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Part C

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Annex C-5

Phytoplankton primary production

Annex 2

Light measurements and intercalibration of standard ICES incubators (second draft)

ANNEX C-5 PHYTOPLANKTON PRIMARY PRODUCTION

ANNEX 2 LIGHT MEASUREMENTS AND INTERCALIBRATION OF STANDARD ICES INCUBATORS (SECOND DRAFT)

L.P.M.J. Wetsteyn[1], L. Edler[2], M.M. Steendijk[1], G.W. Kraay[3], F. Colijn[4] & R.N.M. Duin[5]

[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] Forschungs- und Technologiezentrum Westküste, Hafentörn, D-25761, Büsum, Germany.
[5] National Institute of Marine and Coastal Management (RIKZ), P.O. Box 20907, 2500 EX Den Haag, The Netherlands.

(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).

MATERIALS 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 $mmol.photons\text{-}m^{-2}.s^{-1}$) 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^o 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 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 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^{o}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
Exp1	A	B	C	D
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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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	

Table 2. Mean values, standard errors and coefficients of variation (defined as mean/standard deviation) of several measured parameters. pe=Ei- lers-Peeters model; jp=Jassby-Platt model; pgh=Platt-Gallegos-Harri-son 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	1.7	0.045	8
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
ape	0.089	0.0089	29.9
ajp	0.061	0.0027	13.4
apgh	0.076	0.0041	16

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 diffe- rent result when compared with the two other models). All values are mean values for the three users. Legend: see Table 2.

	ape	ajp	apgh
EXP2	0.109	0.068	0.0873
EXP1	0.094	0.062	0.0777
EXP3	0.064	0.055	0.0677
INC1	0.087	0.066	0.0827
INC3	0.104	0.064	0.082
INC2	0.076	0.054	0.068

Table 4. The results of the fourth user. * points to a very high value resul- ting 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	0.27	23.1
Pmaxpgh	2.142	0.357	28.9
Pobs	1.86	0.069	6.5
Ioptpe	*	*	*
Ioptpgh	180	67.9	65.4
Ikpe	54.6	21.5	68.2
Ikjp	63	22.6	62.2
Ikpgh	58.7	24.5	72.3
ape	0.051	0.0141	48.2
ajp	0.038	0.0094	42.4
apgh	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
a	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 (xoooooooox) or 10 TL tubes.
Figure 5. Absolute irradiance distribution with coated bottles and two 10 TL- sets parallel (P) and crossed (C).

Experts to add figures