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Annex C-5
Phytoplankton primary production

Annex 1
Design and tests of a novel P-I incubator
to be used for measuring the phytoplankton primary production
in ICES monitoring studies

ANNEX C-5 PHYTOPLANKTON PRIMARY PRODUCTION

ANNEX 1 DESIGN AND TESTS OF A NOVEL P-I INCUBATOR TO BE USED FOR MEASURING THE PHYTOPLANKTON PRIMARY PRODUCTION IN ICES MONITORING STUDIES

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This paper has been developed during a period of several annual meetings of the ICES Working Group on Phytoplankton Ecology and its predecessors. Field and laboratory studies were made in successive years.

We would like to acknowledge many colleagues who gave the possibility to test the method on board their ships or in their labs, like the meeting in Helsinki, Finland in 1989; the additional measurements made in the Indian Ocean in 1992-1993; and in the German Wadden Sea in 1995.

Abstract

An inexpensive and simple incubator for primary production measurements is presented along with a protocol (see Working Manual) to achieve 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 , German 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 observed.

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 levels. These experiments show that actual P-I relations can be measured with a good fit of the P-I curve parameters, like initial slope ±, I_k, I_{opt} and P_{max} values.

A series of measurements were performed for a period of 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 P_{max}levels. Correlations between chlorophyll and primary production were high.

Daily primary production values have been calculated based on the P-I relations after integration over time and depth on selected series of data and compared with a simple empirical equation based on P_{max}, attenuation coefficient, daylength and daily insolation. The agreement between both methods was 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 needs 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.

Based on the measurements in the German Wadden Sea daily primary production has been calculated according to a strict format. This format will be proposed to standardize calculations.

Application of a known irradiance in the incubation bottles is still one of the most difficult parameters in this method, whereas the application of the tracer method itself is easy and straightforward.

1. INTRODUCTION

Results of the Hirtshals intercalibration (c.f. Richardson, 1991) 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, incubation handling, 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 and therefore were never stored. This paper describes the use of an incubator and develops a strict protocol with as few steps as possible, and contains recommendations about the use of materials, to reach highest comparability of results.

Originally, 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 ±, and a remotely sensed biomass field. Such estimates might be possible for the North Sea within the near future if both P_{max} and ± are known.

Stimulated by the discussions and comments 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 de Keijzer (1994) (Annex 3). 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 µE.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 2À-sensor and therefore are substantially lower than the earlier measurements 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 µE.m^{-2}.s^{-1}, respectively as maximum irradiances. A full description of the irradiance distribution is given in (Annex 2) where several options for illumination were tested.

During one of the meetings 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 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 how to obtain different irradiance levels in the incubation bottles has been solved by using a epoxy-resin layer of different attenuation (Annex 2).

Incubations are carried out in disposable tissue (ultraclean) culture flasks (e.g. Greiner, tissue culture flasks, 690160) containing 50 ml of sample. These flasks can be used several times without deterioriation of the vessel walls and are suited to adhere the epoxy-resin layers.

Temperature is controlled to within ± 0.1 °C by a suitable thermostat with enough capacity (Lauda, Colora). 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 complete system is built by Hydrobios (Kiel, Germany), whereas the calibrated incubation bottles are sold by ZEMOKO (the Netherlands) (full adresses of both companies are given at the end). The cost per unit can be reduced if several incubators are built/ordered 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 Biddulphiaaurita, B. sinensis, Coscinodiscus concinnus, Skeletonemacostatum 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 µE.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 according 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}/ µE. 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 µE. 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 measurements will be used, in combination with irradiance and attenuation measurements to calculate the daily and annual primary production at station Büsum (Tillmann 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 Working Manual is followed, data obtained in this way are directly comparable.

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. The results (not given) showed 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 have further evaluated this procedure and finally present a calculation mode with the Working Manual. The calculation is also available on disk through SMHI in Sweden.

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 have calculated the daily primary production in Büsum as an example. The same calculation has been 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 an alternative to the 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. A moment there is uncertainty because we have not tested it.

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

Filter type	Incubation time (h)	DPM	x ± sd cv
GF/F	1	45163	43724 ± 1757 4.0
GF/F	1	44243	
GF/F	1	41765	
GF/F	2	78384	79183 ± 2469 3.1
GF/F	2	81953	
GF/F	2	77212	
Sartorius	2	71228	67348 ± 5154 7.6
Sartorius	2	69316	
Sartorius	2	61500	
GF/F	2 (in dark)	1142	1424
Sartorius	2 (in dark)	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	2565
2530	2515
2518 2514 ± 17 0.6	2602 2553 ± 78 3.1
2523	2441
2514	2541
	CPM/h irradiance
	257 5%
	425 10%

	991	25%
	1967	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

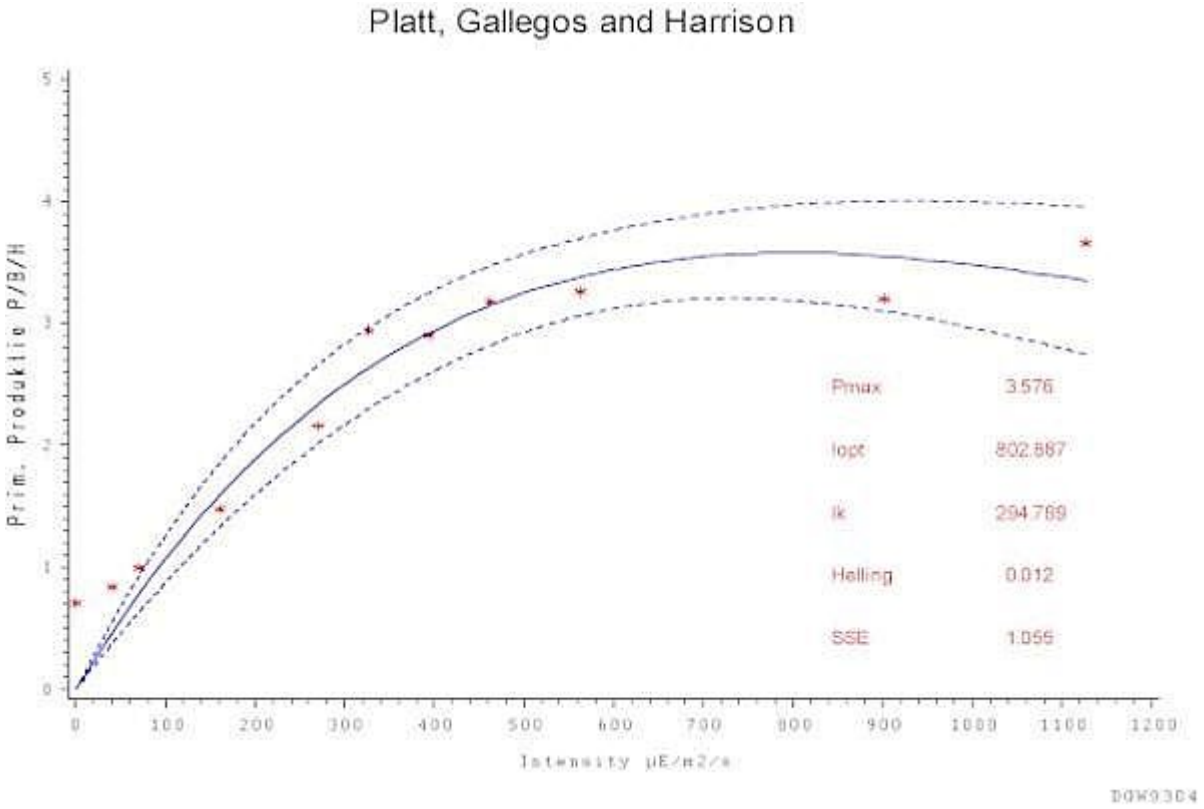
Table IV. Example of results of experiments conducted in the Indian Ocean, location off Kenya and Somalia (Veldhuis & Kraay, in prep.) to show daily inequality.

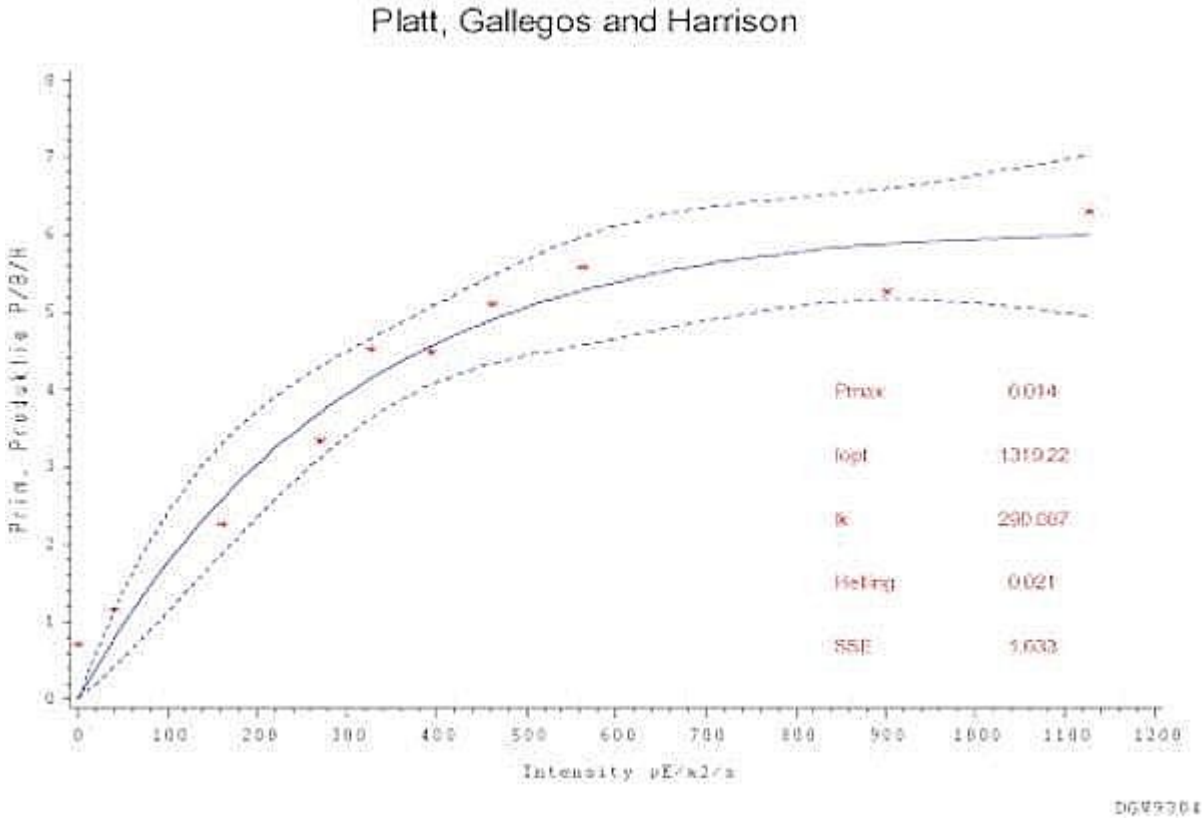
Same sample was used for both incubations; parameters estimated by the equation of Platt et al. (1980). Calculation of daily primary production is based on ke = 0.1, daylength = 12 hrs., and mean surface irradiance = 1000 mE.m-2.s-1. SSE is the error sum of squares of the fitted model.

	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 Production	260	465	$mgC.m^{-2}$

Legends to figures

Figure 1. Photograph of ICES incubator (see text).
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).
Figure 4. Examples of P-I curves measured at Station büsum; all curves were normalised to chlorophyll-a; fits were made with the equation of Platt (1980)
Figure 5. Seasonal course of P-I parameters at Station Büsum in 1995; all parameter calculations based on Platt et al. (1980)
Figure 6. Relation between assimilation number (P^b_{max}) with temperature for the measurements conducted in Büsum in 1995
Figure 7. Calculation of daily and annual primary production based on daily insolation , vertical attenuation and P-I curves from the ICES incubator


Platt, Gallegos and Harrison
Prim. Produktie P/B/H
Intensity µE/m2/s
Pmax 3.576
Iopt 802.887
Ik 294.769
Helling 0.012
SSE 1.055


Platt, Gallegos and Harrison
Prim. Produktie P/B/H
Intensity µE/m2/s
Pmax 0.014
Iopt 1319.22
Ik 290.067
Helling 0.021
SSE 1.633

Experts to add missing figures and to number the above figures