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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).

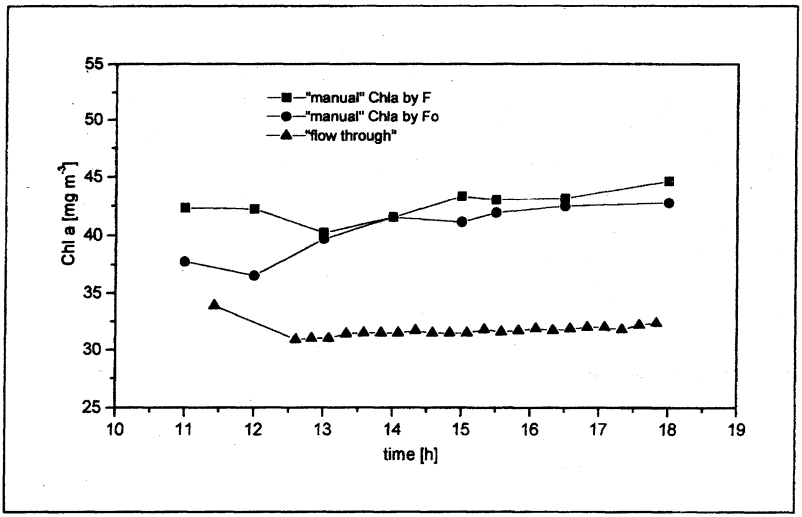

Fig. 1 Chla concentration measured by the 1Hz-fluorometers

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

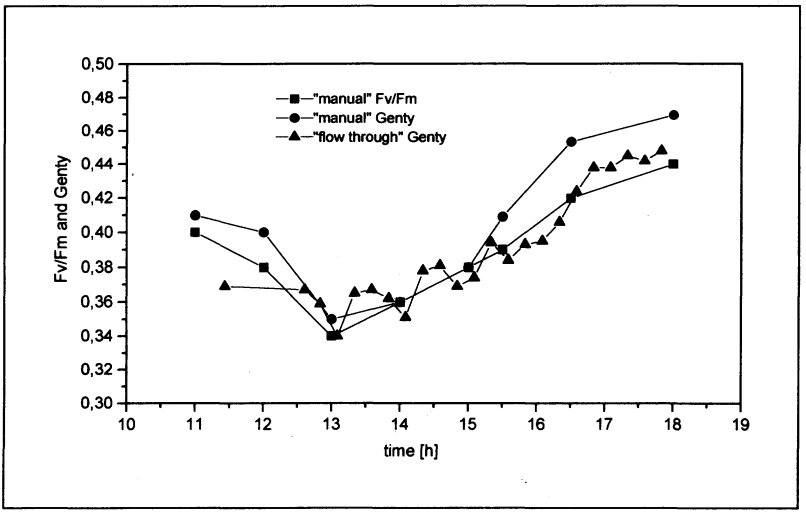

Fig. 2 Maximum and operational quantum yields versus daytime

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.

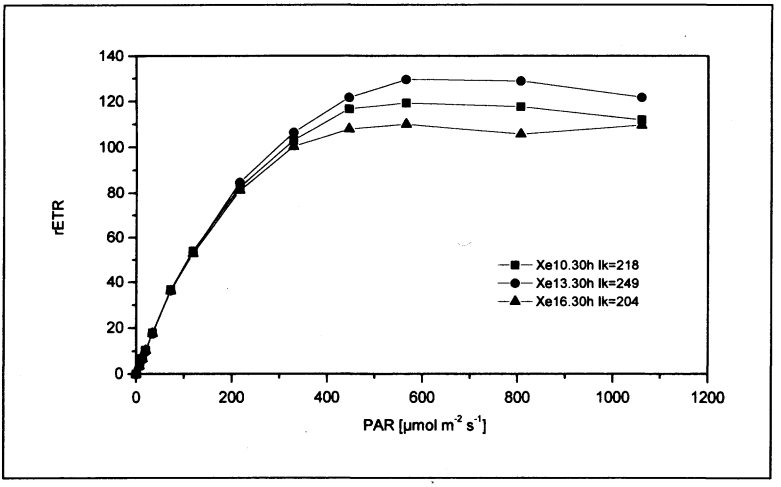

Fig. 3 rETR versus incident PAR

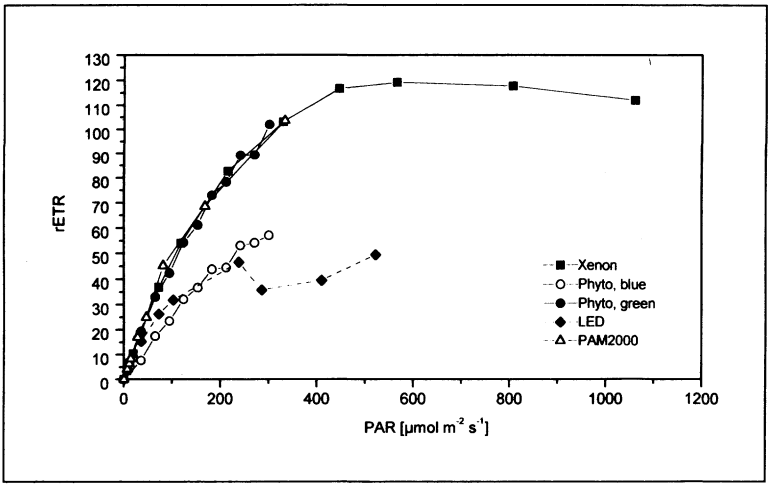

Fig. 4 rETR versus irradiance

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).

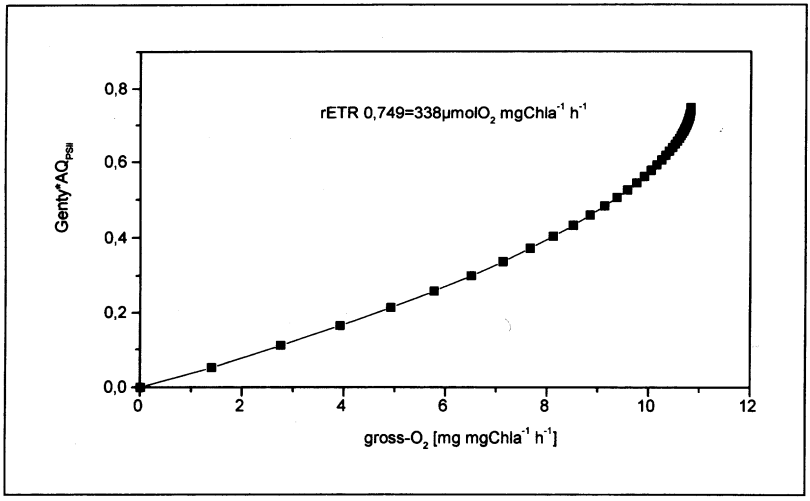

Fig. 5 Fitted correlation of rETR based on PSII-absorbed photons and O_2-production

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} [$\mu E\ m^{-3}\ s^{-1}$]	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

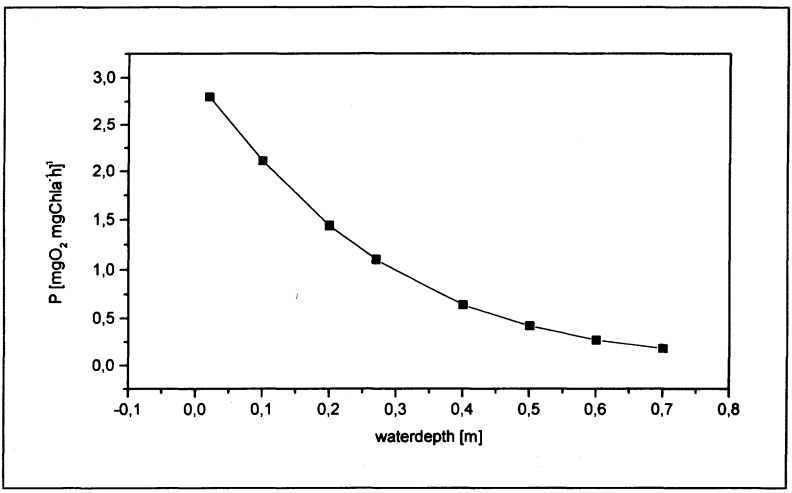

Fig. 6 Calculated photosynthetic rate [$mgO_2\ mgChla^{-1}\ h^{-1}$] versus depth [m].

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}.

References

BIDIGARE, R.R., SMITH, R.C., BAKER, K.S., MARRA, J. (1987) Oceanic primary production estimates from measurements of spectral irradiance and pigment concentrations. Global Biogeochemical Cycles 1, 171-186

GEEL, C., STEENDJIK, M., PEETERS, J. C. H., SNEL, J. F. H. (1995) The use of chlorophyll fluorescence in the determination of photosynthetic capacity of phytoplankton. In: Photosynthesis: from Light to Biosphere, vol. V, pp. 885-888, P. Mathis, ed. Kluwer Academic Publishers, Netherlands

GEEL, C., VERSLUIS, W., SNEL, J. F. H. (1997) Estimation of oxygen evolution by marine phytoplankton from measurement of the efficiency of PSII electron flow. Photosynthesis Research 51, 61-70

GENTY, B., BRIANTAIS, J.-M., BAKER, N. R. (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence.. Biochimica et Biophysica Acta 990, 87-92

KIEFER, D.A., MITCHELL, B.G. (1983) A simple, steady state description of phytoplankton growth based on absorption cross section and quantum efficiency. Limnol. Ocean. 28, 770-776

KOLBOWSKI, J., SCHREIBER, U. (1995) Photosynthesis: From Light to Biosphere. In: Computer-controlled phytoplankton analyzer based on a 4-wavelengths PAM chlorophyll fluorometer, vol. V, pp. 825-828, P. Mathis, ed. Kluwer Academic Publishers, Netherlands

MOLDAENKE, C., VANSELOW, K.H., HANSEN, U.P. (1995) The 1Hz-fluorometer: a new approach to fast and sensitive long-term studies of active chlorophyll and environmental influences. Helgoländer Meeresuntersuchungen 49, 785-796

SCHREIBER, U. (1994) New emitter-detector-cuvette assembly for measuring modulated chlorophyll fluorescence of highly diluted suspensions in conjunction with the standard PAM fluorometer. Zeitschrift Fur Naturforschung C - A Journal of Biosciences 49, 646-656

SCHREIBER, U., NEUBAUER, C., SCHLIWA, U. (1993) PAM Fluorometer Based on Medium-Frequency Pulsed Xe-Flash Measuring Light - A Highly Sensitive New Tool in Basic and Applied Photosynthesis Research. Photosynthesis Research 36, 65-72

SCHREIBER, U., SCHLIWA, U. AND BILGER, W. (1986) Continous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer.. Photosynthesis Research 10, 51-62

SEATON, G.C.R. AND WALKER, D.A. (1990) Chlorophyll fluorescence as a measure of photosynthetic carbon assimilation.. Proc. Royal Society London B 242, 29-35
VAN KOOTEN, O., SNEL, J. F. H. (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology.. Photosynth. Research 25, 147-150
VANSELOW, K.H., HANSEN, U.P., RUESCH, B., MOLDAENKE, C. (1992) 7. Symposium für Maritime Elektronik. In: Anwendung von Rauschnalyse und Doppelkorrelation in der Öko-Technik, vol. 39, pp. 44-47, H. Albrecht und O. Wild, ed. Universität Rostock, Rostock

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