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REPORT OF THE WORKING GROUP ON PHYTOPLANKTON ECOLOGY

Copenhagen 23-26 March 1994

*General Secretary
ICES
Palægade 2-4
DK-1261 Copenhagen K
DENMARK

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1 OPENING OF THE MEETING

The meeting was opened by the Chairman, Dr F. Colijn, at 10.00 hours on March 23, 1994. The meeting was attended by 12 scientists representing 10 countries. A list of participants is given in annex 2. J. Pawlak, the ICES Environment Secretary, attended a part of the opening session. The draft agenda was discussed and adopted unchanged by the Working Group. This is attached as annex 1. Dr. O. Lindahl was appointed as rapporteur. J. Pawlak outlined the structure of the ICES organisation and emphasised the importance of ICES as input for monitoring studies within the framework of organisations like OSPARCOM. She also emphasised the importance of studies on nutrient phytoplankton interactions because of eutrophication problems in several parts of the ICES area, the relations between eutrophication and monitoring programmes and the relevance of making recommendations on current techniques. This also includes the evaluation of the ^{14}C-method.

2 TERMS OF REFERENCE

The chairman informed the Working Group on Phytoplankton Ecology regarding the C.Res. 1993/2:56, which states:

"A Working Group on Phytoplankton Ecology will be established under the chairmanship of Dr. F. Colijn (Netherlands) and will meet at ICES Headquarters from 23-26 March 1994 to:

a) investigate the incorporation of new techniques (e.g., nutrient uptake measurements, algal culturing, satellite colour imagery) in phytoplankton ecology studies;
b) consider ways of developing an understanding of nutrient/phytoplankton interactions;
c) assess current techniques used in the measurement of algal biomass, growth rate and productivity;
d) examine the mechanisms behind processes such as seasonal succession and long-term development of phytoplankton in relation to natural variability and anthropogenic influence;
e) report to ACME on progress made by the former Working Group on Phytoplankton and the Management of their Effects on planning a ^{14}C method evaluation exercise, and provide advice on future action.

The Working Group will report to the Biological Oceanography Committee and be referenced to the Marine Environment Quality Committee."

The terms of reference were accepted as a starting point for the new working group and were used to guide the discussions, although it was felt that the terms were too broad and extensive for a full discussion for only three days. The Chairman explained and gave his ideas about the terms of reference and gave a short overview of the background of the new working group and also on the relation of this working group to the Working Group on Harmful Algal Bloom Dynamics (WGHAB).

3 GENERAL DISCUSSIONS OF TERMS OF REFERENCE

(a, b, c, d and e refer to the terms of reference)

a. Incorporation of new techniques.

Dr Bode from Spain gave a short introduction on the relation between nitrogen compounds and productivity.

Nitrogen is usually the primary limiting macronutrient in the seawater. The study of nitrogen productivity in ecosystem research is of interest to many areas. Among them are: the measurement of biogeochemical cycles (particularly in relation to the carbon cycle), the study of the mixed layer productivity, and the evaluation of the nitrogen control of carbon fixation.

In the past years, many measurements of uptake and release of different nitrogen forms have been performed using the stable isotope technique. Most of the work was done in the context of the model of the NEW versus **regenerated** production of Dugdale and Goering developed for the oligotrophic ocean. However, the extension of the measurements to many different ecosystems and the continuous modifications of the incubation techniques and models for calculating the rates made for comparisons of results rather difficult. Three critical aspects emerge as subjects of immediate analysis. The first one is conceptual: Are the classical definitions of **new, regenerated and export** production suitable for all kinds of pelagic ecosystems, as conceived for the oligotrophic ocean? Some systems, like the coastal ocean or the Southern Ocean (Antarctic) exhibit apparent deviations from the classical concepts of new and regenerated nutrients. The second aspect is an operational one: How to deal with the dissolved organic nitrogen (**don**) forms and exchanges? Which are the forms of **don** that need a particular study of their dynamics? How to separate the dynamics of **don** in different planktonic compartments (e.g. bacteria, phytoplankton). Finally there are some methodological questions. Are the available models and experimental designs (incubations) adequate enough to study the nitrogen dynamics at the required time-scales? Should always the simultaneous measurement of uptake and regeneration rates be included? Do we need estimates of the nutrient status (physiological indexes) of the phytoplankton cells, species composition or the degree of nutrient limitation during the measurement of the nitrogen dynamics?

To answer these, and many other, questions, the future work may progress in two directions: Can we work out a set of procedures to be applied to field work at different space and time scales? Alternatively: Can we suggest which are the main unknowns in the study of nitrogen dynamics in plankton ecology that need further (urgent?) study both in the laboratory and in the field?

Dr Smayda stated that there is no clear definition of the term production. Dr Sakshaug wanted to restart the discussion, stating that first the group should look at measuring strategies instead of methods. The group concluded that discussions on methods and techniques for monitoring are too restrictive and therefore that research perspectives should be included as well. Dr Hickel suggested that the discussions should be directed towards problems in phytoplankton ecology and he mentioned the example of changes in plankton composition through eutrophication. The example shows that different methodological approaches are needed to study different size fractions of the phytoplankton.

This resulted in a round table discussion on the views of the working group members on the most important problems in phytoplankton ecology in relation to the terms of reference. The following problems were identified:

* nutrient uptake/supply including regeneration;
* new versus regenerated production;
* food chain aspects:
  a) microbial loop
  b) match versus mismatch
  c) coupling to benthos;
* size-related production and biomass;
* primary production and biomass and their variability in time and space;
* autonomous systems (buoys, moorings, ferries, satellites);
* physical forcing of pelagic systems;
* long-term changes in nutrients, production, species composition;
* what do methods really measure?

A small group was appointed to place these problems into a coherent set of priorities in a matrix for further discussion.

Two other examples of new applications and techniques to study temporal and spatial variability of phytoplankton were given by Dr Leppanen and Dr Sakshaug. The first presentation dealt with the use of automatic equipment on board of ferries crossing the Baltic Sea.

In order to reliably monitor the changes in the plankton community and as an early warning tool for potentially harmful algal blooms, the Finnish Institute of Marine Research has installed flow-through analyzers on board several ferries in the Baltic Sea. The water for the sensors is pumped continuously from a fixed depth ($\sim$5 m) while the ships are moving. The frequently measured parameters are chlorophyll fluorescence, temperature and salinity. The positions of the measurements are determined with a GPS navigator. The system is controlled and data logged by a personal computer. The system is equipped with an automated water sampler in order to obtain material for the analyses of phytoplankton species composition and nutrient concentrations. Once a week, 24 water samples are taken during one voyage of the ship and kept refrigerated and in the dark before the analysis in the laboratory. These samples are used for chlorophyll analysis, as well as to allow conversion of the fluorescence values to chlorophyll concentrations. The whole system works unattended on board the ferries. The recorded data are transferred via mobile telephone connection when the ships are visiting the Helsinki Harbour.

The system makes possible the analysis of the surface layer variability with very high spatial (100-200 m) and temporal (1-3 days) resolution. The data have been complemented with satellite images to give supplemental information on the horizontal extent of the blooms. The system has been tested in the Baltic Sea for 3 years. The unattended sampling method on ferries has been found to be an effective tool in collecting data on algal blooms. In the early warning stage of harmful blooms, it is possible to select the samples for analysis, if the simultaneously measured chlorophyll fluorescence values are high, indicating bloom formation. The number of samples analyzed is reduced but the necessary information on the bloom-forming species is still obtained.

The second presentation was devoted to the use of moored buoys (Seawatch, OCEANOR, Norway). The Seawatch system includes equipment for temperature, salinity, currents, wave-height, NO_3 and PO_4 and an "algal sensor" using a three wavelength attenuation meter (red, green, blue). The red band may yield estimates for chlorophyll a, while the ratios of attenuation in red, green, and blue may yield information on the composition of the algal community, e.g. prymnesiophytes vs. diatoms. Such bio-optical discrimination is possible because of differences in pigmentation between algal groups. To minimize errors and interferences by non-algal matter the development of a sensor which instead measures fluorescence excited in the red, green, and blue bands has just started. This system has proved to be reliable even in the roughest weather. Buoys are now distributed along the Scandinavian coasts from the Kattegat to Novaya Zemlya, off Thailand and Tonga, for monitoring purposes.

The ideal monitoring programme should cover both the spatial and temporal aspects. This requires the use of remote sensing techniques (e.g. satellite imagery) as well

as the use of autonomous systems on moored or drifting buoys, ferries etc. The autonomous systems may be particularly relevant in areas with prevalent cloudiness.

b. Nutrient-phytoplankton interactions

The present status of nutrient-phytoplankton interactions in the Dutch coastal zone was presented by Dr Colijn.

To understand the impact of eutrophication on the Dutch coastal zone several studies were performed. These include a review with a first trend analysis on nutrient concentrations and chlorophyll. Microzooplankton was identified as a major gap in knowledge (Klein & van Buuren, 1992).

During a three year field study cruises were made to investigate primary production, limiting factors and species composition (Peeters et al., 1993). The results show that there is a large spatial and interannual variation in primary production, and that dense algal blooms occur in the coastal zone, a zone of about 50 km from the shores. Multifactor limitation by nutrients and light were observed in different regions and during different periods. Silicate and phosphate, and light limited production in the nearly coastal zone, whereas offshore nitrogen limits phytoplankton production.

Subsequent studies were devoted to modelling and experimental work in mesocosms (Peeters et al., 1993). In these mesocosms the light and nutrient conditions of the coastal zone can be adequately mimicked. Depending on the season, different blooms (diatoms, Phaeocystis) were observed in the mesocosms. In future experiments the effects of nutrient reductions on the phytoplankton should be deduced, including the answer to which degree reduction should be applied to avoid eutrophication effects. The impact of eutrophication on higher trophic levels (herbivorous zooplankton, suspension feeders) is being studied in joint projects with other Dutch institutes, as well as the effects of different nutrient reduction scenarios.

Results of studies on effects on nutrient enrichments in microcosms were presented by Dr Smayda. He presented a brief overview on the responses of natural phytoplankton communities in Boston Harbour to nutrient enrichment. The rates of uptake, primary production, biomass (chlorophyll) yield, and species responses to various concentrations of ammonia, nitrate, phosphate and silicate present in secondarily treated effluent and in chemical enrichment were considered. It was shown that primary production, chlorophyll yield and the assimilation index (C fixed per unit chlorophyll per unit time) increased with nutrient levels for ammonia, nitrate, phosphate and silicate. Uptake of ammonia and silicate was directly related; the percentage utilization of added ammonia was a direct function of silicate availability. The contributions of the diatoms and non-siliceous phytoplankton species to total carbon production were also dependent upon the availability of silicate, and correlated with the ratio of uptake of NH_4 :Si.

The various experimental results indicate that in nutrient enriched coastal regions the regulation of carbon production, biomass (as chlorophyll-a) yield and species composition by nitrogen (traditionally considered to regulate marine phytoplankton dynamics) is modified by silicate availability. This suggests that silicate, which is not dependent upon direct anthropogenic inputs, unlike N and P and therefore usually ignored, should be included in assessments of the influence of nutrification on the modification and regulation of phytoplankton dynamics in coastal waters, undergoing progressive nutrient enrichment.

Several of the members tried to fit in their results and experiences on the results Dr Smayda had presented. It was concluded that the nutrient to growth relations in many areas showed similar responses compared with the microcosms experiment described. However, the nutrient to growth relations are very dynamic and a large number of question marks still remains.

c. Current techniques in measurement of algal biomass, growth rate and productivity.

As a new technique for measuring biomass flowcytometry was mentioned specially for small phytoplankton cells. Also, many problems still exist with the ordinary chlorophyll measurements: rapid degradation of chlorophyll occurs after filtration and differences between spectrophotometric and fluorometric measurements have also been observed. The group decided to study the report of SCOR WG 78 by Mantoura and Jeffrey concerning measurement of pigments and to report on this at the next meeting of the WGPE. Dr Williams explained that major stress could occur during vacuum filtration of phytoplankton. Therefore, he stressed that gravitational fractionating or pressure filtration are less susceptible to this form error.

A next presentation was given by Dr Williams on the interpretation of production data and methods.

A review of the past: in the early 1970s suggestions were made of major (i.e. 10-fold) errors in the ^{14}C technique. The source of the purported error could fall into one of three levels of hierarchy:

1. Isotope or computation errors.
2. Errors associated with *in situ* procedures.
3. Errors arising from lack of understanding of the physiological processes associated with the net fixation of $^{14}CO_2$.

The first error type was the substance of the study by Richardson. The second class of error was examined thoroughly during the late 1970s and early 1980s. From these studies the general conclusion was.

1. There was no evidence for major (i.e. 10-fold) errors *in situ* procedures.
2. Contamination problems exist, they can be severe, but given careful attention to detail these errors may be contained.
3. Extreme clean techniques do not seem to be obligatory to obtain satisfactory results.

Current problems associated with the ^{14}C technique: the error of understanding now is probably less than a factor or two. At this level the physiological model used to interpret the net ^{14}C uptake measurement becomes critical. Two basic models exist: one which takes account of the respiration of the newly fixed carbon, the second acknowledges that recycling of respiratory carbon dioxide will reduce the specific activity of the CO_2 at the site of enzyme activity. These two models give rise to profoundly different expectations over what net ^{14}C uptake determines in terms of the physiological process of gross and net production and its development with time.

	Short term incubations (< <3-6h)	Long term incubations (> >6h)
Consequence of respiration of ^{14}C labelled carbon	Gross production	Net production
Consequence of recycling of respiratory CO_2	Net production	Not clear

The generalization provided a basis to examine the controlling physiological process. The first experiments were undertaken with Skeletonema which showed net ^{14}C fixation close to net C production, which implies that recycling is the major determinant.

The future: There is a hierarchy of production measurement techniques:

1. Remote sensing - discussed elsewhere in report.
2. *In situ* methodologies - not discussed.
3. Unequivocal determination of production rate.

Gross production can not be measured directly. It is measured as the sum of net oxygen production or carbon fixation and respiration. Whereas net production can in principle be measured unequivocally, current approaches to determining respiration involve dark incubations, over which uncertainties exist. In principal unequivocal measurement of respiration can be obtained in the light using the determination of $^{13}CO_2$

Another presentation was given by Dr Sakshaug on biooptical measurements in the sea which are related to the modelling of photosynthesis in the sea.

Bio-optical models for the photosynthetic and growth rates of phytoplankton have been increasingly used in the last 15 years, for instance in conjunction with the calculation of the photosynthetic rate on the basis of satellite images of chlorophyll-a. This approach in principle requires knowledge of: the vertical profile of spectral irradiance and chlorophyll-a, and a set of appropriate parameter values for the P-E function. One important parameter is a*, the chlorophyll-a normalized light absorption spectrum of phytoplankton. Multiplied by the maximum quantum yield, ϕ_{max}, it forms a^B, the so called photosynthetic efficiency which determines the photosynthetic rate in weak light. A large "library" of a* data is being built up globally. Although a* data are the more relevant for modelling the submarine light field, they admittedly may overestimate absorption of photosynthetically "usable" light because they include absorption/scattering by non-algal matter as well as absorption by photoprotective algal pigments. To estimate only the fraction of light which is photosynthetically usable, the use of scaled fluorescence excitation spectra has been suggested. Such spectra (sigme*) are conveniently measured. However, more research is needed with regard to the scaling of such spectra because the raw data are relative.

A profiling absorption meter measuring at 676, 650 and 712 nm is now commercially available (Wet Labs Inc). This meter provides the "red peak" of a*, to which the fluorescence excitation spectrum can be scaled. By using, in addition, an appropriate value for ϕ_{max}, a^B can be estimated - as well as its spectral dependence which may cause a variation of a factor of 2 depending on "water colour".

A most promising approach in the field is the use of the "pump and probe" fluorometer (profiling version commercially available in 1994) and PAM fluorometry (field version not yet developed). These approaches are based on the progressive closure of photosystem II reaction centres, and the subsequent increase in fluorescence, by a brief series of strong and weak excitation flashes. In this fashion the quantum yield of fluorescence as well as the absorption cross section of photosystem II can be estimated. In essence, the two new instruments and the Wet Labs instrument in combination with spectral fluorescence excitation measurements can provide in principle

P-E parameters on a profiling basis, making incubations unnecessary except for calibration purposes.

After these discussions on the different topics from the terms of reference, the chairman identified the terms and priorities of the problems agreed upon by the members. He summarised the following categories: autonomous systems like buoys, ferries and satellites to study time/space scales and variability of phytoplankton distribution and dynamics. Further, the complex of nutrient uptake supply and regeneration in eutrophied coastal areas was stressed as an important field of interest for the group. New and regenerated production is also of interest. Evaluation of long-term changes in nutrients, productivity and species composition should have a high priority in the coming years (see recommendations).

At this time, less priority is given to food-web aspects as well as to physical forcing of pelagic systems as well as "what do methods measure".

In the subsequent discussion Dr Smayda suggested a workshop or symposium to be held in two to three years dealing with long-term trends in e.g. primary production, nutrient concentrations and phytoplankton species. The meeting should include a study on the balance of natural and anthropogenically induced effects. Effects of climate changes and its variability should be included. The working group decided to recommend this topic to BOC (Biological Oceanographic Committee) and consider it further at next years' meeting.

d. Mechanisms behind processes such as seasonal succession and long-term development of phytoplankton

Dr. Hickel reported on time-series from Helgoland in the German Bight (this presentation is put under d. but has strong links with b.). These measurements are carried out since 1962 every working day. This is one of the longest time-series in the North Sea which includes inorganic nutrients and qualitative as well as quantitative phytoplankton measurements.

It was found that phosphate concentrations doubled during the first decade of the time-series, staying at this level for another decade and then declined. The measures taken for phosphate reduction thus could be detected at Helgoland. The nitrate eutrophication of the German Bight became evident much later, since 1979 only, but then increased faster to reach three times the former level within a few years. P- and N-eutrophication thus were not coupled; as high nitrate levels still prevail- as shown by salinity-normalized nitrate data- a shift of N:P- ratios is observed in the German Bight. Now a large N-surplus (as compared with the Redfield-ratio) is recorded the whole year round.

The consequences of the eutrophication for the phytoplankton are less clear in the German Bight than in other eutrophied coastal areas. Though an increase (about 3 times) of total phytoplankton biomass (as carbon) could be found during the last 3 decades, no trend can be seen for the diatom stocks nor for the larger dinoflagellate populations. The largest increase occurred with the nanoflagellates, since 1979. It appeared together with the rise of nitrate and hence of N:P ratios, but the real causes of the nanoflaggelate increase are still unclear.

Spatial surveys of plankton in relation to hydrography and nutrients supplied additional information for the south-eastern North Sea and showed the dominant influence of the density stratification on phytoplankton blooms. Eutrophication will mainly be effective if vertical stratification allows for better light utilization in the upper (euphotic) part of the water column.

As a consequence, a better analysis of the nano- and picoplankton component must be recommended; this component is quantitatively underestimated, qualitatively poorly known, but most probably very important. Besides better knowledge of interaction between the various plankton components and nutrients, permanently established research groups are needed to continue the observation of temporal trends in plankton as natural and man impacted factors might change. Most information presented here has been published in Hickel et al.(1993).

In the discussion on this presentation several questions were raised: how representative is the Helgoland station for the direct Elbe plume, and the importance of sedimentation and subsequent regeneration of nutrients from the Elbe valley. Also the differences in behaviour of nitrogen and phosphate compounds during different river discharges were mentioned. One of the eutrophication effects observed along other coasts, the bloom character of Phaeocystis is not observed at Helgoland but restricted to the coastal zones along the North and West Frisian islands. Other members commented on the sudden increase in naoflagellates in other areas at the same time.

e. Evaluation of standard ICES ^{14}C incubator method

Dr Colijn reported on the progress made on the intercalibration of the so called standard ICES incubator. He presented a first draft of the paper containing the results of this exercise which was held in the Netherlands from 9 to 11th March, 1994, in Middelburg and which was well organised by his colleague, Mr Wetsteyn. The results of the intercalibration were assessed as very good with minor exceptions and having a precision acceptable for this type of work. The manuscript is added to the report as annex 4. In the following discussion the

authors of the paper were asked to look for more realistic daylight illumination, to find out how the irradiance could be increased to such an extent that photoinhibition could be measured, to make a full description of the application of epoxy-resin to reduce the irradiance in the bottles, and to complete the manuscript with the experimental protocol. If the total production is to be measured, the whole water bubbling method could be applied instead to reduce filtration errors. The WGPE welcomed and concluded that the engineering of the ICES standard incubator is now complete and following some considerations of the light source ready for application in monitoring studies. These conclusions will be reported to ACME. The Working Group plans to consider further ways of promoting the incubator at its next meeting.

4 STRATEGIC DISCUSSION

The working group had a final 'strategic discussion' on the future topics. This was done with the help of a matrix with context and problems/approaches on both axes. In general the interests of the group were rather consistent: long-term changes, interannual variability and eutrophication were identified as the most prominent context items. Of the problems and approaches most interest was expressed towards a) nutrient supply and uptake, b) time and space scales in variability of primary production and biomass, c) autonomous systems and d) physical forcing of pelagic systems.

Therefore the terms of reference for next year should be focussed on these items and topics.

5 ANY OTHER BUSINESS

The WG agreed to accept the invitation of the chairmen to have the next meeting in the Netherlands (the Hague) in about the same period, to avoid conflicting interests for those who are involved in phytoplankton spring bloom studies.

6 RECOMMENDATIONS

1. The Working Groups stresses the importance of collection of long-term series of phytoplankton and related parameters in view of global or local changes in the marine environment. Governmental institutes should perform, or make funds available for monitoring studies. This could be done in a low-budget, simple fashion, but also series should set up with more sophisticated new instrumentation. Eventually both types of series should be linked.

2. The Working Groups emphasizes that a workshop-/symposium should be held on the interpretation of long-term series with respect to anthropogenic impacts on the marine environment within 2-3 years. Both biological, chemical and physical parameters should be included.

3. As soon as the standard ICES incubator with the protocol and other facilities has become available, it should be disseminated to the institutes cooperating within the framework of ICES.

4. The Working Group on Phytoplankton Ecology (Chairman: Dr F. Colijn, Netherlands) will meet in The Hague (Netherlands) from 29-31 March 1995 to:

   a) consider ways of promoting the use of the ICES standard incubator;

   b) evaluate possible new techniques for the measurement of pigments and primary production;

   c) develop an understanding of nutrient to growth relationships in eutrophic coastal areas;

   d) summarise the first results on the use of automatic equipment on buoys and ferries for monitoring the spatial and temporal distribution of phytoplankton and chlorophyll;

   e) develop plans for a possible future workshop/-symposium to evaluate the use of long term time series in primary production etc., in order to partition natural from man-induced environmental effects.

The Working Group will report to the Biological Oceanography Committee, referenced to the Marine Environmental Quality Committee.

Justifications for the proposed agenda items for this meeting is given in Annex 6.

7 CLOSING OF THE MEETING

The meeting was closed at 16.10 h on Friday 25th of March by the chairman after he had acknowledged all members for their active participation in this meeting. He mentioned that it had taken some effort to convene the meeting, because several potential members were unable to attend. He nevertheless concluded that the meeting had been successful and inspiring, and he hoped to see everybody next year.

ANNEX 1

Agenda

1. Opening of the meeting
2. Terms of reference
3. General discussions of terms of reference
4. Strategic discussion
5. Any other business.
6. Recommendations to the Council
7. Closing of the meeting.

ANNEX 2

List of participants

Name	Address	Telephone	FAX	E-mail
Franciscus Colijn (**Chairman**)	National Institute for Coastal and Marine Management/RIKZ Koningskade 4, P.O. Box 20907 2500 EX Den Haag Netherlands	+3170 3745208	+31 70 3282059	
Antonio Bode	Instituto Español de Oceanografia Centro Costero, Apdo 130 15080 La Coruña Spain	+34 81 205362	+34 81 229077	
Lars Edler	SMHI Doktorsgatan 9D 26252 Ängelholm Sweden	+46 431 80854	+46 431 83167	
Kristinn Gudmundsson	Marine Research Institute Skúlagata 4 121 Reykjavik Iceland	+354 1 20240	+354 1 623790	kristinn@ hafro.is
Wolfgang Hickel	Biologische Anstalt Helgoland Notkestrasse 31 22607 Hamburg Germany	+49 40 89693-203	+49 40 89693-115	
Juha-Markko Leppänen	Finnish Institute of Marine Research P.O. Box 33 00931 Helsinki Finland	+358 0 331044	+358 0 331376	jukkis@fimr.fi
Odd Lindahl	Kristineberg Marine Research Station Kristineberg 2130 450 34 Fiskebäckskil Sweden	+46 523 18500	+46 523 18502	
Terry Mc Mahon	Fisheries Research Centre Department of the Marine Abbotstown Dublin 15 Ireland	+353 1 8210111	+353 1 8205078	
Francisco Rey	Institute of Marine Research P.O. Box 1870, Nordnes 5024 Bergen Norway	+47 55 238500	+47 55 238584	internet: pancho@imr.no omnet: f.rey
Egil Sakshaug	Trondheim Biological Station University of Trondheim Bynesv. 46 7018 Trondheim Norway	+47 73 591583	+47 73 591597	
Ted Smayda	Graduate School of Oceanography University of Rhode Island Kingston, R.I. 02881 USA	+1 401 7926171	+1 401 7926682	
Peter Williams	School of Marine Sciences University of Wales, Bangor Wales LL57 2UW United Kingdom	+44 248		

ANNEX 3

Action List:

- Wetsteyn, Edler, Colijn: to complete the manuscript on the incubator with additional information on irradiance quality, epoxy resin application and protocol.

- Sakshaug, Bode, Rey: to comment on chlorophyll, pigment analyses based on SCOR-report.

- Smayda, Hickel, Lindahl: to comment on nutrient-phytoplankton interactions in (eutrophical) coastal areas.

- Leppänen, Sakshaug, Colijn: to report on the use of ferries, buoys to study temporal/spatial scales of phytoplankton, nutrients etc.

- Williams; to comment on progress made in the PRIME programme

ANNEX 4

Report on light measurements and intercalibration of standard ICES incubators.

L.P.M.J. Wetsteyn[1], L. Edler[2], M.M. Steendijk[1], G.W. Kraay[3], F. Colijn[4] & R.N.M. Duin[4]

[1] National Institute of Marine and Coastal Management (RIKZ), P.O. Box 8039, 4330 EA Middelburg, The Netherlands.
[2] Swedish Meteorological and Hydrological Institute (SMHI), Doktorsgatan 9D, S-26252 Ängelholm, Sweden.
[3] Netherlands Institute of Sea Research (NIOZ), P.O. Box 59, 1790 AB Den Burg, Texel, The Netherlands.
[4] National Institute of Marine and Coastal Management (RIKZ), P.O. Box 20907, 2500 EX Den Haag, The Netherlands.

(Results 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)

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 of four incubators became suddenly possible giving the opportunity to do some light measurements and to perform a reproducibility experiment before the next meeting.

Here we will present 1) some of the results of extensive light measurements in the standard incubators and 2) 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.

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. 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 and the copper tubes were parallel connected to the thermostat (Colora). In this way we reached acceptable differences in water temperature between the 4 incubators without the risk of contaminating the cooling device or 4 incubators at the same time.

Different levels of irradiance were created by applying different layers of epoxy-resin as neutral density filters on the surfaces of the incubation bott-

les. The side walls and the necks of the bottles were covered with black epoxy-resin. The reason that we chose for 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 daylight-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	0	0	0
1.0	1.1	1.5	1.5
2.5	2.6	2.9	2.9
9.4	9.8	9.9	9.9
18.0	18.9	19.1	19.3
22.9	23.5	23.6	24.3
28.5	28.7	30.5	31.4
31.5	31.6	32.9	35.7
42.5	42.8	43.2	43.3
51.0	51.5	53.1	54.1
70.6	71.0	72.1	72.9
100	100	100	100

Figure 1 shows the relative transmission of 3 and 1.5 % filters of the used epoxy-resin. It is clear that the behaviour of this material is extremely good in the very low transmission range (thick epoxy-resin layer) and in the high transmission range it must be even better.

Irradiance measurements

Knowledge on irradiance measurements is of utmost importance for P-I measurements. Therefore, a new small 4π sensor was constructed around a Si-detector. With a stopper, through which the wire passed, it can be fixed in the centre of an incubation bottle. 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 4π sensor was calibrated using a tungsten strip lamp and a LICOR-1000 lightmeter. The obtained calibration factors (multipliers to get $W.m^{-2}$ or $\mu E.m^{-2}.s^{-1}$) hold only for the combination of this sensor and TLD33.

Figures 2-5 give examples of light measurements performed with the 4π 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 4π sensor approaches the vertical parts of the copper tubing. Figure 2 illustrates little difference between the four TL-sets (with PS-layer and coated bottles). Figure 3 gives the absolute irradiance distribution with clear bottles and with and without PS-layer. It can be seen that adding the PS-layer 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.

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 μg/l, was used. A tenfold dilution with 0.2 μm filtered Oosterschelde water was done 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 μM; Si-SiO_2: 18 μM; N-NH_4: 1.5 μM and N-NO_3+NO_2: 48 μM. The low phosphate concentration and very high N/P ratio suggests 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 μl with 2 μCi was added. The bottles were always incubated for two hours. After incubation they 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 aquadest 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 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. ΣCO_2 was measured by titration according to standard procedures; the measured ΣAlkalinity in some of the samples was 2.263. From each sample 20 ml was taken for cell counts (if needed) and preserved with 50 μl Lugol.

Experimental set-up

A Standard Latin Square Design as experimental set-up was chosen in such a way that it was possible to deal as well as possible with the following sources of variability:
-variability as a consequence of subsampling,
-variability by the use of different, but in principle identical incubators,
-variability by different users,
-variability introduced by the inevitable differences in times of starting the incubations.

This can be illustrated with the following scheme:

	Incl	Inc2	Inc3
Expl	A	B	C
Exp2	B	C	A
Exp3	C	A	B

A,B and C are the different users. Incl, Inc2 and Inc3 the different incubators and Expl, Exp2 and Exp3 the 3 consecutive experiments. Allocation of the incubators 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 disturbing effects of incubators and experiments, in such a way that a better test of a possible user effect can be performed.

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

A statistical test was used to determine the variability between the different sources of error. To do this P_{max}, I_{opt}, I_k and α were used. These parameter values 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 values except one were ca. 1 % of the maximal photosynthetic rate.

It was also meant to check the reproducibility of a measurement. This was done by one user always using the same incubator. Unfortunately these results deviated so much from the results of the other three users that an apart 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 μg/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 determined with the pgh-model) there was no user effect after correction of the 'disturbing' factors incubator and time. 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 are depicted in Table 2.

Furthermore it appeared that differences could be found for the three P-I models according to the number of the experiment and of the incubator; see Table 3. This table presents the over the users averaged values. The differences are minimal, 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 accurate. Iopt is most variable, while Ik seems to be much more stable; especially for the pgh-model. The values for Pmax, Ik and α 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 measured different when compared with the three other users. Just during the third measurement a similar result as from the other users is obtained.

Table 5 gives the mean values with the standard errors and coefficients of variation for all used P-I models. 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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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
Incl	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 $W.m^{-2}$; α 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 with the different 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 αjp, 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.

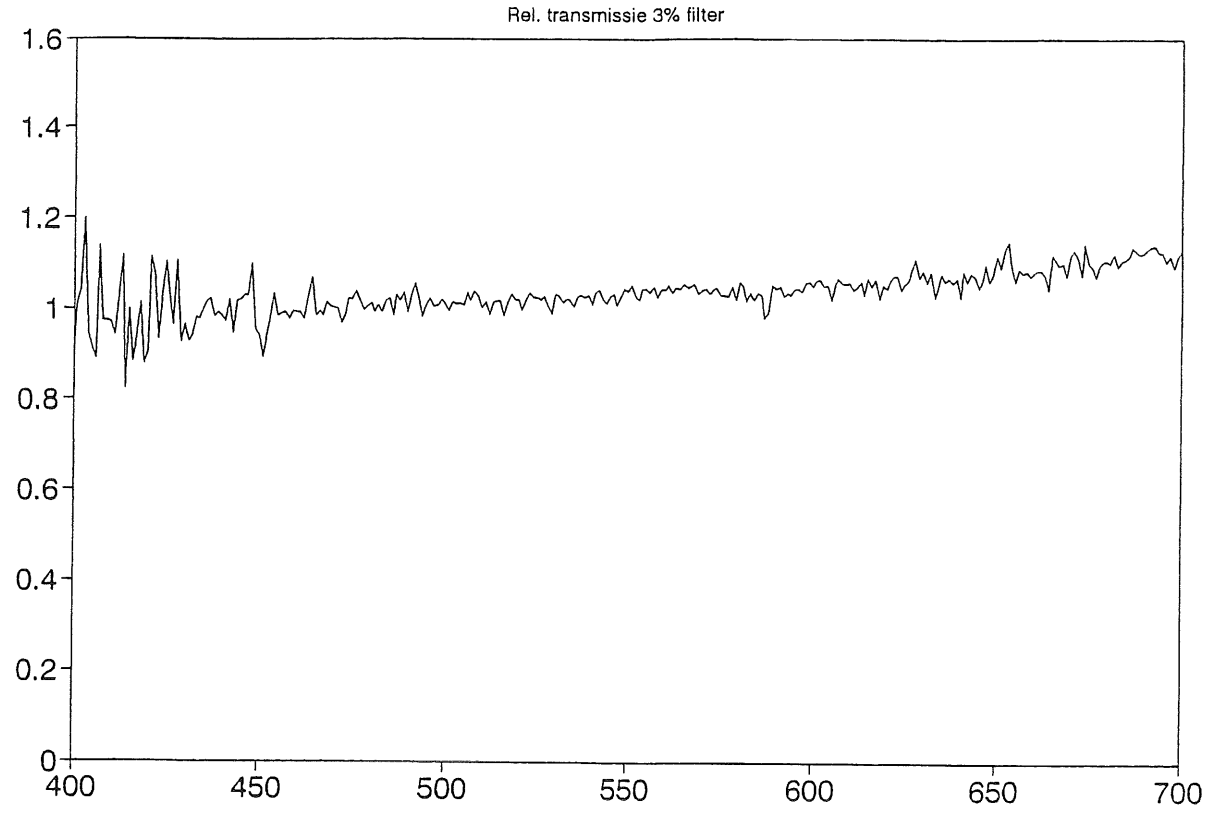
	αpe	αjp	αpgh
EXP2	0.1093	0.0677	0.0873
EXP1	0.0937	0.0617	0.0777
EXP3	0.0637	0.0547	0.0677
INC1	0.0867	0.0663	0.0827
INC3	0.1037	0.0637	0.0820
INC2	0.0763	0.0540	0.0680

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



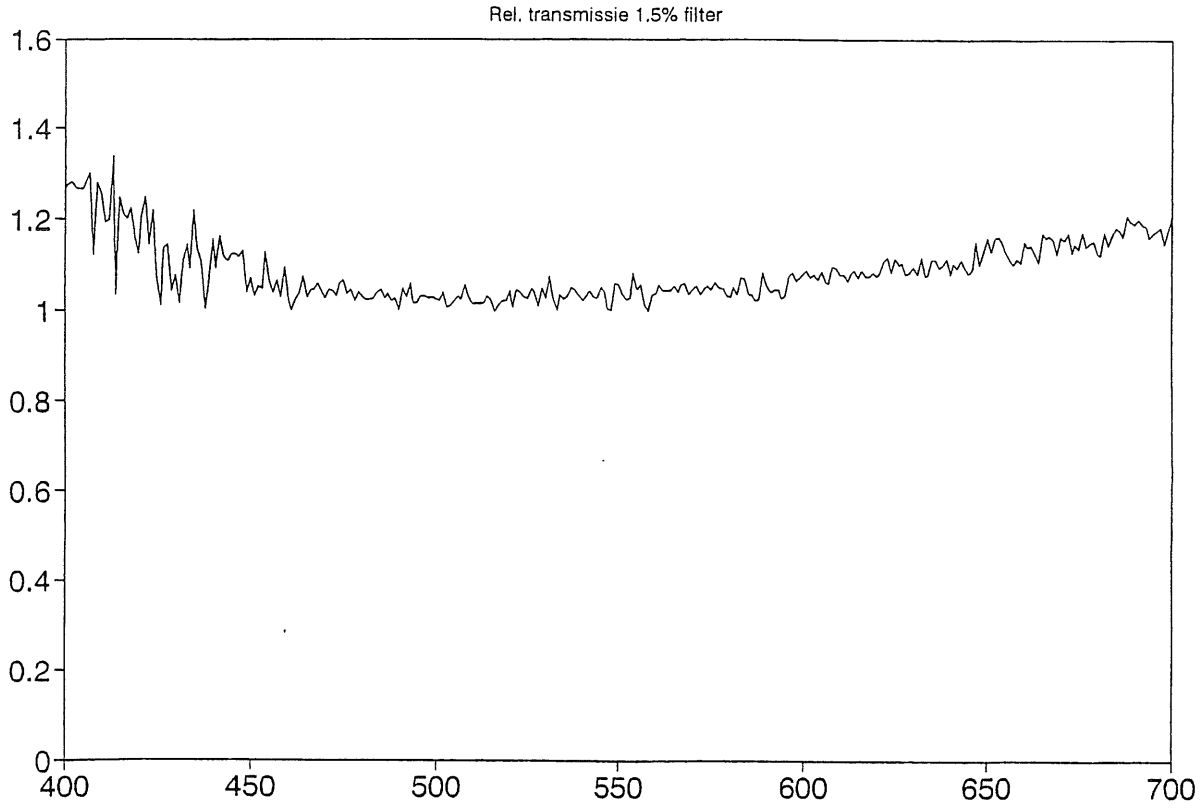


Figure 1. Relative transmission of 3 and 1.5 % epoxy-resin filters in the 400-700 nm range.

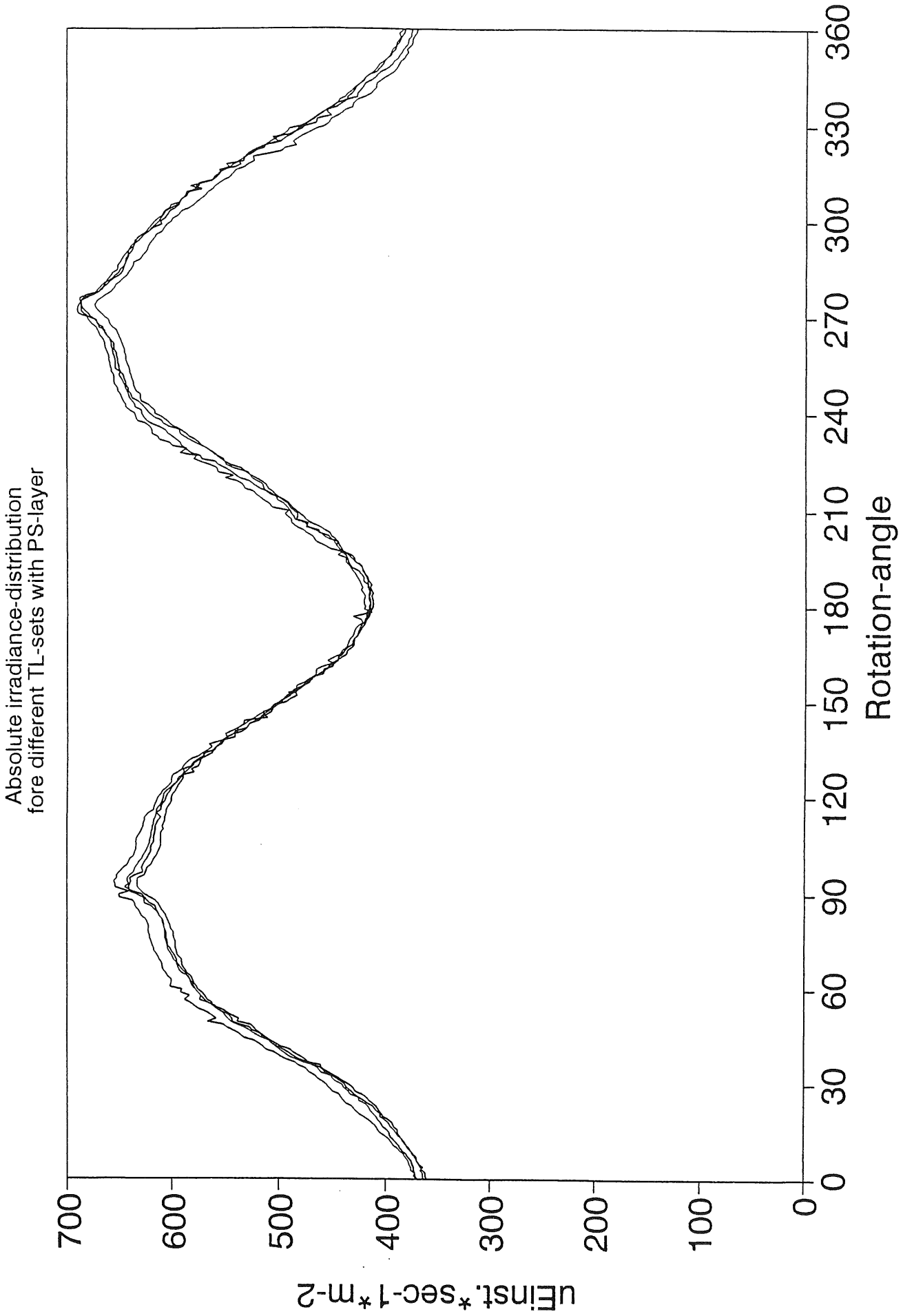


Figure 2. Absolute irradiance distribution of four different TL-sets, 10 TL tubes, with PS-layer and with coated bottles.

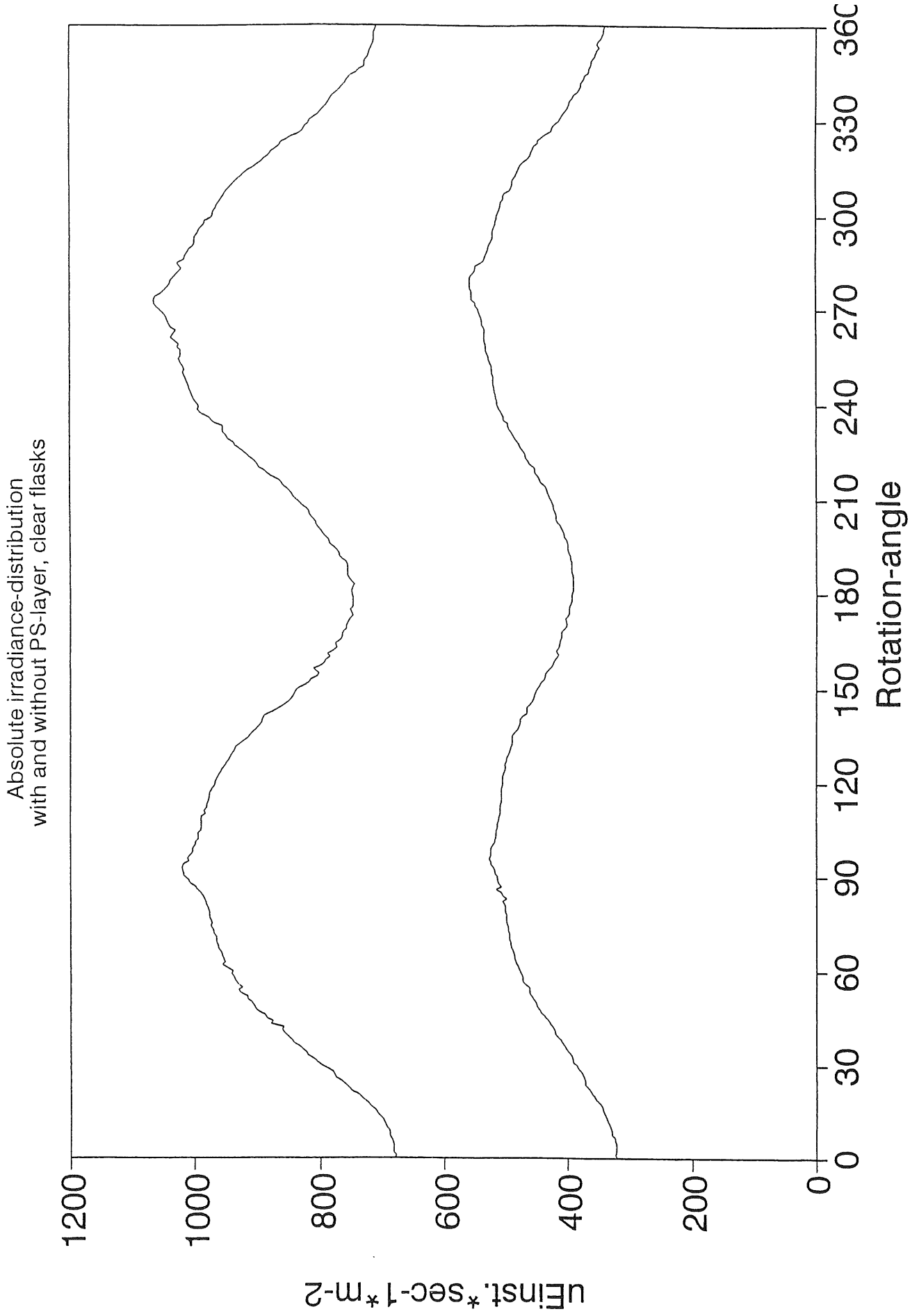


Figure 3. Absolute irradiance distribution with and without PS-layer, clear bottles and 10 TL tubes.

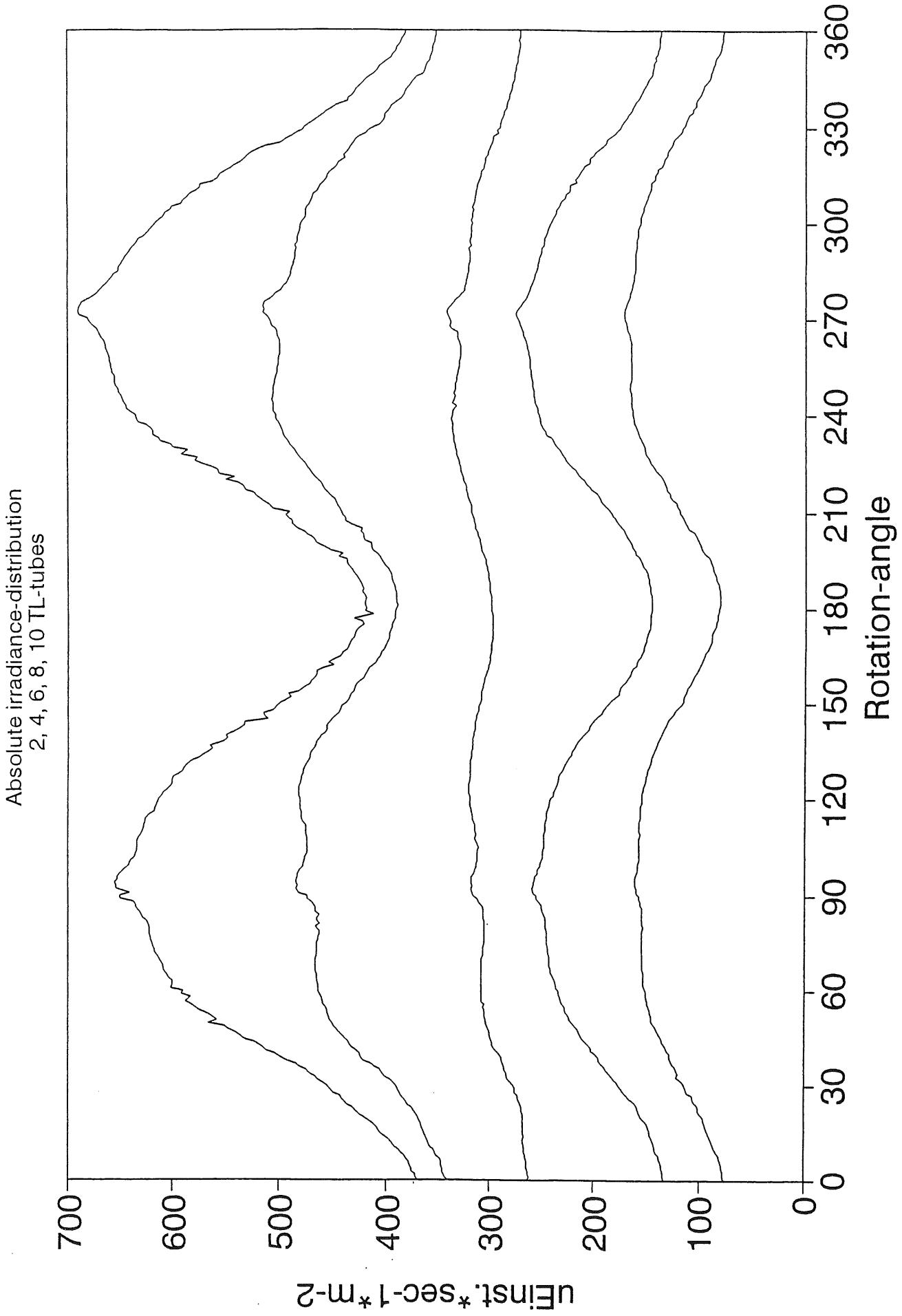


Figure 4. Absolute irradiance distribution with PS-layer, with coated bottles and 2 (xxoxxxxoxx), 4 (xoxoxxoxox), 6 (xoxooooxox), 8 (xooooooox) or 10 TL tubes.

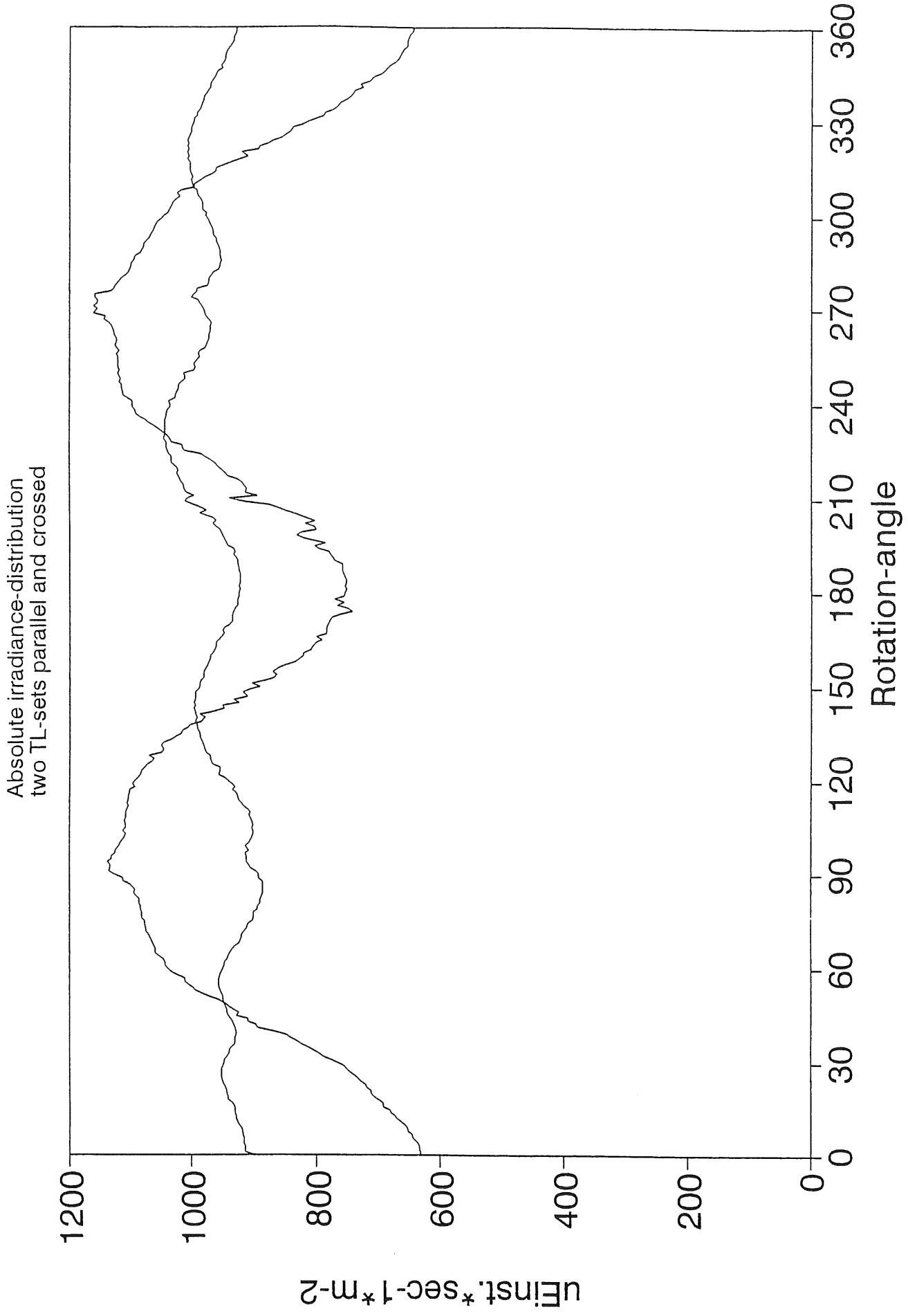


Figure 5. Absolute irradiance distribution with coated bottles and two 10 TL-sets parallel and crossed.

ANNEX 5

References

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Annex 6

Justification for the agenda items for next Meeting of the Working Group (see Section 6)

a) the engineering of the ICES standard incubator is now complete, and is ready for application in monitoring studies. In addition a successful intercalibration exercise has been undertaken, so now it is important that those concerned with such monitoring are encouraged to use it.

b) The report of SCOR WG 78 by Mantoura and Jeffrey concerning measurements of pigments may help to solve some of the many problems with ordinary chlorophyll measurements. The group will study and discuss this report in detail. New developments are underway to measure primary production in the sea. An attempt will be made to follow these developments closely to see if they can be incorporated in the long term as an alternative to current O_2 and ^{14}C - methods.

c) Although nutrient to growth relations in many areas show similar responses compared with microcosm experiments, the details of this relation are extremely dynamic and a large number of questions must be addressed. The inclusion of this item represents only a start in the answering of these questions.

d) Recent technological advances have provided the capability of using autonomous equipment for monitoring phytoplankton equipment. Schemes for monitoring using this equipment are already in use, especially in the Baltic and in the Skagerrak/Kattegat. Such a capability will greatly benefit our ability for example to have an early warning for harmful algae blooms.

e) It is considered that the evaluation of long-term changes in nutrients, productivity, and species composition should have a high priority in the coming years because of the need to separate climate-induced variability from man-made changes. In anticipation this need, and to stimulate interest in it, an appropriate meeting fora should now be considered.