Biological Oceanography Committee

ICES CM 1996/L:3
Ref.: C + E + Env

REPORT OF THE
WORKING GROUP ON PHYTOPLANKTON ECOLOGY

Reykjavik, Iceland

28–30 March 1996

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

Palægade 2–4 DK–1261 Copenhagen K Denmark

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1 OPENING OF THE MEETING

The meeting was opened by the Chairman, Prof. Franciscus Colijn at 9.00 hours on 28 March 1996. The chairman gave the floor to Mr. Jakob Jakobsson of the Icelandic Institute of Marine Research, who welcomed the participants of the Working Group Meeting to his institute and gave a brief overview on the tasks of the Institute. The meeting was attended by 9 scientists representing 7 countries. Although this amount of scientists for a working group meeting is disappointing, no long discussion was held to see whether there are specific reasons for the low attendance. One reason is probably that currently more interest is given by member countries (or scientists) to attend the WG HAB, because it covers more politically interesting topics (see below). A list of participants is given in Annex 2. The chairman presented the agenda, which was left unchanged by the Working Group.

The agenda is attached in Annex 1. Dr. O. Lindahl was appointed as rapporteur. The chairman gave an overwiev of the different Tasks of the WG and commented the main issues of this meeting, a.o. the finalization of the manuscript on the incubator, a working manual for the incubator and the organization of the ICES-symposium on „The temporal variability of Plankton and their Physico-Chemical Environment" in Kiel in spring 1997.

He also emphasised the input asked from the working group, together with the WG on Shelf Seas Oceanography, on the effects of anthropogenic nutrient inputs in particular areas.

The following members were absent with notice: Bert Wetsteijn (the Netherlands), Egil Sakshaug (Norway), Katherine Richardson (Denmark), Stephen Bates (Canada), Lars Edler (Sweden) and Juhu-Markku Leppänen (Finland). The chairman made the following announcements:

The Working Group on Harmful Algal Blooms (WGHAB) will meet in Brest, France, from 17-20 April. Regarding the functioning of both WG's, the WGPE is of the opinion that a future collaboration of both WG's is needed to avoid overlap and strengthen the further development of new techniques to study the ecology of phytoplankton in general and of toxic algal species in particular. This collaboration could be improved by having the next WG-meetings at the same place and in an overlapping time-frame, however without the *a priori* intention to merge the groups.

The chairman will contact the WG HAB chairman (P. Gentien) before their meeting, so that the item can be discussed during their meeting in Brest.

-The chairman further announced the second ICES/IOC workshop on the "Development of *in situ* growth rate measurements for Dinoflagellates", at Kristineberg Marine Research Station in Sweden from 9-15 September 1996. This workshop will test several different techniques to measure growth rates of Dinoflagellates. Members of the WG are encouraged to participate in the workshop in case they can offer additional techniques. The chairman will ask one of his colleagues P. Hartig, (FTZ, Büsum) to offer cooperation with the PAM Method as an alternative measurement of primary production.

2 TERMS OF REFERENCE

The chairman informed the Working Group on Phytoplankton Ecology regarding the Council Resolution 1995/2:48, which states:

The Working Group on Phytoplankton Ecology (Chairman: Prof. F. Colijn, Germany) will meet in Reykjavík, Iceland from 28 March to 30 March 1996 to:

a). finalise a descriptive account and a Working Manual for the ICES Standard Incubator for measuring Primary Production with a view to their publication by ICES;

b). consider improvements to the ICES Standard Incubator, including improvements to the measurement of irradiance in the incubator;

c). propose new pigment procedures for measurements of chlorophyll a, taking into account recommendations contained in the SCOR report on phytoplankton pigments;

d). continue the evaluation of new techniques for the measurement of primary production and biomass with the aim of producing a systematic review of relevant instrumentation;

e). elaborate plants for the forthcoming ICES symposium on the variability of plankton;

f). review the literature on nutrient manipulation experiments in terms of the ratio and cycling of N and P in relation to maximum cell quota and species composition of phytoplankton, and report to the Advisory Committee on the Marine Environment (OSPARCOM 1.4);

g). prepare, in communication with the Working Group on Shelf Seas Oceanography, multidisciplinary descriptions of the response of the marine environment to anthropogenic nutrient inflows in some example areas (e.g. Kattegat, German Bight) and report to the Advisory Committee on the Marine Environment (OSPARCOM 1.4);

h). examine the feasibility of, and potential contributions to, an Environmental Status Report for the ICES Area on an annual basis, and report to the Advisory Committee on the Marine Environment by the end of 1995.

The Working Group will report to the Biological Oceanography Committee (Reference Marine Environment Quality Committee and hydrography Committee). The chairman distributed an annotated agenda and a copy of the section from the 1995 ACME report on TOR's f) and g).

3 GENERAL DISCUSSION OF TERMS OF REFERENCE

a). finalise a descriptive account and a Working Manual for the ICES Standard Incubator for measuring Primary Production with a view to their publication by ICES;

The former ICES WG on Primary production had as a term of reference for its meeting in 1986 the task to carry out an intercomparison of the different approaches by the various ICES country members for the measurement of primary production by the ^{14}C method. An intercomparison exercise was designed and carried out during June 1987 at Hirtshals, Denmark, under the leadership of Dr. Katherine Richardson. The main outcome of this exercise was that several potential problems in the practical application of the method existed, because coefficients of variance in the experimental results were high (Richardson, 1991).

Two of the major sources of error identified were the different types of incubators in use within (and outside) the ICES community and the way they were operated, including the manipulation of samples and postincubation treatment. Based on these observations the former ICES Working Group on Primary Production proposed to ICES to develop a standard ICES Method using an artificial light

incubator for measuring phytoplankton photosynthetic activity or primary production for monitoring purposes. The task of designing and testing the incubator was given to Dr. Franciscus Colijn, at that time working at the Tidal Waters Division, now National Institute of Coastal and Marine Management (RIKZ) in the Hague, together with colleagues from the Netherlands Institute of Sea Research, Mr. Gijs Kraay and Dr. Marcel Veldhuis. The incubator was constructed and tested under a variety of conditions: Marsdiep (tidal inlet to the Dutch Wadden Sea), Baltic Sea near Helsinki, North Sea, and Indian Ocean. The measurements near Helsinki were successfully used to intercompare with the Baltic incubator. During later meetings, unfortunately with varying members of the WG, two aspects were identified that needed improvements. One referred to the need to have higher irradiance levels in the incubator to be sure that P_{max} can be properly measured and the wish to be able to measure complete P-I relationships. The latter improvement after all was outside the original terms of reference **to develop a cheap and easy to use incubator**. Nevertheless, the incubator procedure has been adapted in such a way that P-I relations can be measured for 12 points, including a dark incubation, simultaneously. These improvements, c.q. extensions, were incorporated together with a Working Manual for the incubator, based on the terms of reference of the 1995 meeting.

During the present meeting, a final discussion on the standard ICES incubator method was held. It was emphasized that users should realise that the standard method has a set of restrictions (these will be mentioned in the paper as well):

1). The incubator with the accompanying Working Manual is only intended to make standardized measurements of phytoplankton photosynthetic activity for monitoring purposes, and not intended to replace all other types of incubators used by individual scientists for particular (eco)physiological measurements.

2). Preferentially the incubator should be used for measurements of primary production in mixed water bodies only. Should the incubator be used for measurements on samples from stratified water bodies, the sampling strategy recommended in the working manual has to be followed, which results in using a second incubator set-up under a different temperature regime.

3. The calculation of primary production on an annual and area basis can only be done if additional information on daily irradiance, vertical attenuation of irradiance and chlorophyll concentrations at the sampling depth(s) are measured. Suggestions for these calculations are listed in the Working Manual.

The design, construction and tests of the incubator are described in Colijn *et al.* (in prep., see annex 4 and below, TOR b), which paper will be extended by results of a year round monitoring series at the station Büsum in the German Wadden Sea (unpublished results U. Tillmann *et al.*).

The irradiance characteristics and the preparation of the incubation bottles with their transmission characteristics are described in Wetsteijn *et al.* (1996) (see annex 3).

The Working Group on Phytoplankton Ecology feels that, with the presentation of the above cited papers including the Working Manual, and which will be submitted for publication to the ICES Journal of marine science or in TIMES, it has fulfilled the terms of reference given to the WG.

b). consider improvements to the ICES Standard Incubator, including improvements to the measurement of irradiance in the incubator;

Due to the absence of one of the participants involved in tasks to complete the incubator manuscript and the description of the irradiance improvements, the chairman announced that he was informed by Mr. Bert Wetsteijn by correspondence. The WG agreed that the only part missing in the finalization

of the improvement of the light climate is the proper description of the preparation of the epoxy-resin cover of the incubation flasks, to obtain a series of different irradiances.

The chairman will ask Mr. Bert Wetsteijn to present additional information on the procedure and manufacturing, which will be added to the manuscript on light measurements (see Annex 3).

NOTE of chairman: The epoxy resin procedure has been developed by a commercial company through a contract to RIKZ in the Netherlands. This company is not willing to publish their procedure, but offers the preparation of series of bottles with different attenuation characteristics. I do not think that this is a good step to obtain a standard method but I understand the policy of the company. How to cope with this problem?

Further improvements on the irradiance levels and distribution are not planned, because for the purpose of monitoring and standardisation these are not needed.

The chairman informed the WG-members on the use of the incubator at his home institute in Germany. The incubators have kindly put at his disposition by Mr. Bert Wetsteijn of RIKZ in the Netherlands. Since the beginning of 1995 weekly P-I measurements have been performed on samples taken from the pier in Büsum (German Wadden Sea). P_{max} values obtained showed a good correlation with the chlorophyll-a concentrations over a wide range (see Figure 4, Annex 4). Also a series of P-I measurements were presented, which show the ability of the incubator to measure over the whole range of irradiances occurring in the field. (see Figure 5 Annex 4).

The WG agreed to incorporate a part of these results in the draft manuscript, because they support the well functioning of the incubator. However, the limitations of the incubator-method should be indicated in the manuscript (see above).

Odd Lindahl presented a first draft of the Working Manual for the incubator. Especially the sampling strategy was discussed in detail. A decision was taken to come up with one sampling strategy, with an option for stratified waters. Also the incubation procedure was standardized. However, it was also agreed that not all steps need to be exactly prescribed , but in case people deviate from the standard procedure they should properly calibrate their procedures. The Working Manual is annexed as Annex 6 and has been finalised and edited by Lindahl and Colijn.

c). propose new pigment procedures for measurements of chlorophyll a, taking into account recommendations contained in the SCOR report on phytoplankton pigments;

This TOR could not be fulfilled, because the SCOR-Manual "Phytoplankton pigments in Oceanography: guidelines to modern methods", edited by S. W. Jeffrey, R. F. C Mantoura and S. W. Wright was not yet available. The chairman had corresponded with Dr. Mantoura in Plymouth to find out the present status of the Report. Dr. Mantoura replied by sending the contents of the SCOR/UNESCO Manual which was distributed to the WG participants. The final Manual will consist of 17 Chapters, and several appendices, altogether 637 pages.

The same TOR will be suggested as a task for next years meeting, and was distributed to F. Rey and E. Sakshaug.

d). continue the evaluation of new techniques for the measurement of primary production and biomass with the aim of producing a systematic review of relevant instrumentation;

New methodology for measuring primary production has been discussed at almost every WG meeting since 1986. A first milestone in this work was the organization of the ICES sponsored International Symposium on the Measurement of Primary Production from the Molecular to the Global Scale held

in La Rochelle, France in 1992 (ICES, Marine Science Symposia, eds. Li & Meastrini, Vol. 197, 1993).

Reviewing the outcomes of the symposium the WG emphasizes that many of the new approaches were still in a developmental stage and would only be ready for use in several years to come. This made them unsuitable at the present time for use in monitoring programmes by country member states. A thorough overview of these methods is focused in the symposium proceedings and in the report of the WG meeting in 1992 (CM 1992/Poll:4). It was felt by the WG that what is needed in the future, more than continuous reviewing new methods/approaches, is to gather experience on the application of the methods in actual development with special attention to the kind of questions for which the methods could give answers. The WG would like to encourage all users of these new methodologies to report to the WG on their experience, so that this information can be used as a basis for their evaluations for future use in ICES monitoring programmes.

Following up, the chairman presented the first results of measurements made with the PAM fluorescence technique (Pulse-Amplitude-Modulated) as originally developed by Schreiber (1986), by one of his colleagues Dr. Hartig in Büsum. A draft paper on the use of this method is in Annex 5.

The conclusion of this presentation is that the PAM method only offers an alternative for the present ^{14}C incubation technique after several problems like the measurement of the specific cross-sectional absorption have been solved. Its merits are the short measuring time, and the possibilities for temporal and spatial coverage of large areas. Also profiling is a future option, on which work is already in progress. The members of the WG are not informed whether Falkowski's profiler is now under construction and can be delivered. Possibly, Chelsea Instruments in the UK is working on it.

After the discussion on new technical means to measure specific processes on phytoplankton, a discussion was held to direct the work of the WG in the near future. Based on this discussion the following paragraph has been compiled.

The traditional approach to phytoplankton ecology has a biomass perspective in which the community is collectively reduced to its chlorophyll component without regard to species composition or functional group dominance. Processes such as primary production are ratioed to biomass, such as the assimilation number (ratio of P_{max} over chlorophyll), and the nutrient field is measured in search of nutrient limitation effects. Sometimes the community is size-fractioned to establish biomass and production rate processes in these categories. This biomass and mass balance approach has been enormously helpful in establishing first order bloom dynamics, global production maps and providing biogeochemical insights, all of which are highly relevant to current concerns over global warming, gas balance, anthropogenic nutrient impacts, etc. Much of our insight into the major features of food web structure and trophodynamics is also based on biomass, limiting nutrient and mass balance approaches. Techniques have been developed from this conceptual basis, and based partly on the need for a high biomass signal. Thus, diatom blooms, and specifically the winter-spring and upwelling blooms, have been focused upon. Blooms of other seasonal occurrences, magnitude, or of different phylogenetic groups have been ignored for the most part. This conceptual approach has been fostered by the applied needs of fishery biologists, and eutrophication models using biomass/mass balance data. To a large extent, such applied needs, beginning already with Victor Hensen's monumental work in 1872, have driven approaches to and technique development in phytoplankton ecology.

There is growing evidence, however, that this historical approach is inadequate for many of the increasing needs to quantify the factors regulating phytoplankton processes in the sea. Moreover, this approach is often peripheral to, and ill equipped to resolve many basic problems within phytoplankton ecology. Resolutions are also essential to the applied needs of the other disciplines which must incorporate phytoplankton dynamics into their studies on fisheries dynamics, pollution/eutrophication, etc. The need to incorporate an organismally-based approach into

phytoplankton ecology is therefore essential, since many of the unresolved issues and controversies reflect species based impacts, processes and dynamics, rather than biomass-based aspects. While it is appropriate to establish the relationships between biomass and nitrogen levels, for example, the form (= species, functional groups) in which particulate N occurs is often more significant. Selective grazing on size based and species based templates, for example, is a well known example of this food quality effect and the need to recognize organismal differences. Nutrient-enhanced enrichment stimulating diatom growth can be beneficial to the food web unlike antagonistic species and bloom stimulation. This is another example of organismal importance. There are numerous related examples of the need to restructure phytoplankton ecophysiological studies, from an organismal focus, to run parallel with traditional biomass approaches.

It is suggested that the Working Group on Phytoplankton Ecology begins to focus on such neglected organismal approaches inquiring into the fundamental issues of phytoplankton ecology with regard to blooms, the role of nutrients, grazers, microbial food loop, species successions, bloom species selections, life cycle strategies etc. as well as to nurture newer conceptual and methodological approaches more suitable to the required assessment of cellular, population and community growth. The distinction between applied phytoplankton ecological approaches and basic phytoplankton approaches is made here. Most efforts of the WG on Phytoplankton Ecology to date have been directed towards helping/guiding other working groups in applying biomass-based results and approaches and preparing technique manuals. This has deflected focus of this Working Group from more basic phytoplankton ecology issues. The terms of reference of the WG on Phytoplankton Ecology and ICES directives to it forwarding requests for applied information should be reconfigured allowing the WG to develop a more balanced approach and increasing focus on organismally-based ecological issues to be discussed, evaluated, methodologically defined and incorporated conceptually in newer approaches needing to be developed. This would also facilitate providing for the growing applied needs for quantitative ecological data on phytoplankton that ICES and its working groups would like to have.

e) elaborate plans for the forthcoming ICES International Symposium on the temporal variability of plankton and their physico-chemical environment.

A general discussion on the scientific goals of this symposium were held in view of the earlier set objectives. A possible time frame and a list of invited speakers was discussed. Also speakers were mentioned to cover particular points which are related to the analysis of long term time series such as its statistical properties, and reliability. An attempt will be made to interest groups of scientists to present their data on plankton and abiotic parameters in a joint effort e.g. the Dutch monitoring data, or the Helgoland data. This could stimulate scientists to come up with more general ideas on the theory of changes in marine systems or areas. The co-conveners of the Symposium will set up a more definitive list during the meeting, with the purpose to obtain a good geographical coverage. An outcome of the Symposium hopefully is that long term series can be continued and that advise on the way how to continue could be given. Therefore a session on the present status of national monitoring networks and their main results should be presented. The flyer of the symposium is now being prepared by ICES and will be available in May. The chairman reminded the members of the Working Group of their expected input to make the Symposium a success, also by acting as invited speakers during the meeting. He will give effort to get more sponsors for the Symposium like the German Research Foundation.

f and g) Prepare a multidisciplinary (physical, chemical and biological) description of the response of the marine environment to anthropogenic nutrient inflows in some example areas.

The idea that it is possible to define theoretical ratios of N:P at which changes in species composition, mean population cell size, foodweb structure, toxin production etc. start to appear, and as expressed by ACME, is problematical. Complications include: measured nutrients are residual levels; turnover

rates which are not derivable from residual nutrient levels have different ratios; cellular quotas of N and P, and interspecific differences in cellular ratios of *in situ* populations which occur in response to variable nutrient levels, are almost never measured; nor can be, because of detrital contributions. Moreover, *in situ* ratios are in continuous change on a diel, daily, and longer basis. Efforts to verify „theoretical" projections, let alone the theory itself, are exercises which have not yet led to results. It is true that experimental chemostat studies indicate nutrient resource competition which can lead to exclusion, or replacement of one species by another (best data for diatoms) (Sommer, 1995). However, the times of replacement *in vitro* are usually so long in contrast with *in situ* species replacement times, that other factors, i.e., non-nutrient, are not only implicated, but seem to be more likely to override any potential nutrient ratio effects. These appear to be grazing, microbial loop effects and hydrographic mechanisms. Therefore, at the present stage of knowledge only general predictions can be formulated when dealing with different scenarios of nutrient loadings and nutrient ratios. As far as we know, there are no simple relationships between the existing nutrient concentrations and phytoplankton communities in marine waters, that are of general applicability.

Mesocosm experiments generally show that in the case of winter-spring diatom bloom components, the impact in nutrient enhanced communities is that the „rich get richer". That is, the dominant diatom species become even more abundant. Mesocosm experiments are often compromised by experimental shortcomings, such as failure to provide representative nutrient recycling rates; herbivorous grazing rates or pelagic-benthic coupling. Enrichments are usually single dose or multiple dose but at fixed ratios which do not take into account actual utilization (*cf.* Prins *et al.* 1995, Escaravage *et al.* 1995). Nutrient ratios have been shown to affect phytoplankton species composition and succession in these experimental enclosures. However, the effect of varying nutrient ratios is difficult to demonstrate directly in the field.

These artifacts and inadequate mimicking of natural ecosystems compromise extrapolations to *in situ* conditions. The merit of such experiments is primarily in providing evidence that when nutrient concentrations or ratios do influence species selection having ecological analogues this occurs primarily at functional group levels, i.e. diatoms vs. flagellates (both N:Si and N:P ratios), cyanobacteria vs. diatoms (N:P), Phaeocystis vs. diatoms (N:P) and diatoms vs. antagonistic species (N:P, N:Si) (Smayda, 1996; cf. Sommer, 1995 on effects of nutrient ratios on the species level within a functional group).

Empirical evidence from *in situ* populations generally supports the occurrence of such nutrient ratioed selections. However, the mechanisms of selection are unclear; such as the extent to which these represent responses primarily to chronic or acute nutrient enhancement, or to parallel trends in grazing processes. Stochastic, unpredictable or so far incomprehensible phytoplankton bloom species selection, magnitude, duration and ecosystem effects are characteristic of a given nutrient ratio. That is, at a given ratio in a given system or time in the annual cycle, the bloom species can not be predicted.

Therefore, the ACME and WGPE view that automated, unattended, sampling devices to detect the effects of anthropogenic nutrients provide an efficient tool for early warning of plankton blooms is supported but needs further development.

Eutrophication (increase of nutrient concentrations and nutrient ratio changes) is expected generally to result in increase in biomass and shifting species composition of phytoplankton. In order to test this hypothesis, one must be more specific in questioning, as has been exemplified with long term time series in the German Bight at Helgoland Roads.

At this monitoring station since 1962, the response to the (significant) increase of nitrate and phosphate as well as of shifting N:P -ratios was not so clear as might be expected. This is caused by the diverse water masses and hyrographical structures which are characteristic for the area. They

account for different growth conditions at the same period of the year within the German Bight. The (most of the time) strongly stratified convergence zone represents a habitat for dinoflagellates, while at the same time some 20 miles eastwards, the growth conditions favour diatom populations. Both water bodies are affected by eutrophication in different ways, so that the question of anthropogenic impact to the German Bight must be specified.

Not only the succession of different plankton populations (according to different growth conditions) during the annual cycle, but also the close proximity of such different growth conditions at the same time can lead to better insights in the functioning of the pelagic ecosystem. Given the relatively small size of the region, there is a chance to synoptically investigate how eutrophication might alter pelagic foodweb relations. To support these general findings Hickel presented a series of newer data from his studies in the German Bight. He showed the very high nitrate values and the reduction of P, which gives further shifts to very high N/P ratios now well over 150 during several months of the year. Even during flood events causing very high nitrate run off, no direct effects on the phytoplankton species composition could be observed. The question which species can use these high nitrate values or why they are not used (Antonio Bode mentioned his studies on high nitrate levels in the Texas Shelf off Louisiana in the Mississippi Delta where the general relations of enhanced nutrient inputs-->high phytoplankton biomass--> increased sedimentation--->hypoxia did not occur) could not yet be answered, which certainly also has to do with the complicated hydrographic conditions in the inner German Bight. A similar differentiation of problems in other areas is also recommended and leads to more qualified questions and finally to a better monitoring strategy.

The chairman also noted the work under progress in the EU funded project NOWESP (NOrth West European Shelf Project). Analyses on the trends in nutrient concentrations in several areas of the North Sea (Belgian-, Dutch coast, German Bight, Skagerrak/Kattegat and off Norway) are being performed. An interesting aspect in this project is the attempt to correlate the behaviour of nutrients and a series of biological parameters /chlorophyll, primary production, zooplankton) between these different areas (boxes). The results of this project will be available during next autumn.

Comparable subsystems should therefore be water masses not just areas- such as the subareas in the QSR 1993. They can be characterized by their depth, proximity to the land-based sources of nutrients, salinity, and their dominant plankton populations. The question is, how such comparable water masses are affected by eutrophication (or pollution) in various sea areas. Gradients of changes in the functioning of ecosystems might finally result from such comparisons.

It is recommended that by evaluating the literature and an appropriate network of monitoring stations, a study of comparable pelagic systems could be attempted. These studies should focus on functional groups of (phyto)plankton instead of complete species composition. Experimental work should be added to arrive at causal relationships. These should encompass synoptic measurements in the areas affected by following nutrient concentrations and ratios, nutrient uptake rates, phytoplankton species composition and if possible be combined with buoys equipped with continuous registration and remote sensing.

Future studies should carefully consider the multiple relationships between nutrient fluxes and ratios, light field and mixed layer physics in relation to phytoplankton growth and succession. The conclusions obtained from mesocosm studies as mentioned above can be used as preliminary hypotheses to be tested in the field.

Smayda presented results of nutrient (N, P) loadings to 20 l mesocosms (microcosms?). Daily nutrient measurements were made and species composition was followed. During these experiments diatoms flourished; only during lower Si-availability a predominance of flagellates was observed. He also mentioned several other studies by Riegman *et al.* (1992) and Stolte (unpubl.) showing the effects of N/P ratios on e.g. the growth and competition of Phaeocystis.

Estimations of phytoplankton parameters like biomass and production from models using nutrient fluxes and inputs can be used to evaluate the outcome in different scenarios of nutrient enrichment or reduction scenarios. The ERSEM Model developed within the framework of the EU/MAST programme is a good example of what models can achieve nowadays(Baretta *et al.* 1995). Such model results must be calibrated against real nutrient measurements in the field (Radach and Lenhart, 1995).

The chairman presented material which had been discussed before at the meeting of the WGSSO in Lisbon and kindly provided by its chairman Einar Svendsen. The results of the 3-D transport model including the fluxes of nitrate and the mean current speed are very illustrative for the overall water transports and fluxes in the North Sea and at its boundaries. However, these model results also showed the lack of resolution for areas like the German Bight. The complex hydrodynamic and hydrographic structures in this area are not resolved to such a degree that calculations of fluxes for phytoplankton growth could be made by the WGPE. Therefore as an first attempt to use the German Bight as an example area for the calculation of nutrient fluxes the paper presented by Beddig *et al.* (1995) can be used. They showed the importance of riverine, advective and atmospheric nitrate fluxes for the inner German Bight (their Fig. 2). The overall net annual nitrogen budget for the water column of the German Bight in 1990 and 1991 showed an increase of about 100.000 tonnes/year, which ,however, was not observed as an increase in the water column concentrations. The authors suggest that this surplus amount is lost to the sediments and to the Wadden Sea. However, the inspection of the errors in the calculations shows that they have a large degree of uncertainty Thus model calculations are not yet good enough to calculate possible increases of phytoplankton biomass and turnover.

The example presented during the WGSSO meeting on the effects of a flood of the Norwegian river Glomma in 1995 is mainly in accordance with the observations in the German Bight, as far as the behaviour of nitrogen and phosphorous is concerned. Similar effects of effects of silicate however are not available for the German Bight. It would be interesting , but obviously this was not discussed, to see whether comparable observations are available for the outflow of the River Rhine during the winter of 1994-1995 along the Dutch coast. Although the nutrient reduction efforts have strongly affected the load of phosphorous from the river Rhine up to now no direct effects on chlorophyll concentrations have been observed, which is not in agreement with what one would expect. It is however in complete accordance with the statement by Pohlmann (Draft WGSSO report) that nutrient input reductions do not give significant reductions in vertically averaged chlorophyll concentrations throughout the year.

Another opportunity to solve this problem of effects of nutrient fluxes on phytoplankton growth and species composition will be the Workshop in the Netherlands on the use of Eutrophication Models for the North Sea under the auspices of OSPARCOM. These selected group of experts is well prepared to give a more quantitative reply to the question on the response of the marine environment to anthropogenic nutrient inflows.

h). examine the feasibility of, and potential contributions to, an Environmental Status Report for the ICES Area on an annual basis, and report to the Advisory Committee on the Marine Environment by the end of 1995.

The discussion on this TOR showed a diversity of opinions. In several countries attempts are underway to present environmental status reports, but their contents is very different as well as the group of potential readers. A question was put forward whether this would be a revival of the former Annales Biologiques. Lindahl mentioned the annual report which is written for the Swedish EPA. Obviously, the WG is not able, without a more clear definition of the contents to contribute to such a report. A problem raised is the kind of data used: only data available to ICES, in the ICES data bank,

or a more or less accidental set of data available to the WG members? The chairman was unable to answer all these questions and therefore needs support of ACME or BOC to clarify what is really needed and wanted.

4 ANY OTHER BUSINESS

An overview of long term primary production data from the Icelandic region were presented by Kristinn Gudmondsson and Thorun Thordardottir. Large differences in annual primary production are observed depending largely on the mixing and upwelling patterns around the island. Because of the strong variability a higher sampling strategy as intended was needed. In some areas correlations between primary production and zooplankton biomass were observed. A primary production model has been developed which includes several environmental parameters.

A discussion was held on a possible venue next year. Bode offered to have the meeting in La Coruna at his institute. However, no decision was taken because the appropriated date to meet would be at about the same time as the International Symposium in Kiel. Therefore the meeting could also be held directly after the Symposium in Büsum (Germany), because most members would already be in Kiel, and reduce travel expenses, or the meeting could be held together with the WG HAB, but their meeting place is yet unknown. A final opportunity would be to have the meeting , in case more discussion is needed in combination with the WG SSO, at their venue. For the moment the point is left open, but needs discussion in ACME; BOC or during the Annual Meeting in Reykjavik.

The chairman announced that he would like to hand over the chairmanship to somebody else because he has now fulfilled this job for several years and new input could be given by a new chairman. Due to strong pressure by the members of the WG to continue for another year, the chairman agreed to be on duty for one more year, but next year a successor will be needed.

After this discussion a short tour through the Biological Oceanography Department was made, where some details of the work of the group were shown, including the instrumentation available.

5 ACTION LIST FOR NEXT YEAR

The action list of next year contains the following points: a discussion on the SCOR report, prepared by Rey and Sakshaug to see whether recommendations on chlorophyll-a measurement to be used in standard oceanographic studies can be forwarded to ICES; to continue the discussion on the effects of nutrient enrichment and fluxes on the functioning of marine systems. This question however, should be curtailed to specific areas and parts of the foodweb to prevent too much generalisations; to set up a discussion on new approaches in phytoplankton ecology on the basis of organismal functioning of the planktonic system; to discuss the results of mesocosm experiments which have been performed to study the direct effects of nutrients inputs(enhanced, reduced) on the phytoplankton composition, primary production and biomass (Dutch studies by RIKZ in their Middelburg mesocosms (Smaal, Peeters *et al.*), NIOZ in their laboratory mesocosms (Riegman), Narragansett Bay mesocosms(Smayda *et al.*), Norwegian studies within the framework of the MARICULT program, Swedish studies (Graneli *et al.*), and others) and the results of studies on interactions of phytoplankton with other trophic levels (grazers, microbial loop); and finally to organize and carry through the International Symposium in Kiel. In stead of continued review of new techniques it was suggested to limit this item to a particular topic as e.g. biosensors.

6 RECOMMENDATIONS REFERRING TO NEW TOR'S

To be extracted from the action list.

7 ADOPTION OF THE WG REPORT

Only part of sections of the report were available at the end of the meeting for inspection by the WG members. These were adopted by the meeting. Most other parts were available in a draft form on diskette and have later been compiled and edited by the chairman.

8 CLOSING OF THE MEETING

The meeting was closed by the chairman after acknowledging the local organizer Kristinn Gudmondsson for the organization of the meeting and the Director of the institute for his hospitality at 13.00 hrs on Saturday 30 April.

ANNEX 1

Agenda of the meeting

1. Opening of the meeting, announcements of the chairman, adoption of the agenda, appointment of rapporteur

2. Terms of reference

3. General discussion of terms of reference

4. Any other business

5. Action list for next year

6. Recommendations referring new TOR's

7. Adoption of the WG report

8. Closing of the meeting

ANNEX 2

List of participants

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ANNEX 3

Report on light measurements and intercalibration of standard ICES incubators
(second draft).

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(Results from earlier performed light measurements in standard ICES incubators and from a workshop held on 9-11 March 1994 in Middelburg, presented at the meeting of the ICES WG on Phytoplankton Ecology in Copenhagen, 23-26 March 1994; additional revisions made after the meetings in Copenhagen, 23-26 March 1994 and in The Hague, 29-31 March 1995)

INTRODUCTION

Since 1987 some of us have worked in a changing configuration on the construction and experimental performance including a standard protocol of a newly designed 'simple' and inexpensive incubator for primary production measurements. The original term of reference was to develop a simple and inexpensive incubator for use in monitoring studies.

During one of the meetings of the former ICES WG on Phytoplankton and the Management of their Effects, the original set-up was criticized because no P-I relations were measured. Therefore the design was adapted enabling the measurement of P-I relations at a range of 12 (including dark) irradiance levels. The incubator has been used as a P-I incubator during Indian Ocean cruises in 1992-1993 by NIOZ-workers (some results were presented in Colijn et al., 1993).

In the last report of the WG on Phytoplankton and the Management of their Effects (C.M.1993/ENV:7 Ref.:L) it was stated that the Dutch workers would be asked to explore the possibility of convening an evaluation workshop in The Netherlands. One of the objectives of this workshop would be to evaluate the reproducibility of measurements using the standard incubator and protocol in the hands of different users. At the end of 1993 funding for the manufacturing of four incubators, four filter/flask series (each with an irradiance gradient), some irradiance sensors and the execution of light measurements by an optical expert became possible, giving the opportunity to perform a reproducibility experiment before the next meeting.

In this report we will present 1) information on the used epoxy resin coating, 2) information on the used irradiance sensor, 3) some results from earlier performed extensive light measurements in the standard incubators and 4) the results from an intercalibration experiment with four incubators to check the comparability of identical incubators and the variability due to manipulation of the samples by different users. Information with respect to 1), 2) and 3) was taken from ZEMOKO (1994).

MATERIAL AND METHODS

Incubators and incubation bottles

A short description of the incubator has been taken from Colijn et al. (1993). The incubator is constructed as a rectangular perspex tank (h*b*w=33*33*9 cm) with a turning wheel (max. 10 rpm, 18 cm in diameter) on which 12 experimental bottles (Greiner, tissue culture flasks, ca. 55 ml, 690160) are clamped. Water is recycled within the incubator by an aquarium pump causing the revolution of the turning wheel, with the bottles acting as paddles. On board ship the incubator should be closed accurately with a perspex cover to avoid overflowing and short-circuiting.
Illumination is provided by 10 Philips 8 W fluorescent tubes (TLD 8W J8, no. 33) which can be switched off/on separately.
Water temperature can be controlled using an external cooling device or with a running seawater system. Because we wanted to cool 4 incubators simultaneously a copper tube outside the light field along the narrow vertical walls and the bottom of each incubator was used; the copper tubes were parallel connected to the thermostat (Colora). In this way we reached similar levels of water temperature in the 4 incubators (see Table 1) without the risk of contaminating the cooling device or the 4 incubators at the same time.

Sensor construction and calibration

Knowledge on irradiance measurements is of great importance for P-I measurements. Therefore, a new small spherical irradiance sensor was constructed, consisting of a Si photodetector in front of which a green filter is mounted and surrounded by a spherical collecting element made of diffuse epoxy-resin. With a stopper, through which the wire passed, it can be fixed in the centre of an incubation bottle.

Detailed information of the measured typical spectral and spatial sensitivity of this type of sensor is given in ZEMOKO (1994).

For the absolute calibration of the sensor in $W.m^{-2}$ or $mmol.photons.m^{-2}.s^{-1}$ a spectroradiometersystem was used, consisting of a spherical collecting element, an optical fiber, a Jarrell Ash gratingmonochromator and a Si photodetector. Furthermore a standard tungsten striplamp as a wellknown radiance source was used.

The obtained calibration factors (multipliers to get $W.m^{-2}$ or mmol.photons-$m^{-2}.s^{-1}$) hold only for the combination of this sensor and TLD33.

With the sensor clamped to the turning wheel it was easy to make a complete rotation-angle of 360° and to calculate the average irradiance and standard deviation. The 4p sensor was calibrated using a tungsten strip lamp and a LICOR-1000 lightmeter. The obtained calibration factors (multipliers to get $W.m^{-2}$ or mmol.photons.$m^{-2}.s^{-1}$) hold only for the combination of this sensor and TLD33.

Neutral density filtercoating

Different levels of irradiance were created by applying different layers of epoxy-resin (in which dark pigments are mixed in different ratios) as neutral density filters on the surfaces of the incubation bottles. The side walls and the necks of the bottles were covered with black epoxy-resin. The reason that we chose this material is our experience that nettings, grids, and even some neutral density filters seriously influence the relative transmission between 400-700 nm. Determination of transmission values in the 400-700 nm range was performed by means of a halogen lamp with day-light-filter and a monochromator. The tubes have the lowest absolute irradiance in the blue and green parts and the highest absolute irradiance in the yellow and orange parts of the 400-700 nm range (data not presented here).

Four series of bottles were available with the following transmission values (in %):

0 1.0 2.5 9.4 18.0 22.9 28.5 31.5 42.5 51.0 70.6 100
0 1.1 2.6 9.8 18.9 23.5 28.7 31.6 42.8 51.5 71.0 100
0 1.5 2.9 9.9 19.1 23.6 30.5 32.9 43.2 53.1 72.1 100
0 1.5 2.9 9.9 19.3 24.3 31.4 35.7 43.3 54.1 72.9 100

Figure 1 shows the relative transmission of 3 and 1.5 % filters of the used epoxy-resin. This material is most suitable in the very low transmission range (thick epoxy-resin layer). In the high transmission range (thin epoxy-resin layer) it must be even better.

The procedure to make the desired epoxy-resin/dark pigment composition and to fix the layers on the incubation bottles is not given here. The reason is that this work was done by a consulting firm that spended some research on this subject. On request the firm is willing to construct on a commercial basis (a restricted number of) series of incubation bottles with known irradiance levels (ZEMOKO, Maritiem technisch bureau, Dorpsplein 40, 4371 AC Koudekerke, The Netherlands, Tel/Fax 0031-0118-551182).

Irradiance measurements

Figures 2-5 give examples of light measurements performed with the 4p sensor. In these figures rotation-angle 0 corresponds with the highest position on the turning wheel. The small and negligible nipple-shaped structures at the tops in Figures 2-5 are measured when the 4p sensor approaches the vertical parts of the copper tubing. Figure 2 illustrates the insignificant difference between the four TL-sets (with coated bottles and white polystyrene foam against one of the outer walls). Figure 3 gives the absolute irradiance distribution with clear bottles and with and without polystyrene foam. It can be seen that using the polystyrene foam substantially increases the amount of available irradiance in the incubator. Surprisingly, however, the difference between minimum and maximum values increased. Figure 4 illustrates the light-absorbing effect of all coated bottles in position on the turning wheel with 2, 4, 6, 8 and 10 TL tubes used. The most flat irradiance distribution was obtained using 6 TL tubes. Finally, Figure 5 gives the results with coated bottles and two sets of 10 TL tubes in parallel and crossed position. In parallel position the mean irradiance during one rotation is ca. 940 mmol.photons.$m^{-2}.s^{-1}$ and in crossed position ca. 960 mmol.photons.$m^{-2}.s^{-1}$, see Table 3 in ZEMOKO (1994). It should be preferable to have also one or two higher irradiance values in the more inhibiting part of the P-I curve. Higher (and more uniform distributed) irradiance values might be obtained by using circular fluorescent tubes at both sides of the incubator. Using a white epoxy-resin instead of black epoxy-resin to reach higher irradiance values might be possible. In that case attenuation is achieved by diffuse scattering/reflection instead of absorption. However, the spectral properties (relative transmission in the 400-700 nm range, see also Figure 1) of black epoxy-resin seem to be better than those of white epoxy-resin.

Incubations

A series of 3 consecutive incubations were performed in all 4 incubators with changing users per incubator. A culture of *Phaeodactylum tricornutum*, grown in a 2000 l indoor pond with enriched seawater under continuous light (6 * Philips 60 W) at Chl-a concentrations of ca. 150 mg/l, was used. It was diluted tenfold with 0.2 mm filtered Oosterschelde water 24 hours before the experiment. Water temperature in the indoor pond was ca. 11°C, but is known to fluctuate during day and night. At the experimental day nutrient concentrations were P-o-PO_4: < 0.03 mM; Si-SiO_2: 18 mM; N-NH_4: 1.5 mM and N-NO_3+NO_2: 48 mM. The low phosphate concentration and very high N/P and Si/P ratio's suggest phosphate-limited conditions.

Protocol

For the experimental procedure we followed the standard protocol with a few modifications due to the lab facilities. Thus the incubation bottles were filled with 55 ml of the sample and to each 20 ml with 2 mCi was added. The bottles were always incubated for two hours. After incubation the samples were filtered over 47 mm GF/F at a reduced suction pressure of < 15 kPa. The filters then were put in scintillation vials. Up till here all manipulations were done by the different users; the rest (preparing the scintillation vials) by one user. To each scintillation vial 10 ml demineralized water was added. After addition of 0.5 ml 2 N HCl they were bubbled with air for 20 minutes. Previous experiments had shown that this period is long enough to remove all the inorganic ^{14}C. After addition of 10 ml Instagel^R the samples were counted for 10 minutes or to 1 % accuracy. Added activity was counted in the same mixture without addition of HCl.

Additional methods

In all samples a Chl-a value was determined using the HPLC method of the laboratory in Middelburg. Filtration was done over 47 mm GF/F at a suction pressure of < 12.5 kPa. SCO_2 was measured by titration according to standard procedures; the measured SAlkalinity in some of the samples was 2.263. From each sample 20 ml was taken for cell counts (if needed) and preserved with 50 ml acid Lugol's solution.

Experimental set-up

The objective was 1) to examine the error in measured primary production parameters if a certain protocol was used by different users working in identical incubators and 2) to check the reproducibility of a measurement.

When determining the error one should take account of different sources of variability:

-variability as a consequence of subsampling,
-variability by the use of different, but in principle identical incubators,
-variability introduced by the inevitable differences in times of starting the incubations (Exp1-3, see below),
-variability by different users.

To attain the first objective a standard Latin Square Design as experimental set-up was chosen. This set-up can be illustrated with the following scheme:

	Inc1	Inc2	Inc3	Inc4
Expl	A	B	C	C
Exp2	B	C	A	D
Exp3	C	A	B	D

A, B, C and D are the different users. Inc1, Inc2, Inc3 and Inc4 the different incubators and Exp1, Exp2 and Exp3 the 3 successive experiments. Allocation of the incubators (except Inc4) was ad random as was also the case with the distribution of the samples between the users. With this set-up it is possible to take full account of possible error effects within incubators and within experiments, in such a way that a possible user effect can be distinguished.

The first series of measurements (Exp1) started between 9 and 10 a.m., the second (Exp2) between 12 and 13 p.m. and the third (Exp3) between 15 and 16 p.m. In between samples were kept in the dark in cool boxes.

The photosynthetic parameters P_{max}, I_{opt}, I_k and a were derived after fitting the data to the equations of Eilers & Peeters (1988), Jassby & Platt (1976) and Platt et al. (1980). Dark values were not subtracted in the productivity calculations; all dark values except one were ca. 1 % of the maximal photosynthetic rate.

To attain the second objective, reproducibility of a measurement, one user (D) always used the same incubator during Exp1-3 (see scheme above). Unfortunately these results deviated so much from the results of the other three users that a separate consideration was necessary.

RESULTS AND DISCUSSION

Some general information on water temperatures and speed of the turning wheels during the experimental day is given in Table 1. It follows that these characteristics hardly changed during the experimental day.

The mean chlorophyll-a concentration of the nine used samples was 25.6 mg/l and the coefficient of variation 6 %. We thus can conclude that subsampling did not contributed much to variability.

From the analysis of the Latin Square Design it appeared that (except for the slope a determined with the Platt-Gallegos-Harrison model) the incubator (INC) effect was not significant ($p>0.05$) as was also the case for the time (EXP) effect. After correction of the 'disturbing' factors incubator and time there was no user effect ($p>0.05$). This means that for determination of the magnitude of the different parameters from the different P-I models the general mean can be used and that the magnitude of the error can be calculated from all measurements. The results (averaged values for all users) are depicted in Table 2.

Furthermore it appeared that differences could be found in a derived from the three P-I models both according to the number of the experiment and the number of the incubator; see Table 3. This table presents the averaged values for all users. The differences are small, but can be demonstrated with a design like this. For the other parameters the variation after correction for the 'disturbing' factors is to such an extent that differentiation is not possible.

From Table 2 it appears that Pmax has the smallest coefficient of variation and thus can be determined most accurately. Iopt is most variable, while Ik seems to be much more stable; especially for the Platt-Gallegos-Harrison model. The values for Pmax, Ik and a are reasonably comparable for the different P-I models.

Table 4 gives the results of the fourth user. Comparison with Table 2 shows clearly that this user's measurements differed from those of the other three. Only during the third measurement results were similar.

Table 5 gives the mean values with the standard errors and coefficients of variation for all P-I models used. These results were obtained from Table 2.

The general conclusion is: by handling of a fixed protocol a very precise production measurement can be performed.

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Colijn, F., G.W. Kraay, R.N.M. Duin & M.J.W. Veldhuis, 1993. Design and tests of a novel P_{max} incubator to be used for measuring the phytoplankton primary production in ICES monitoring studies. Annex 5 in Report of the Working Group on Phytoplankton and the Management of their Effects, Copenhagen, 28-30 April 1993, C.M.1993/ENV:7 Ref.:L.

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Jassby, A.D. & T. Platt, 1976. Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. Limnol. Oceanogr. 21: 540-547.

Platt, T., C.L. Gallegos & W.G Harrison, 1980. Photoinhibition of photosynthe-sis in natural assemblages of marine phytoplankton. J. Mar. Res. 38: 687-701.

ZEMOKO, 1994. Irradiance Differentiation and Control in the ICES incubator. Needed in order to be able to accomplish reliable P-I measurements with respect to phytoplankton primary production studies. Unpublished report.

Table 1. General information on water temperatures and speed of the turning wheels during the experimental day.

	Water temperature (°C)			Speed (rpm)		
	Mean	SD	n	Mean	SD	n
Inc1	11.48	0.04	12	8.6	0.6	3
Inc2	11.54	0.08	12	7.8	0.3	3
Inc3	11.72	0.07	12	7.5	0.5	3
Inc4	11.78	0.11	12	8.9	0.9	3

Table 2. Mean values, standard errors and coefficients of variation (defined as mean/standard deviation) of several measured parameters. pe=Eilers-Peeters model; jp=Jassby-Platt model; pgh=Platt-Gallegos-Harrison model. Pobs is measured maximal production. Pmax and Pobs in $mgC.mg^{-1}Chla.h^{-1}$; Iopt and Ik in $W.m^{-2}$; a in $mgC.mg^{-1}Chla.h^{-1}.W^{-1}.m^{2}$.

	Mean	Standard error	CV (%)
Pmaxpe	1.70	0.045	8.0
Pmaxjp	1.67	0.052	9.4
Pmaxpgh	1.69	0.047	8.3
Pobs	1.75	0.045	7.7
Ioptpe	102.3	12.2	35.8
Ioptpgh	179.9	92.9	154.9
Ikpe	21.1	2.79	39.5
Ikjp	27.6	1.65	17.9
Ikpgh	22.2	1.27	17.2
αpe	0.089	0.0089	29.9
αjp	0.061	0.0027	13.4
αpgh	0.076	0.0041	16.0

Table 3. The slopes of the P-I curves calculated for the different experiments and incubators. EXP stands for the number of the experiment and INC for the used incubator. The measurements are arranged in order of magnitude (except for the incubators under ape, these gave a different result when compared with the two other models). All values are mean values for the three users. Legend: see Table 2.

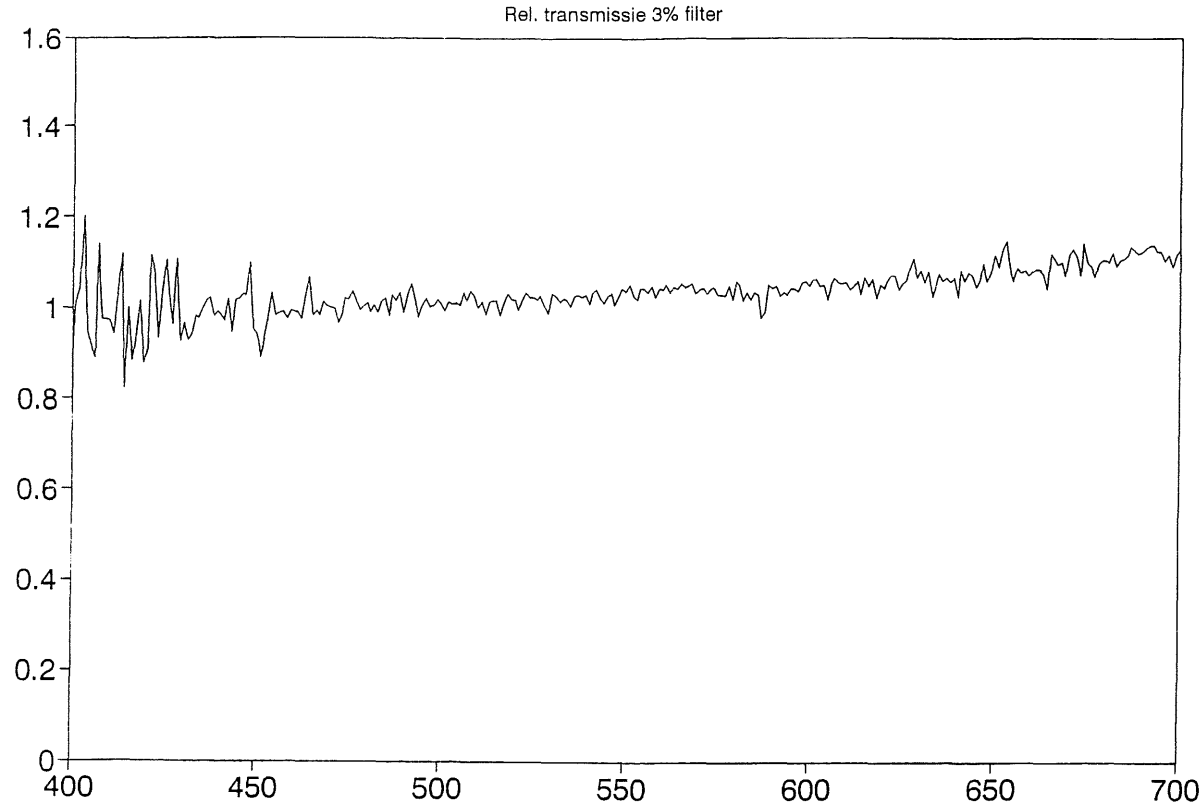
	αpe	αjp	αpgh
EXP2	0.1093	0.0677	0.0873
EXP1	0.0937	0.0617	0.0777
EXP3	0.0637	0.0547	0.0677
INC1	0.0867	0.0663	0.0827
INC3	0.1037	0.0637	0.0820
INC2	0.0763	0.0540	0.0680

Table 4. The results of the fourth user. * points to a very high value resulting from not-saturated P-I curves. The figures are based on three measurements performed simultaneously with the three other users. Legend: see Table 2.

	Mean	Standard error	CV (%)
Pmaxpe	2.163	0.221	17.7
Pmaxjp	2.027	0.270	23.1
Pmaxpgh	2.142	0.357	28.9
Pobs	1.860	0.069	6.5
Ioptpe	*	*	*
Ioptpgh	180.0	67.9	65.4
Ikpe	54.6	21.5	68.2
Ikjp	63.0	22.6	62.2
Ikpgh	58.7	24.5	72.3
αpe	0.051	0.0141	48.2
αjp	0.038	0.0094	42.4
αpgh	0.046	0.0012	45.4

Table 5. The mean values for the three different users and the different P-I models used. Legend: see Table 2.

	Mean	Standard error	CV (%)
Pmax	1.68	0.048	8.6
Iopt	141.1	66.25	140.9
Ik	23.6	2.01	25.6
α	0.075	0.0059	23.6



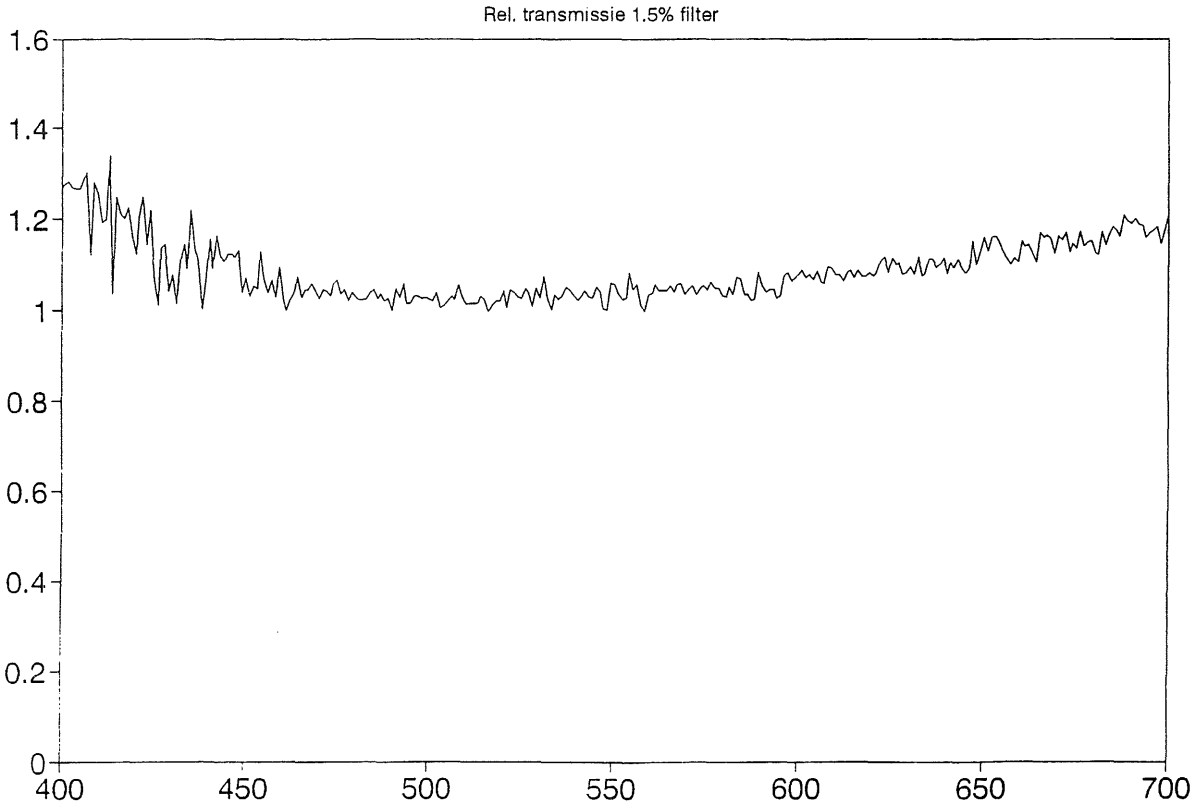


Figure 1. Relative transmission of 3 and 1.5 % epoxy-resin filters in the 400-700 nm range.

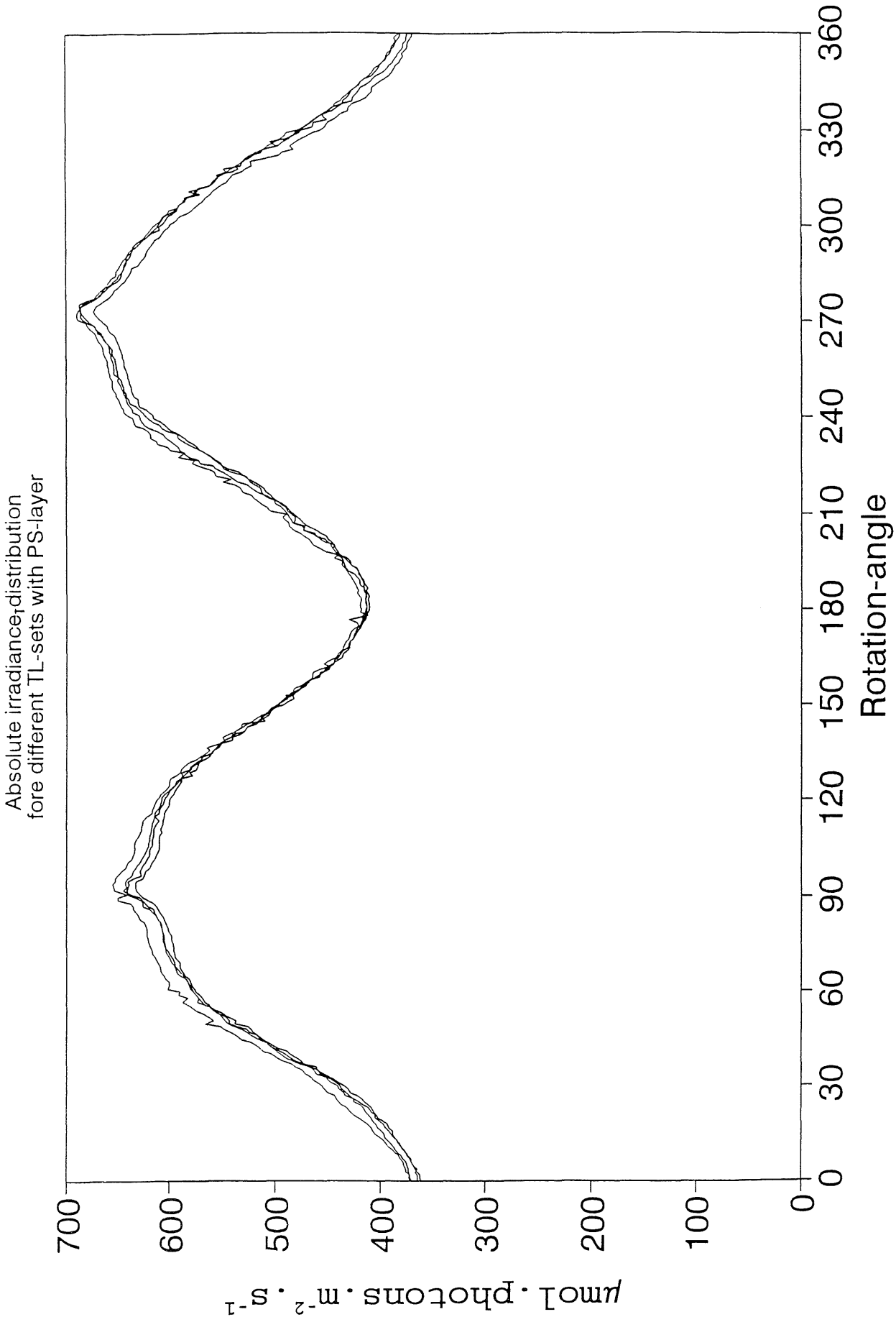


Figure 2. Absolute irradiance distribution of four different TL-sets, 10 TL tubes, with polystyrene (PS) foam layer and with coated bottles.

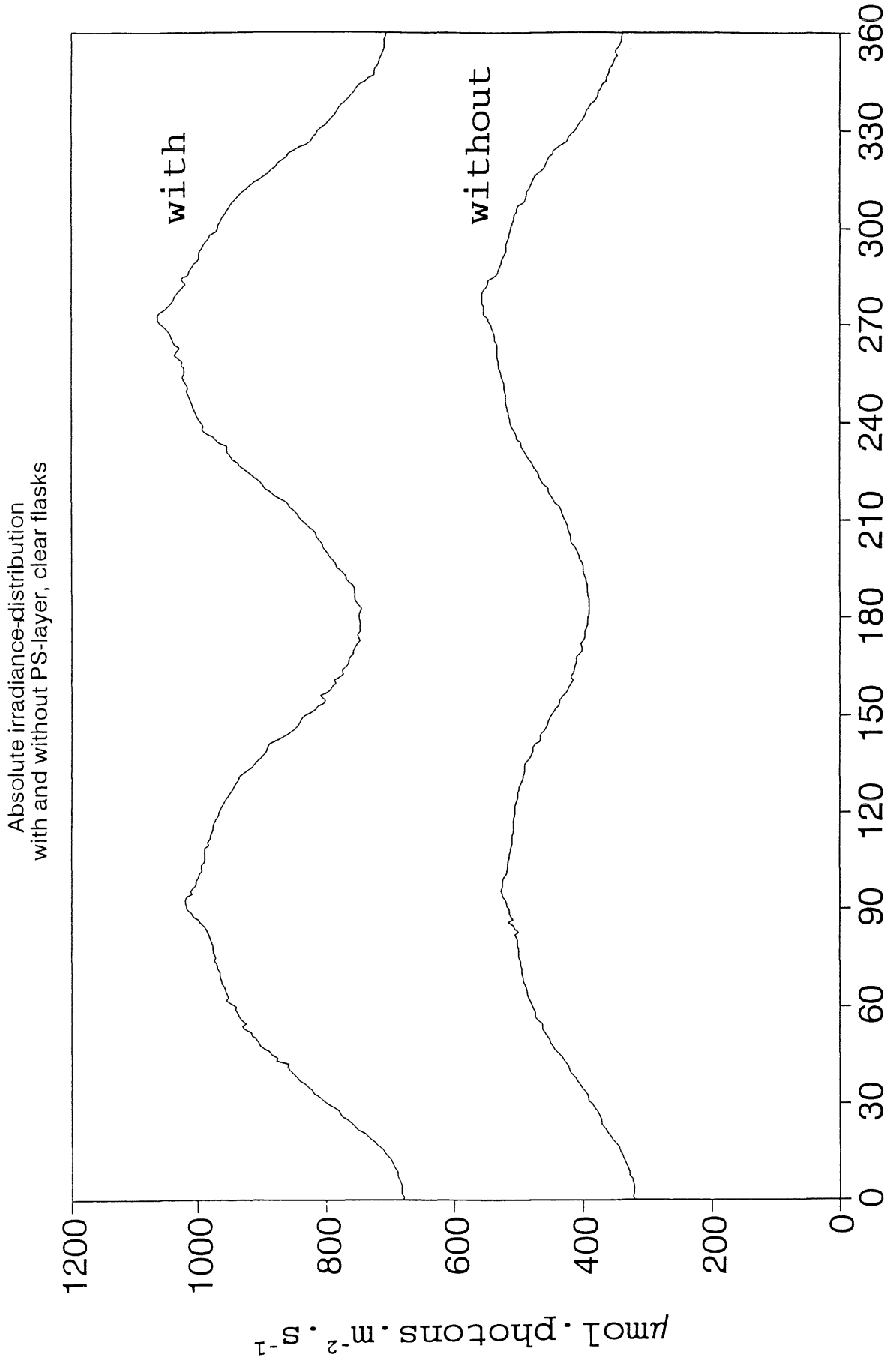


Figure 3. Absolute irradiance distribution with and without polystyrene (PS) foam layer, clear bottles and 10 TL tubes.

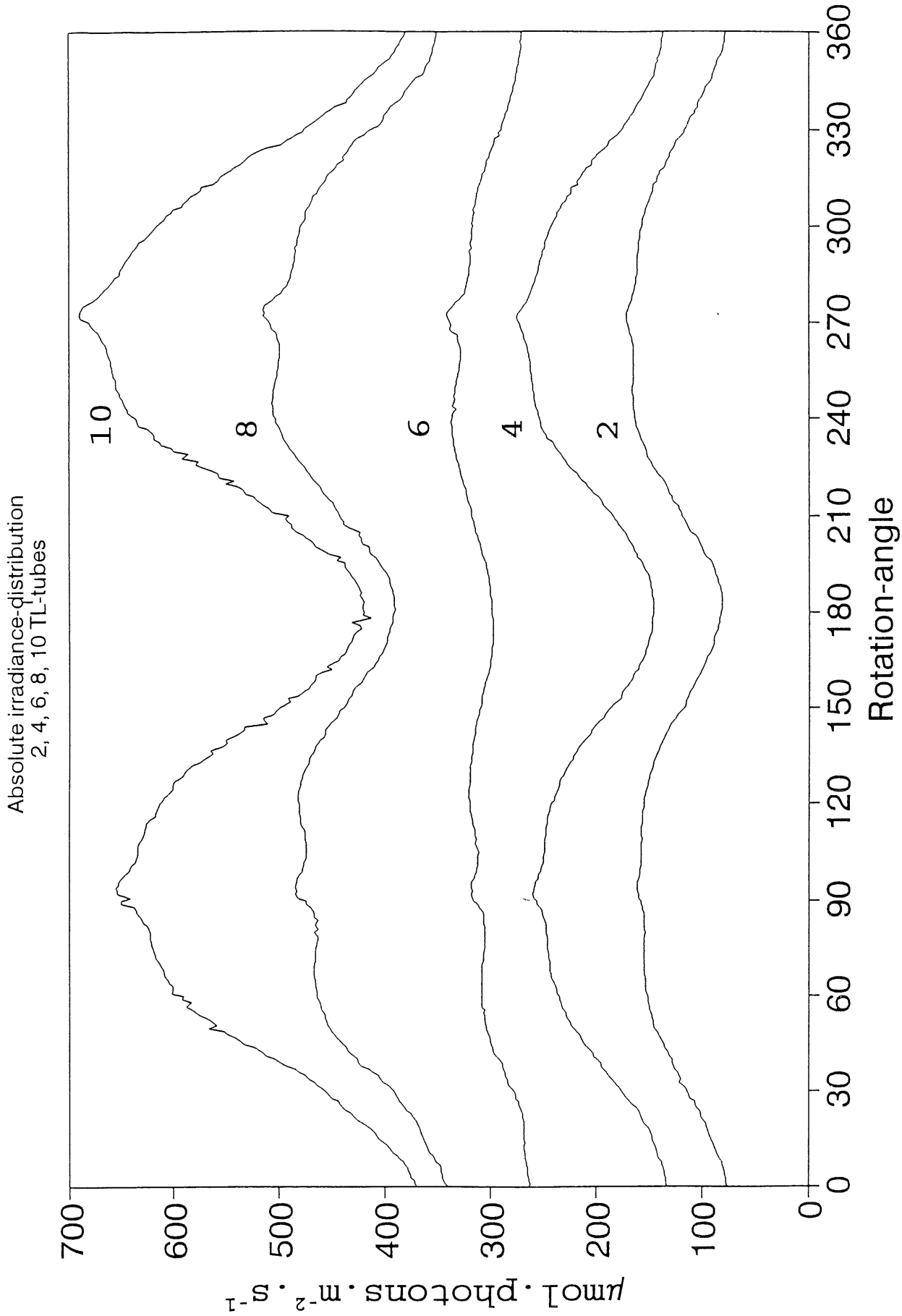


Figure 4. Absolute irradiance distribution with polystyrene foam layer, with coated bottles and 2 (xxoxxxxoxx), 4 (xoxoxxoxox), 6 (xoxooooxox) 8 (xooooooox) or 10 TL tubes.

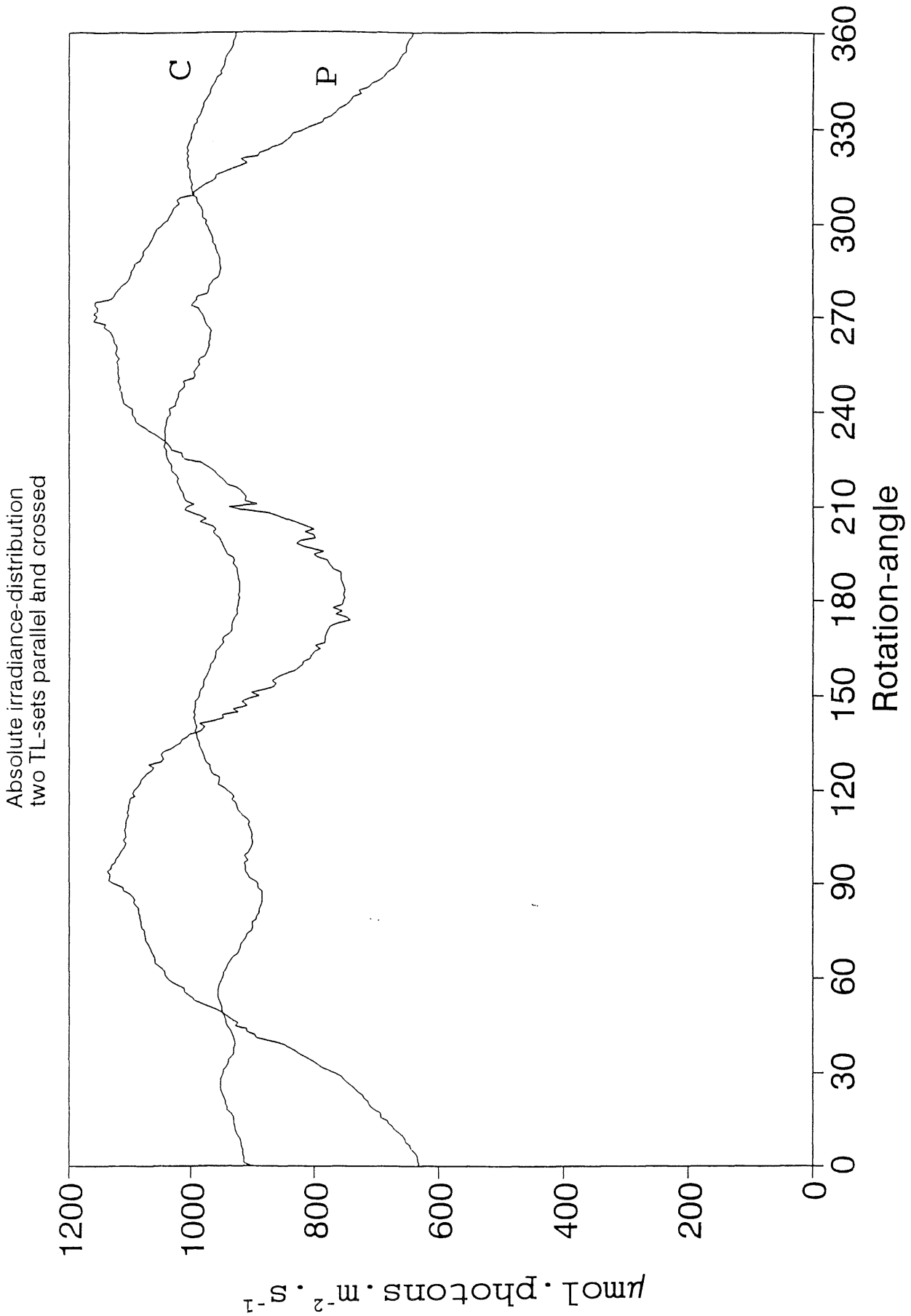


Figure 5. Absolute irradiance distribution with coated bottles and two 10 TL-sets parallel (P) and crossed (C).

ANNEX 4

(revised version May, 7, 1996)

DRAFT

Design and tests of a novel P_{max} incubator to be used for measuring the phytoplankton primary production in ICES monitoring studies

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(paper presented at the meeting of the ICES Working Group on Primary Production, Helsinki, Finland 4-7 July 1989; revised and additions made on August 16th 1989; additional revisions made at the ICES Working Group on Phytoplankton and the Management of their Effects, Oban, Scotland, 3-7 April, 1990; additions made for the meeting of the ICES WG on Phytoplankton and the Management of their Effects in Copenhagen, 28-30 April 1993, to be presented by L.P.M.J. Wetsteijn, last update after the meeting of the Working Group on Phytoplankton Ecology in Reykjavik, 29-31 March 1996)

Contents

Abstract

An inexpensive and simple incubator for primary production measurements is presented along with a protocol for achieving strictly comparable and reliable ^{14}C-fixation rates of phytoplankton. The incubator, based on Steemann-Nielsen and Aabye Jensen (1957), is comprised of incubation bottles revolving in a temperature controlled water bath at a fixed irradiance. The recommended protocol and incubator have been tested in different water types, such as Dutch and Finnish coastal waters, in the North Sea and in the Indian Ocean, and give reliable estimates of the photosynthetic rate at the fixed irradiance used. Coefficients of variation were between 0.6 and 7.6 in incubation experiments with three and five samples. No difference between P_{max} measured in the Baltic incubator and the ICES incubator was found.

The incubator has been used as a P-I incubator during cruises in the Indian Ocean by providing a series of bottles with different transmittance characteristics. These experiments show that actual P-I relations can be measured with a good fit of the P-I curve parameters, initial slope (a), I_k, I_{opt} and P_{max} values.

A last series of measurements were performed for over one year at a monitoring station in the German Wadden Sea. These measurements showed the typical characteristics of P-I incubations with almost stable alpha values and temperature controlled Pmax levels. Correlations between chlorophyll and primary production was good.

Daily primary production values on selected series of data have been calculated based on the P-I relations after integration over time and depth and compared with a simple empirical equation based on P_{max}, attenuation coefficient daylength and daily insolation. The agreement between both methods was still rather poor, and variable. Dependent on the calculation mode all values were roughly 1.5 to 2 times too high as compared to the integrated values based on one of the fitted P-I curve parameters. Further work has to be done to improve this empirical formulation. The three equations used to calculate the daily primary production were comparable. Calculations not based on a sinoidal light function but on a rectangular mean irradiance level were 5-20 % higher.

1. Introduction.

Results of the Hirtshals intercalibration were discussed during the workshop of the ICES Working Group on Primary Production in Copenhagen (June 1988). The meeting adopted the following recommendation: "... that there is a need for a standardized primary production method to be used in monitoring studies with special coded data in the ICES data bank". The authors have accepted to comply with the request by building a simple and inexpensive incubator and proposing an appropriate protocol.

At present several procedures are available to measure daily depth-integrated primary production ($mgC.m^{-2}.d^{-1}$). Most of these methods are based on measurement of P (photosynthesis) vs. I (irradiance) relationships, of vertical attenuation coefficients, and solar irradiance (Aertebjerg Nielsen & Bresta, 1984; Gargas & Hare, 1976; Richardson, 1987).

The results of the Hirtshals intercalibration workshop (Anonymous, 1989; Richardson, 1991, 1993) have shown that calculation of integral daily primary production may contain a whole series of errors or assumptions which cause large differences in the final result. Substantial errors arise from handling of samples, incubation time, the incubation approach, liquid scintillation counting, and calculation methods, but the main difference was due to the different types of incubators used (measurement of irradiance, differences in light quality etc.). Therefore, data offered to the ICES data bank are not comparable. This paper describes the use of an incubator and a strict protocol with as few steps as possible, and with recommendations about the use of materials, to reach directly comparable data.

Our task, however, has been limited to this specific point and therefore no attempt has been made to propose a method to calculate integral daily production from single P_{max} ($mgC.m^{-3}.h^{-1}$) measurements, assuming that the incubator has the possibility to measure P_{max} at light saturation within a large range of irradiances. Several other assumptions have to be made to calculate daily primary production, including a vertically homogeneous distribution of algal biomass, similar photosynthetic characteristics of the phytoplankton and the same species composition throughout the water column. Also data on vertical attenuation and daily irradiance should be available. As shown by Riegman and Colijn (1991) calculations based on surface samples alone can underestimate areal primary production by 17%. As pointed out by Platt and Sathyendranath (1988) oceanic primary production might be well estimated from an irradiance model based on measurements of P_{max} and α, and a remotely sensed biomass field. Such estimates might be possible for the North Sea within the near future if both P_{max} and α are known.

Stimulated by the discussions in the ICES WG we finally have attempted to use the ICES-incubator as a P-I incubator and to compare daily primary production values measured in the ICES-incubator with fully integrated values over time and depth, using P-I relations.

2. Description of the incubator.

The incubator strongly resembles the one originally used by Steemann Nielsen & Aabye Jensen (1957),(cf. Postma & Rommets, 1970; Cadée & Hegeman, 1974). It is constructed of a rectangular perspex tank (h x b x w= 33 x 33 x 9 cm) with a turning wheel (max. 12 rpm, 18 cm in diameter) on which experimental bottles (max. 12) are clamped. Illumination is provided by 10 Philips 8 W fluorescent tubes (TLD 8W J8, no.33) which can be switched off/on separately (Figure 1). Irradiance should in all cases be measured with an appropriate light sensor (e.g. LICOR, $\mu E.\ m^{-2}.s^{-1}$ or $W.m^{-2}$) or the special sensor developed by Wetsteijn et al. (1996). Our experimental set up gave a mean irradiance of 360 $\mu E.m^{-2}.s^{-1}$, providing a saturating ^{14}C fixation rate (see results section). However, the light field is not homogeneous but ranged from 140 to 530 $\mu E.m^{-2}.s^{-1}$ depending on position of the flasks during revolution. The homogeneity of the light field can be easily improved by using a backscattering white polystyrene foam layer opposite to the fluorescent tubes. These irradiance measurements were done with a 2π-sensor and therefore are substantially lower than the earlier measurements than the values measured in the incubator during the Indian Ocean cruise with a spherical sensor: with 10, 8, 6, 4 and 2 tubes and this polysterene layer we measured 1100, 850, 650, 300 and 250 $\mu E.m^{-2}.s^{-1}$, respectively as maximum irradiances.

During the workshop it was discussed whether this incubator could be used to measure P-I relations. Indeed, this can be done by covering the incubation bottles with neutral density filters (e.g. Flash Light Lee), available in several transmission classes. An alternative is painting the bottles in different black intensities. Such tests have been performed recently during cruises in the Indian Ocean in 1993. However, this procedure did not fall into our primary goal as stated above in the recommendations of the 1988 meeting. Thus the incubator now no longer acts as a simple incubator again introducing several of the "old" uncertainties and errors, especially as far as irradiance levels in the bottles is concerned. During a later stage the problem who to obtain different irradiance levels in the incubation bottles has been solved by using a epoxy-resin layer of different attenuation (see Wetsteijn et al., 1996)

Incubations are carried out in disposable tissue (ultraclean) culture flasks (Greiner, tissue culture flasks, 690160) containing 50 ml of sample. These flasks can be used several times without deterioriation of the vessel walls.

Temperature is controlled to within $\pm$ 0.1 °C by a Lauda thermostat. Water is recycled within the bath by an extra pump which also causes the revolution of the wheel, with the flasks acting as paddles. If only a few samples are incubated the open positions should be filled with flasks containing water to attain a constant turning of the wheel. A running seawater system on board the ship could be used instead of the thermostated water bath. The estimated cost of the apparatus (materials only and without the cooling device) is about US $500, half of which is due to the illumination system. The cost per unit could further decrease if several incubators are built simultaneously.

3. Results of test runs on five locations.

Several tests by independent workers have been conducted with the apparatus in its former and improved form.

3.1. Test at the Netherlands Institute of Sea Research (NIOZ).

During the typical spring bloom of phytoplankton in Dutch coastal waters (plankton dominated by the diatoms Biddulphia aurita, B. sinensis, Coscinodiscus concinnus, Skeletonema costatum and colonies of Phaeocystis sp.), an incubation experiment was performed, according to the protocol (see Appendix). Incubation periods of 1 and 2 hours were tested, along with two filter types: Whatman GF/F (approximate pore size 0.7 µm, 47 mm) and Sartorius cellulose acetate 11106 (pore size 0.45 µm, 47 mm).

After filling the experimental bottles, 0.1 mL $NaH^{14}CO_3$ (Amersham) from a stock solution prepared with superclean distilled water containing one pellet of Ultrapure NaOH (pH =9), was added. Ampoules have been cleaned with 6N HCl. Total activity added, to be determined for each experiment, was $11.46 .10^6$ dpm/ 0.1 ml. Precautions should be taken to use a pure ^{14}C-bicarbonate solution, especially when release of extracellular dissolved organic carbon has to be measured (Bresta et al., 1987).

After incubation, samples were filtered within a few minutes through the two filter types. After fuming over concentrated HCl for 5 min in a desiccator, samples were counted in 10 ml Instagel in 20 ml glass scintillation vials. Cells on the filters were disrupted in a Bransom Ultrasonic device during 15 min. Without this disruption, counts can be up to 50% lower. Cpm's were converted into dpm's with a quench curve and the external standard channels ratio method. Results of the first experiment are compiled in Table I.

The results show a good reproducibility of the ^{14}C fixation rates, an almost linear uptake over the 2h period, and a lower recovery and a higher variability of ^{14}C on Sartorius cellulose acetate filters compared with GF/F filters (cf. Hilner & Bate, 1989). Dark values were about 2% of the light values.

3.2. Test at the Finnish Institute of Marine Research (Helsinki)

During an ICES workshop, the new incubator was tested on board the research vessel Aranda by making a direct comparison between the ICES incubator and the Baltic Sea incubator on July 6, 1989. A surface water sample containing cyanobacteria and several other species without dominance of a particular one was taken from the Baltic and divided into 14 bottles. To each bottle 0.1 ml of 2 mCi $NaH^{14}CO_3$ was added. Samples were incubated 2 h 25 min and filtered onto GF/F filters, and fumed over concentrated HCl for 10 min. Filters were disrupted by sonification and counted as above. Five samples were incubated in the ICES incubator, 5 in the Baltic Sea incubator at full light (400 $\mu E.m^{-2}.s^{-1}$), and another four samples were incubated at 50%, 25%, 10% and 5% of full light, respectively. Reduction of irradiance was obtained with neutral density filters.

Results are given in Table II. The full light samples in both incubators showed the highest fixation rates. The reproducibility was very high in both incubators. The single point

measurements at the attenuated irradiances showed a good linearity, indicating that in this case four measurements suffice to estimate the photosynthetic efficiency a. Despite the difference in maximum irradiance in the two incubators, the same maximum fixation rate was measured, suggesting that photosynthesis was saturated at an irradiance of about 300 $\mu E.m^{-2}.s^{-1}$.

3.3. Tests in the North Sea by the National Institute of Coastal and Marine Management (RIKZ), formerly Tidal Waters Division at Middelburg (NL)

A similar but completely independent set of experiments was conducted during one of our regular sampling surveys of the North Sea within the EUZOUT (Eutrophication of the North Sea) project. Samples were taken at different stations in the North Sea (Fig. 2), covering both coastal and offshore waters, up to 370 km from the Dutch coast during a cruise from 25 to 27 July, 1989. Surface, thermocline and subthermocline samples were also incubated at the stratified stations. To 50 ml samples 10 μCi in 0.1 ml was added. In this case the results are also compared with P_{max} values calculated from P-I measurements on the same samples incubated simultaneously but in another incubator (Peeters et al., 1991; Klein & van Buuren, 1992). Two comparisons of short (2 h) versus long (6 h) incubation times were made. All samples were filtered onto Whatman GF/F filters; after addition of 10 ml HCl, samples were bubbled with air for 20 min and counted as described in Peeters et al. (1991).

The results are given in Table III. Depending on the station a wide range of photosynthetic activities was observed. Coastal eutrophied stations showed rates up to 40 times higher than in the oligotrophic central part of the North Sea. Vertical profiles showed high rates in the thermocline or subthermocline layers. The long-term incubations showed an almost linear uptake over the 6 h period. Duplicate incubations generally showed a maximum difference of 10%.

Comparison of the P_{max} in the ICES incubator with the P_{max} in the P-I incubator shows that the ICES P_{max} is somewhat higher than the latter P_{max}. This confirms our findings in Helsinki which also showed that the ICES incubator measures a value close to P_{max}. However, samples in the P-I incubator were run for about 6 h instead of 2 h in the ICES incubator.

3.4. Tests during Indian Ocean cruises (JGOFS) in 1992-1993 by NIOZ (Texel) east of African coast off Somalia and Kenya

During these cruises of which the results will be presented elsewhere a series of experiments were performed with bottles painted black with different degrees of transmittance resulting in a range of c. 4% to 100%. Irradiance in all individual bottles however had to be measured. Thus the incubator has now been used as a real P-I incubator. To increase the irradiance levels the backside of the incubator was covered with white polystyrene foam which gave a range of 40 to 1100 $\mu E.m^{-2}.s^{-1}$ in the bottles. A large sample of about 10 l has been taken from the surface during an evening cast at 18 h. LT. From this sample a P-I relation has been measured for 2h, including chlorophyll-a concentrations. Part (about 7 l) of the sample has been stored overnight in a dark cool box and incubated in a similar way the next morning at 6.00 h LT. The results of three of such series are given in Fig. 3 and Table IV. The P-I curves were analysed according to equations given by Jassby & Platt (1976), Platt et al. (1980) and Eilers & Peeters (1988). The first two equations showed comparable results whereas the third one showed higher P_{max} values for both incubations. The former P_{max} values were within 5% difference. Calculation of daily

production also showed good agreement for the former two equations. However the P_{max} and daily production values showed large differences between the two incubations (evening vs. morning) , mainly due to the higher P_{max} values of the morning incubation due to a circadian rhythm (chlorophyll had slightly increased during the storage period) whereas also the initial slope a increased by 25%. More data of the Indian Ocean cruises are available but will be published elsewhere (Veldhuis and Kraay, in prep.).

3.5. Tests at the Station Büsum, along the German Wadden Sea in 1995

Within the framework of our monitoring studies in the German Wadden Sea, weekly incubations were made using the standard incubators, kindly provided by Mr. Bert Wetsteijn of the RIKZ in Middelburg. Contrary to the standard procedure, we used a direct cooling of the incubator in the lab by a Lauda cooler instead of the closed circuit with the copper tubing. This was done to be able to obtain very low incubation temperatures during winter time and does not have any further consequences for the measurements. The samples were illuminated from both sides to obtain sufficiently high irradiances up to 800 $\mu E.m^{-2}.s^{-1}$ for proper P_{max} determination. Throughout these measurements we used the new incubation bottles and the improved irradiance setup as described in Wetsteijn et al. (1996). As a standard incubation time 2 hours were used, but in winter during low activities up to 4 hours were used. TL tubes were arranged to perform a homogeneous light field. Irradiance was measured inside the incubation bottles with the same equipment as developed by Wetsteijn et al.(1996). Mean irradiance values were based on twelve measuring points during one revolution of the wheel. The special incubation bottles prepared by ZEMOKO (see Wetsteijn et al., 1996) were used throughout the measurements. For one P-I measurement 8 bottles including one dark were used. Dark values were low but always subtracted from the light values. Added activity ranged from 0.5 to 3 μC in winter (volume 50 to 300 μl). Samples were filtered over 0.45 μm membrane filters (not GF/F) under reduced suction pressure (200 mm Hg), washed with 10 ml 'cold' filtered seawater and dried. Counting took place in Filter-count (Packard). Added activity was counted after dilution in 55 ml of sample and pipetting 50 μl of the mixture in counting vials. Calibration occurred according to the external standard ratio procedure of the liquid scintillation counter.

Primary production values were normalised to chlorophyll-a measured spectrophotometrically accoridng to Lorenzen (1967).

The results are presented in figures 4 to 6. In Fig. 4 four representative examples of P/I curves are shown from different seasons. Curve fitting and calculation of P/I parameters was made according to the equation of Platt and Gallegos (198). The seasonal variations in P-I parameters is shown in Fig. 5. Chlorophyll specific maximum photosynthetic rates (P^b_{max}) ranged from 2.0 to 9.9 μG C./ μg Chlor/ h^{-1} and showed a large variation over the year and was highly significant correlated with water temperature (Fig. 6). In contrast, the slope of the P/I curves ranged from 0.0150 to 0.0375 μg C/ μg Chlor. h^{-1}/ $\mu E.\ m^{-2}.s^{-1}$ (Fig. 5) and proved to be less variable and irrespective of water temperature. During the whole year no strong light inhibition at high irradiances could be observed. I_k values, used as a parameter of light adaptation, were relatively high throughout the year varying between 81 and 453 $\mu E.\ m^{-2}.s^{-1}$ (Fig. 5). Thus in spite of the low light conditions in the Wadden Sea due to high turbidity, no signs of low light adaptation of the phytoplankton could be detected. Further we conclude that based on the measured high P^b_{max} values and the natural mean low light levels in the Wadden

Sea , the phytoplankton of the turbid inner parts is light limited and not nutrient limited throughout the year.

The results of these P/I measuremnts will be used, in combination with irradiance and attenuation measurements to calculate the annual primary production at the station Büsum (Tillman et al., in prep.).

4. Discussion, recommendations and problems.

To a great extent the task accepted during the 1988 ICES meeting in Copenhagen has been fulfilled: a simple and inexpensive incubator has been built and tested. The tests so far show that the incubator works well, that it is simple to use, and that it also has the potential to measure P-I curves. However, it is not recommended as a P-I incubator, due to the fact that these already exist in a wide variety with more sophisticated irradiance regulation. Reproducibility and linearity of uptake rates are within the expected limits. Problems arising from different photosynthetic characteristics like a daily disparity or circadian rhythm in the same sample can not be solved. Because such differences can be quite large there is no simple solution except to incubate samples several times during the day. To reduce this kind of variability a practical and pragmatic solution could be to incubate all samples around noon.

The series measured at Station Büsum during 1995 show the consistent results which can be obtained with the incubator. Apart from minor changes such as the cooling device at low temperatures, we followed the protocol as described for the continuously mixed water mass. The series will be used to calculate the annual primary production, whereas we intend to continue the measurements to get a series for several years to see whether nutrient reductions influence the primary production in this part of the Wadden Sea. At the moment light limitation is the most important regulating factor.

Apart from the results obtained so far, there is a need for concurrent work with two types of incubators: an ICES type of incubator for monitoring studies and a more sophisticated type where P-I relations can be measured for physiological studies. Comparisons between this simple and maybe more complex types of incubator should be made by the individual scientists as part of an intercalibration study. Nevertheless, the limited amount of methodological steps is of great advantage and reduces several of the common errors. If the protocol listed in the annex is followed, data obtained in this way are directly comparable. (This should finally be replaced by the Working Manual, but needs a little bit of editing).

Discussions both in the ICES working group and with several colleagues have shown that there is a need for a further standardization step leading to the calculation of values per m^2 from these P_{max} measurements. As a first approach, empirical formulations like the one used by Cadée & Hegeman (1974) and DiToro et al.(1971) are useful. In Helsinki we decided that such a formulation should be derived, which then could be used to calculate a value per m^2. A first attempt has been made to use such an empirical equation by comparing daily primary production calculated by integration and based on P-I parameters with this empirical estimate of daily primary production (Tabel V). The results show that daily primary production calculated according to the equations given by Eilers and Peeters, Jassby and Platt, and Platt and Gallegos and in all cases with a sinusoidal irradiance give almost equal results. If daily

primary production is calculated with a rectangular light distribution using one mean irradiance level the daily values are about 5 to 20% higher. If we use the empirical equation of Ditoro et al. (1971) we obtain values up to 1.5 to 2 times as high. Probably the calculation is not yet very realistic and we will further evaluate this procedure.

One should, however, realize that in all cases this value is only an estimate, due to physiological characteristics of phytoplankton (Neale & Marra, 1985; Savage, 1988; Vandevelde et al., 1989), and to an uneven vertical distribution of phytoplankton in the sea (Riegman & Colijn, 1991). Calculation of primary production under such circumstances can only be achieved if samples from different depths are incubated and their light-, temperature- and time-dependant fixation rates are known.

Based on a larger data set comprising P_{max} data and simultaneous P-I measurements, we will try to further evaluate the possibility to such empirical formulation. This formulation will be suggested to ICES for the calculation of primary production per m^2 in different areas. A further step in modelling primary production could be the incorporation of time-dependent adaptation responses as described by Neale and Marra (1985). However, this was not the primary goal of the working group and therefore falls beyond the scope of this paper.

A recent paper of McBride (1992) also compiles several equations to calculate daily photosynthesis, one of which may be adopted by ICES as a standard. The present method to calculate daily primary production is based on an numerical integration over time and depth which is very rapid and simple with modern PC's.

A problem which is not solved sofar is the irradiance needed to measure P_{max}. In our opinion a procedure should be developed to relate the saturating irradiance for P_{max} to the geographical latitude and the time of the year. Then a standardized incubation irradiance could be prescribed.

5. Acknowledgements.

The authors would like to thank Juha-Markku Leppänen for the opportunity to work on board the R.V. Aranda during the ICES workshop in Helsinki and Louis Peperzak for his help during the EUZOUT cruise in July, 1989. Useful comments on the manuscript were given by several members of the working group, and by C. Peeters.Steve Bates is acknowledged for his efforts to improve the english version of the manuscript and Ken Jones for his long lasting stimulus to complete this manuscript.

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Tables

Table I. Sample from the Marsdiep tidal inlet of the Wadden Sea (cf. Cadée & Hegeman, 1974).

Filter type	Incubation time (h)	DPM	x ± sd cv
GF/F GF/F GF/F	1 1 1	45163 44243 41765	43724 ± 1757 4.0
GF/F GF/F GF/F	2 2 2	78384 81953 77212	79183 ± 2469 3.1
Sartorius Sartorius Sartorius	2 2 2	71228 69316 61500	67348 ± 5154 7.6
GF/F Sartorius	2 (in dark) 2 (in dark)	1142 1706	1424

Table II. Samples from the inlet to the Helsinki harbour.

ICES Incubator			Baltic Incubator		
CPM/h	x ± sd	cv	CPM/h	x ± sd	cv
2486 2530 2518 2523 2514	2514 ± 17	0.6	2565 2515 2602 2441 2541	2553 ± 78	3.1
			CPM/h	irradiance	
			257 425 991 1967	5% 10% 25% 50%	

Mean irradiance in ICES incubator: 297 $mE.m^{-2}.s^{-1}$;
Full irradiance in Baltic incubator: 400 $mE.m^{-2}.s^{-1}$.

Table III. Results from the North Sea cruise (25-27 July 1989); for location of stations see Fig.2. s=short term(c. 2 h), l=long term(c. 6 h) incubation; sur=surface, ther=thermocline, subther=subthermocline sample; P_{max} derived from P-I measurements based on 6 h incubations

DPM/2 h				DPM/2 h			
Station	ICES		P_{max}	Station	ICES		P_{max}
NW100 sur	3385	4656	2242	TS100 sur	4590	5282	3982
NW70 sur	6951	6777	5532	TS100 ther	7541	7293	6213
TS370 sur/s	3293	2970	2699	TS100 subther	1478	1415	1085
TS370 sur/l	2755	---	---	TS10 sur	6583	6646	3454
TS275 sur/s	1741	1624	---	TS4 sur	59062	56027	53403
TS275 sur/l	1897	1870	1503	NW20 sur	17984	20844	15411
TS175 sur	2336	1906	1328				
TS175 ther	2565	2740	1452*				
TS175 subther	7712	8141	3527*				

* samples showed strong photoinhibition

Table IV. Example of results of experiments conducted in the Indian Ocean, location off Kenya and Somalia (Veldhuis & Kraay, in prep.) to show daily inequality.
Same sample was used for both incubations; parameters estimated by the equation of Platt et al. (1980). Calculation of daily primary production is based on ke = 0.1, daylength = 12 hrs., and mean surface irradiance = 1000 mE.m-2.s-1. SSE is the error sum of squares of the fitted model.

	Evening Incubation	Morning Incubation	Unit
P_{max}	3.55	5.93	$mgC.m^{-3}.hr^{-1}$
I_{opt}	802	1319	$mE.m^{-2}.s^{-1}$
I_k	294	290	$mE.m^{-2}.s^{-1}$
a	0.012	0.021	$mgC.mgChl\text{-}a^{-1}.hr^{-1}$
SSE	1.055	1.633	
Daily Pro-duction.	260	465	$mgC.m^{-2}$

Table V. Calculated daily primary production in mgC.m-2. using different equations: Pdp, Pdj and Pdg are values calculated using P-I parameters and sinusoidal irradiance distribution :p=Eilers and Peeters, 1988, j=Jassby and Platt,1976 and g=Platt et al., 1980; Pdpm, Pdjm and Pdgm are values calculated using a rectangular mean irradiance distribution, further as before; Pdem and Pdeh: calculations based on DiToro et al., 1971 with Pmax at mean irradiance and at half of mean irradiance, respectively.

sample	Pdp	Pdj	Pdg	Pdpm	Pdjm	Pdgm	Pdem	Pdeh
13-even	0.206	0.189	0.191	0.242	0.222	0.226	0.460	0.399
13morn	0.414	0.379	0.387	0.482	0.445	0.456	0.873	0.707
14-even	0.188	0.330	0.330	0.216	0.393	0.411	0.613	0.488
14morn	0.528	0.529	0.535	0.628	0.619	0.631	0.969	0.783
15-even	0.308	0.301	0.300	0.372	0.358	0.366	0.477	0.482
15morn	0.301	0.273	0.280	0.356	0.324	0.333	0.564	0.481
115-even	0.558	0.554	0.556	0.674	0.659	0.673	0.889	0.864
115mor	0.789	0.746	0.757	0.932	0.882	0.901	1.421	1.240
116-even	0.510	0.478	0.494	0.606	0.570	0.588	0.839	0.820
116-morn	0.866	0.879	0.891	1.039	1.038	1.064	1.573	1.456
march	0.578	0.597	0.603	0.596	0.610	0.625	0.206	0.120
april	0.417	0.392	0.400	0.477	0.453	0.455	0.952	0.765
may	4.027	3.649	3.770	4.659	4.266	4.329	8.084	6.484
june	0.691	0.554	0.454	0.778	0.592	0.476	1.400	0.604
july	1.183	1.021	1.053	1.347	1.135	1.178	3.239	2.698
aug.	0.983	0.976	0.996	1.037	1.026	1.057	2.339	0.915
sept.	0.170	0.120	0.138	0.180	0.121	0.143	0.417	0.224
oct.	0.724	0.609	0.628	0.790	0.643	0.671	0.251	0.144

Figure 1 . Photograph of ICES incubator (see text).

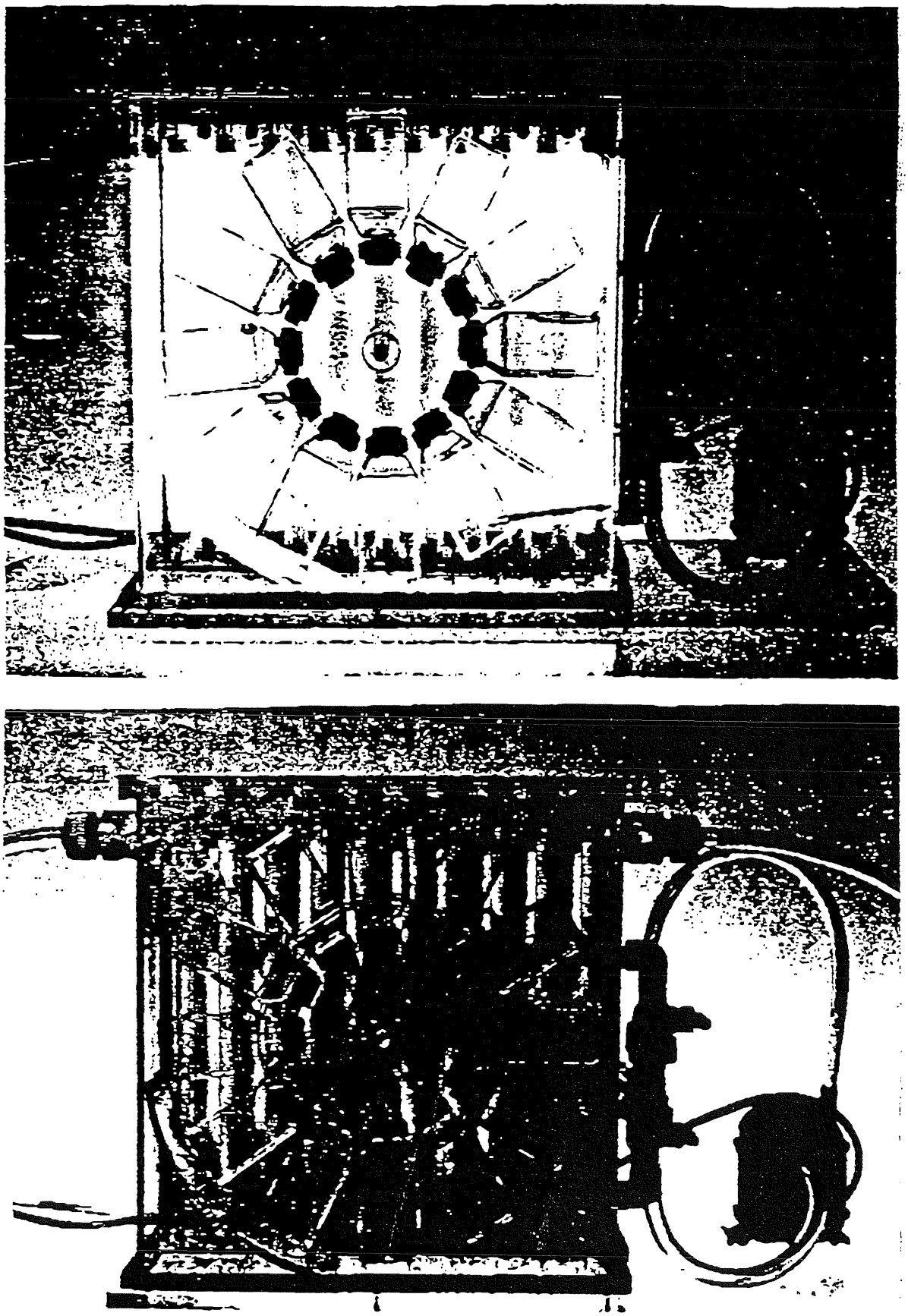
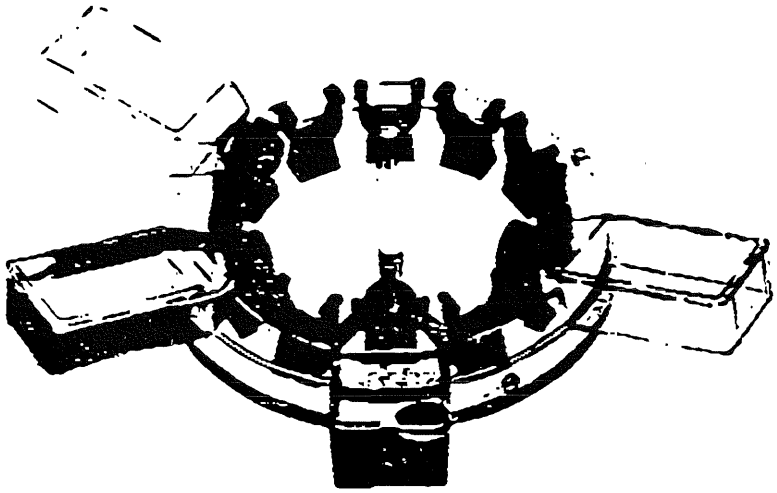


Figure 1



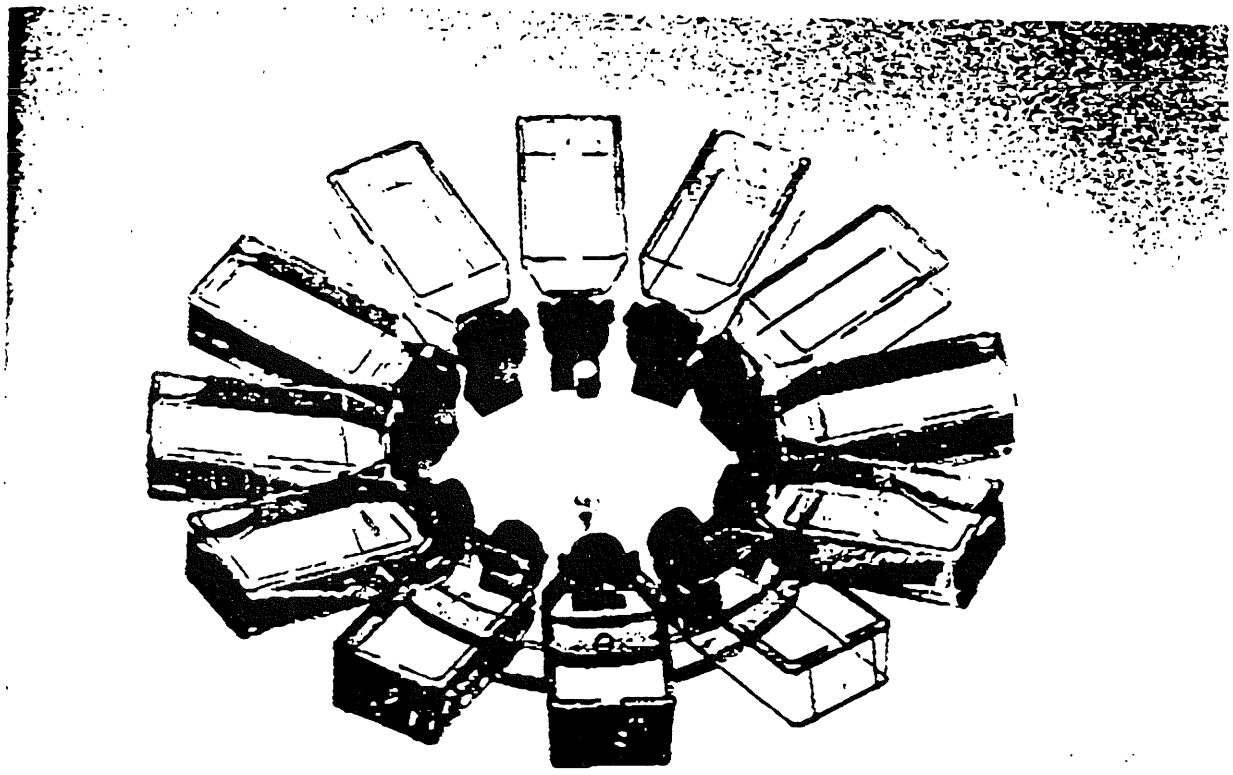


Figure 2. Map showing location of sampling stations during the July cruise in the North Sea (Peeters et al., 1991).

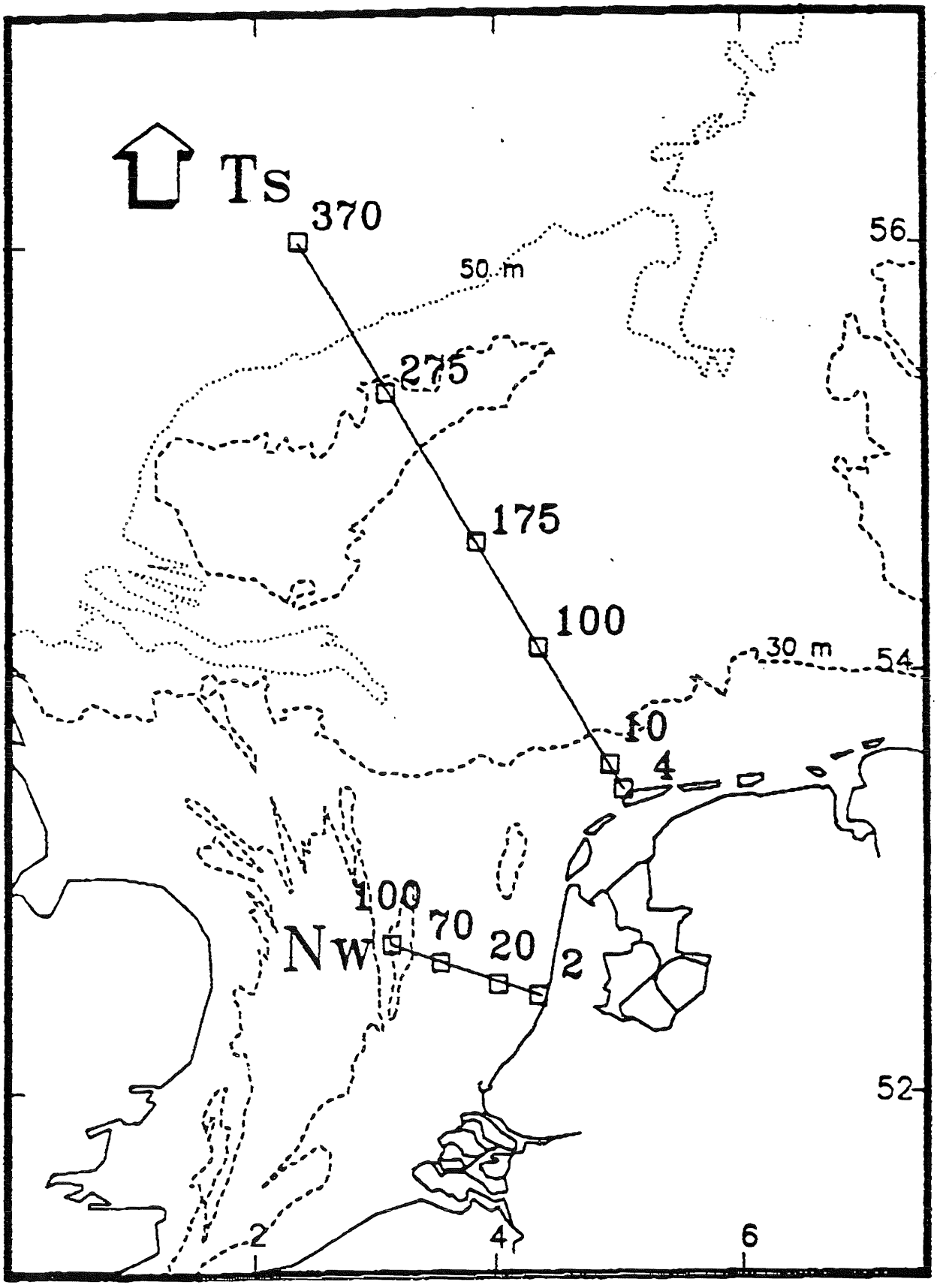
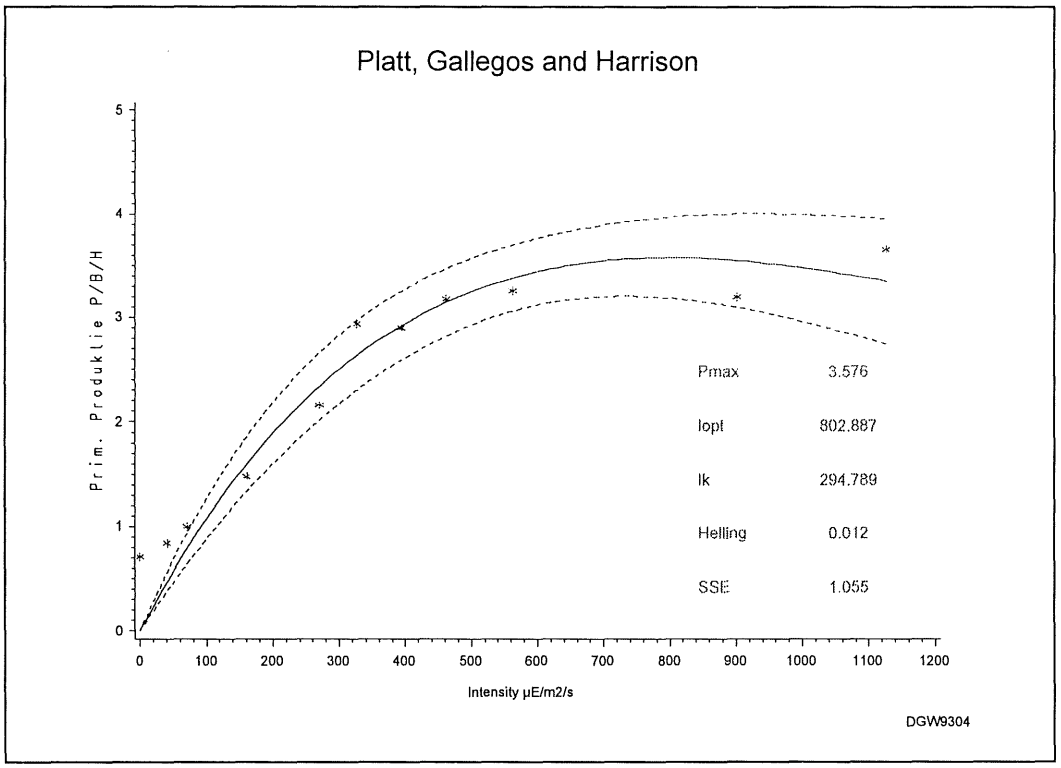


Figure 3 P-I curves for two incubations on the same sample; a) in the evening, b) in the morning. Fitted curve is equation of Platt et al. (1980).

A



B

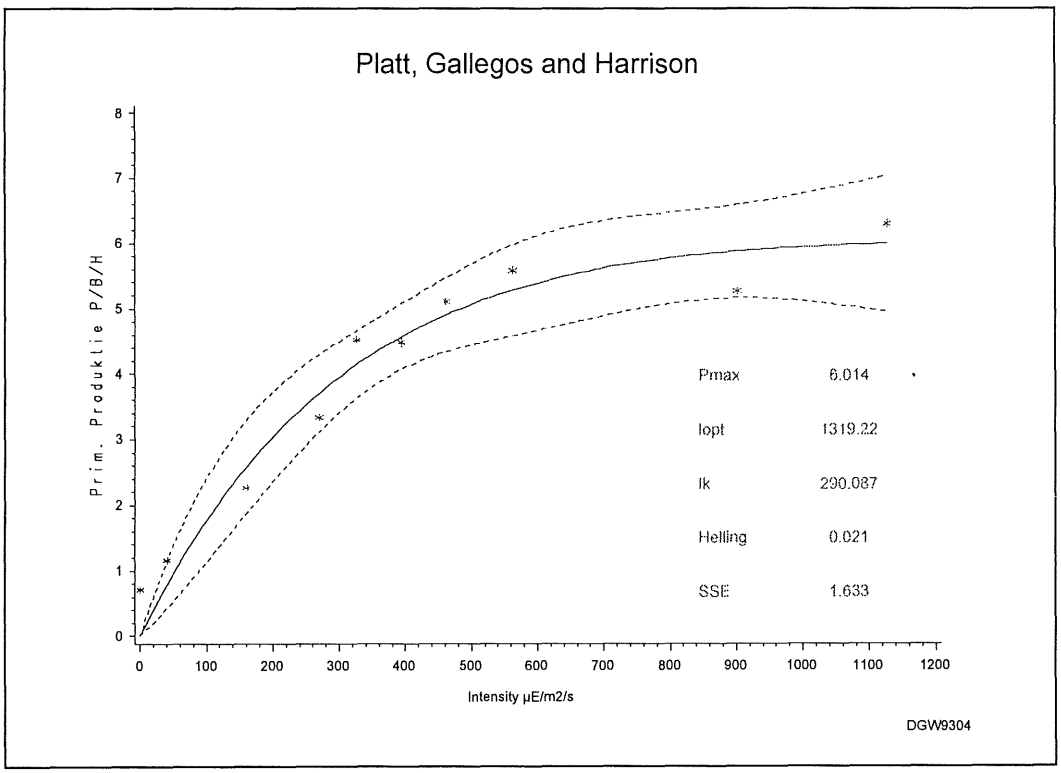


Figure 4 Examples of P-I curves measured at the Station Büsum, all curves normalised to chlorophyll-a; fits were made with the equation by Platt

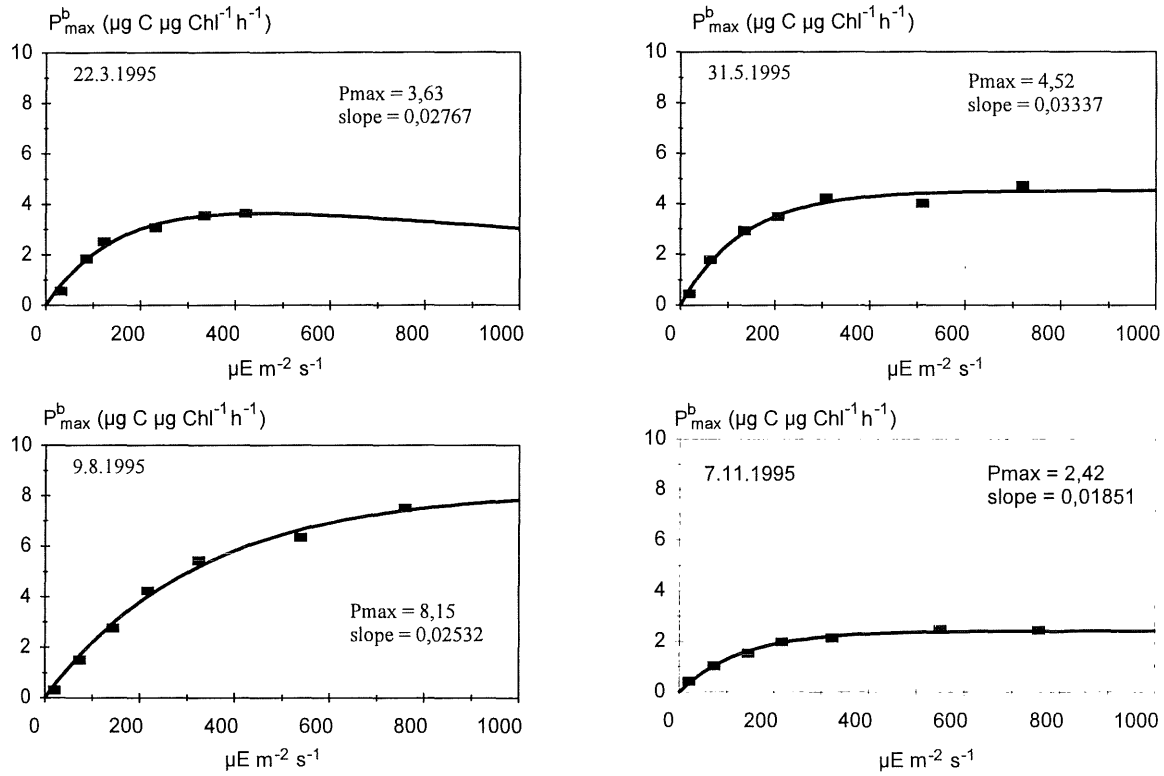
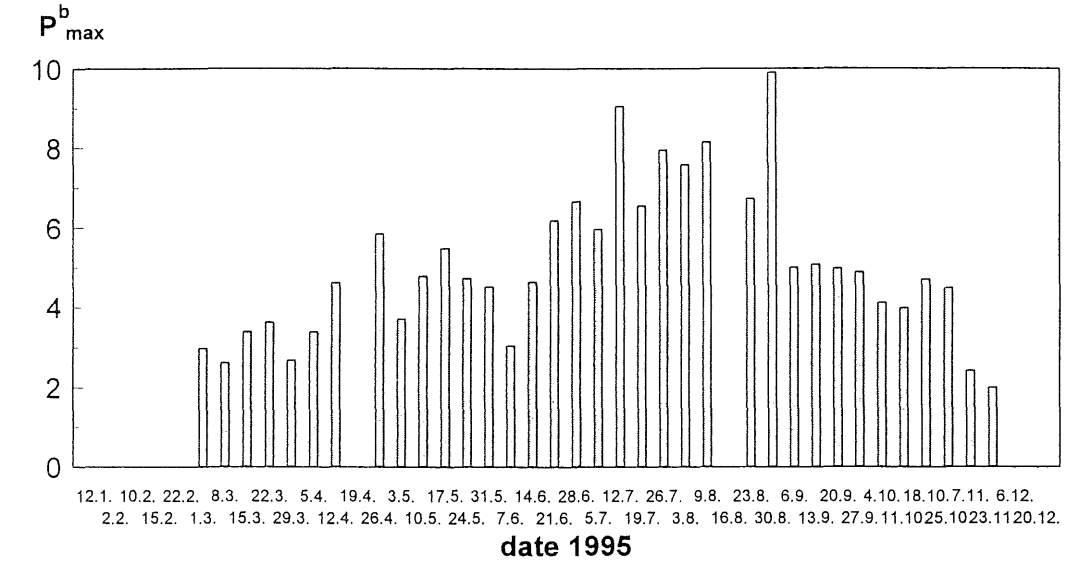
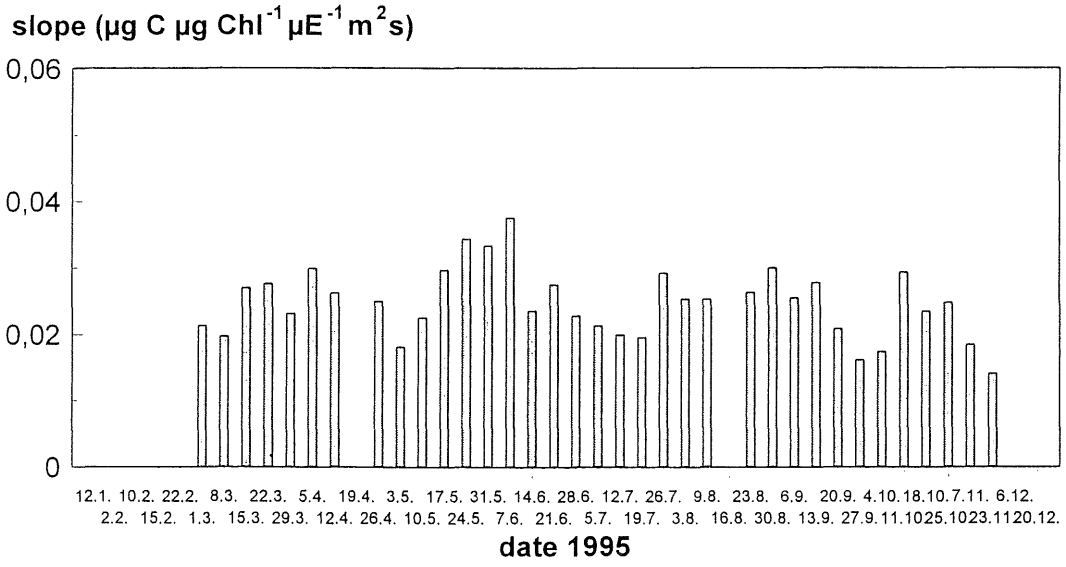


Figure 5 Season course of P-I parameters at the Station Büsum, in 1995; all parameter calculatons based on Platt *et al.*





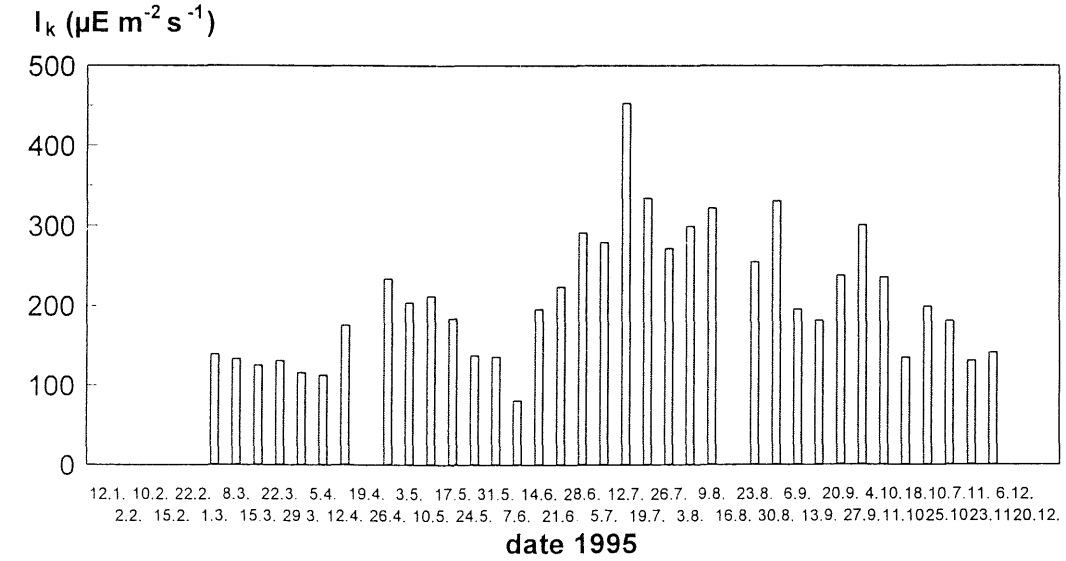
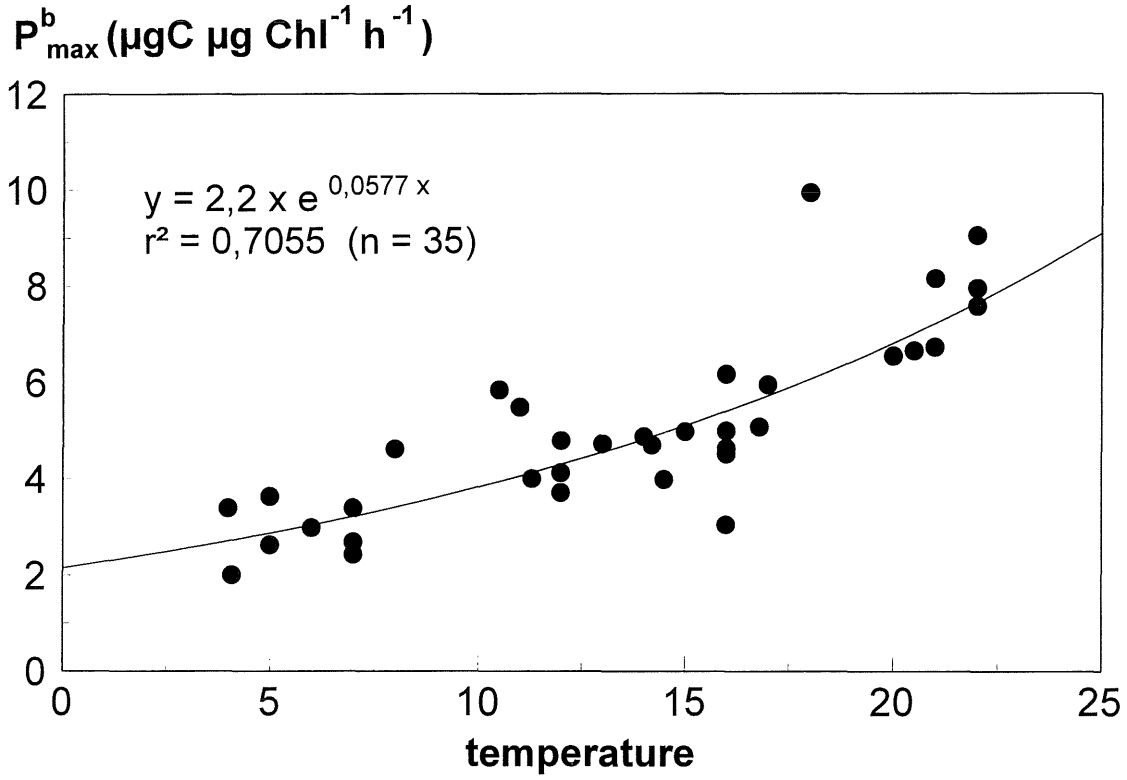


Figure 6 Relation between assimilation number (PB/max) with temperature for the measurements at Station Büsum in 1995.



4.2.2 Outline of the Experimental Protocol

The Working Group discussed details of the experimental protocol and makes the following suggestions for inclusion in a standard method:

(i) Sampling should take place during the day, preferably around noon. However, it is recognised that constraints on ship-time may affect this. Water should be sampled from mid-way within the mixed layer or at the discretion of the user when faced with complicated physical oceanographic circumstances, as determined by CTD profiling.

(ii) Sample collection bottles should have any parts made of toxic, rubber materials removed and replaced with non-toxic, silicone parts. All containers used to hold water samples prior to filling the incubation flasks should conform to this standard and should be thoroughly cleaned to the same standard as the incubation bottles.

(iii) All transfers of water samples should take place in subdued light to avoid light-shock to the contained phytoplankton. Special care should be taken to avoid mechanical damage to phytoplankton cells. Incubation flasks should not be filled directly from water sampling bottles. The water sample should be gently mixed in another clean container before gently dispensing (by siphon) to incubation flasks.

(iv) An appropriate choice of incubator irradiance levels will have to be made by the operator for individual areas and circumstances, in order to ensure that a sufficient number of points falls within the regions of limited and saturated photosynthesis to allow reliable estimation of P-I parameters or, when using one irradiance level, this should be related to geographical latitude and season to obtain saturation of photosynthesis.

(v) The ^{14}C incubation should start as soon as possible, preferably within 0.5 h after sample collection.

(vi) The amount of ^{14}C activity added will depend on the biomass level present, but 1mCi per 50 ml aliquot should be sufficient in eutrophic coastal waters. At least one dark bottle and one time-zero, control sample should be run and reported but not subtracted from light bottle values. The isotope should be added to each incubation bottle using a precise, calibrated micro-pipette. It is crucial that the stock isotope should be free of contaminants. It is recommended that the isotope with acceptable quality with regard to contaminants, be purchased already at the desired dilution for dispensing, to avoid the possibility of contamination during any dilution step in the laboratory. The ^{14}C activity

added to each incubation flask should be determined by first adding an aliquot of the isotope to phenylethylamine in the scintillation vial, in order to trap the $^{14}CO_2$, prior to counting. As an alternative a fraction of the sample after addition of ^{14}C may be counted.

(vii) Samples should be incubated for 2 h. The incubation temperature should be within 0.5° C of the temperature at which the sample was collected. After 2 h, the contents of the bottles should be filtered immediately through 25 mm CF/F filters on a vacuum manifold fitted with enough filter units to filter all incubated samples simultaneously. The vacuum used should not exceed 0.3 Kp cm^{-2}.

(viii) After filtration, unassimilated inorganic ^{14}C should be removed from the filters by adding 0.1 ml of 0.1 M HCl to the filter in the scintillation vial and leaving for 24 h in a well-ventilated environment or the filters are fumed over concentrated HCl in a desiccator for 5 min.

(ix) The radioactivity of filters should be measured using liquid scintillation counting. The particular scintillation cocktail chosen will depend on the user, but the appropriateness of cocktail type to the samples counted should be investigated by each researcher, as factors such as pH of the sample might affect the efficiency of the cocktail system. Following addition of the scintillation cocktail, vials should be left in the dark for at least 3 h to reduce any chemiluminescence.

Sufficient counts should be accumulated such that the counting error is not more than 5% for each sample. Counting efficiency should be determined either by external-standards channels-ratio method or international standardisation and corrections applied to obtain the DPM (disintegrations per minute) value for each sample. The possibility of colour quenching by algal pigments should also be taken into account and corrections applied, particularly in eutrophic waters where phytoplankton biomass might be high. Disruption of filters after addition of the scintillation cocktail in an ultrasonic waterbath for 15 min. facilitates and increases counting efficiency.

(x) The chlorophyll *a* and TCO_2 (weight of total carbonate present in the seawater) concentration of the sea water should be determined at the time of the ^{14}C incubations.

Chlorophyll *a* concentration should be determined by the fluorometric method of Strickland and Parsons (1972). The sample (10-100 ml) is filtered through a 25 mm GF/F filter at a vacuum not exceeding 0.3 Kp cm^{-2}. Alternatively, HPLC may be used to obtain precise measurements of chlorophyll-a.

TCO_2 is determined by measuring the total carbonate alkalinity as described by Strickland and Parsons (1972). Alternatively, TCO_2 can be measured using modern instrumentation (e.g., infra-red gas analysis).

(xi) The following formula is used to calculate the rate of the carbon uptake, P (mg C m^{-3} h^{-1}):

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where DPM_{LB} is the DPM in the light bottle; the isotope (^{12}C:^{14}C) discrimination factor is 1.05; TCO_2 is the weight of total carbonate carbon present in the seawater (mg m^{-3}); and t is the incubation time in hours.

The value of P can be normalised to the concentration of chlorophyll a (mg m^{-3}) present in the same sample of water, in which case the units of photosynthesis for P^B become mg C mg Chla^{-1} m^{-3} h^{-1}.

The relevant parameters of the P-I curve (P^B_{max} and a) are computed using an appropriate curve fitting programme with equations (2) and (3) (Platt et al (1980):

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where $a = aI/P^B_s$, $a = ßI/P^B_s$, I is the irradiance level and P^B_s (maximum rate of photosynthesis, normalised to chlorophyll a, if there were no photoinhibition); the parameter a is the initial slope of the P-I curve and ß is a photoinhibition parameter.

The value of P^B_{max} (the maximum rate of photosynthesis, normalised to chlorophyll a, at light saturation is given by equation (3) which corrects for any photoinhibition.

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Other alternatives for analysis and calculations of P-I curves are available (see manuscript).

(xii) In addition to chlorophyll *a* and TCO_2 determinations which are essential for calculations of biomass- normalised ^{14}C uptake the following accompanying measurements and observations should be made and recorded for storage in a primary production data base:

- Station position, date, time and depth of water collection, start and end times of incubation;
- Seawater temperature at depth of sample collection, incubation temperature;
- Daily irradiance (hourly means) at the station;
- Secchi disk reading or vertical downwelling attenuation coefficient;
- Irradiance level in each incubation flask;
- *DPM* and *P* (calculated as above) for light incubations at each irradiance level and in dark and time zero bottles. It is important to record raw data for DPM estimates so that recalculations can be made if necessary;
- Estimates a and P_{max} with corresponding standard error estimates for each parameter.

ANNEX 5

Pulse-amplitude-modulation-fluorescence (PAM) - a tool for fast assessment of primary productivity in the sea?

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Abstract

Analysis of the kinetics of chlorophyll fluorescence quenching can give qualitative information on the functioning and the organisation of the photosynthetic apparatus. For higher plants a linear relation between fluorescence yield and electron transport has been observed. Edwards & Baker (1993) concluded that under a wide range of conditions the fluorescence yield can be used to predict accurately and rapidly CO_2 assimilation rates in maize.

Up to now it is an open question whether it is also possible to calculate the production rates of phytoplankton by analysis and measurement of fluorescence yield. This would give us a new tool for fast measurements of primary productivity in the sea.

In order to assess the usefulness of the PAM (Pulse-Amplitude-Modulated) - fluorescence method to estimate primary production in marine phytoplankton we have examined the relation between the rate of relative photosystem II electron transport determined with the PAM-fluorescence technique and the rate of carbon fixation as measured with the conventional ^{14}C-technique for different marine phytoplankton species (e.g., *Skeletonema costatum*, *Thalassiosira weissflogii*, *Prorocentrum redfieldii*, *Dunaniella spec.*) and microphytobenthos communities under different irradiance levels. A short overview about the PAM-fluorescence technique is presented to understand the main principles of this new technique.

Introduction

Up to now there exist different methods to estimate primary productivity in the sea. Among these the most common method for measuring primary productivity is based on the radioactive tracer technique with ^{14}C as originally described by Steemann Nielsen (1952) and modified for scintillation counting by several authors. This method allows to measure carbon fixation up to very low production rates. Artifacts which may arise when using this method (use of radioactive material, so-called bottle effects, measuring gross or net photosynthesis etc.) are still a matter of controvers. For us the main disadvantage of this method is a logistical one: one can only make a few measurements a day and therefore one cannot use this method for rapid estimations of spatial and temporal distribution patterns with high resolution of phytoplankton production and also

one cannot use it for monitoring applications. Oxygen measurements, which may be used for in situ applications, are mostly not sufficiently sensitive for marine use.

To overcome the logistical problems and in order to measure primary productivity *in situ* frequently, there is a strong need for introducing new methods. In the past chlorophyll fluorescence has evolved as a very useful and informative indicator for photosynthetic electron transport in intact leaves and chloroplasts. Fluorescence measurements can be made rapidly, conveniently and continously without a long incubation time, thereby eliminating bottle effects. However, interpretation and use of fluorescence signals are not straightforward.

In the last decade the biggest advantage in estimating electron flow by fluorescence was done with the modulated fluorescence technique mainly for higher plants

Modulated fluorescence technique

The modulated fluorescence technique allows fluorescence monitoring in the presence of continuous light and therefore the investigation of photosynthetic rates of plants and algae in a natural illluminated state (Schreiber and Bilger, 1987). Modulated fluorometers allow the determination of the photochemical (q_P) and non-photochemical quenching (q_N), coefficients of fluorescence quenching, as well as determination of photochemical efficiency ϕ_{P0} and the determination of the regular Kautsky curve (further information see below)

In the following chlorophyll fluorescence nomenclature and abbreviations follow van Kooten and Snel (1990).

Contrary to chlorophyll in solution, chlorophyll in vivo displays large changes in fluorescence yield upon illumination (Kautsky and Hirsch 1931). Fluorescence emission competes with photochemistry and heat dissipation. Therefore two basic types of fluorescence quenching, photochemical and nonphotochemical can be distinguished. A simplified model shows the major fluorescence excitation mechanism that happens in a green photosynthetic organism after illumination (Fig.1).

Incident irradiance (E) is absorbed by the light harvesting chlorophyll complex (LHCII). When all reaction centers (RC2) are open (Q_A fully oxidized), the minimal fluorescence yield (F_o) is observed, whereas the maximal fluorescence yield (F_m) is found when all reaction centers (RC2) are closed (Q_A fully reduced). The difference between F_o and F_m is called variable fluorescence (F_v). The fluorescence signal F observed at irradiance E is an average of F_o and F_m weighted by the fraction of open and closed reaction centres. Fluorescence emission competes with photochemistry and heat dissipation. Two basic types of fluorescence quenching can be distinguished: photochemical quenching, which is an approximate measure of the fraction of the open PSII centres and non-photochemical quenching, which mainly reflects the transthylakoid proton gradient (Krause et al. 1982)

The rationale of the saturation pulse method is simple: upon application of a sufficiently strong light pulse, Q_A is fully reduced and hence photochemical fluorescence quenching becomes suppressed. This means that fluorescence is maximal when there is no non-photochemical quenching. This state should exist when the algae are dark adapted. For problems with dark adaptations, see below.

On the basis of the saturation pulse method, Ulrich Schreiber developed ten years ago in 1986 the so-called PAM-method (Pulse-Amplitude-Modulation-fluorescence), which allows separation of the different forms of fluorescence quenching. The rapid introduction of this method into the broad field of photosynthesis research for higher plants has opened the way for rapid assessments of photosynthesis yield and capacity *in situ* by fluorescence measurements. Weis and Berry (1987) and Genty et al. (1989) first showed that for a variety of higher plants the relative rate of photosynthetic electron flow can be determined from fluorescence measurements alone.

In practice this method requires a particular measuring technique, with an exceptional selectivity and sensivity, which we describe below.

PAM-Method

The PAM fluorometer (PAM-100, Fa. Walz, Effeltrich, Germany) is a fluorometer based on a new modulation principle. It tolerates a ratio of $1:10^6$ between modulated fluorescence and nonmodulated background signal. That means that the fluorescence yield can be measured even in full sunlight.

As we mentioned before, fluorescence measurements in the past have been mostly applied to the study of higher plant photosynthesis, using intact leaves or chloroplasts, where the signal amplitudes are rather large because of the high chlorophyll concentrations.

In order to work with dilute suspensions of unicellular algae the sensivity of the standard PAM-100 Fluorometer had to be increased. With the introduction of the Emitter-Detector-Unit ED-101-US the limit for fluorescence quenching analysis was already lowered to suspensions containing 20-30 µg chlorophyll l^{-1} and sensitive measurements of chlorophyll fluorescence in "dilute" suspension became possible (Schreiber, 1994). Because of the development of a new photomultiplier system the sensivity could be increased again and therefore measurements on suspensions containing < 1 µg chlorophyll l^{-1} can be made.

Principles of the PAM-method

Algae in a small cuvette are exposed to continuous light ranging in the natural, moderate light intensities (AL, actinic light). This induces autofluorescence of chlorophyll which is detected by a photodiode (ML, measuring light). The fluorescence intensity of the cells depends on the relative numbers of the opened and closed photosynthetic reaction centers at this moment (see model). Subsequent exposure to a very short (0.5-1 s)

intense light impulse (SP; saturating light pulse) leads to a complete closure of all photosynthetic reaction centers in the algae: the fluorescence is at maximum. The very short exposure to high irradiance is required in order to avoid light adaptation processes. The increase of fluorescence intensity at high light pulse (all reaction centers are closed) relative to that at moderate light intensities (only part of reaction centers are closed) is divided by maximum fluorescence intensity at high light pulse (all reaction centers are closed). This allows the determination of the photochemical efficiency (fluorescence quantum yield) of the algae during the prevailing light conditions (see section calculation and Genty-Parameter). Photochemical efficiency under moderate intensities gives an estimate of relative electron flow.

The following block diagram shows the experimental set-up we used in our experiments (Fig. 2):

- LED-emittering light source for the measuring light (ML). It is controlled by the LED-driver. The LED measuring beam has a peak wavelenght of 655 nm. It is passed through a short pass-filter to remove long wavelenght components. It emitts µs light pulses at frequencies of 1.6 or 100 kHz. 1.6 kHz should be used if possible, because it lowers the amount of the measuring light intensity and thus preventing non-photochemical quenching. To increase sensivity sometimes it is necessary to use 100 kHz.

- The saturation pulse lamp (SP), equipped with a 650 nm short pass filter, intensity 1500-2500 µE m^{-2} s^{-1}.
- Branched fiberoptics connecting to the various light sources. The fibers are statistically mixed at the end for homogenous illumination of the sample.
- Actinic light (AL) source to drive photosynthesis (a halogen lamp fiber illuminator).
- Detector unit housing a PIN-photodiode (Hamamatsu S 3590-01).
- 10x10mm double sided mirrowed cuvette.
- Perspex rods (instead of fibre optics) in the Emitter-Detector-Unit ED-101-US are applied for guiding exitation light to the mirrowed cuvette and from there at 90 °angle to the photodetector
- Main control unit housing the LED-driver and an synchronous pulse signal amplifier.

Fig. 3 shows a schematic trace of a typical PAM-measurement we used for our experiments: First the sample is still in the dark adapted state. In this state the minimal and maximal yields (F_o and F_m) are determined. The ratio Fv/Fm is a convinient measure of the potential maximal PSII fluorescence quantum yield of a given sample (see also section "Potential photochemical efficiency as determination of photoinhibition").

During illumination (actinic light, AL) the fluorescence yield F undergoes complex changes.
The intensity of continuous illumination should be in the natural range (5-2000 µE m^{-2} s^{-1}) and should be choosen after the previous light history of the phytoplankton cells. In

practice it is very difficult, if not impossible, to get precise information about the light history. Therefore there is a strong need for a profiling PAM-fluorometer with which one can measure directly in the water column. Under continous illumination (AL), photosynthetic organisms reach a steady state of low fluorescence yield, which is governed by different quenching mechanisms. On one hand, there is photochemical quenching caused by charge seperation at PSII centers. On the other hand, so-called non-photochemical quenching persists when all PSII centers are closed.

With the help of saturation pulses (normally in 10s frequence) the changed levels of maximal yields (F_m') are determined. Then F_m-F_m' reflects non-photochemical quenched fluorescence and F_m'-F reflects photochemical quenched fluorescence.

Far-red illumination can be used to ensure complete reoxidation of the primary stable electron acceptor of PS II (Q_A) in between the light flashes. But during our measurements, F_0 was similar to F_o' and for that reason we did not give far-red light.

Calculations

Recently, it has been argued that the photochemical efficiency (ϕ_{Po}) which is also named in literature as quantum yield of PS II photochemistry (ϕ_{PSII}) can be determined from the fluorescence yield (F) and maximal fluorescence yield (F_m') under illumination determined during steady state photosynthesis (Genty et al., 1989). F_m' is achieved for an algae or a leaf at steady state photosynthesis by an exposure to a brief pulse (ca. 0.5-1s) of light sufficiently intense to maximally reduce the primary quinone acceptors of the PS II (normally between 2000-4000µE m^{-2} s^{-1}). The model of Genty et al. (1989) predicts that ϕ_{P0} equates to $(F_m'-F)/F_m'$, since ϕ_{Po} is determined by the product of the efficiency of capture by 'open'reaction centers (defined by $F_m'-F_0'/F_m'$, where F_0' is the fluorescence yield at steady state photosynthesis when the PS II acceptors are maximally oxidised) and the fraction of 'open' PS II reaction centers, which is estimated by the coefficient of photochemical quenching, q_p, which equates to $(F_m'-F)/(F_m'-F_0')$.

This so-called Genty-Parameter $[(F_m'-F)/F_m']$ has a great advantage from a practical point of view. It does not require knowledge of F_o', of which the measurement may be problematic particulary under field conditions (see section "Problems with dark adaptation of samples"). F_o' can be determined only upon sample darkening and application of weak far-red background light for PSI-driven QA oxidation. But knowledge of F_o' is indispensable in order to obtain information on the extent of PSII "openness" via q_P calculation. In order to estimate nonradiative dissipation (NPQ) we do not need F_o', because it is possible to describe NPQ with the following expression: NPQ = $(F_m-F_m')/F_m'$. The actual mechanism of non-photochemical quenching is still controversial.

For definitions of all relevant quenching coefficients we used in our experiments, see Fig. 3.

Potential photochemical efficiency as determination of photoinhibition

Photoinhibition can be estimated from the ratio of the variable and maximum fluorescence $F_v/F_m = (F_m-F_o)/F_m$. This ratio is a measure of the photochemical efficiency in open reaction centers (Björkman, 1987) and has been shown to be an indicator of photosynthetic efficiency (Genty et al., 1989; Demmig and Björkman, 1987). During photoinhibition primarily the PS II reaction centres are damaged, which leads especially to a reduction of the variable fluorescence. Therefore decreased F_v/F_m ratios seem to be an indicator of the damage of PS II reaction centers. Photoinhibtion can be determined only when all transient quenching processes have been allowed to relax. Hence, following transfer of the samples from the incubation chambers, the samples should be dark adapted for a short time. Hofstraat et al. (1994) argued for their experiments that 15 min are sufficient to remove any photochemical or energy-dependent quenching. But the time one needs for removing any quenching is dependent on light history, physiological conditions and species composition. There seem to be organisms, especially dinoflagellates, which need a small amount of light to recover from photoinhibition.

Problems with dark adaptation of samples

Interpretation of F_v/F_m ratio in particular, relies on the assumption, that the photosynthetic apparatus is completely dark-adapted when investigated. The time of darkness required for dark adaptation in leaves is usually considered 15-30 min (Bolhar-Nordenkampf et al, 1989):

To overcome the problems arising from dark adaptation of samples, as stated in the former chapter, fluorescence parameters which do not require F_o determination, and therefore can be measured in light-adapted plants and algaes, are probably the most suitable for field measurements. Of these the most useful is ϕ_{Po} which has been shown to be an accurate predictor of photosynthetic assimilation rates(Edwards and Baker, 1993). Yet most of the data which show a good correlation between ϕ_{Po} and photosynthetic assimilation rates are from plants and it is not clear yet if this holds for algae.

Material and Methods

We incubated 4 different marine phytoplankton species (e.g., *Skeletonema costatum*, *Thalassiosira weissflogii*, *Prorocentrum redfieldii*, *Dunaliella spec.*) and mixed multispecific field populations of microphytobenthos in a photosynthetron at 15° at 10 different light intensities for one hour.

The phytoplankton species are kept before incubation for 14 to 30 days under semicontinuous conditions in a light incubator. Light intensities are 20 µmol m^{-2} s^{-1} and temperature was 15°C. Light was given with fluorescence tubes in a 14h light and 10h dark regime. The algae are growing exponentily.

Radioactive $Na^{14}CO_3^{2-}$ was added to glas vials with algal suspension (2,5 ml) and known anorganic carbon content and the suspensions were then exposed to the different irradiances in the photosynthetron. After incubation for one hour, the phytoplankton cells were filtered onto a membrane filter, washed and the radioactivity in the phytoplankton cells was measured with a liquid scintillation counter. The uptake of radioactive carbon, as a fraction of the whole, is assumed to measure the rate of photosynthesis.

Before the start of the fluorescence measurements with the PAM, samples were light-adapted to the same actinic light intensity for at least 10 min in the photosynthetron. Because of the preincubation time fluorescence measurements could start already after 30 s.

Chlorophyll fluorescence was measured with a PAM-101 fluorometer using the accessory module PAM-103 for saturation pulse control (Walz Effeltrich, Germany). The basic system was extended by a new emitter detector-cuvette assembly (ED-101, USA) which allows sensitive measurements with dilute suspensions of algae (Schreiber, 1994).

Calculation of fluorescence parameters as made are described in section on calculation. Photosynthetically active photon flux density (PPFD) was measured by a microquantum sensor in the suspension. Mean values for $(F_m'-F)/F_m'$ are obtained during 3-6 min depending on actinic light intensity.

Absorption spectra of cell suspensions were measured in an Uvicon dual-beam spectrophotometer with filtrate f/2-medium as reference. Cell suspension was measured in 1 cm quartz glass cuvettes, that were placed dircetly before an intregrating sphere (Ulbrichtkugel). The spectral values were integrated and averaged over the wavelenght range 400-700 nm. We normalize the absorption to the concentration of Chla.

Results

From the data with the conventional ^{14}C-technique photosynthetic irradiance curves (P-I-curve) were calculated for all experiments. Fig. 4 shows a typical P-I-curve as obtained with *Thalassiosira weissflogii*.

Photochemical efficiency was high under low light intensities, because most reaction centers are open under these intensities. With higher light intensities the photochemical efficiency decreased due to closing of reaction centers (Fig. 5). Similarly saturation curves as obtained with the conventional ^{14}C-technique could be calculated also from the PAM-method with the data of the photochemical efficiency and the incident light intensity as basis (Fig. 6). These are expressed as relative electron flow versus incident light intensity. When comparing the relative electron flow and the carbon fixation rates a very high correlation was observed (Tab. 1).

From data of both methods we calculated also the light saturation parameter (I_K) (Tab. 2), a parameter which is often used for calculation of photosynthesis in combination

with P_{max} on daily basis. Mostly the same I_K- values could be obtained by the ^{14}C-technique and the modulated fluorescence technique for *Thalassiosira*, *Prorocentrum* and microphytobenthos communities. But for *Dunaliella* and *Skeletonema* the I_K-values derived with the fluorescence method were two times larger than the I_K-values derived with the ^{14}C-technique (Tab. 2 and Fig.7).

The ratio of estimated relative electron PSII flow and carbon fixation varies more than a factor of 3 between species (Fig. 8). Reasons for these differences could be, among other things, different C/Chla- ratios. Dinoflagellates, as *Prorocentrum weissflogii*, seem to have higher C/Chla-ratios than diatoms. This may hence caused the lower slope between the relation of relative electron PSII flow and carbon fixation, because carbon fixation is expressed on Chla basis. On the other hand, different absorption cross sections could be responsible for the observed differences. But when the overall absorption cross sections were taken into account, we were not able to find a lower variability between species (Fig. 9). That indicates that other factors than the potential light harvest absorption characteristics must be responsible for the variation between species or that our measurements of the absorption cross section is not correct. Eventually, it is important to measure the changes of the absorption cross section of PSII and not the overall absorption cross section as we did. Changes in the absorption cross section of PSII could be caused by rapid state transitions in algal cells (1 μs) (Ferris and Christian, 1991). In order to draw reliable conclusions on the photosynthetic performance of algae from fluorescence measurements we need more information on the regulatory mechanisms of the absorption cross section. As photosynthetic electrons are not only used in CO_2 reduction but also in the reduction of nitrate to ammonium, the nitrogen source also might affect the slope of the relation between the rate of PSII electron transport and the rate of carbon fixation.

Conclusions

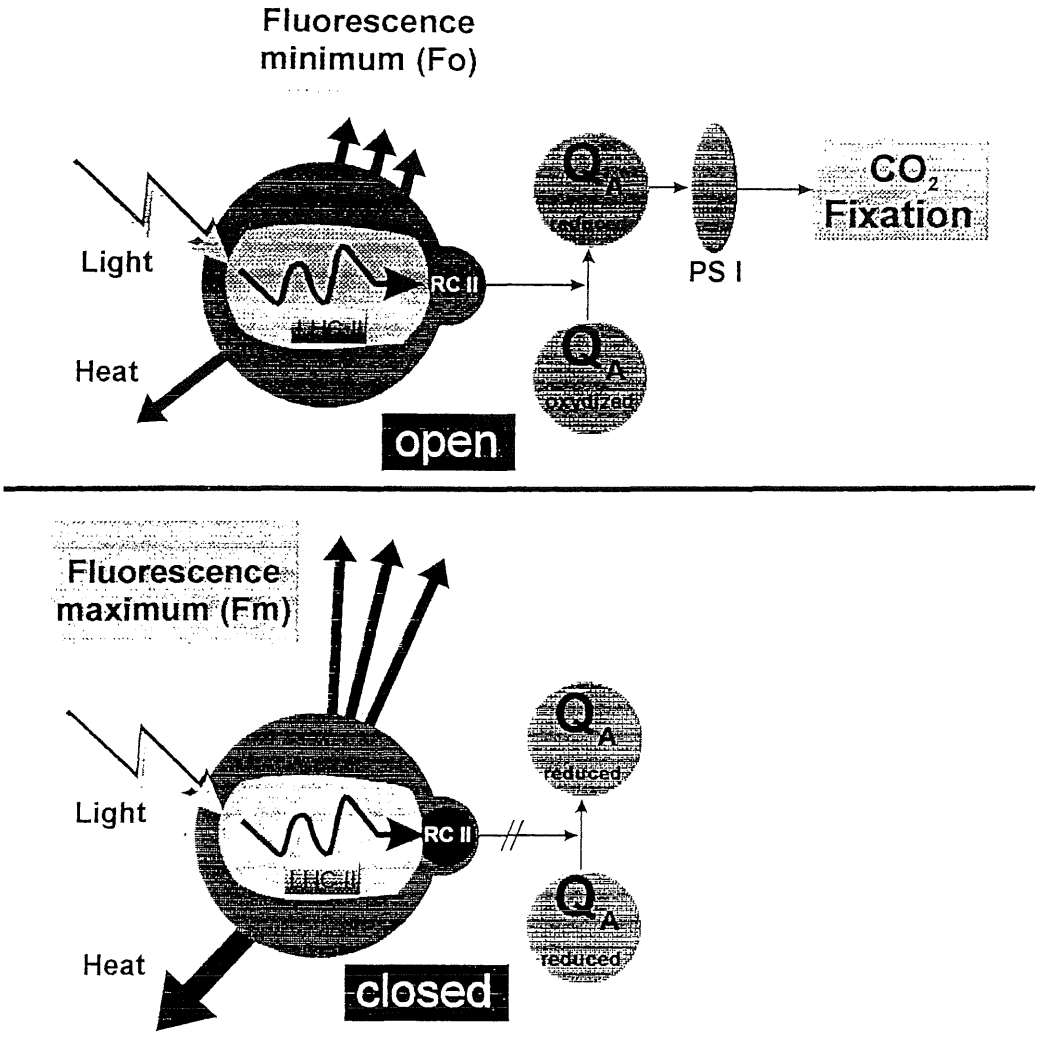
Photosynthetic carbon fixation and relative PSII electron flow as measured by the PAM-method are highly correlated at intensities up to 600 μE m^{-2} s^{-1} in a number of marine species and natural microphytobenthos communities. This is in the range of mean irradiances in the water column. Furthermore, the linearity extends to an irradiance which is 30 times higher than the irradiance at which the species was grown.

The highly significant relation between the PAM-method and the ^{14}C-technique allows one to make rapid, comparative PAM- measurements in order to obtain information on different physiological states of phytoplankton cells, futhermore to get informations on spatial and seasonal patchiness of phytoplankton communities and at least to use the PAM-method for rapid assessment of primary production in the sea.

The ratio of estimated relative electron PSII flow and carbon fixation varies more than a factor of 3 between species. Our next challenge will be to find out the causative mechanisms for these differences.

Figures:

Fig 1: Model of fluorescence emission. For further explanations, see text.



Model of fluorescence emission

Incident irradiance is absorbed by light harvesting chlorophyll complex (LHCII) and randomly encounter a reaction center (RC2)

When all reaction centers (RCII) are open (QA fully oxidized) the minimal fluorescence yield (Fo) is oberserved, whereas the maximal fluorescence yield (Fm) is found when all reaction centers (RCII) are closed (QA fully reduced)

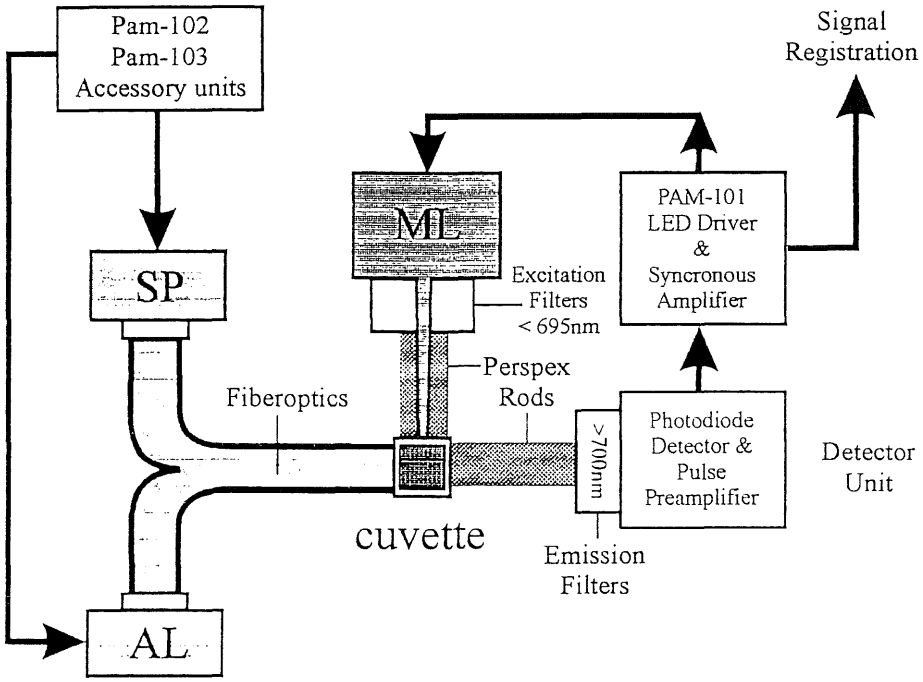
The difference between Fo and Fm is called variable fluorescence (Fv)

The fluorescence signal F observed at irradiance is an average of Fo and Fm wheigted by the fraction of open and closed reaction centers

Fluorescence emission competes with photochemistry and heat dissipation. Therefore two basic types of fluorescence quenching, photochemical and nonphotochemical can be distinguished

Fig.2: Block diagram of the experimental set-up for measurements of light-induced changes of fluorescence with the PAM-fluorometer

Block diagram of the experimental set-up for measurements of light-induced changes of fluorescence with the PAM-fluorometer (Pulse-Amplitude-Modulation)



SP Saturation light pulse
ML Modulated measuring light
AL Actinic light

Modulated fluorescence is measured with a PAM-fluorometer, consisting of the main control unit PAM-101 and the accessory modules PAM-102 and PAM-103, using a new emitter- detector cuvette assembly for ultra-sensitive measurements with diluted suspensions.

after Schreiber et al., 1995

Fig. 3: Schematic traces of a measurement of modulated chlorophyll fluorescence are shown, with the characteristic fluorescence levels and quenching coefficients being defined in agreement with accepted nomenclature (van Kooten and Snel, 1990).

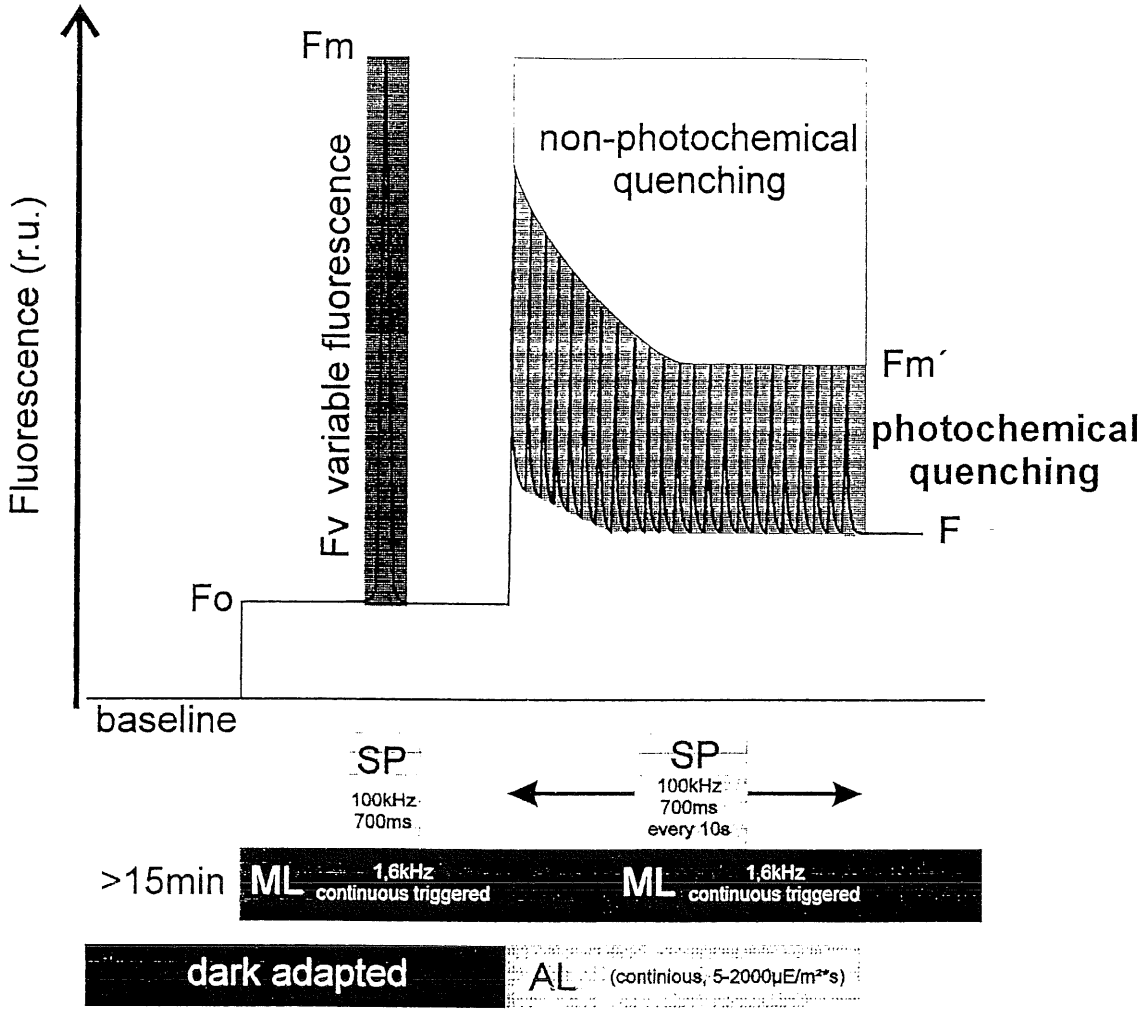


Illustration of pulse amplitude modulation (PAM) - method

Definition of relevant quenching coefficients:

Dark condition: **Potential photochemical efficiency**

$$\phi P = \frac{Fm - Fo}{Fm} = \frac{Fv}{Fm}$$

Light condition: **Effective photochemical efficiency**

$$\phi Po = \frac{Fm' - F}{Fm'} = \frac{\Delta F}{Fm'}$$

Relative PSII electron flow: $\phi Po \cdot E$

Fig. 4: Photosynthetic C-fixation dependence of actinic light intensities (P-I-curve) with light saturation parameter (I_K) for *Thalassiosira weissflogii*.

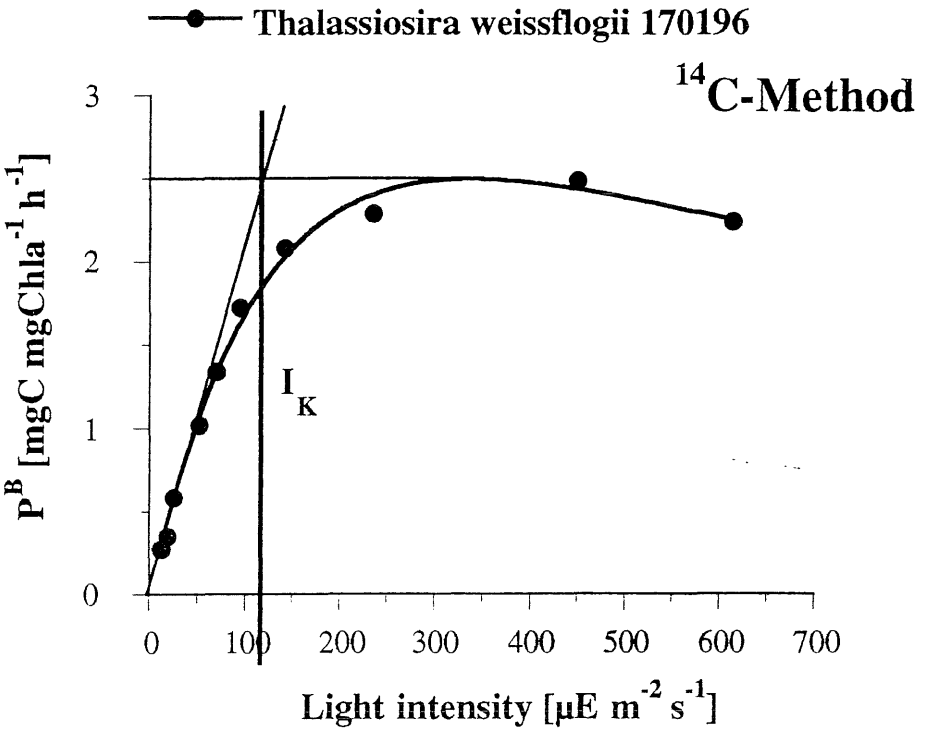


Fig. 5: Actinic light-intensity dependence of effective PSII quantum yield. Values for the photochemical efficiency [$(F_m'-F)/F_m'$] in relation to actinic light intensities.

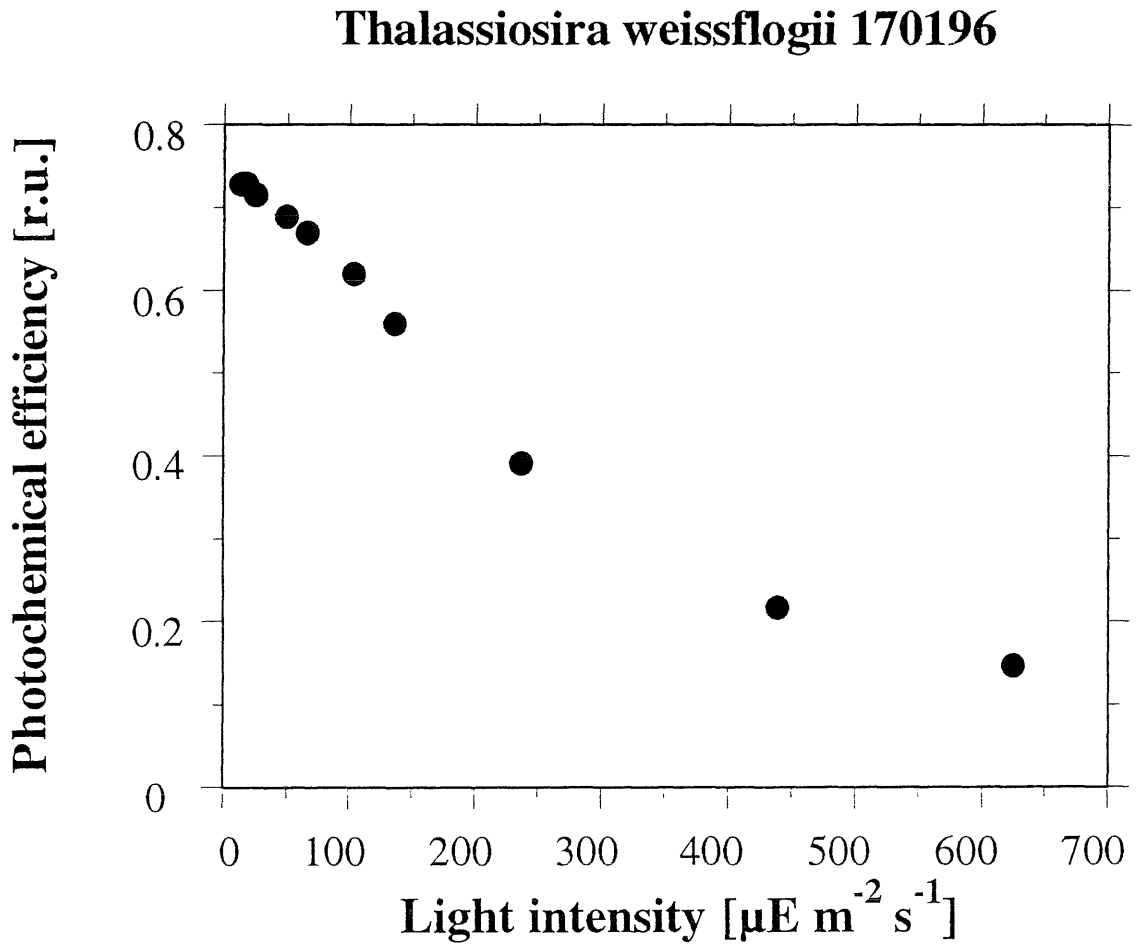


Fig. 6: Light saturation curves of relative electron transport rates calculated from fluorescence parameters on the basis of the data in Fig. 5. Relative electron transport rates are the product of effective PSII quantum yield [$(F_m'-F)/F_m'$] and incident photon flux density, PPFD.

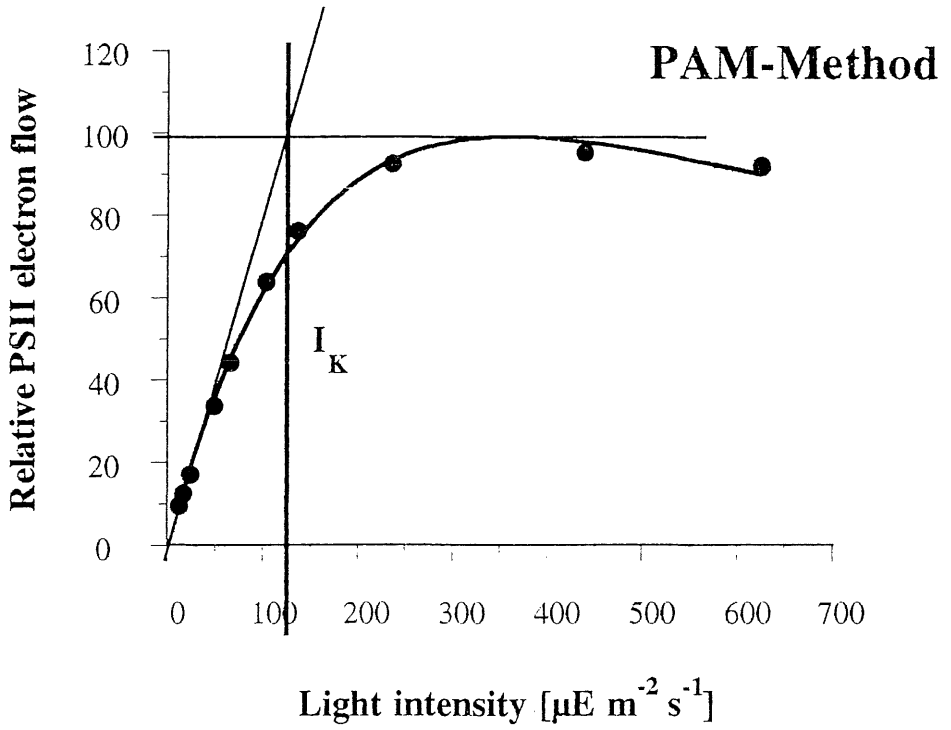


Fig. 7: Relative PSII electron flow versus specific carbon fixation for *Thalassiosira weissflogii.*

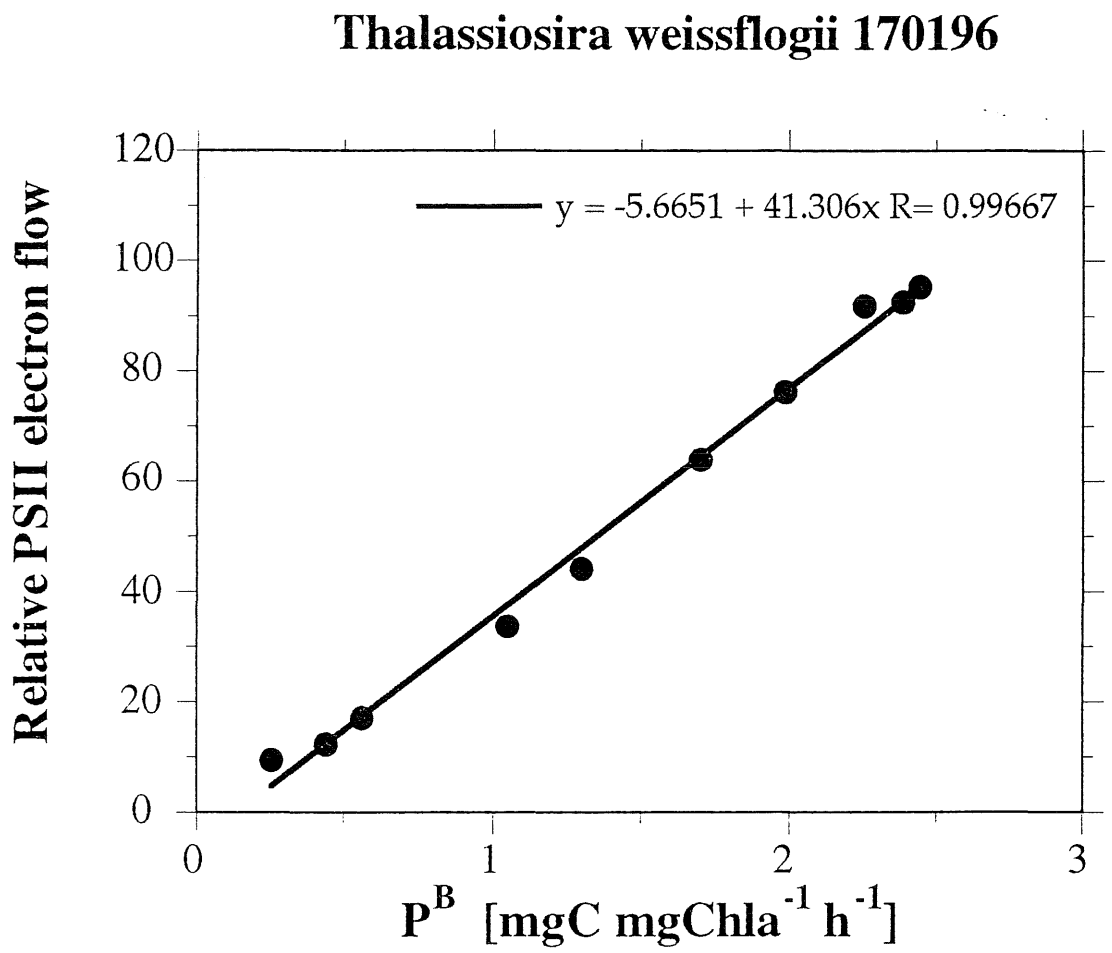


Fig. 8: Relative PSII electron flow versus specific carbon fixation for different algal species and microphytobenthos communities

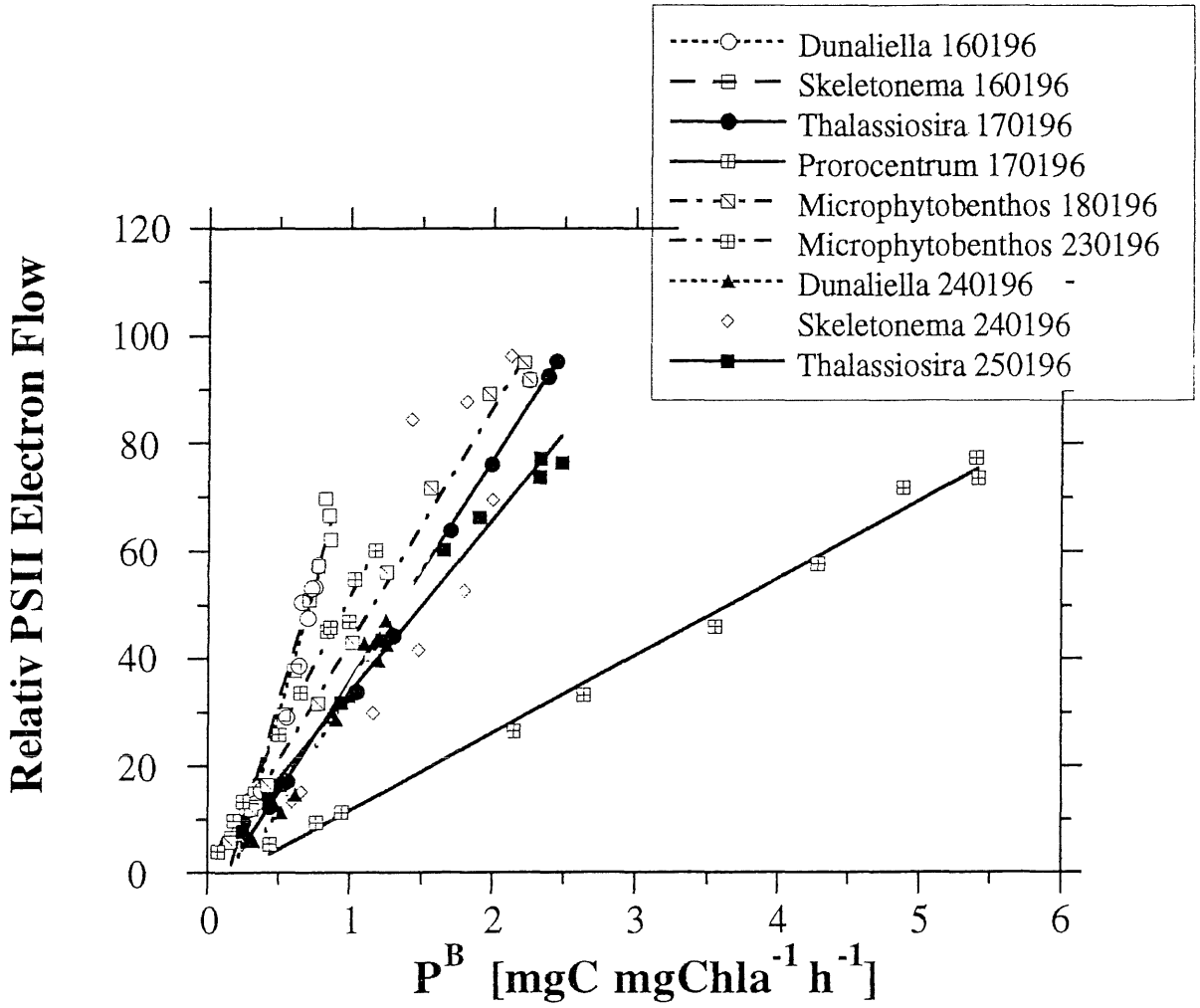
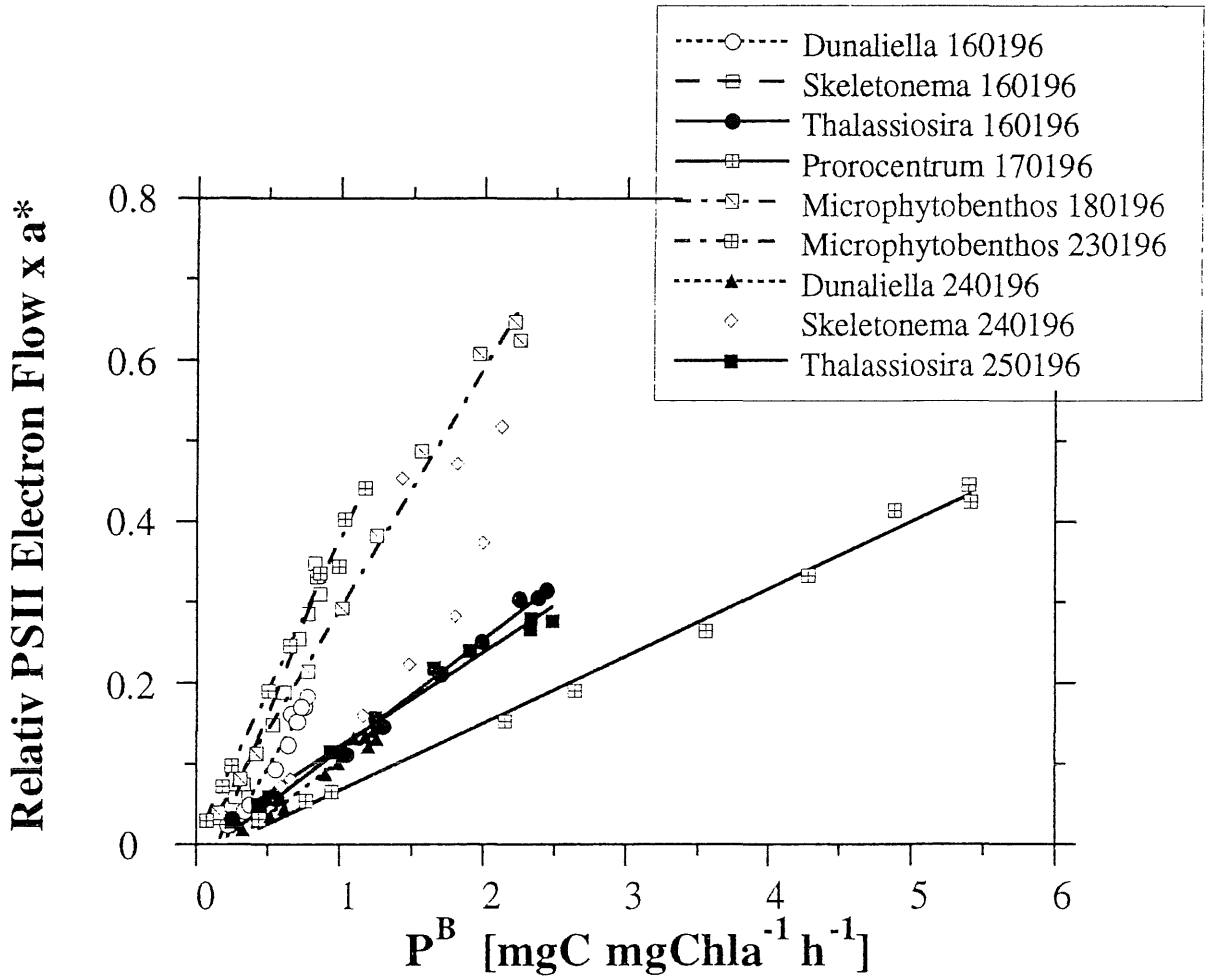


Fig. 9: Relative PSII electron flow normalized to overall absorption cross section (a*)versus specific carbon fixation for different algal species and microphytobenthos communities



Tab. 1: Relation between specific carbon fixation (P^B) and relative PSII electron flow ($\phi_{Po} * E$)

Organism	Date	r-Value
Dunaliella sp.	16.01.	**0,981**
Skeletonema costatum	16.01.	**0,986**
Thalassiosira weissflogii	17.01.	**0,997**
Prorocentrum redfieldii	17.01.	**0,997**
Microphytobenthos	18.01.	**0,996**
Microphytobenthos	23.01.	**0,996**
Dunaliella sp.	24.01.	**0,987**
Skeletonema costatum	24.01.	**0,887**
Thalassiosira weissflogii	25.01.	**0,994**

Significance is given for r-Values > 0.8

Tab. 2: I_k-values derived from both methods (PAM and ^{14}C-technique)

Organism	Date	I_K (^{14}C) [µmol m^{-2} s^{-1}]	I_K (Fluor.) [µmol m^{-2} s^{-1}]	I_K (^{14}C)/ I_K (Fluor.)
Dunaliella sp.	16.01.96	31	59	0,53
Skeletonema costatum	16.01.96	46	87	0,53
Thalassiosira weissflogii	17.01.96	112	119	**0,94**
Prorocentrum redfieldii	17.01.96	129	142	**0,91**
Microphytobenthos	18.01.96	122	129	**0,95**
Microphytobenthos	23.01.96	106	101	**1,05**
Dunaliella sp.	24.01.96	32	63	0,51
Skeletonema costatum	24.01.96	71	149	0,48
Thalassiosira weissflogii	25.01.96	113	105	**1,08**

Legend:

a^*	=	overall absorption cross section (400-700 nm)
F_m	=	maximum fluorescence yield after dark adaptation
F_0	=	minimum fluorescence yield after dark adaptation
F_v	=	variable fluorescence (F_m-F_o)
F_m'.	=	maximal fluorescence yield under actinic illumination
F	=	steady state fluorescence yield under actinic illumination
I_k	=	light saturation parameter
NPQ	=	nonradiative dissipation (F_m-F_m')/F_m'.
PAM	=	Pulse-amplitude-modulation-fluorescence
PPFD	=	incident photon flux density
P^B	=	specific carbon fixation (expressed in mgC mgChla^{-1} h^{-1})
ϕ_{Po}	=	photochemical efficiency (Genty-parameter) under actinic illumination (F_m'-F)/F_m'
ϕ_{Po} * E	=	relative PSII electron flow
q_P	=	photochemical quenching
q_N	=	non-photochemical quenching
Q_A	=	the primary stable electron acceptor of PS II

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ANNEX 6 – Report WG Phytoplankton Ecology 1996

DRAFT

WORKING MANUAL FOR
THE ICES PRIMARY PRODUCTIVITY INCUBATOR
(eds. Odd Lindahl and Franciscus Colijn)

1. Introduction

It is recommended that the "P/I curve method" should be performed. With this method the ^{14}C uptake is measured at a range of irradiance levels in the incubator, in order to get a estimate of the photosynthesis versus irradiance, which then can be parametrised. P_{max} (maximum photosynthesis) as well as α (the slope of the linear increase of photosynthesis against irradiance) can be calculated by using this method.

The method for estimating primary production by the ICES-incubator proposed in this manual (Colijn et al., 1996) is mainly intended for monitoring purposes and it should be possible to carry out the measurements from a small as well as from a large vessel. Thus, some simplifications are necessary from what could be considered to be the ideal method. It should be pointed out that the ICES incubator method is not meant as a replacement of other P/I curve techniques. It has mainly been designed to provide a reliable and comparative measurement of P_{max} by using a simple incubator and a standard protocol.

If the incubator is not equipped with filters or other arrangements creating different irradiance levels, the more simple "potential production method" can be used. However, this method will give a restricted result of the production but can be valuable for studying the regional degree of trophy of waters. When the potential production is to be converted into actual daily production, additional data on attenuation and daylength are needed. It is recommended that measurements of the potential production are carried out from at least the upper and the lower part of the photic zone to get information on the sun and shade adaptation of the plankton algae.

The advantage of the P/I curve method is the ecophysiological information which can be derived from the P/I curve. The disadvantage is that only one sample from a particular depth can be studied at a single station and that the production results must be normalised with chlorophyll in order to calculate the areal production. This may introduce unknown errors. The irradiance measurements in the sea and the irradiance levels used in the incubator are important and critical measurements which easily may introduce errors.

2. Sampling strategy

Mixed water columns

In areas where the euphotic zone is mixed and the phytoplankton community is uniformely distributed, one representative subsurface sample is sufficient.

Stratified water columns

In stratified areas or at stations were the phytoplankton community is not homogenously distributed, it is suggested that water samples from a number of depths are taken with water bottles and mixed. An alternative to mixing samples from water bottles is the hose sampling method, which gives an integrated sample from all depths of interest including pycnocline populations, subsurface chlorophyll maxima etc. Or, if preferred; samples from different depths can be taken with bottles and incubated seperately at temperatures similar to temperatures from the sampling depths. In that case more incubators are needed , or subsequent incubations to be made. The hose sampling method can also be used as an alternative to sampling with water bottles as the complete sample can rather easily be divided by depth for induvidual incubations by using a clamp. A silicon (non-toxic) hose is recommended (Lindahl, 198?). In conclusion: measurements of primary production in stratified water bodies is much more complicated and normally will fall beyond 'simple' monitoring strategies.

3. Measuring protocol

1. Placement of the incubator.

The incubator must be placed where the outside light conditions do not disturb the light climate inside the incubator. The incubator needs to be thermostatically controlled, so that the temperature of the water in the incubator has the average temperature of the water being sampled. For samples from stratified waters which differ in temperature, it is recommended that that two separate incubators are used, or two consequetive incubations are carried out.

2. Light levels and gradients in the incubator.

A set of incubation bottles with different attenuation levels should be used covering irradiances from at least 0 to 500 $\mu E.m^{-2} s^{-1}$ .with emphasis on enough measuring points to obtain a good estimate of α and $P_{max.}$ Thelevels are not prescribed here, because they depend on the availability of the range of light bottles.

3. ^{14}C solution.

Dilution of the commercially available ^{14}C solution should be avoided due to the risk of contamination. The standard activity of every batch of ^{14}C solution should be controlled by the liquid scintillation technique (see point 11). It is recommended to use ampoules which contain the amount of 14C needed for one incubation series. This reduces the number of measurements on the added activity.

If ^{14}C solution is made from stock solutions it is recommended that only high grade (p.a.) chemicals and UHQ water should be used for the preparation of the ^{14}C solution. The final carbonate concentration of the solution should agree with the average carbonate concentration of the sea area which is being studied and the pH of the solution should be in the range of 9.5 - 10.0.

4. Incubation flasks.

Tissue culture flasks of 25 cm^2 style (50 ml) are recommended. These flasks work well as paddles for the water-jet driven rotation of the flask-wheel.After every incubation, the flasks should first be rinsed with diluted HCl (10%) and then several times with fresh water. If the incubator is used under oligotrophic conditions, the flasks should at last be rinsed with UHQ water. Finally the flasks should be dried in a drying oven at 70 °C.

5. Field measurements.

Before sampling the water for the incubation experiment, a CTD-cast of at least the top of the water column should be made in order to establish the position of pycnoclines and the depth of the mixed surface layer. It is recommended that a profile of the fluorescence over depth also is measured so that the vertical distribution of phytoplankton is known before sampling. Finally, a measurement of the under-water irradiance (PAR, 4π collector) at least at the depths of sampling is necessary to calculate the attenuation coefficient. As a cheap alternative Secchi disc readings can be applied to estimate the euphotic zone. If the daily production is going to be calculated, the total daily surface irradiance (PAR, 2π collector) must be measured. In order to get a representative daily mean, readings should be taken at least at 15 minute intervals.

6. Sampling.

Non-transparent and non-toxic sampling (no rubber cords in water bottles) devices are necessary. Sampling should take place during the day, preferably so that the incubation can be carried out around noon, in order to increase comparability between stations. However, within monitoring programmes this is not always possible. For mixed water bodies one subsurface sample is incubated. In case of stratified waters, the samples from different depths should gently, but carefully, be mixed in a clean container.The incubation should start as soon as possible, preferably within half an hour after sampling. The flasks should be rinsed with water from the appropriate sample. The flasks should be filled up to the neck, leaving a little air in the flask. One dark flask for each series is

recommended. All transfers of water samples should take place in subdued light in order to avoid light-shock to the phytoplankton.

7. Total CO_2 concentration.

It is recommended to calculate the total CO_2 concentration of the sample water according to the formulas of Buch (1945) or according to other standard methods using titration of carbonate (Parsons et al., 1984, A manual of chemical and biological methods for seawater analysis.). Devices to measure temperature, salinity and pH must then be available.

8. Addition of 14C.

The ^{14}C solution should be added to the experimental flasks in such concentrations that statistically sufficient counts of the radioactivity in the plankton algae can be obtained. However, it is important that the added volume is small and that a precise, calibrated micro-pipette is used. Depending on whether filtration or bubbling is performed at the termination of the measurement (see 10.), 1μCi respective 4 μCi of ^{14}C will be sufficient in eutrophic coastal waters when added to each flask. As an alternative, the ^{14}C solution could be added to the containers with the subsamples and carefully mixed and then poured into the incubation flasks. In this case, the ^{14}C activity of the water to be incubated must be determined by a time-zero sample.

9. Incubation.

An incubation time of 2 hours and a rotation speed of about 10 rpm are recommended.

10. End of incubation.

There are two ways to terminate the incubation: by filtration or bubbling. The filtration method will give only particulate production, while the bubbling method will also include exudates and therefore can be designated as total primary production. In both cases the termination should be done as soon as possible and samples kept in the dark until further processing. Preservation of the samples should not be used.

Filtration: Glass-fibre filters (GF/F, Ø 25 mm) are recommended since these filters are cheap, become opaque and are known not to disturb the counting procedure of the radioactivity. The suction pressure should not exceed 30 kPa. After filtration the filters are immediately placed in scintillation vials and 2 drops of 80% HCl is added to each filter. This procedure should not exceed 30 minutes for the entire series of samples. The vials are left open to dry, thereafter a scintillation cocktail dissolving tissue is added.

Bubbling: From each incubated sample (volume must be known), a sub-sample of 10 ml is pippetted into a scintillation vial and 0.2 ml of 80 % HCl is immediately added. In a ventilated cupboard, the vials are thereafter bubbled with a fine jet of air bubbles for 20

minutes or are left open for 24 hours. 10 ml of scintillation cocktail (e.g. Instagel) is added and the vials are shaken by hand for some seconds.

The bubbling method has some advantages compared with filtration. For example, there is no loss due to the breakage of cells or cells passing through the filter which sometimes may happen with nanophytoplankton. A disadvantage is that a higher ^{14}C concentration is necessary when bubbling is performed, because only a part of the sample is counted and therefore it is suggested that triplicate samples are counted.

11. Counting of the radioactivity.

Only the liquid scintillation technique should be used when counting the radioactivity.In case GM counting is used, this should be occassionally calibrated against scintillation counting. It is recommended to count 10 000 DPM or counting for 20 minutes in order to get a result of 3% accuracy. Quench curves should be established and the measuring efficiency by the liquid scintillation counter should be checked by adding an internal standard.

12. Calculation of carbon uptake.

The total carbon uptake is calculated from the equation:

$$mgC.m^{-3}.hr^{-1} = \frac{dpm\ (a)\cdot total\ ^{12}CO_2\ (c)\cdot 12\ (d)\cdot 1.05\ (e)\cdot 1.06\ (f)\cdot k1\cdot k2}{dpm\ (b)}$$

where:

(a) = sample activity (minus back-ground), dpm
(b) = the activity added to the sample, dpm
(c) = total concentration of $^{12}CO_2$ in the sample water, mM/l
(d) = the atomic weight of carbon
(e) = a correction for the effect of ^{14}C discrimination
(f) = a correction for the respiration of organic matter during the experiment
k1 = subsampling factor
k2 = time factor

The result will be in $mgC.m^{-3}.hr^{-1}$ or $\mu gC\cdot l^{-1}\cdot h^{-1}$ (Steemann Nielsen, 1952).

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ANNEX 7

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Legends to figures:

Figure 1: Correlation between Pmax and Chlorophyll-a concentration for the measurements in 1995 at Station Büsum

Figure 2: Series of P-I measurements at Station Büsum during summer 1995