_F - \psi_P(\nu)\}\ A_{th}^{max}(\nu) \qquad \text{(Eq. 1)}$$

where ψ_F and $\psi_P(\nu)$ are the fluorescence yield and the photochemical yield, respectively, in percent of the absorbed light energy (energy yields, but not quantum yields). The fluorescence yield, ψ_F, is relatively small in photosynthetic systems and therefore often neglected (but see also DAU

& HANSEN 1990). The photochemical yield, $\psi_P(\nu)$, is sometimes denoted as 'photochemical loss' or 'L'.

In photochemically inactive samples (e.g. inhibitor-treated samples) the thermal photoacoustic signal can be used as a measure of the absorption of the sample. Absorption measurements by PA-techniques can be advantageous in the case of highly scattering or non-transparent or optically inhomogenous samples.

Neglecting the contribution of fluorescence, the quantity A_{th}^{max} can be determined by application of non-modulated, saturating light. Because this 'background light' (BL) saturates the photochemical reactions, the yield of photochemistry approaches zero and the photoacoustic signal is approximately equal to A_{th}^{max}. Thus,

$$\psi_P(\nu) = L(\nu) \approx \{A_{th}^{max}(\nu) - A_{th}(\nu)\} / A_{th}^{max}(\nu) . \qquad \text{(Eq. 2)}$$

The A_{th}^{max} depends on the frequency of the modulated light because the involved heat-transfer processes are frequency dependent. Furthermore, the energy is dissipated in form of heat in different times after light absorption because the heat release is coupled to different steps in the chain of electron transfer reactions. Thus, also ψ_P is frequency dependent.

Analysis of the thermal PA signal could provide the means for determination of the following parameters:

(1) absorbance of light-energy by photosynthetic organisms and all other substances in the test-volume $A_{th}^{max}(\nu)$;
(2) straightforward determination of the total ($\psi_P A_{th}^{max}$) and relative (ψ_P) extent of photosynthetic light-energy usage even for samples with a highly heterogeneous population of photosynthetic organisms;
(3) for excitation at various wavelengths (various λ_{ML}), energy usage by the individual groups of photosynthetic organisms in the sample volume (green algae, cyanobacteria, ..). However, intrinsic and ambient acoustic noise are presently a significant obstacle to profitable *in-situ* measurements. Reasonable signal-to-noise ratios are only obtainable for extremely concentrated suspensions or by filtration and measurement on the filter containing the adsorbed photosynthetic organisms.

(ii) Superimposed photobaric contribution

The modulated light causes a modulation of the photosynthetic oxygen evolution resulting in pressure changes in the sample cell. Due to damping of the oxygen diffussion waves by the aequous medium, at modulation frequencies above 300 Hz the photobaric contribution to the PA signal is fully negligible. The oxgyen evolution contribution has been utilized for kinetic investigations on short-term light adaptation (e.g., DAU & HANSEN 1989).

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Comparison of photosynthetic measurements based on active fluorescence (PAM)- , O_2- and ^{14}C – methods

Traditionally measurements of phytoplankton photosynthesis are performed based on the detection of released oxygen (O_2) or fixed carbon (C). The latter is based on the time dependent incorporation of radiocarbon into particulate matter and has been used excessively since the work of Steemann-Nielsen (1952) in phytoplankton ecology. This method can be regarded as a highly sensitive classical standard method for the determination of phytoplankton photosynthesis. Unlike to the radiocarbon measurements the detection of the released O_2 is limited due to the unsatisfactory sensitivity of this method and therefore has not reached the same acceptance as the radiocarbon method in phytoplankton photosynthesis research. In addition, both methods are limited by several other methodological problems: e.g. long incubation times (0.5-24h), bottle effects, differentiation between net and gross photosynthesis and changes in phytoplankton assemblages due to concentration procedures.

In order to overcome these practical and methodological problems and to measure phytoplankton phytosynthesis *in situ* with a high frequency and/or on a relevant spatial scale a strong need to introduce new methods exists. One promising approach is the detection of the fast active chlorophyll fluorescence with the PAM-method developed by Schreiber which is described by Dau et al. in this volume. It has to be mentioned that another active fluorescence method (pump and probe, PNP) has been in use for several years by Falkowski and coworkers for phytoplankton research. Because we only used several PAM-equipments (see Domin et al. this volume) during the workshop in Zingst, we focus the following article on the PAM-method. To clarify to which extend this method can be applied for the estimation of

phytoplankton photosynthesis a comparision with the above mentioned classical methods (^{14}C, O_2) is strongly needed.

In Fig. 1 it is shown at which steps of the photosynthetic process the information for the assessment of phytoplankton photosynthesis is gathered by the different methods.

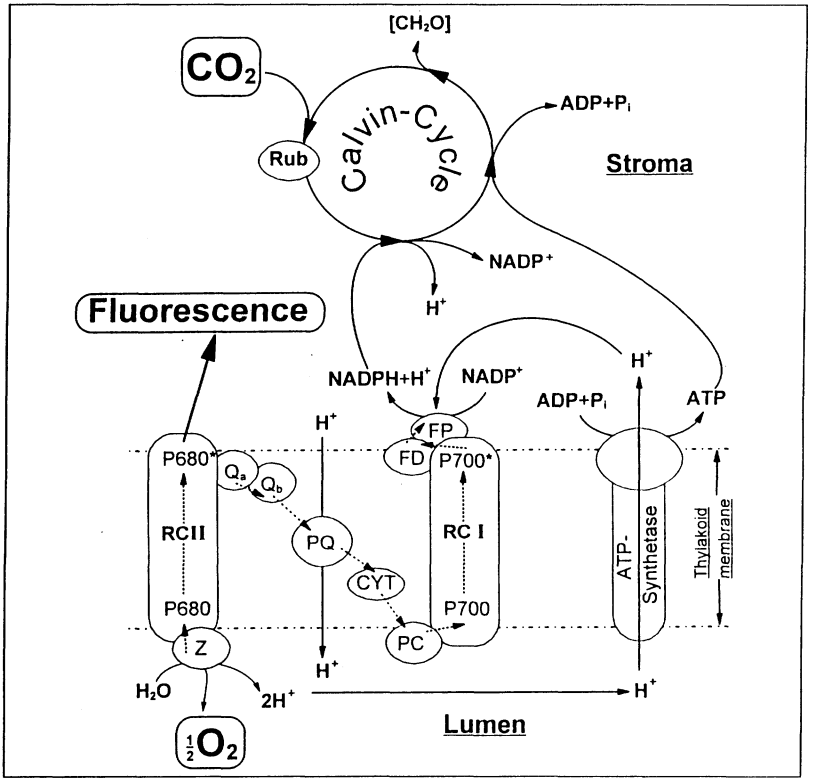

Fig. 1 Simplified, schematic presentation of the photosynthetic processes at which the information for photosynthetic measurements is gathered by the different techniques. The dotted arrows show the formal pathways of the electrons generated during the watersplitting process. Z: watersplitting complex; P680/P700: special chlorophyll molecules that can be excited to P680*/P700* due to light absorption; RCII/RCI: reaction centers of photosystem I/II; Q_a/Q_b: primary and secondary electron acceptor of RCII; PQ: plastoquinone; CYT: cytochrome b6f complex; PC plastocyanine; FD: ferredoxin; FP: ferredoxin-NADP-oxidoreductase; Rub: ribulose-1,5-bis-phosphate carboxylase; [CH_2O]: glucose (formal)

It becomes clear that fluorescence-emission and O_2-release are closely connected at the reaction center of photosystem II (RCII), whereas the CO_2-fixation takes place at a subsequent step in the Calvin cycle. This already implies that a stonger correlation of O_2 than of ^{14}C with the fluorescence-measurements can be expected. The most important differences between O_2-release and carbon-fixation arise from the fact that not all electrons evolved in the watersplitting process at RCII are strictly used for C-fixation. Alternative electron sinks are e.g. NO_3^- - reduction, photorespiration, Mehler reaction and reactions to repair damages caused by high irradiances. Since the PAM-method principally detects electron flow rates in RCII (Hofstraat et al. 1994, Hartig and Colijn 1996, Hartig et al. 1998) one can only expect a close correlation between the fluorescence- and the ^{14}C- based measurements if all electrons are used for C-fixation and not for other processes.

However fluorescence itself is highly variable due to different quenching mechanisms, which are not strictly connected with C-fixation or O_2-release. Without going into detail quenching includes all processes which lead to a reduction of fluorescence yields resulting in an inequality between fluorescence signals and O_2-release or C-fixation.

Relationships between classical- and active fluorescence-based methods of photosynthetic measurements conducted with higher plants and algae in different studies are presented in Tab. 1. It should be noted that this overview does not claim to be complete. Most of the studies that are intended to provide a comparison between the different methods of photosynthetic measurements have been carried out on higher plant leaves or on isolated chloroplasts. Only a few researchers have applied PAM fluorescence techniques on unicellular algae and phytoplankton for this purpose (Kroon et al. 1996, Hartig and Colijn 1996, Geel et al. 1997, Hartig et al. 1998). Because PNP has found a wide application in phytoplankton research we also present the relationship to the classical methods obtained with this technique. The results of the different studies on the relationship between PSII electron transport rates and carbon fixation respectively oxygen release in higher plants and algae showed different results (Tab. 1). Even when conducted under a variety of experimental conditions, most comparisons show a linear correlation under moderate irradiances, whereas a non-linear correlation was found under low light intensities and in some cases under high light intensities. Therefore irradiance seems to be an important factor which influences the assessment of photosynthesis based on fluorescence measurements.

Tab.1 Relationships between classical- and active fluorescence-based methods of photosynthetic measurements conducted with higher plants and algae in different studies. Abbreviations: HL: high light; LL: low light; IL: intermediate light, AL: actinic light, SD: spectral differences.

Author (Year)	Methods	Organisms	Relationships	Reasons for non-linearity
Boyd et al. (1997)	PNP, ^{14}C	Phytoplankton	Linear (for I_k, P_m); Non-linear (for ??	SD of AL sources
Falkowski et al. (1986)	PNP, O_2	Algae (cultures)	Linear (IL); Non-linear (LL and HL)	HL: cyclic electron flow around PSII, LL: quenching processes
Geel et al. (1997)	PAM 101, Xenon-PAM, O_2	Algae (cultures)	Linear (LL and IL); Non-linear (HL)	Oxygen consumption (not photorespiration)
Genty et al. (1989)	PAM 101, CO_2 IRGA	Higher plants (C3, C4)	Linear	
Genty et al. (1992)	PAM 101, $^{16}O_2/^{18}O2$	Higher plants (C3)	Linear	
Harbinson et al. (1990)	PAM 101, O_2	Higher plants (C3)	Linear; Non-linear (at 20% O_2)	Photorespiration
Hartig & Colijn (1996)	PAM 101 (PM), ^{14}C	Algae (cultures and microphytobenthos)	Linear	
Hartig et al. (1998)	PAM 101 (PM), ^{14}C	Algae (microphytobenthos)	Linear (IL); Non-linear (LL and HL)	LL: SD od AL sources, HL: electron sinks
Hofstraat et al. (1994)	PAM 101, μ (growth)	Algae (cultures)	Linear	
Horton (1989)		Higher plants	Linear	
Keiller & Walker (1990)		Higher plants	Linear	
Kolber & Falkowski (1993)	PNP, ^{14}C	Phytoplankton	Linear (r 0.86)	
Krall & Edwards (1991)	PAM 101, O_2	Higher plants (C4)	Linear	
Kroon et al. (1996)	PAM 101, O_2	Algae	Linear ; Non-linear (HL)	Adapt. Phenom. (Baumert 1996)
Oberhuber et al. (1993)	PAM 101, CO_2 (IRGA)	Higher plants (C3,C4)	Linear; Non-linear (LL)	Respirat. or presence of inactive PSII centers
Rees et al. (1992)	PAM 101, O_2	Algae (cultures)	Non-linear	Non-assimilatory electron flow
Seaton & Walker (1990)	PAM 101, O_2	Higher plants (C3,C4)	Linear; Non-linear (LL)	

For the observed deviations under low and high light conditions the authors offered possible explanations similar to those we already mentioned above and some additional reasons for non-linearity like e.g. spectral differences of light sources, oxygen consumption and cyclic electron flow around PSII.

Most of the studies who revealed a non-linear relationship between the classical methods and the active fluorescence methods where carried out with algae. Apart from the already given explanations for the observed non-linearity another important factor is the higher plasticity of the photosynthetic machinery of phytoplankton than that of higher plants. This plasticity is mainly reflected by a different pigment composition and the intracellular arrangement of the pigments, which are furthermore highly variable under different environmental conditions (see Forster et al. this volume, Cleveland & Perry 1987, Herzig & Falkowski 1989). Therefore light absorption of phytoplankton is different to higher plants, where it is considered to be very constant. For calculation of fixed carbon or released oxygen based on fluorescence measurements a definite knowledge about the light absorption of phytoplankton is needed (Hartig et al. 1998).

It has been shown that the final conversion of irradiance into fixed C by phytoplankton is impaired due to different effects (e.g. different electron sinks, variable light absorption etc.). This complicates the comparision of photosynthetic measurements based on PAM-, O_2- and ^{14}C – methods. However in some studies carried out with phytoplankton a good correlation between O_2- or ^{14}C-based methods and the active fluorescence based methods (PAM and PNP) was found (Tab.1). Therefore we assume that the active fluorescence methods offer the potential for rapid estimation of phytoplankton photosynthesis with high spatial and temporal resolution, which is needed particularly for phytoplankton communities due to their patchy distribution.

Abbreviations

PAM: Pulse Amplitude Modulated Fluorometer
PNP: Pump and Probe Fluorometer
PM: Photomultiplier
IRGA: Infra Red Gas Analyser

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Experimental conditions accompanying the measurements of photosynthesis

Introduction

A successful comparison of primary production measurement techniques demands carefully-controlled conditions and accurate recording of relevant parameters at all steps between sample collection and final calculation of results. Sakamoto et al. (1984), in the proceedings of the 1st Group for Aquatic Productivity Workshop in Konstanz, noted that differences observed between different techniques could have been due to "...the handling of the experimental samples, especially in the care which was taken in shielding them from excess or stray light". A recommendation of the GAP authors was that "Attention to methodological details must be emphasised if accurate assessments of parameters such as I_k and compensation depth are to be made".

The following article describes the experimental conditions accompanying the comparison of photosynthesis measurements during "Pri-Pro 1997". Relevant parameters are described, together with the main causes of error, and suggestions are made for future workshops of this kind. The summary is divided into three parts describing

(1) collection of the water sample and subsequent handling before the measurements
(2) design of the experimental chambers used for the incubations, with reference to control of temperature and irradiance
(3) measurement of the additional optical parameters which are required for comparisons of photosynthesis measurements made under different spectral irradiance conditions.

Sample collection, handling and characterisation

Collection and handling

The collection depth should be clearly defined for field samples e.g. by use of Niskin bottles with automatic opening and closure, alternatively an integrated water column sample can be taken by means of a long plastic cylinder. For the Zingst workshop, a large experimental mesocosm containing natural phytoplankton was deployed (Schubert *et al.* 1998, this volume). The mesocosm container was continually mixed during the whole day by means of small centrifugal pumps situated at the bottom of the tank. Additional mixing was performed manually shortly before the water samples were removed for measurements, thus the sample was representative of the whole container. The volume of sample collected from the population should be large in order to provide sufficient amounts for all groups, and to minimise container effects and changes in temperature during the time between collection and the start of measurements. The sample may be filtered during collection to remove larger zooplankton. At the Zingst workshop, the water had already been filtered through 200 µm gauze during the filling of the 1000 l mesocosm. The time between collection from the water body, or in this case 1000 l mesocosm, and use in experiments should be kept as short as possible, with the sample stored under controlled irradiance and temperature conditions. At Zingst, a 10 l subsample was taken at each time point and stored in a plastic bucket in dim light at room temperature (approx. 10 °C higher than the mesocosm temperature). The sub-samples used by each group in the intercomparison should be taken after a thorough mixing, then introduced into the various measurement chambers as quickly as possible, and not allowed to stand under uncontrolled conditions in the laboratory where they may be subject to warming. The start time for the photosynthesis-irradiance curves should be synchronised for all groups, and should ideally be within 1 h for all. The actual start times of P-I measurements in "Pri-Pro 1997" varied from 0.5 to 4 h, as considerable time was needed to concentrate samples for some of the experients.

Photosynthesis measurements using Clark-type oxygen electrodes, or with certain types of fluorescence detection equipment may not be sensitive enough to function with water samples containing algal populations with less than 100 µg chl a l^{-1}. Concentration is necessary in

order to ensure a sufficient signal-to-noise ratio when measuring P-I curves over short time periods. The method used for concentration of the sample should ideally be tested beforehand in separate experiments to ensure that photosynthetic rates are not altered by the treatment. Samples in Zingst for the Light Pipette (HRO) and LED-PAM fluorimeter (FTZ) were concentrated by repetitive centrifugation at moderate speed, without cooling, until chl a concentrations of > 500 µg l^{-1} were achieved. The Light Pipette was also used to measure a P-I curve of an unconcentrated mesocosm sample. A run time of 120 min was used and the rates obtained were similar to those of the concentrated samples.

Nutrient concentrations and algal biomass.

Large difference in nutrient concentrations between the original water body (mesocosm) and the experimental subsamples are not likely to occur if zooplankton are absent and the precautions listed above for handling samples are observed e.g. storage time is kept short and handling stress is avoided. Measurement of the macronutrient concentrations is desirable, and may assist in the calculation of carbon fixation from fluorescence-based electron flow rates (nitrate assimilation is an important sink for electrons), and in the interpretation of photosynthetic and respiratory quotients (Davies & Williams 1984). Nutrient concentrations in the 1000 l mesocosm were not measured at Zingst, but measurements in the water column adjacent to the field station on the date that the compartment was filled showed values of 0 µg l^{-1}, 0.31 µg l^{-1}, 0.85 µg l^{-1} and 2.31 µg l^{-1} for nitrate, nitrite, ammonium and phosphate respectively.

Chlorophyll concentration was used as a proxy measurement for algal biomass in the subsamples. Measured volumes of sample were filtered at ~400 mbar suction pressure onto Whatman GFF filters. The filters were extracted at room temperature overnight in 100% dimethylformide (DMF). In a recent comparison by Wright et al. (1997), DMF was found to be the most effective solvent for phytoplankton extraction. (N.B. 100% methanol was however recommended for general use due to the toxicity of DMF). The equations of Porra et al. (1989) were used to calculate concentrations of chl a and chl b. Concentrations are given in Table 2.1.1 of Domin (1998, this volume). To date, no extinction coefficients for chl c in DMF have been published, so the possible contribution of this pigment cannot be estimated. Additionally, no distinction is made in this method between chlorophyll and its breakdown products such as phaeophytin. A recommendation for future

workshops is to use HPLC for pigment analysis rather than spectrophotometric methods (e.g. Jeffries et al. 1997).

Design of the experimental chambers and measurement of P v I curves

Control of temperature

Both respiration (R) and the dark reactions of photosynthesis (which determine P_{max}) are enzymatic and are strongly dependent upon temperature. The temperature setting for the P-I curves should be agreed on by the investigators before the experiments, and then be controlled accurately by use of suitable water baths, Peltier elements etc. It is advantageous to have online measurements of temperature in the cuvettes during fluorescence and oxygen measurements, especially when small volumes of sample are exposed to strong actinic irradiances. In the case of *in situ* bottle experiments, the temperature in the water column should be recorded for comparison with the laboratory measurements. Temperature differences ranging from 13 °C (in situ bottle experiments) to 23 °C (fluorescence cuvettes) existed between the various measurements systems used in Zingst. In the absence of temperature control, the results of samples measured at two different temperatures should be related using suitable Q_{10} values from the literature.

Control of irradiance

Accurate measurements of irradiance are essential before any attempts are made to critically compare the methods used for generating P-I curves. In addition, the estimation of carbon fixation rates from fluorescence parameters alone (e.g. Kolber & Falkowski 1993) also depends upon the accuracy of the irradiane measurements.

As a first step in determining the actinic irradiances used in such a workshop, the irradiance meters and spectroradiometers used by each group should be directly compared against a stable light source of known output. Previous experience of such intercalibrations has shown that irradiance meters may vary considerably, with deviations of up to 50% for machines of the same model being occasionally reported (results of Hanse Tag in Hamburg, 1995). Irradiance meters which deviate by more than 20% from the group mean could be excluded from measurements. The spectral and angular distribution of the light source should be known, and

the calibration of the standard light source should be confirmed in advance with a spectroradiometer operated by qualified optical specialists (e.g. Deutsche Wetterdienst). Sufficient time (1 day) should be allowed for these initial comparisons, for measurement of the irradiance incident upon the various photosynthesis cuvettes, and for subsequent discussions, before the start of the primary production measurements.

One problem which emerged during irradiance measurements at Zingst was that some cuvette set-ups were illuminated with fibre-optic cables which produced narrow, concentrated light beams. The full light-collecting surface of the available irradiance meters (Li-Cor LI-192) or spectroradiometer (Macam) were only partially illuminated by these beams, and thus could not be used for measurements. Use of miniaturised flat (cosine) and spherical (4 ^{1}) sensors (such as the instruments produced by Zemoko, Koudekerke, NL) together with micromanipulators is recommended to alleviate this problem and also to allow the measurement of light gradients within algal suspensions in the cuvettes.

Ideally, experimental vessels should be designed in order to minimise the problems listed above, e.g. good temperature control is essential, together with a stirring mechanism and a simple construction which allows the actual irradiance inside the cuvette to be measured. Use of cylindrical cuvettes containing internal focal points, or reflective side walls or mirrors which cause multiple pathlengths through the cuvette should be avoided.

Increasing the chlorophyll concentration of the sample is often necessary to improve the signal-to-noise ratio, but a serious disadvantage of using concentrated algal samples is that strong attenuation of irradiance within the cuvette occurs. In extreme situations, e.g. with chl concentrations of over 5000 µg l^{-1} the incident irradiance (I_o) may be reduced by over 50% within a 10 mm cuvette. The spectral quality is also shifted under these conditions to favour wavelengths which are not strongly absorbed by the algal pigments. Furthermore, the algae are subject to a rapidly fluctuating light field instead of constant conditions. A correction must be applied to determine the mean irradiance within the cuvette. The following equation can be used for monochromatic irradiation of algal suspensions:

Van Liere & Walsby (1982)

$$I_m = \frac{(I_o - I_b)}{\ln(I_o / I_b)} \qquad \text{(Equation 1)}$$

where I_m = mean irradiance in the chamber, I_o = irradiance at the front face of the chamber, I_b = irradiance at the back of the chamber. This equation can also be used with very little error for estimating the mean broadband PAR in a photosynthesis chamber (Bright & Walsby 1998). Irradiance values for all photosynthesis measurements made with concentrated samples at Zingst were corrected by using a spectrally-resolved version of Equation 1. $I_{o(l)}$ was measured for all actinic light sources with a spectroradiometer at a position just in front of the cuvette. $I_{b(l)}$ was calculated indirectly using spectral attenuation coefficients for the concentrated algal samples measured with a spectrophotometer, and assuming that the irradiance was attenuated exponentially in accordance with the Lambert-Beer law. In future, it would be advisable to avoid these uncertainties either by using concentrated algal suspensions only in combination with short optical pathlength cuvettes, or by making direct measurements of the light gradient within the algal suspension. No correction was made for measurements on unconcentrated samples at Zingst (for a 10 mm cuvette, and 35 µg chl a l^{-1}, $I_b > 0.95*I_o$).

I have given prime importance in this chapter to accurate measurement of irradiance, but there are also other factors to consider when comparing P-I curves. The number of irradiance steps, and the spacing of the irradiance steps, are important factors in the experimental design. Too few irradiance steps in the light-limited part of the curve will reduce the accuracy with which alpha can be determined. Sufficient high irradiance points must be included to clearly show that light saturation has been reached. Recommendations for designing P-I curves and mathematical fitting of the data are given in a review by Henley (1993). The length of each irradiance step may also influence the photosynthetic rates. Longer irradiance steps at the higher irradiances will increase the likelihood of photoinhibition being observed, but too short a step length may also alter photosynthetic rates e.g. by incomplete activation of RUBISCO if the sample has been dark-acclimated (Macintyre & Geider 1996). Comparisons of P-I curves obtained with widely different incubation times should only be done with caution (Macedo et al. 1998)

Optical parameters required for comparison of photosynthesis-irradiance curves

In addition to the variations in cuvette design, and the problems with strong attenuation within the cuvette mentioned above, there were also large differences in the spectral distribution of the various actinic light sources used at the workshop (Figure 1).

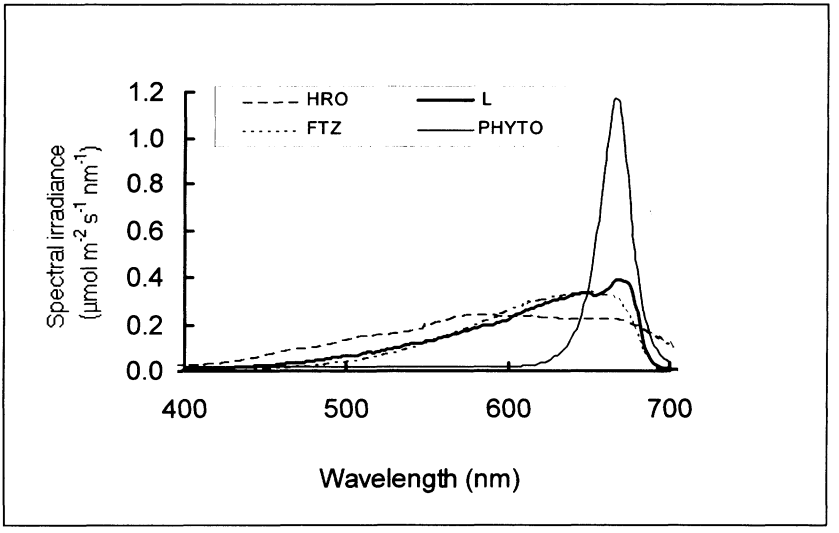

Fig. 1 Comparison of four different actinic light spectra at low irradiance. **HRO** = halogen lamp of the Light Pipette (Uni. Rostock) at 49 µmol m^{-2} s^{-1}; **L** = halogen lamp of a Schott KL-1500 (Uni. Leipzig) at 43 µmol m^{-2} s^{-1}; **FTZ** = halogen lamp of a Schott KL-1500 (FTZ Busum) at 40 µmol m^{-2} s^{-1}; **PHYTO** = red LED (Büro für Umweltmesstechnik) at 35 µmol m^{-2} s^{-1}. Spectra were measured at the I_0 position with a Macam SR-9910 spectroradiometer. The shape of the **HRO** and **PHYTO** spectra was constant at all irradiances, whereas the **L** and **FTZ** spectra were blue-shifted at higher irradiances. When working with concentrated algal suspensions, the mean spectral irradiance would differ from these spectra due to attenuation within the cuvette.

The spectra produced by the various light sources are also different in shape to natural solar radiation and to the *in situ* light climate of the mesoscosm. These spectral differences can potentially affect photosynthetic rates, as photosynthesis in the light-limited range of the curve is influenced by the rate at which algae absorb light:

(Kirk 1994) $$\alpha = \phi_{max} . a^*$$ (Equation 2)

where a = photosynthetic efficiency as measured by the initial slope of the P-I curve, f_{max} = maximal quantum efficiency of photosynthesis, and a^* = absorbtion cross-section of the photosynthetic apparatus.

Of these parameters, a^* is spectrally dependent, whereas f_{max} is generally assumed not to depend upon the wavelength of the actinic irradiance (but see Schofield et al. 1996). Differences in the photosynthetic efficiency, a, between different actinic light sources e.g. between a laboratory cuvette and *in situ* water column incubations, can be predicted if both the actinic irradiance spectra and absorption spectra of the algae are known:

(Arrigo & Sullivan 1992): $$\alpha_a = \alpha_b . \frac{\bar{a}^*_a}{\bar{a}^*_b}$$ (Equation 3)

where a_a = initial slope in light field *a*, a_b = initial slope in light field *b* , $\bar{a}_a^*$ = spectrally-weighted absorption in light field *a*, $\bar{a}_b^*$ = spectrally-weighted absorption in light field *b*. The usefulness of this correction method was confirmed by Schofield et al. (1996).

The mean spectrally-weighted absorption coefficient is a measure of the effectiveness of algae at absorbing quanta from a given spectral irradiance distribution, and is an important component of bio-optical models of productivity. It is derived from a combination of the algal absorption spectra and the spectral distribution of the actinic irradiance:

(Bannister 1974; Berner et al. 1987) $$\bar{a}^* = \frac{\sum_{400}^{700} (a^*_{(\lambda)} * I_{(\lambda)} * \Delta\lambda)}{\sum_{400}^{700} (I_{(\lambda)} * \Delta\lambda)}$$ (Equation 4)

where $a_{(l)}^*$ is the optical cross-section of the algae, and $I_{(l)}$ is the scalar irradiance at wavelength l. Normally, cross-sections are expressed per unit chlorophyll, but may equally well be expressed per cell, or per unit C if accurate measurements of these are available. In the following calculations I have chosen chlorophyll as the biomass parameter for calculating the amount of light absorbed by the algae under different actinic irradiance conditions with Equation 4. This assists our comparisons with the literature, as virtually all published data for phytoplankton is in the chl-specific form. The actual amount of quanta absorbed at any moment in time from a light stream is given by the upper part of Equation 4:

(Kroon et al. 1993) $$AQ = \sum_{400}^{700}(a^*_{(\lambda)} * I_{(\lambda)} * \Delta\lambda)$$ (Equation 5)

The optical cross-section referred to in Equations 4 and 5 can be either the total light absorbed, or the light absorbed which is used in photosynthesis. I will differentiate between these two forms of cross-section by adding subscripts as in Sosik & Mitchell (1995):

a^*_{ph} refers to the total photons absorbed by the algae, and a^*_{ps} is in most cases more useful, and refers only to the photons absorbed by photosynthetically-active pigments.

a^*_{ph}, the total absorption cross-section of the algae can be estimated either by concentrating the algae on a filter and scanning the filter in a suitable designed spectrophotometer [Quantitative Filter Technique developed by Kiefer & SooHoo (1982), Mitchell & Kiefer (1984)], or by scanning the sample inside, or at the inlet port of an integrating sphere (Maske & Haardt 1987; Nelson & Prezelin 1993). Spectra obtained with the QFT method must be corrected for the increase in pathlength caused by multiple scattering within the wet filter (ß-correction; Mitchell 1990). True phytoplankton absorption is derived by correcting scans for the absorption due to dissolved organic material, and for non-phytoplankton detrital material (Kishino et al. 1985, Roesler et al. 1989). Figure 2 shows the a^*_{ph} spectra for the concentrated phytoplankton samples used at Zingst,

with distinctive peaks and shoulders at 438 nm (chl a), 460-500 nm (carotenoids, chl b), 630 nm (phycocyanin) and 680 nm (chl a). The mean unweighted absorption was 0.012 m^2 (mg chl a)$^{-1}$, which is at the lower end of values typically reported for field samples. The signal-to noise ratio with unconcentrated samples was too low to give an accurate spectra.

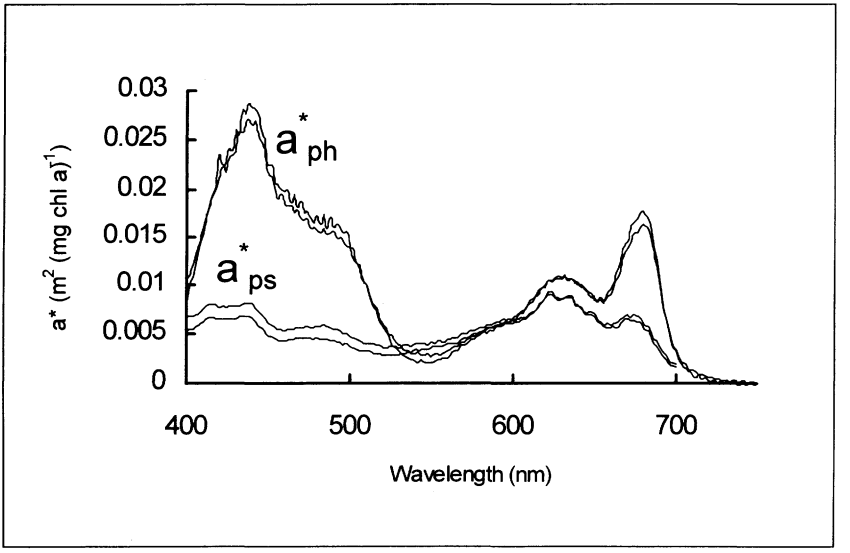

Fig. 2 Chl-specific *in vivo* absorption spectra (a^*_{ph}) measured for concentrated phytoplankton samples compared to scaled, quantum-corrected, DCMU-enhanced fluorescence excitation spectra (a^*_{ps}) of the same samples. Samples were collected from the mesocosm and concentrated by centrifugation before use in photosynthesis experiments at 10:30 and 16:30. The absorption spectra were measured in the integrating sphere attachment of a Lambda 2 spectrophotometer, and are corrected for absorption due to dissolved organic material, but not for detrital absorption. Residual scattering effects have been removed by subtracting the absorption value at 750 nm
The F_{DCMU} spectra, measured in a Hitachi 4010 spectrofluorimeter, were first quantum-corrected, then scaled to 35% of the red absorption peak.

Several methods exist for estimating a^*_{ps}, the absorption cross-section of only the photosynthetically-active pigments. One solution is to use HPLC either to measure the amount of absorption due to non-photosynthetic

pigments (such as zeaxanthin and diadinoxanthin) and then subtract the contribution to these from the total absorption spectra, another is to completely „reconstruct" the *in vivo* absorption of only the photosynthetically-active pigments. These methods have been pioneered by Prezelin and co-workers (Nelson & Prezelin 1990; Johnsen et al. 1994, but have been criticised due to the difficulties of converting from *in vitro* to *in vivo* absorption. The alternative approach is to use the *in vivo* excitation spectra for photosystem II fluorescence to estimate the shape of the photosynthetically-active absorption spectra for this photosystem (Neori et al. 1988). PSII fluorescence emission is measured in the far-red region (> 720 nm) in order to include the whole range of photosynthetically-active pigments in the scan (350-700 nm). It is recommended to add DCMU to the algal suspension in order to close all PSII reaction centres, and to raise the fluorescence yield of "active" chlorophyll relative to "dead" chlorophyll breakdown products (Johnsen & Sakshaug 1993). Before use, the raw F_{DCMU} spectra require correction for changes in the quantum output of the excitation lamp. Examples of quantum-correction techniques are given by Mitchell & Kiefer (1984), and Lutz et al. (1998).

The fluorescence method has the advantage of being relatively insensitive to scattering, but has the disadvantage that the F_{DCMU} spectra have no units, and cannot be used directly in models of algal absorption. A scaling procedure is used to solve this problem, in which the F_{DCMU} spectra is matched to the corresponding a^*_{ph} spectra. Techniques and problems associated with scaling of the F_{DCMU} spectra, in particular the problems associated with phycobilin-containing algae, are discussed by Johnsen & Sakshaug (1996), and Lutz et al. (1998). The F_{DCMU} spectra measured in Zingst showed activity at 420-440 nm (chl a), 460-500 nm (chl b and carotenoids), 620 nm (phycocyanin), and 680 nm (chl a). In this example, the F_{DCMU} spectra of the concentrated samples were scaled so that the red peak of the F_{DCMU} spectra was equal to 35% of the a^*_{ph} spectra. This is an empirical factor which I have chosen to account for the fact that most of the chlorophyll in this mixed algal sample is in cyanobacteria, and is therefore associated with the non-fluorescent PSI. Use of a higher scaling factor would have resulted in the a^*_{ps} spectra "overshooting" the a^*_{ph} spectra, which is not physiologically possible. Scaled in this way, a^*_{ps} has a mean unweighted value of 0.005 m^2 (mg chl a)$^{-1}$, or 45% of the total absorption. The remaining 55% of the absorption would be accounted for by PSI and photoprotective pigments (zeaxanthin is known to be present in high

concentrations in Bodden samples). Clearly, the scaling of the F_{dcmu} spectra to obtain cross-sections in absolute units is highly subjective and is an area of considerable uncertainity. Nevertheless, in cases where only the relative shape of the algal spectra are needed, e.g. with Equation 3, then the quantum-corrected fluorescence excitation spectra can be used with confidence.

I have calculated $\tilde{a}^*$ the mean spectrally-weighted algal absorption for the 4 actinic irradiances shown in Figure 1, using both types of a^* spectra (Table 1), together with the percentage difference in comparison with a perfect "white" light source (e.g. weighted with a value of 1 throughout the spectrum).

Tab. 1 Variation in mean spectrally-weighted absorption ($\tilde{a}^*$) arising from the differences in actinic light spectra shown in Fig. 1, and compared to a an unweighted "white" light (=100%). Absorption cross-sections are calculated for both total phytoplankton absorption (a^*_{ph}) and for PSII-specific photosynthetically-active pigment absorption (a^*_{ps}) as in Figure 2. The effective absorption in each actinic light spectra is given as a percentage of the unweighted spectra. Actinic light source abbreviations and descriptions are given in the legend to Figure 1. "Lo" = irradiance settings between 35 and 49 µmol m^{-2} s^{-1} ; "Hi" = irradiance settings between 400 and 800 µmol m^{-2} s^{-1}.

Name	Light source		a^*_{ph}		a^*_{ps}	
"White"	Equal quanta at all wavelengths	-	0.0115	100%	0.0052	100%
HRO	Light Pipette	Hi	0.0092	80%	0.0058	112%
	Halogen lamp	Lo	0.0092	80%	0.0058	112%
L	Halogen lamp	Hi	0.0094	82%	0.0061	117%
		Lo	0.0093	81%	0.0064	123%
FTZ	Halogen lamp	Hi	0.0085	74%	0.0061	117%
		Lo	0.0086	75%	0.0064	123%
PHYTO	Red LED	Hi	0.0117	102%	0.0064	123%
		Lo	0.0117	102%	0.0064	123%

For the a^*_{ph} spectra, the three halogen lamp spectra produced a mean effective absorption which was between 19% and 26% lower than that with theoretical "white" light. This is because these spectra contained substantial amounts of quanta at wavelengths which were minimally

absorbed by the Zingst algae. The red LED of the PHYTO-PAM resulted in higher effective absorption, as it matched the red absorption band of chl a and b. Increasing the power supply of the halogen lamps (L, FTZ) caused a blue-shift of the spectrum, but there was only a small effect on effective absorption. The results obtained with the spectra for a^*_{ps} differ in that all 4 actinic light sources showed 12-23 % higher effective absorption in comparison to "white" light, and that there was very little difference between the effectivity of all 4 light sources.

The large contribution of orange-red absorbing pigments to the a^*_{ps} spectra matched better the distribution of the actinic spectra. In this case, the blue shift caused by changing the L and FTZ halogen lamps from low irradiance (approx 40 µmol m^{-2} s^{-1}) to high irradiance (approx. 400-800 µmol m^{-2} s^{-1}) caused a 4% drop in the absorption efficiency. Thus, assuming that the a^*_{ps} is most appropriate weighting spectrum for measurements of PSII activity via fluorescence and oxygen evolution, then very minor differences in α would be expected from the use of different actinic light sources at the workshop.

As intercomparisons of the optical methods listed here have rarely been reported in the literature, one productive area for future "Pri-Pro" workshops would be to include a series of such tests e.g. determination of a^*_{ph} spectra with the filter technique compared to with an integrating sphere.

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The mesocosm approach for direct comparison of photosynthesis measurements based on active fluorescence (PAM)-, O_2- and ^{14}C- methods

The Mesocosm-System used for a direct comparison of the different methods for determination of primary production consisted of a polyethylene-container of approximately $1m^3$ volume (0.9m x 1.1m x 0.98m). The white walls of the container were shaded by black sheets, resulting in a steeper irradiance gradient compared to the field situation, and complicating calculations of obtained light doses for all incubations. The container was filled with a field sample of water from the Darss-Zingst-estuary in the evening hours of the 7.10.1997. Salinity, measured as electrical conductivity of the sample, was 7,2 °/00.Permanent mixing was obtained by means of 2 radial pumps with a throughput of approx. 3l min^{-1} each.

Irradiance above the surface (incident irradiance) was monitored continously by means of a cosine-corrected PAR-sensor (LiCor 190, LiCor Inc., USA) mounted alongside the mesocosm. A spherical underwater sensor (LiCor 193, LiCor Inc., USA) installed at 0.68m depth was used as a reference for verification of the calculations of light doses at the individual incubation depths. Data from both detectors were automatically recorded with a datalogger. Figure 1 shows the irradiance data measured on the 8.10.1997 with the two sensors mentioned above. The mesocosm was used for several different types of in-situ incubations on the 8.10.1997. The exact positioning of the various vessels are listed in Table I.

During the incubation period oxygen concentration, temperature and pH of the mesocosm were measured every 30 minutes. The course of these parameters are shown in figure 2.

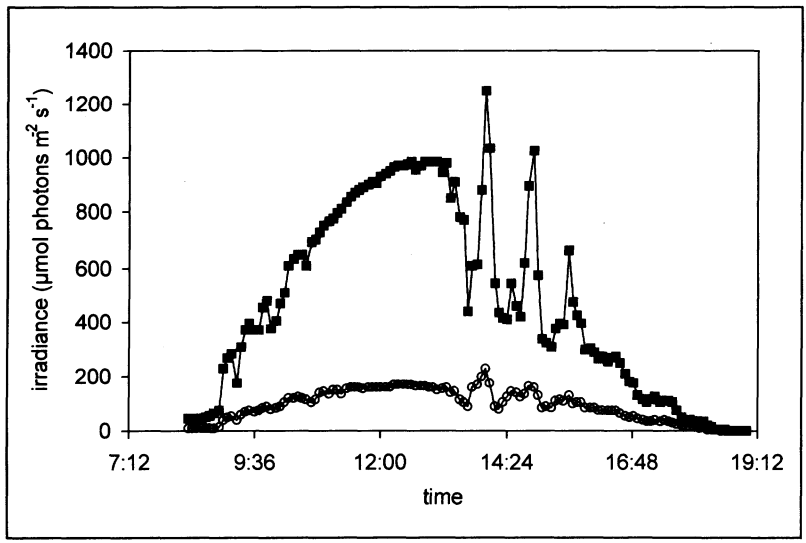

Fig. 1 Surface and underwater irradiance measured in and alongside the mesocosm at Zingst, 8.10.1997. Filled squares: surface irradiance (cosine-readings), open circles: spherical sensor readings at 68cm depth.

Tab. I Location of the in-situ incubation vessels within the mesocosm. Corners and walls were facing to: corner I East, wall A South-East, corner II South, wall II South-West, corner III West, etc..

Inkubation	position (distance in cm from)					
	corner	first wall	cm	second wall	cm	depth (cm)
Dialysis chamber	II	A	18	B	18	variable
Lift system, small vials	III	B	29	C	30	variable
Lift system, 250ml vials	IV	C	35	D	31	variable
static incubation	I	A	59	D	19	25cm steps, starting at 2cm
LICOR 193UW	III	B	25	C	43	68

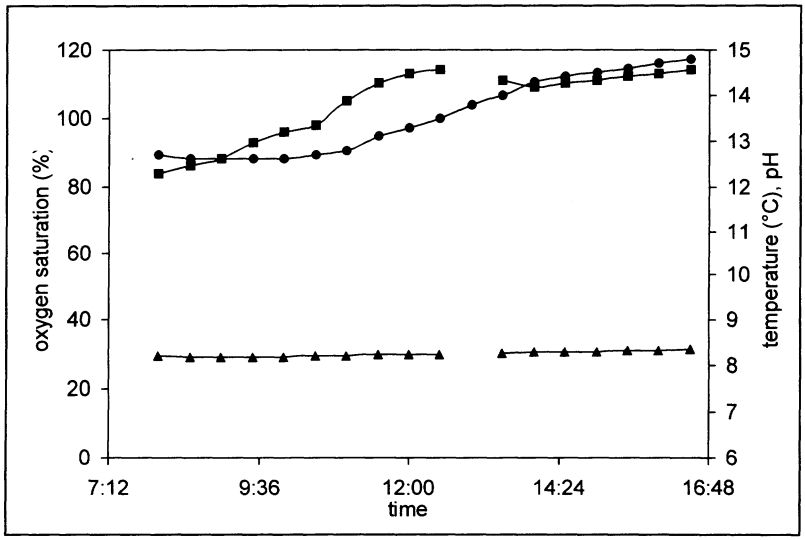

Fig. 2 Course of abiotic parameters of the mesocosm during 8.10.1997
Filled squares: oxygen saturation (%, left y-axis), filled circles: temperature (°C, right y-axis), filled triangles: pH (right y-axis).

For calculating the total light doses received by each of the in-situ incubations, attenuation spectra were measured in the shaded and unshaded part of the mesocosm. There were almost no differences found in slope and absolute values between the two spectra obtained, therefore all calculations were performed on the basis of the spectra obtained in the unshaded part (Figure 3). For calculating irradiances in the shaded part from the surface data, the following algorithm was used:

1) calculation of sub-surface irradiance from cosine-corrected just-above-surface readings (adjusted for losses due to reflection) using the equations published by Walsby (1997),
2) calculation of underwater irradiance at the depth at which shading starts by means of the attenuation spectrum,
3) calculation of the alteration of the irradiance climate within 10 cm of leaving the unshaded part by means of the "conversion spectrum", obtained by averaging 10 measurements across the shadow-line in

the mesocosm (each reflecting the irradiance difference within 10cm above and below the shadow line) at different depths, and

4) calculation of the irradiance at the incubation site, using the attenuation spectrum as in B) for the remaining depth until incubation depth.

Attenuation and conversion spectra are shown in Figure 3, and calculation of the shadow depth at any particular time point was performed by means of a matrix basing on the equations given by Walsby (1997).

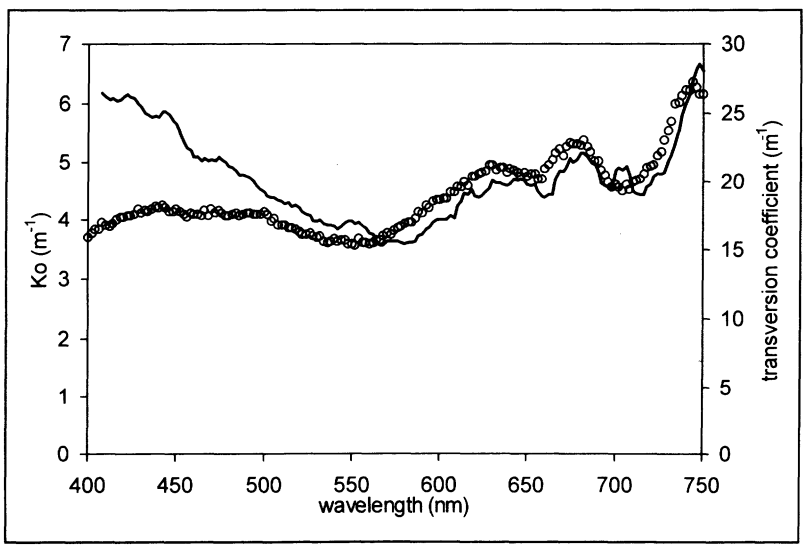

Fig. 3 Attenuation and conversion spectrum
Solid line: attenuation spectrum, measured in the unshaded part of the mesocosm, open circles: "conversion spectrum" obtained from 10 measurements of the alterations of the light climate within 10cm of the shadow line at different depths. The measurements for both spectra were performed with a MACAM SR 9910 spectroradiometer (MACAM Inc., Scotland). The procedure and calculations are described in detail in Schubert et al. (1995).

This matrix was designed to calculate the depth until shading started by comparing 3D-location of the sample with solar position for any time, taking the geometry of the mesocosm into account. It is part of a spreadsheet which is able to calculate the incubation irradiance for dynamic and static incubations and can be obtained free from the authors (german version).

Simultaneously with the in-situ incubations, samples were taken out of the mesocosm and processed for the laboratory investigations, as will be described in the following articles.

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Comparison of different types of chlorophyll fluorometers

1 Instruments used

We worked on 6 different fluorometers, four of which used the PAM-(pulse-amplitude-modulated) technology (Walz GmbH, Effeltrich, Germany) developed by SCHREIBER et al. (1986), and two which used the 1Hz-Technology (bbe Moldaenke, Kiel, Germany) developed by VANSELOW et al. (1992) and MOLDAENKE et al. (1995).

PAM-fluorometers

Three of the PAM-fluorometers, i.e. the „Xenon-PAM" (SCHREIBER et al.1993), the „Phyto-PAM" (KOLBOWSKI et al. 1995) and the „LED-PAM" (SCHREIBER et al.1986) were connected to the ED101-US-cuvette holder with perspex rods as optical pathways to the emitter and detector and a standard 10×10×45mm quartz cuvette mirrored on one side (SCHREIBER 1994), one machine (LED) using an additional handbuilt cooling system and a stirrer. The „PAM2000"-fluorometer was connected via its glass fiber optics to the side of a specially-built plexiglass cuvette for a MK2 Light Pipette (Illuminova, Uppsala, Sweden) for simultaneous measurement of O_2-production and fluorescence yields. For every fluorometer three samples were taken every 3 h starting at 10.30a.m., from 10l mesocosm-subsamples. Samples of the LED and PAM2000 had to be concentrated by centrifugation (Chla >300mg m^{-3}) resulting in a time delay of up to 4h. Xenon- and Phyto-PAM measurements used unconcentrated samples immediately We used the fluorescence nomenclature of van KOOTEN and SNEL published in 1990. Every fluorometer measured the dark-

adapted maximum quantum efficiency Fv/Fm and the operational quantum yield or Genty-parameter $\Delta F/F'm$ (GENTY et al. 1989) after several minutes of light-adaptation at succesively increasing light intensities. The relative PSII electron transport rate (rETR) was calculated as the product of the Genty-parameter and the incident irradiance in the measuring chamber. The α-slope was calculated by linear regression in the light-limited part ($\leq 65 \mu E\ m^{-2}\ s^{-1}$) of a PI-curve. The actinic light irradiance in the measuring chamber of the fluorometers using the ED101-US unit was measured with a spherical sensor (Zemoko, Koudekerke, NL) of 8mm diameter giving PAR. The MK2-Light Pipette cuvette used built-in 2π-sensors also giving PAR. The actinic light intensities of the LED- and Xenon-PAM were adjusted by glass filters of different transmission grades (Schott Glaswerke, Mainz, Germany) and electronic dimming of the halogen light source resulting in spectral differences. The actinic light of the lightpipette and the Phyto-PAM was spectrally independent of the intensity. In vivo absorption a_{ph} (400-700nm) spectra were measured in concentrated and unconcentrated samples in a 10mm pathlength quartz cuvette close to the entrance of a Taylor-type integrating sphere connected to a spectrophotometer (Perkin Elmers). DCMU enhanced fluorescence spectra a_{PSII} (400-700nm) were measured on concentrated samples at an emission wavelength of 720nm. The total spectral irradiance absorbed by PSII (AQ_{PSII}) was calculated as the product of the measured DCMU enhanced fluorescence spectrum and the spectral irradiance $Q(\lambda)$ of either the actinic light source measured with a Macam-spectroradiometer or the underwater light field measured with a Eldonet-spectroradiometer, as in Equation 5 of Forster (1998, this volume).

Tab. 1 Technical data of the PAM-fluorimeters used

PAM-device		„LED"	„Xenon"	„Phyto"	„PAM 2000"
ML	[nm]	LED: 650	Xenon: 400-600, peak at 500	LED: 450; 590; 620; 650	LED: 650
frequency	[kHz]	1.6 or 100	0.002	1.2	1.6 or 100
cuvette holder		ED101-US	ED101-US	ED101-US	Illuminova
volume	[ml]	1.5	1.5	1.5	8
AL		halogen	halogen	LED 660	halogen
intensity	[$\mu E\ m^{-2}\ s^{-1}$]	0-630	0-1060	0-300	0-380
light-adaptation	[min]	5	5	1	2.5
cooling		yes	no	no	yes
temperature	[°C]	15	23	23	15
stirrer		yes	yes	no	yes

1Hz-fluorometers

The 1Hz-fluorometers were developped for on-line, in-field measurements of chlorophyll concentration and physiological fitness of algae using the Genty-parameter and/or dark-adapted maximum PSII efficiency Fv/Fm. Only the one called „flow-through" was able to take samples automatically using a peristaltic pump, which also served to mix the sample in the cuvette in order to prevent sedimentation. The fluorescence signal was corrected for transmission and temperature to calculate the chlorophyll concentration. Both devices used two LEDs with emission peaks in the blue (450nm) and orange (±590nm) as light sources of measuring light in order to account for excitation of Chl a+b (Chlorophyceae etc.) and phycobiline-containing (Cyanophyceae) algae.

Tab. 2 Technical data of the 1Hz-fluorometers used

bbe-device		„manual"	„flow-through"
dark adaptation	[min]	30	10
ML for F	[nm]	LED: 450; 585	LED: 461; 591
intensity	[µE m^{-2} s^{-1}]	both: 10	blue: 38; orange: 9
ML for F´m	[nm]	halogen: biassed	laser: 655
duration	[min]	<1	<1
stirrer		yes	pump every 30s
probe every	[min]	60, manually	15, automatically

2 Results

1Hz-fluorometers

Fig. 1 shows the Chla concentration measured by the 1Hz-fluorometers from 11:00 a.m. until 6:00p.m. versus daytime.

The Chla concentration measured by the 1Hz-fluorometers (32 and 40.5 resp. 42.6mg m^{-3}) rises slightly (+5%) through the day suggesting a phytoplankton growth and shows good correllation with the concentration calculated from DMF extraction (33.3 mg m^{-3}) (Tab. 3) although there are a lot of humic acids in the water of the Bodden estuary complicating the measurements by absorption in the blue-green (400-500nm).

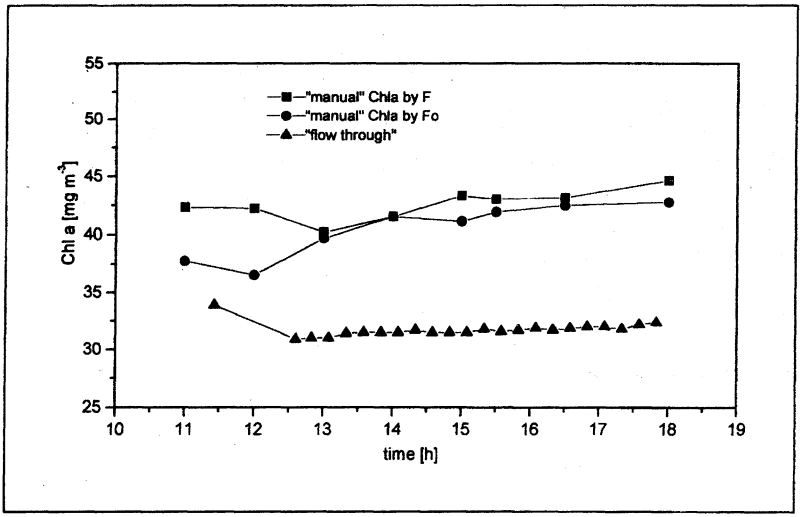

Fig. 1 Chla concentration measured by the 1Hz-fluorometers

Tab.3 Chla concentration [mg m^{-3}], Fv/Fm and ΔF/F´m measured by 1Hz-fluorometers:

	„flow through"		„manual"				extraction in DMF
time	Genty	Chla	Chla from Fo	Chla from F	Fv/Fm	Genty	Chl a
8:30							35,2
10:30							34,7
11:00			37,78	42,33	0,4	0,41	
11:26	0,37	33,9					
12:00			36,58	42,24	0,38	0,4	
13:05	0,34	31	39,7	40,2	0,34	0,35	
14:05	0,35	31,5	41,55	41,55	0,36	0,36	
15:05	0,37	31,5	41,15	43,34	0,38	0,38	
15:35	0,38	31,6	41,95	43,03	0,39	0,4091	
16:35	0,42	31,9	42,5	43,18	0,42	0,4535	30
17:50	0,45	32,4					
18:00			42,79	44,65	0,44	0,4696	
mean	0,38	32	40,5	42,6	0,39	0,4	33,3

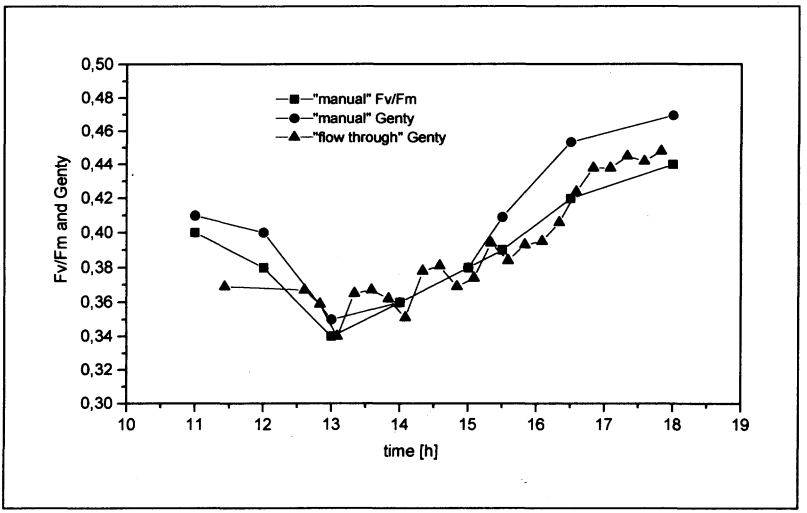

Fig. 2 Maximum and operational quantum yields versus daytime

The quantum yields measured by both fluorometers show a similar time dependency with a distinguished noon depression at 1:00 p.m. (0.35) recovering until 6:00p.m. up to 0.47. Operational quantum yield at a actinic light intensity of 10µE m^{-2} s^{-1} is higher than maximum dark adapted quantum yield measured in the „manual"-fluorometer (Fig.2).

PAM-fluorometers

Plots of rETR based on incident light versus irradiance (Fig. 3) of 3 samples using the Xenon-PAM obviously show differences only in the light-saturated part of the PI-curves, α is almost identical. There is no depression at noon, rather an enhancement of photosynthesis at 1:30p.m., compared to 10:30a.m.

Fig. 4 shows rETR versus irradiance of all PAM-fluorometers at 10:30a.m. and is typical for the 3 measurements over the day of the different fluorometers. P_{max} could only be detected by the Xenon- and the LED-PAM at irradiances of 565 and 521µE m^{-2} s^{-1}, because the Phyto- and PAM2000 used maximum irradiances of 300 and 380µE m^{-2} s^{-1} (see table 1).The α-slope (0.43) for the LED-PAM was significantly smaller than the slopes of the other 3 fluorometers, with good correlations of 0.51 for Phyto- , 0.55 for Xenon- and 0.6 for the

PAM2000 (see table 4). 620nm excitation of the Phyto-PAM revealed cyanobacteria photosynthesis with a much smaller α of 0.27.

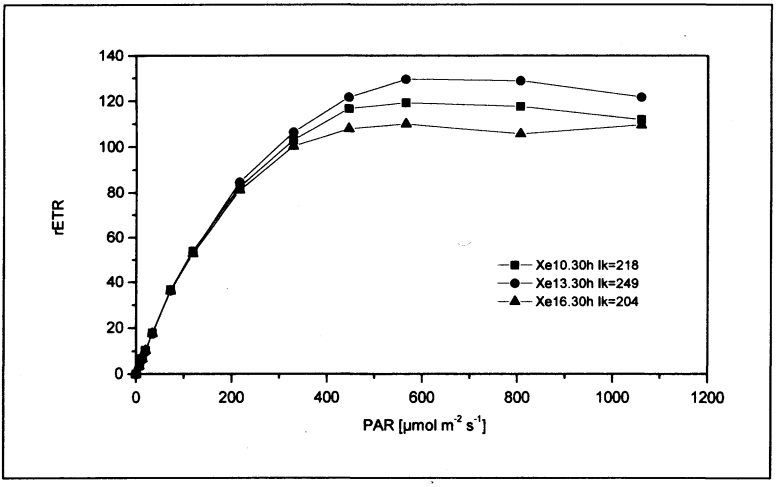

Fig. 3 rETR versus incident PAR

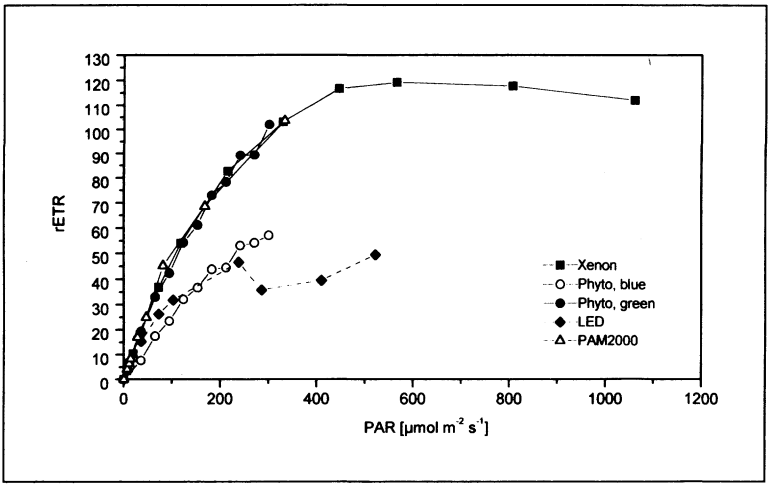

Fig. 4 rETR versus irradiance

Dark adapted maximum quantum efficiency Fv/Fm at 10:30a.m. varied between 0.44 (LED), 0.56 (PAM2000), 0.58 (Phyto) and 0.57 for the Xenon-PAM.

Tab. 4 linear regression (α-slope) and Fv/Fm

PAM; time	range for α	A	error	B	error	n	sd	Fv/Fm	P
Xenon; 10.30h	0-19	-0,01	0,02	0,55	0,001	4	0,02	0,57	<0.0001
Xenon; 13.30h	0-19	-0,03	0,04	0,52	0,004	4	0,05	0,51	<0.0001
Xenon; 16.30h	0-19	-0,01	0,01	0,54	0,001	4	0,01	0,57	<0.0001
Phyto; green, 10.30h	0-65	0,43	1,07	0,51	0,025	3	1,15	0,58	0,03126
Phyto; blue, 10.30h	0-65	-0,53	1,34	0,27	0,031	3	1,44	0,17	0,07417
Phyto; green, 13.30h	0-65	0,43	1,07	0,44	0,025	3	1,15	0,54	0,03620
Phyto; blue, 13.30h	0-65	0,21	0,53	0,23	0,013	3	0,58	0,15	0,03464
PAM 2000; 10.30h	0-14	0,00	0,03	0,60	0,003	4	0,04	0,56	<0.0001
PAM 2000; 16.30h	0-13	-0,02	0,04	0,56	0,049	4	0,08	0,55	<0.0001
LED; 10.30h	0-36	0,09	0,12	0,43	0,005	3	0,14	0,44	0,00809
LED; 12.30h.	0-14	0,04	0,12	0,51	0,012	4	0,13	n.d.	0,00055

Comparison of fluorescence and O_2-data

Maximum gross light-saturated oxygen production averaged 11.8 mg $mgChla^{-1}$ h^{-1} (n=3) and was 10.8mg $mgChla^{-1}h^{-1}$at 10.30a.m. The mean maximum quantum yield was 0.12 O_2 (absorbed photon)$^{-1}$ (0.098 at 10.30a.m.). We found a good correlation between Chla-specific gross oxygen production and fluorescence-based ETR. The correlation was linear for incident irradiances up to 200µE m^{-2} s^{-1}, and became slightly curved at higher irradiances as ETR continued to increase without further proportional increases in oxygen evolution (Fig. 5).

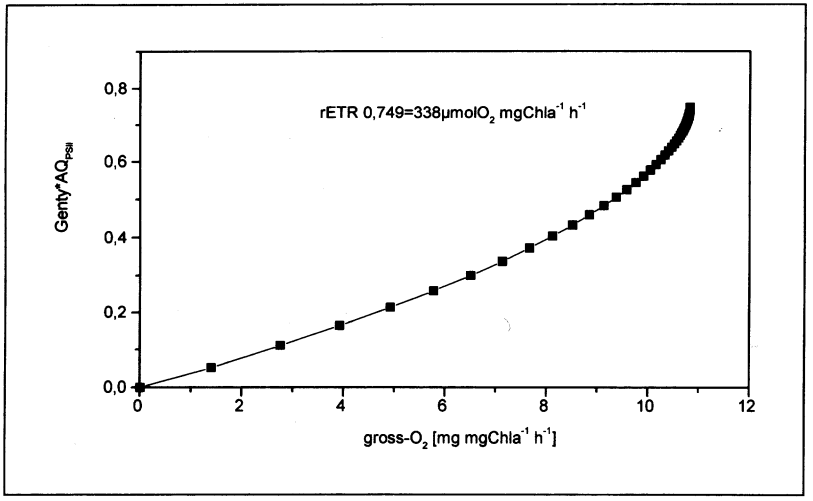

Fig. 5 Fitted correlation of rETR based on PSII-absorbed photons and O_2-production

Calculation of primary productivity in the mesocosm

Using the equation of BIDIGARE et al. 1987 (eq.1) and the photosynthetic model of KIEFER et al. 1983 (eq.2) we were able to calculate gross O_2-production at any depth.

$$\Phi(z) = \Phi_{max} \times \frac{P_{max}/\Phi_{max}}{P_{max}/\Phi_{max} + Q_{phar}(z)} \quad (1)$$

$$P(z) = \Phi(z) \times 1200 \times Q_{phar}(z) \quad (2)$$

P = operational or maximum photosynthetic rate [mgO_2 $mgChla^{-1}$ h^{-1}]

Φ = operational or maximum quantum yield [O_2 (absorbed photon)$^{-1}$]

Q_{phar} = AQ_{PSII} = total spectral irradiance absorbed by PSII [µE m^{-3} s^{-1}]

z = depth [m]

We calculated the photosynthetic rate at different depths for an incubation time of 2h (starting at 10.30a.m.) in order to faciliate the comparison between O_2 and radiocarbon measurements (Tab. 5, Fig.6).

Tab. 5 Calculated photosynthetic rates

z [cm]	time [h]	PAR incident	AQ_{PSII} [µE m^{-3} s^{-1}]	P [mgO_2 $mgChla^{-1}$ h^{-1}]	P [mgO_2 m^{-2} h^{-1}]
2	10.30-12.30	460	11.39	2.80	74.6
10	10.30-12.30	321	7.91	2.11	54.7
20	10.30-12.30	205	5.02	1.44	37.3
27	10.30-12.30	150	3.67	1.1	28.5
40	10.30-12.30	85	2.06	0.64	16.6
50	10.30-12.30	55	1.32	0.42	10.9
60	10.30-12.30	36	0.85	0.27	7
70	10.30-12.30	23	0.553	0.181	4.7
			mean	1.12	29.3

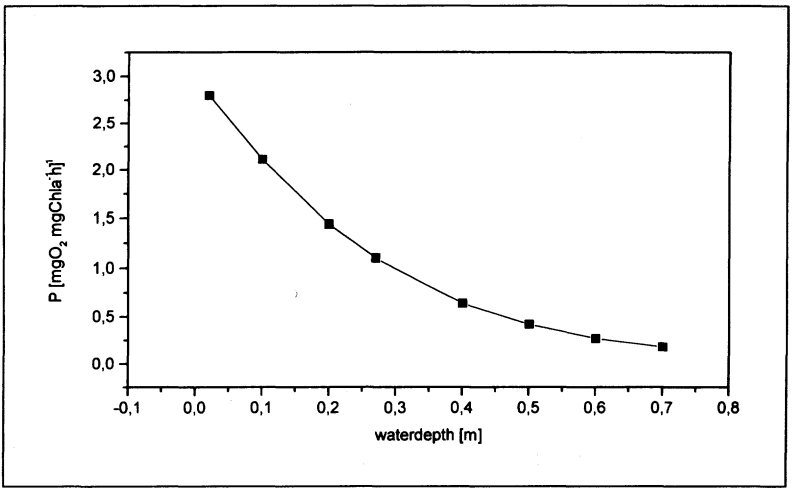

Fig. 6 Calculated photosynthetic rate [mgO_2 $mgChla^{-1}$ h^{-1}] versus depth [m].

3 Discussion

The two 1Hz-fluorometers were able to record the actual Chla concentrations in the mesocosm as well as the operational and maximum quantum yields, which showed a remarkable midday depression as PAR rose up to nearly 1000µE m^{-2} s^{-1} at 1p.m.

The PAM-fluorometers used various set-ups in respect to e.g. stirring, cooling, optical properties and required Chla-concentration (s. Tab. 1). Duration at each light treatment and maximum intensity of actinic illumination differed and were responsible for differences in the time required for measuring a PI-curve. None of the PAM-fluorometers measured a significant reduction in photosynthetic capacity of the algae at noon, which is in contradiction to the results of the 1Hz-fluorometers.

In order to get a PI-curve as fast as possible algae can be quickly (1min) exposed to actinic light intensies in the light-limited part of a PI-curve but have to be exposed to light intensities at light saturation for at least 5min before the steady state fluorescence is reached. The minimum time required to get a fluorescence based PI-curve with 8 light intensities is therefore 30 min. Samples should be temperature controlled and agitated to prevent sedimentation. Differences in the PI-curves measured with the PAM-fluorometers could be due to these variations and would have been exaggerated if every fluorometer would have reached light saturating intensities. Differences in PI-curves of the LED- and Xenon-fluorometer are only partly dependent on temperature effects. Most likely the Chla concentration of 713mg m^{-3} was too dilute for exact measurements with the LED-fluorometer.

We found a good correlation between Chla-specific gross oxygen production and fluorescence-based ETR. The correlation was linear for small irradiances up to 200µE m^{-2} s^{-1}, and became slightly curved at higher PAR intensities. These results are in agreement with the findings of GEEL 1997, SEATON and WALKER 1990 and others. The light intensity at which the correlation becomes curvilinear is dependent on the algae, their nutrition, e.g. nitrogen source, age and growth conditions („light history").

Calculation of primary productivity was done using the in-situ, oxygen-based values for maximum quantum yield and photosynthetic rate and the photosynthetic models of BIDIGARE et al. 1987 and KIEFER et al. 1983 (s. table 5). Because of high concentrations of humic acids in the eutrophic Bodden estuary, resulting in a high attenuation coefficient of the water, the total spectral irradiance available for algae diminishes rapidly with depth. Therefore the product of the total spectral irradiance and the DCMU-enhanced excitation spectrum, AQ_{PSII}, gets

very small at a depth of e.g. 0.7m and $\Phi_{(z)}$ approximates Φ_{max} and $P_{(z)}$ approximates 0. Calculated photosynthetic rates through the water column follow the incident spectral irradiance of the depth and show a sigmoidal decrease of photosynthesis. Integration of primary productivity with integration steps of 0.1m, Chla concentration of 34mg m^{-3} and a mesocosm volume of 0.76m^3 delivers a mean oxygen production of 29 mgO_2 m^{-2} h^{-1}.

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Measurement of primary production in aquatic systems by the radiocarbon method

1 INTRODUCTION

During the workshop "Measurement of primary production in aquatic systems - comparison and assessment of available methods", several different versions of the radiocarbon method were used. While the procedure of filtering and measuring the fixed radiocarbon was almost the same, there were differences in the incubation methods employed. The methods were: classic in-situ static incubations at defined depths, the simulation of vertical mixing by the use of lifts and the measurement of a photosynthesis-light curve in a laboratory incubator. Additionally, different incubation bottles used by the participants of the workshop were compared in order to find possible bottle effects.

2 MATERIAL AND METHODS

The incubations were performed on 8.10.1997 between 10:45 and 14:45. All incubations were based on the same sample from the Bodden near Zingst. The Bodden water was filled into a mesocosm (ca. 1 m^3) one day before the beginning of the experiments. A subsample from the mesocosm was taken and approximately 0.02 µC ^{14}C ml^{-1} were added. Four different types of incubation were carried out:

- measurement of a photosynthesis-irradiance (P/I) - curve after one hour of incubation in the photosynthetron at defined light intensities. The photosynthetron (Tilzer et al. 1993) is a laboratory incubator which facilitates the measurement of primary production under defined

light conditions. Measurements with the radiocarbon method and fluorescence measurement were conducted at the same time. Results of the fluorescence measurements will be given by Hartig (this issue).

- dynamic incubation in the mesocosm with a frequency of 2 minutes and an amplitude of 0.75 m (surface to bottom of mesocosm) in 500 ml and 25 ml bottles. Detailed description of the bottle lift is given by Behrendt (1989). The Incubation time was 1, 2 and 4 hours, respectively, for light and for dark bottles.
- static incubation in the mesocosm in four different depths (0.02 m, 0.27 m, 0.52 m and 0.77 m) in 25 ml vials. Incubation time was 1, 2 and 4 hours for light and dark bottles.

After the incubation, all samples were processed in the same way. Total ^{14}C ($T^{14}C$) was determined in a 2 ml subsample, 2 drops of 1 M NaOH and 3 ml of a scintillation cocktail (Ultima Gold, Packard) were added. The particulate organic fixed ^{14}C($PO^{14}C$) was determined by filtration of 3 ml of the sample on a 0.2 µm membrane filter (Costar, polycarbonate filter) with subsequent storing on filters soaked with 0.01 M HCl for 30 minutes. After drying, the filters were dissolved in 0.5 ml Soluene-100 (Packard), 2.5 ml scintillation cocktail (Hionic Fluor, Packard) was added. All samples were measured within 6 hours after the processing in a liquid scintillation counter (LSC). The measurement was repeated two days later in the LSC Packard Tri-Carb 2100TR with internal quench correction.

Light doses were recorded by means of both underwater and surface quantum radiometers (Eldonet / Licor). Additional measurements with a spectroradiometer were conducted for the calculation of light attenuation (Schubert, this volume).

Primary production rates were calculated according to equation (1).

$$C_{ass} = DIC \bullet \frac{T^{14}C}{PO^{14}C} \bullet 1{,}06 \qquad (1)$$

(C_{ass} = rate of carbon assimilation; DIC = concentration of dissolved inorganic carbon [mg l^{-1}]; $DO^{14}C$ = ^{14}C retained on the filter; $T^{14}C$= ^{14}C concentration in the sample; 1,06 = factor taking into account the discrimination between ^{12}C and ^{14}C by phytoplankton)

The parameters of the photosynthesis-irradiance curve were calculated according to equation (2).

$$P = P_{max} \bullet \left(1 - e^{-\alpha \bullet PFD / P_{max}}\right) + (\beta \bullet PFD) \qquad (2)$$

(P = rate of primary production [µg C l^{-1} h^{-1}]; α = initial slope of the P/I-curve; β = photoinhibition term; PFD = photon flux density [quanta m^{-2} s^{-1}])

Fitting of data to the model was done with the Solver function of the spreadsheet program Microsoft Excel. For the calculation of integral carbon fixation, dark fixation values were subtracted from the results of the respective light incubated samples.

3 RESULTS

The samples obtained from the different incubation methods were measured repeatedly. Although NaOH was added to the samples for the determination of $T^{14}C$ to give a pH >10, a dramatic loss of radioactivity over the time was observed (Fig.1).

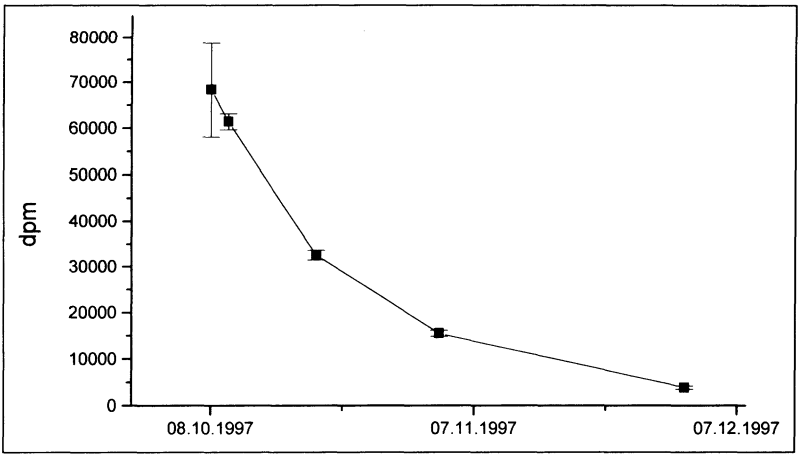

Fig. 1 Loss of radioactivity from the $T^{14}C$-samples during a two month period of storing. pH of sample rose to 10 before addition of the scintillation cocktail, but was then reduced to about 7 because of the high buffering capacity of the scintillation cocktail.

Further investigations showed that the alkalisation of the **mixture** of the sample and the scintillation cocktail to pH > 10 is necessary to prevent the loss of radioactivity. This finding is important for field work because frequently a longer period of time passes by between the processing of the samples in the field and the measurement in the laboratory.

The samples for the determination of $PO^{14}C$ lost only trace amounts of radioactivity, the results were almost stable over the time.

For the calculation of chlorophyll-specific primary production rates a chlorophyll a-concentration of 50 µg l^{-1} was assumed according to first measurements during the workshop. The DIC-concentration was 23.23 mg l^{-1}. The specific primary production rates are shown in Tab. 1.

Tab. 1 Specific primary production rates [µg C (µg Chl a)$^{-1}$ h^{-1}] of dynamic, static and laboratory incubation.

Incubation	1 h	2 h	4 h
dynamic, small bottles	2,2	1,9	1,9
dynamic, big bottles	2,5	2,2	1,9
static, 2 cm depth	1,7	1,0	0,8
static, 27 cm depth	2,5	2,4	2,1
static, 52 cm depth	2,2	2,6	2,3
static, 77 cm depth	1,7	1,4	1,9
Photosynthetron	0,56-3,42	-	-

Highest specific primary production rates were observed in static incubated bottles at 27 and 52 cm, followed by the dynamic incubated bottles, both small and big bottles. Lowest specific primary production rates, probably due to strong photoinhibition, were found in the near-surface (2 cm) static incubated bottles.

Dark fixation rates were almost the same when small and big bottles were compared. Most of the absolute carbon fixation in the dark bottles took place within the first hour of incubation (Tab.2).The highest dark fixation rate of carbon was measured in the photosynthetron, maybe due to insufficient temperature control.

The photon dose-dependent rates of carbon fixation were linear for incubations for a duration of 1 to 4 hours, with no indication of time-dependent changes in photosynthesis, and showed a strong dependency on the irradiance (Fig. 2).

Tab. 2 Dark fixation rates measured with different incubation types.

Incubation	Dark fixation[µgC(µg Chl a)$^{-1}$h^{-1}]
static, 1 h, small bottle	0,62
static, 2 h, small bottle	0,31
static, 4 h, small bottle	0,16
static, 4 h, big bottle	0,17
Photosynthetron, 1 h	0,88

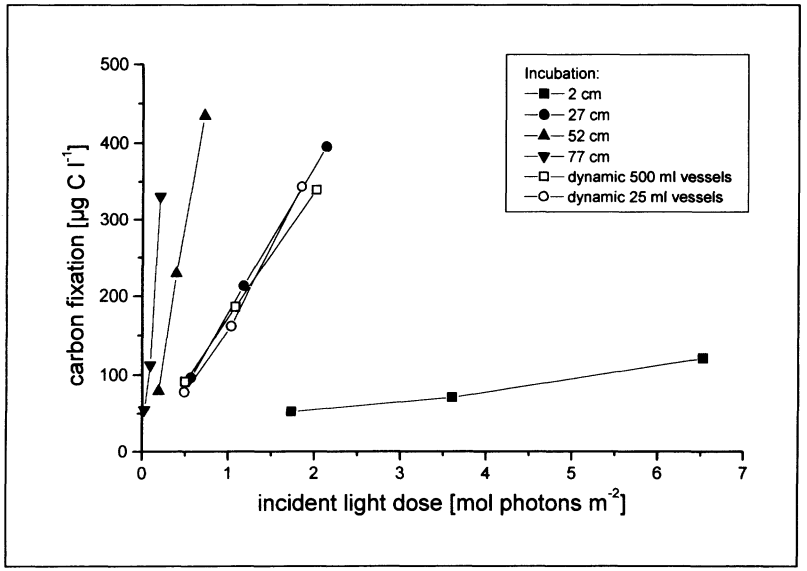

Fig. 2 Carbon fixation rate versus light dose plot for the different ^{14}C-incubations. The figure shows the carbon fixation rates of the static in-situ incubations as well as the rates of the dynamic incubations using vessels of different size for incubation times of 1, 2 and 4 hours, representing different light doses received.

Highest light-specific carbon fixation rates occurred in the static incubated samples at the depths of 52 and 77 cm. Carbon fixation in the dynamic bottles was comparable to those of the static bottle in 27 cm depth.

Plotting the ^{14}C-fixation rates versus irradiance for all incubations (including the laboratory incubations in the photosynthetron) showed

similar rates in the light-limited region but a lower P_{max} for the static incubated samples (Fig. 3).

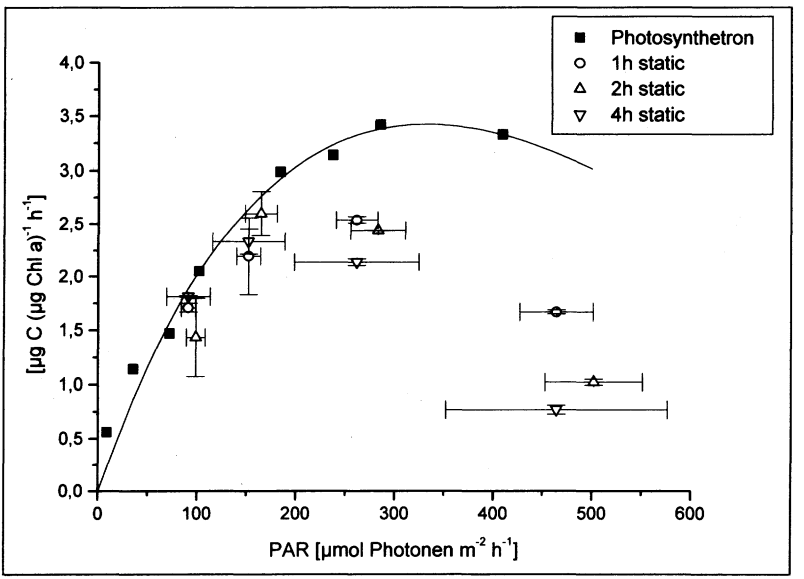

Fig. 3 Photosynthesis-light dependency. The line represents the result of fitting the data obtained with the photosynthetron to equation 2. Error bars represent irradiance amplitude (horizontal) and standard deviation of carbon fixation (vertical).

The lowest rates of photosynthesis were determined in the surface incubated bottles, lower than the rate of the sample exposed in the photosynthetron to comparable light intensities for the same exposure time.

Calculation of integral carbon fixation rates of the mesocosmos for 1, 2 and 4 hours revealed similar results for all incubation methods (Fig.4).

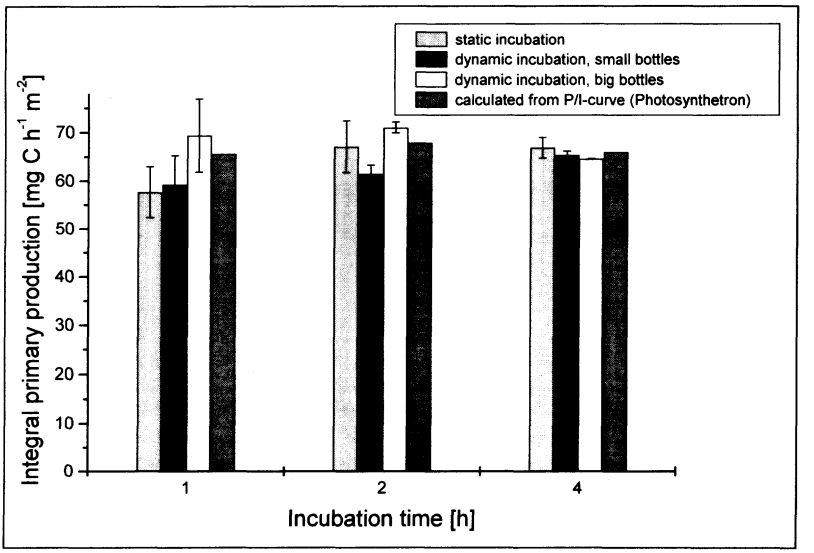

Fig. 4 Integral primary production in the mesocosm calculated from static incubations, dynamic incubations in small and big bottles and from the PI-curve obtained with the photosynthetron

DISCUSSION

Some authors have reported discrepancies between static and dynamic incubations (e.g. Gervais 1997, Lizon & Lagadeuc 1998, Nixdorf 1990). Higher as well as comparable carbon fixation rates for dynamic incubation have been described. In this study we found fairly high agreement between the results of the different incubation methods. The higher carbon fixation rates obtained with the laboratory incubator are probable due to insufficient temperature control. The agreement of the calculated integrated production resulting from the laboratory incubation with the production calculated from the in-situ incubations is a result of the equally elevated dark fixation rates, which were subtracted in each case.

Differences between incubation methods could be explained by photoadaptation occurring in the static incubated bottles but not in dynamic incubated bottles. These differences should increase with longer incubation times. Photoadaptation as well as photoinhibition

occurring in static incubation bottles could enhance or underestimate primary production calculations in comparison to dynamic incubated samples. Whether results from laboratory or in-situ incubations match the „true" values, depends on the possibility to determine the extent of mixing in the field. Therefore, a methodology for the estimation of the extent of mixing is needed. This would also be helpful for the adjustment of frequency and amplitude of dynamic incubation methods to near-natural conditions.

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Results of ^{14}C- and O_2 measurements in relation to PAM-fluorescence measurements

In order to compare the results obtained with the $^{14}C/O_2$-based methods and the fluorescence-based methods (PAM) four experiments were carried out during the workshop (Tab.1). The different PAM devices are described by Domin et al. in this volume. For ^{14}C-based primary production rates (PPR) small aliquots (20ml) of the mesocosm sample at 10:30am were incubated simultaneously in a photosynthetron (Tilzer et al. 1993) at 18° ±1°C at 8 different irradiances for one hour (9; 36; 72; 102; 238; 285; 409; 521 µE m^{-2} s^{-1}). A complete description of the ^{14}C method is given by Krumbeck et al. in this volume. The O_2 measurements were performed with the MK2 Light Pipette (Illuminova) (description in Wolfstein & Hartig 1998) in a custom designed plexiglass chamber, which was equipped with a port for the fiberoptic of the PAM 2000. This allowed simultaneous measurements of fluorescence and O_2 release.

Tab.1 Experiments performed and methods used

Experiment	Sampling time (Mesocosm)	Fluorescence-devices	Classical-method
1	10:30 a	PAM 101 (PM)	^{14}C
2	10:30 a	PAM 2000	O_2
3	10:30 b	PAM 2000	O_2
4	16:30	PAM 2000	O_2

Because the values for the electron transport rates of PSII (ETR) are relative, they were scaled to the value range of the carbon assimilation rates (C_{ass}) and the oxygen production rates (O_2) respectively to provide a better comparison of the values obtained by the different methods according to equation 1:

ETR (after equation 1) = (ETR – y) / m (1)

With: y = offset of the linear regression between ETR and C_{ass} or O_2.
m = slope of the linear regression between ETR and C_{ass} or O_2.

Photosynthesis vs Irradiance (P-I) - curves for experiment 1 derived with the PAM and the ^{14}C method are shown in Fig. 1a .Over the whole investigated incident irradiances both curves (ETR, C_{ass}) show nearly a similar shape (Fig.1b). These observations lead to a very linear correlation between C-based (C_{ass}) and fluorescence- based (ETR) values (r = 0.99) when both methods are compared directly (Fig. 1c). The small deviations at irradiances between 140 and 380 µE m^{-2} s^{-1} could be caused by quenching mechanisms that diminished the fluorescence values without affecting carbon fixation. A possible explanation for the small deviations at irradiances over 410 µE m^{-2} s^{-1} could be due to electron flow into sinks other than CO_2 fixation (see Hartig & Lippemeier in this volume). However the observed differences were very low and in this case the fluorescence values could be used for accurate estimation of ^{14}C fixation rates.

The comparison between the oxygen - and the fluorescence-based values for the sample at 10:30 where also ^{14}C – incorporation was measured (Tab.1) also showed a very good correlation (r = 0.98) between both methods (Fig. 2a-c). The slightly higher ETR-values at irradiances between 70 and 250 µE m^{-2} s^{-1} could be explained by cyclic electron flow around PSII. For experiment 4 we found a nearly perfect correlation (r = 0.998) (Data not shown). A linear analysis (r = 0.985) for all oxygen vs fluorescence experiments leads to the conclusion that the fluorescence values could also be used for accurate estimation of production rates (Fig. 3).

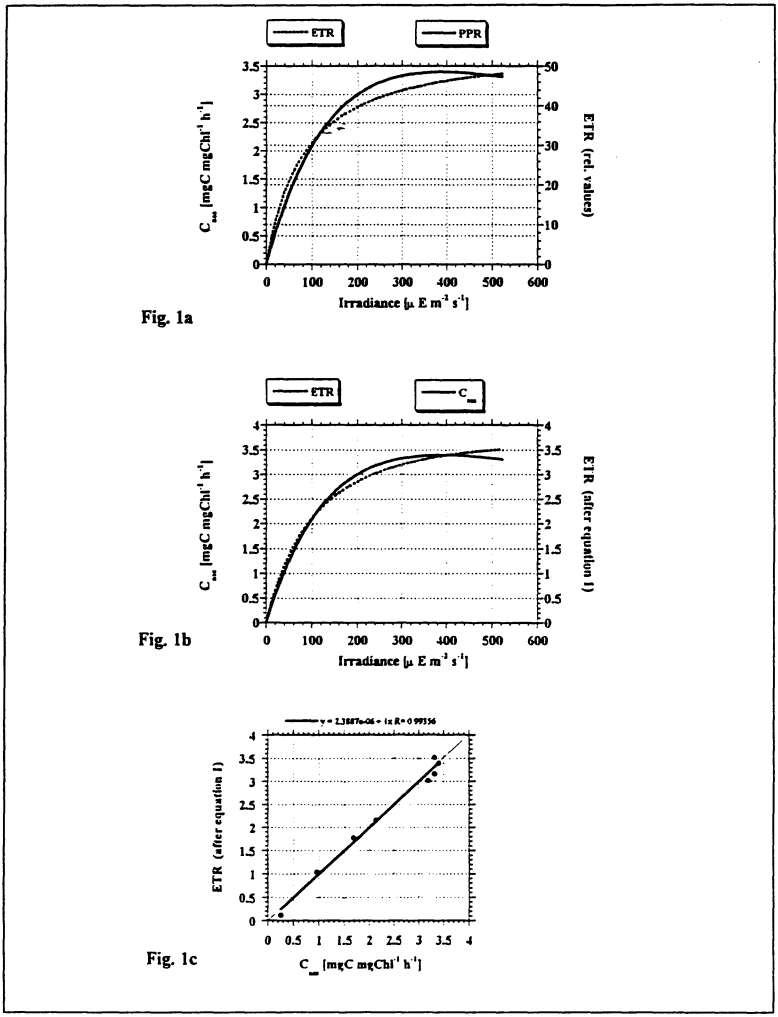

Fig. 1 Comparison of fluorescence and radiocarbon measurements

1a: P vs I – curves for fluorescence based (ETR) and carbon– based (C_{ass}) measurements. ETR was calculated after equation $[(F_m'-F)/F_m' \bullet I]$. For abbreviations see Dau et al. in this volume.

1b: P vs I – curves for ETR (after equation 1) and carbon– based (C_{ass}) measurements. ETR (after equation 1) was calculated after equation 1 see text.

1c: Correlation between ETR (after equation 1) and C_{ass}

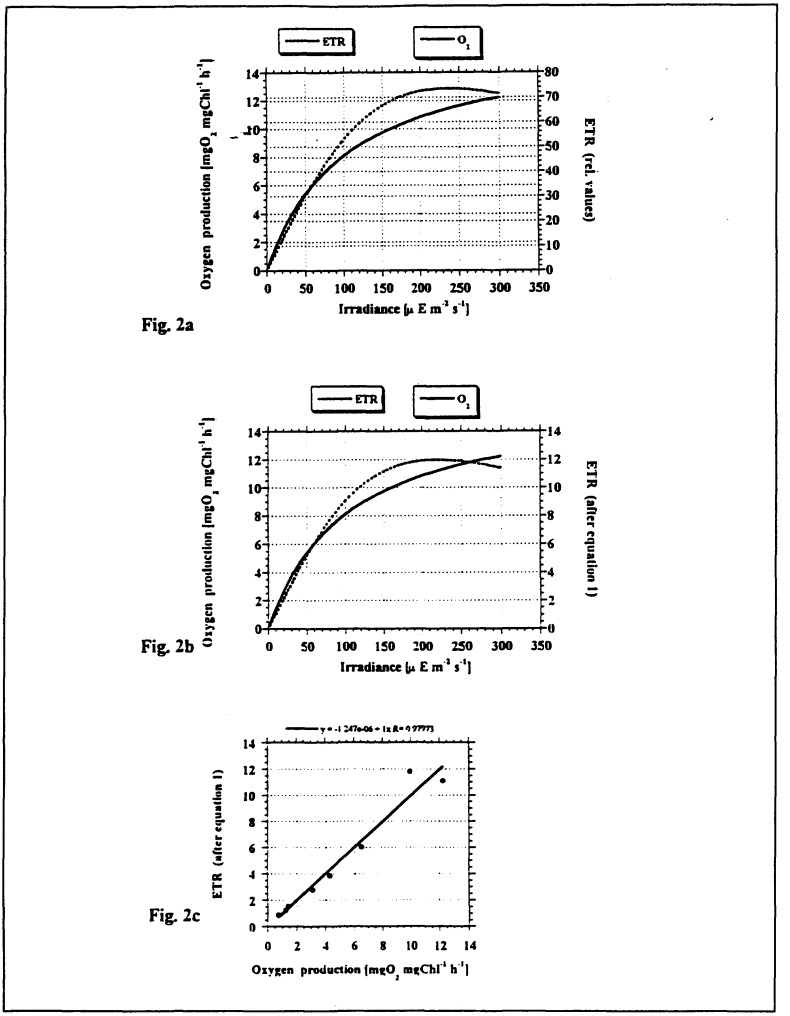

Fig. 2 Comparison of fluorescence and oxygen exchange measurements

2a: P vs I – curves for fluorescence based (ETR) and oxygen– based (O_2) measurements. ETR was calculated after equation [(F_m'-F)/F_m' • I]. For abbreviations see Dau et al. in this volume.

2b: P vs I – curves for ETR (after equation 1) and oxygen– based (O_2) measurements. ETR (after equation 1) was calculated after equation 1 see text.

2c: Correlation between ETR (after equation 1) and O_2

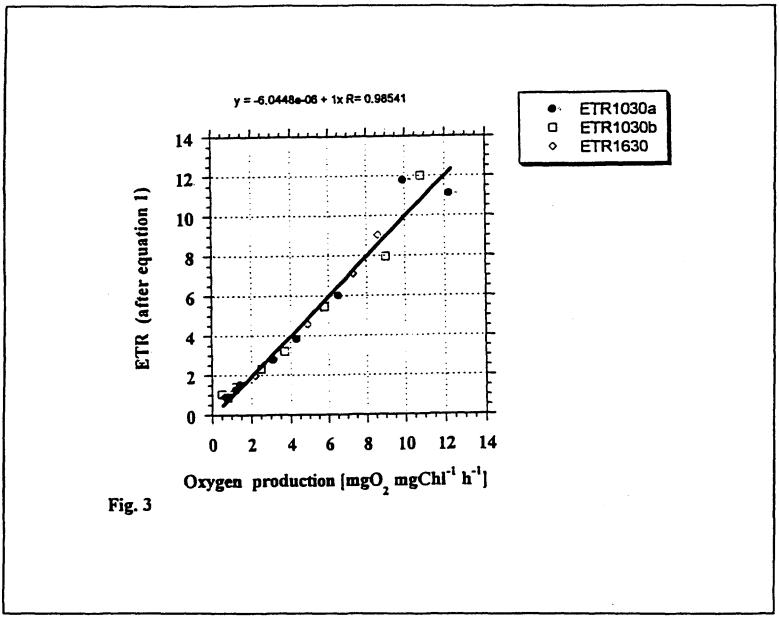

Fig. 3

Fig. 3 Correlation between ETR (after equation 1) and O_2 for all oxygen measurements (experiments 2,3,4).

The good correlation between C- based and fluorescence based values could be due to the fact that we measured rather gross production than net production, because of the short incubation time (1h). It has to be mentioned that some of the observed deviations between the different methods could be due to difficulties of the irradiance measurements in concentrated algal samples (see Forster, this volume). For calculation of absolute values an estimation of the PSII concentration and of the functional absorption cross section of PSII by fluorescence techniques is needed (Kieber & Falkowski 1993, Hartig et al.1998). Development of this technique is already in preparation and calculation of these parameters will hopefully be possible during the next workshop.

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