*COOPERATIVE RESEARCH REPORT*

*NO. 170*

# *REPORT OF THE ICES $^{14}C$ PRIMARY PRODUCTION INTERCOMPARISON EXERCISE*

Hirtshals, Denmark

June 1987

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May, 1990

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# 1. List of Participants in Intercomparison Exercise and related Working Group Meetings

## Table 1. List of Laboratories Participating in Intercomparison Exercise

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U.S. Dept. of Commerce, NOAA, NMFS, *
Northeast Fisheries Center,
Sandy Hook Laboratory,
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The Danish Institute for Fisheries and Marine Research, *
Charlottenlund Castle,
DK-2920 Charlottenlund,
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Tvärminne Zoological Station, *
SF-10900 Hanko,
Finland

* Participated in both parts of the Intercomparison Exercise

Analysis of the data collected during the Intercomparison Exercise was carried out at the 1987 and 1988 Meetings of the ICES Working Group on Primary Production.

Participants in the 1987 Working Group Meeting (held in Copenhagen (2-4 Sept.) were:

| | |
|---|---|
| K. Richardson (Chairman) | (Denmark) |
| K. Gudmundson | (Iceland) |
| S. Demers | (Canada) |
| J-M Leppänen | (Finland) |
| K.L. Sjöblom | (Finland) |
| O. Lindahl | (Sweden) |
| E.G. Thelén | (Sweden) |
| E. Bagge | (Denmark |
| A. Moigis | (FRG) |
| P. Fritsche | (FRG) |
| P. Krischker | (FRG) |

Participants in the 1988 Working Group Meeting (held in Copenhagen (30 May - 2 June) were:

| | |
|---|---|
| K. Richardson (Chairman) | (Denmark) |
| F. Colijn | (Netherlands) |
| G. Kraay | (Netherlands) |
| M. Veldhus | (Netherlands) |
| T. Thórdardóttir | (Iceland) |
| S. Demers | (Canada) |
| J. Lenz | (FRG) |
| J-M. Leppänen | (Finland) |
| O. Lindahl | (Sweden) |
| M. J. Perry | (USA) |

The final report of the exercise was written by K. Richardson following guidelines approved at the 1988 meeting of the Working Group on Primary Productivity.

## 2. Introduction

Following Council Resolution 1986/2:34, an International Exercise for comparing results obtained using the $^{14}C$ Incorporation Method of measuring primary production was carried out during 1987 under ICES regie. The exercise was coordinated and organised by the Danish members of the Working Group on Primary Production (K. Richardson, G. Ærtebjerg Nielsen and L.M. Jensen) and was divided into two parts.

In the first part, 2 types of filters onto which a known concentration of $^{14}C$ containing phytoplankton had been filtered were distributed to 24 laboratories from 14 countries with instructions to determine the amount of $^{14}C$ associated with the filters using the normal procedures employed by each laboratory. At the same time, data from a "typical" North Sea station were circulated and the participants asked to calculate primary production using their own calculation procedures. In this manner, it was possible to compare the counting and calculation procedures specific to the individual laboratories prior to the comparison of experimental procedures which was carried out during the field exercises (Part II) of the Intercomparison. These field exercises were conducted in Hirtshals, Denmark onboard the R.V. Dana (Danish Fisheries Ministry) and in the North Sea Center Laboratories of the Danish Institute for Fisheries and Marine Research from 1-6 June 1987.

A list of laboratories participating in the Exercise is presented in Table 1. In order to participate, laboratories were asked to announce intent prior to 1 February 1987. Advertisement of the Intercomparison Workshop was made by word of mouth through ICES Delegates and Members of the Working Group. It should be noted, however, that a number of laboratories have, subsequent to the Intercomparison, expressed disappointment that they were not aware of the exercise and, thus, unable to participate. Organisers of future intercomparison studies carried out within ICES may want to consider more active advertisement of planned activities.

## 3. Description of Experiments

Part I: Filter and data distribution

### 3.1 Intercomparison of $^{14}C$ Determination by Participating Laboratories

Filters were prepared at the International Agency for $^{14}C$ Determination (Hørsholm) by automatically pipetting a given volume of a culture containing radioactive Isochrysis galbana (grown by introducing $^{14}C$ to the culture medium and following routine procedures for algae culture at the Danish Institute for Fisheries and Marine Research) onto Whatman GF/F and Sartorius cellulose nitrate membrane filters (0.2 $\mu$m). Each laboratory received 6 replicates of each set (treatment) of filters.

| Set | Treatment |
|---|---|
| 1 | Membrane filter/$^{14}$C/low quench |
| 2 | Membrane filter/$^{14}$C/high quench |
| 3 | Membrane filter: blank (no $^{14}$C) |
| 4 | GF/F/$^{14}$C/low quench |
| 5 | GF/F: blank |

All filters with $^{14}$C (with the exception of those sent to the laboratory 24) contained approx. 3500 DPM. "High Quenching" was obtained by filtering non-radioactive Isochrysis onto the experimental filters until the total algal concentration was 10-15 x higher on the high quench filters than on the low. Filters were acid fumed (5 min), dried (60°, 20 min) and packed in plastic containers (1 set filters/container) which were subsequently mailed to the participating laboratories.

## 3.2 Intercomparison of Calculation Procedures

The data presented in Table 3.1 were sent to all laboratories with instructions to calculate primary production in mg C $m^{-2}d^{-1}$.

**Table 3.1. Data and Instructions distributed for Intercalibration of methods for calculation of daily production**

It should be possible from the fictitious data-set below to calculate the daily primary production per $m^2$ water surface (mg C/$m^2$/day) using your normal calculation procedure. In this calculation, the given concentration of total $CO_2$ ($TCO_2$) should be used. No correction factors for isotope discrimination or respiration/reassimilation of marked substances should be used. If you normally calculate the production in more than 6 depths, it might be necessary to interpolate over depth in Table 3. If in situ (or simulated in situ) incubations are usually used in the calculation of daily production, the PI-curve given can be regarded as an in situ (or simulated in situ) incubation of water from 2.5 m depth for 2 hours from noon (12.00) to 14.00 at the irradiances given in Table 2, and with the bottles incubated at: 75%, 43%, 22%, 11%, 6.5%, 4.3%, and 2.2% light depths (0.8 m, 3.2 m, 6.4 m, 9.6 m, 12.2 m, 14.3 m, and 17.7 m). The daily irradiance is regarded as being symmetric with noon as the symmetry axis.

Table 3.1 cont.

The data was generated as follows:

Water was sampled from 6 depths at a station in the North Sea (55°19'N, 07°13.0'E) the 24th August 1986 and incubated for 2 hours (12.00 - 14.00) at optimum irradiances in an artificial light incubator, after adding $^{14}C$ activity to each 25 ml experimental bottle. The relationship between photosynthetic rate and irradiance (the PI-curve) was determined with water from 2.5 m depth. Dark fixation has been subtracted from the given DPM-values, which refer to a 2-hour incubation:

DATA-SET:

$TCO_2$ : 2.10 mM
pH : 8.14 (at all sampling depths)
$^{14}C$-activity added: 4 442 700 DPM (= 2.00 $\mu$Ci = 74.05 kBq)
Irradiance in incubator: 500 $\mu E\ m^{-2}\ s^{-1}$ (=3.01 x $10^{20}$ quanta $m^{-2}\ s^{-1}$)
Temperature in incubator: 16.8°C

Table 1. The PI-curve

| Irradiance $\mu E\ m^{-2}\ s^{-1}$ | 25 | 50 | 75 | 125 | 250 | 500 | 875 |
|---|---|---|---|---|---|---|---|
| DPM (per 2 hours) | 61 | 287 | 569 | 1108 | 2582 | 4068 | 3958 |

Table 2. The irradiance ($Ed_{(z=0)}$ just below the water surface (reflection subtracted) from noon to sunset*

| Time | 12-13 | 13-14 | 14-15 | 15-16 | 16-17 | 17-18 | 18-19 |
|---|---|---|---|---|---|---|---|
| Irradiance $\mu E\ m^{-2}\ s^{-1}$ | 1126 | 1199 | 1057 | 788 | 533 | 269 | 89 |

Table 3. Depth profiles

| Depth m | Irradiance % | Temp. °C | Salinity °/oo | $TCO_2$ mM | Potential production DPM per 2 hour | Chla mg $m^{-3}$ |
|---|---|---|---|---|---|---|
| 0.1 | 95 | 17.7 | 32.6 | 2.10 | 3822 | 1.29 |
| 0.8 | 75 | 17.7 | 32.6 | 2.10 | 3945 | 1.33 |
| 2.5 | 50 | 17.7 | 32.6 | 2.10 | 4068 | 1.37 |
| 5.7 | 25 | 17.4 | 32.9 | 2.10 | 3061 | 1.03 |
| 10.0 | 10 | 16.5 | 33.3 | 2.10 | 3572 | 1.23 |
| 18.2 | 2 | 15.7 | 33.3 | 2.10 | 2578 | 0.81 |

Part II: Field Exercises: Intercomparison of experimental procedures.

The experiments carried out onboard the R.V. Dana and by the group at the North Sea Center are summarized in Table 3.2. Dana experiments are denoted by numbers whereas North Sea Center experiments have been called by letters. The common method referred to in Dana experiments 1, 8 and 9, and North Sea Center experiments C and D is presented in Appendix I. This method was taken from the Guidelines for the Baltic Monitoring Program for the Second Stage 1983 (Pers.com. G. Ærtebjerg Nielsen). Details of the experimental procedures will not be presented here. Information relevant to interpreting the results, however, is presented in footnotes to the table.

## 3.3 Dana Experiments

Experiments onboard Dana were carried out at an anchor station (57°37'N, 10°54'E) where the depth was 28 m. The station was salinity and temperature stratified throughout the study period. Surface salinity ranged from 21.80 - 22.19 °/oo during the study. Bottom salinity ranged from 32.88 - 33.44 °/oo. Surface temperatures ranged from 11.65° - 12.56°C. A sharp pycnocline occurred at ca. 8 m. Fluorescence profiles showed higher chlorophyll concentrations above the pycnocline than below but, in most profiles, there was no evidence of a subsurface chlorophyll maximum. Chlorophyll determinations made below the pycnocline showed concentrations of 0.2 - 0.3 mg chl*a* $m^{-3}$. Above the pycnocline, values ranged from 0.5 - 1.1 mg chl*a* $m^{-3}$. On one occasion, a value of 2.2 mg chl*a* $m^{-3}$ was recorded at the pycnocline. Microscopic examination revealed that the phytoplankton was comprised primarily of small flagellates.

## 3.4 North Sea Center Experiments

Sea water for the experiments A B and C was collected from an outdoor 92 $m^3$ experimental tank. The salinity was about 33.2 °/oo and the temperature between 12 and 13°C. The phytoplankton concentration was rather low (0.5 - 1.2 mg Chl*a* $m^{-3}$) and consisted mainly of dinoflagellates and small flagellates. Approx. 30 min before the experiments, water was collected from the tank and brought to the laboratory. The water was stirred by bubbling with air. The bottles were washed with the experimental water prior to filling by dipping them in the water. All experiments were made with artificial light incubators from the five different laboratories that participated in this part of the intercomparison exercise.

In order to compare the results obtained by the Dana and North Sea Center Groups, a water sample was collected and stored (10°C) onboard Dana until return to harbour. Approximately 18 h after collection, the sample was divided between the North Sea Center and Dana Groups and primary production determinations were made (Experiments 8, 9, D). At the time of these determinations, clorophyll*a* concentration in the water sample was 0.5 mg $m^{-3}$.

For all experiments, only $^{14}C$ incorportion in the particulate fraction was examined. Filters were placed in scintillation vials at the end of experiments and flown to the International Agency for $^{14}C$ Determination (Hørsholm, Denmark) where scintillation fluid was added and the incorporated radioactivity determined.

**Table 3.2. Experiments carried out in the Intercomparison Exercise**

Dana Experiments

| Date | Experiment |
|---|---|
| 1/6 | 1. Comparison of light saturated rates of photosynthesis (Pmax) generated using a common method (a) |
| 2/6 | 2. Comparison of daily or hourly production rates using own method, own incubator and own $^{14}C$ a. Pooled sample. b. Non-pooled sample (b) |
| | 3. Comparison of P vs. I and Pmax on a pooled sample generated using two different natural light incubators (c) |
| | 4. Determination of the effect of isotope source on Pmax |
| 3/6 | 5. Comparison of daily or hourly production rates on a pooled sample determined using own methods but with a common isotope addition |
| | 6. Determination of the residual $^{14}C$ after acidification in the different isotopes (d) |
| | 7. Effect of incubation length on the determination of photosynthetic rates |
| 4/6 | 8. Comparison of P vs. I curves generated using a common method and the same water sample as used in the North Sea Center Expt. D |
| | 9. Comparison of Pmax generated using a common method and the same water sample as in the North Sea Center Expt. D |
| | 10. Examination of reproducibility - 6 replicate measurements conducted by 1 laboratory |
| 2-4/6 | 11. Comparison of light measurements made by different laboratories through the water column |
| 4/6 | 12. Comparison of light measurements made by different laboratories in air |

Table 3.2 cont.

North Sea Center Experiments

2/6 A. Comparison of P vs. I curves generated using own methods; replicates of light saturated photosynthesis (Pmax) (e)

B. Determination of post-"filtration effect handled by one investigator until the end of the incubation phase; each laboratory received four replicated and applied their own post-filtration methods) (f)

3/6 C. Comparison of P vs. I curves generated from four hour incubations using a common method; replicates of Pmax (g)

4/6 D. Comparison of P vs. I curves generated from 2 hour incubations using a common method and replicates of Pmax (see Dana Expts. 8 and 9) (h)

*(a) The limited number of artificial light incubators onboard Dana necessitated that laboratories 4, 17, 18, and 23 used the same artificial light incubator.*

*(b) Participating laboratories were grouped according to the methods used. Each group then conducted a separate water cast. All casts were made between 0615-0759 GMT.*

*Laboratories 4, 8, 17 and 18 incubated in natural light (8+18 in the same incubator) while the remaining labs used fluorescent light incubators.*

*(c) Laboratory 17 used colored (blue) filters to attenuate light in a deck incubator. A similar deck incubator was used by laboratory 18 with the exception that neutral density filters were used to attenuate light.*

*(d) The residual activity in four replicates from each of five 14-C bicarbonate working solutions was determined. The bubbling apparatus was acid cleaned and rinsed several times with deionized water prior to this test for residual activity. Approximately 20 µCi 14-C bicarbonate from each stock were added to glass liquid scintillation vials using a calibrated Eppendorf micropipette. Ten ml deionized water were added. Each vial had 0.5 ml of 0.1 N HCl added to it. Then the twenty samples were purged with 100 cc air vial$^{-1}$ min$^{-1}$ for 60 minutes. Ten ml INSTA-GEL (Packard Inst. Co.) was added to each vial following bubbling.*

*(e) Comparison of PI curves that were measured using the usual method and incubator of each participating laboratory. The Finnish standard method of determining only the Pmax value at 20°C was also included in this experiment. Table 4 describes the methods used by each laboratory.*

*Table 3.2. cont.*

*(f) 20 samples were simultaneously incubated in the incubator of lab 22. Following incubation, each laboratory received 4 samples and conducted their standard post-incubation treatment.*

*(g) Comparison of the PI curves that were measured with each of the incubators, but using the common method (Appendix I). The Finnish standard method was also included but at in situ temperature.*

In the text of the Report, the results of the experiments have been grouped in order to address the following:

1. The between and within laboratory precision of $^{14}C$ determination

2. The variability (and causes thereof) in the determination of Pmax (light saturated rate of photosynthesis) within and between laboratories and

3. The variability (and causes thereof) in the estimation of daily primary production rates between laboratories.

The implications of the results of this intercomparison exercise for the use of primary production measurements as a tool for monitoring changes in the environment as well as some problems associated with the establishment of a data bank for primary production measurements are dealt with in the conclusion of the Report.

## 4. $^{14}C$ Counting Precision

In order to compare results of $^{14}C$ determination made by the different laboratories, the average of the counts obtained on the 6 replicates of each filter type where $^{14}C$ had not been added was assumed to represent the blank (including background) for each laboratory. The blank value (DPM) for the respective filters was then subtracted from the DPMs recorded for those filters onto which $^{14}C$ containing algae had been filtered.

These results are plotted in Figures 4.1-4.3. One laboratory made their determinations using a modified Geiger Counter; all others employed Liquid Scintillation Counting (LSC). The results returned by laboratory 7 lie considerably above the mean. Some months later, this laboratory sent "revised results" explaining that the original results had been obtained on an improperly calibrated machine. The revised results resembled much more the results obtained by all other laboratories. These revised results were, however, received after the 1987 Working Group Members had had access to the data from this experiment. No representative from laboratory 7 was at the 1987 WG Meeting. However, while not meaning to imply the existence of a "leak" from the 1987 WG

**Fig. 4.1 Radioactivity associated with membrane filters**
(DPM - blank; see text)

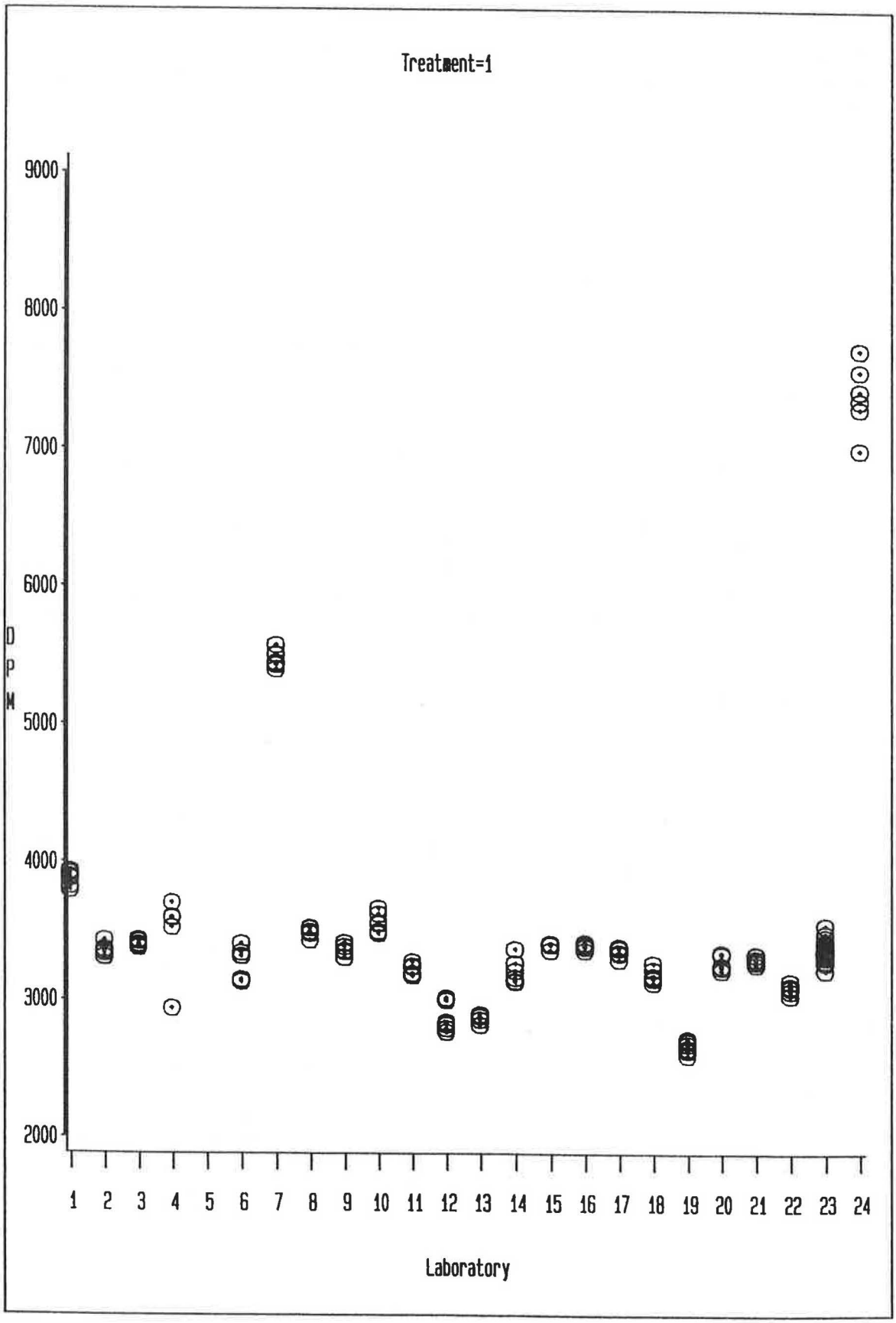


**Fig. 4.2 Radioactivity associated with membrane filters in the presence of a quenching agent**
(DPM - blank; see text)

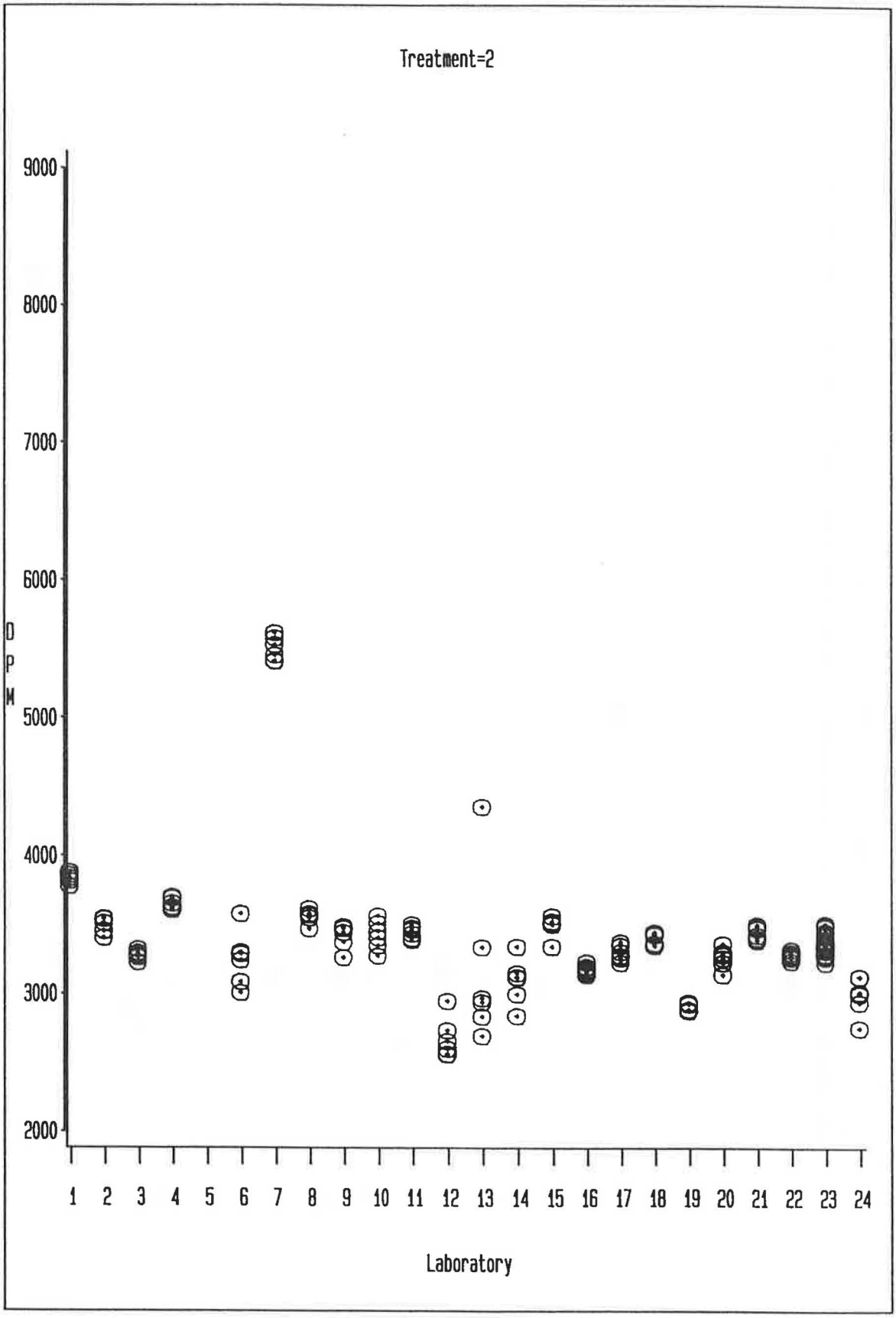
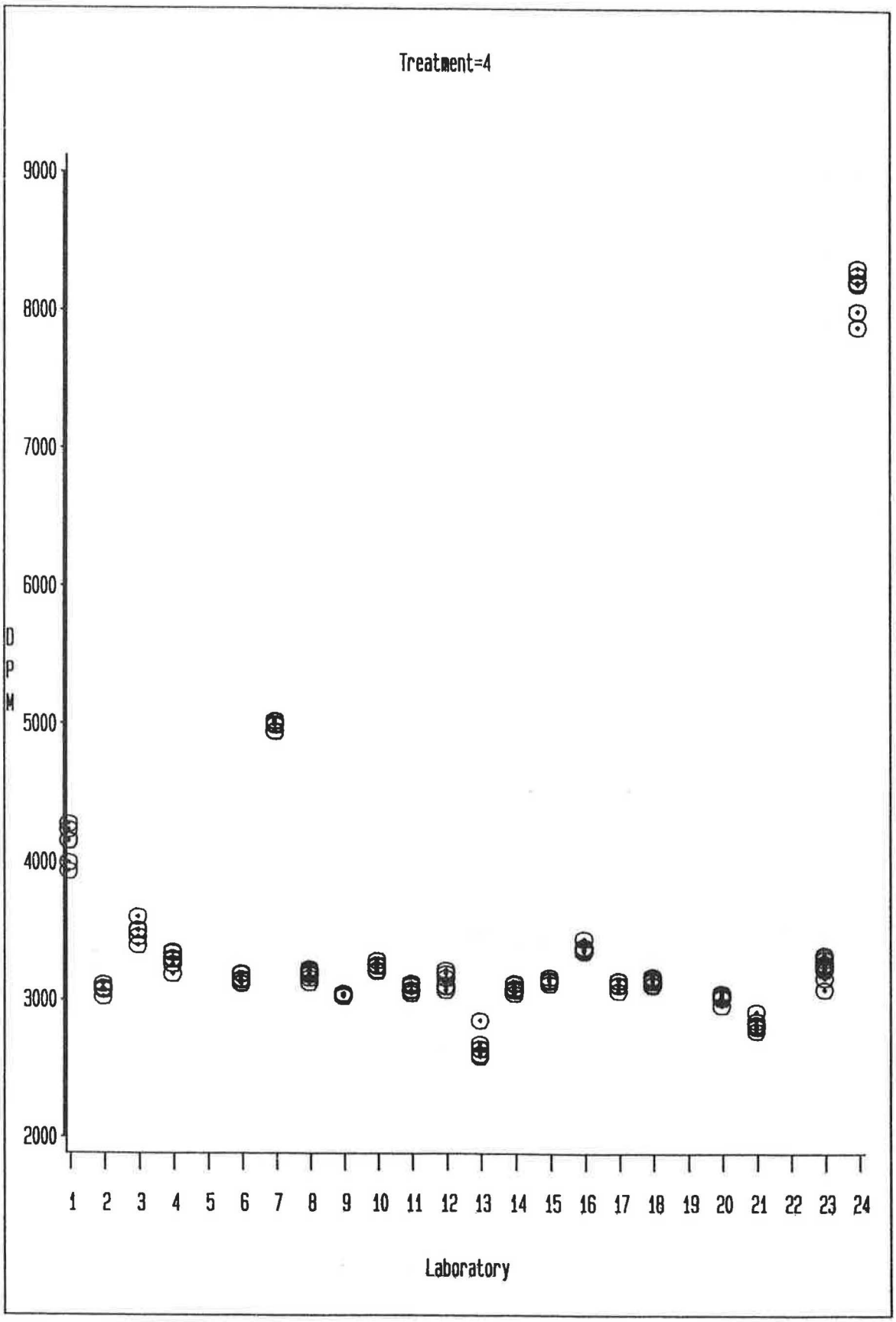


**Fig. 4.3 Radioactivity associated with GFF filters**
(DPM - blank; see text)



meeting, the WG members present at the 1988 meeting decided that the most proper way to deal with Laboratory 7's data was to omit it from the statistical analysis. Therefore, the originally submitted results have been included in the figures but data from Laboratory 7 are not included in the statistical analysis. Laboratory 24 forwarded the filters they received to another laboratory. A second batch of filters was then made and sent to Laboratory 24. However, in view of the statistical problems involved when 2 batches of filters are considered, data from Laboratory 24 have also been omitted. From the figures, it can be seen that this laboratory recorded considerably lower DPMS/filter in the presence of a quenching agent. For other laboratories, the quenching effect, while significant, was not so dramatic.

In the final statistical analysis, the natural log of (DPM-blank) for each replicate and treatment was used. The log transformation of the data was used as the possibility of a multiplicative relationship between the various effects (laboratory, filter type, quenching) was assumed. Statistical analysis was carried out using SAS GLM Procedure.

The results of the analysis (Table 4.1) indicate that laboratory, filter type and quenching are all significant sources of error in the results reported. However, the laboratory effect is large compared to both filter type and quenching.

**Table 4.1**

| Source of Variation | Degrees of Freedom | Sum of squares | F value | Pr > F |
|---|---|---|---|---|
| LABxFILTER | 18 | 0.18968470 | 11.35 | 0.0001 |
| LABxQUENCH | 20 | 0.13884705 | 7.48 | 0.0001 |
| LAB | 20 | 1.37206627 | 73.88 | 0.0 |
| FILTER | 1 | 0.12862910 | 138.52 | 0.0001 |
| QUENCH | 1 | 0.01101426 | 11.86 | 0.0006 |

The latter two sources of error are interesting in that no consistent pattern can be observed between laboratories. Thus, the recorded differences recorded between laboratories cannot be corrected for by distributing standards for all laboratories unless filter type and quench correction procedures are also standardized.

## 5. The variability associated with the Determination of Pmax using $^{14}C$

### 5.1 $^{14}C$ Incorporation recorded at Pmax using a standard experimental procedure

A standard "cookbook" method (see Appendix I) of carrying out primary production determinations was applied by the different laboratories to pooled sample material on three different

occasions during the course of the intercomparison Exercise: North Sea Center Expt C, Dana Expt 1 and North Sea Center Expt D combined with Dana Expt 9. Analysis of the data (DPMS recorded for the incubation bottles incubated at photon flux densities above which Pmax was achieved) was carried out using SAS (GLM procedure).

The means, STD, and number of replicates recorded for the different laboratories in each of these experiments are shown in Tables
5.1-5.3.

**Table 5.1. Results from use of standard method on pooled water sample (North Sea Center Expt C)**

| LAB | DPM | | |
|---|---|---|---|
| | MEAN | STD | N |
| 10 | 3763.50 | 648.99 | 4.00 |
| 14 | 6186.71 | 308.54 | 7.00 |
| 15 | 6681.20 | 127.14 | 5.00 |
| 22 | 4358.00 | 342.63 | 5.00 |
| 24 | 3944.14 | 245.65 | 7.00 |

**Table 5.2. Results from use of standard method on pooled water sample (Dana Expt 1)**

| LAB | DPM | | |
|---|---|---|---|
| | MEAN | STD | N |
| 4 | 1880.00 | 302.64 | 2.00 |
| 8 | 1588.50 | 96.87 | 2.00 |
| 9 | 475.00 | 123.04 | 2.00 |
| 17 | 2394.00 | 390.32 | 2.00 |
| 18 | 2247.00 | | 1.00 |
| 20 | 1523.50 | 113.84 | 2.00 |
| 23 | 2265.00 | 120.21 | 2.00 |

**Table 5.3. Results from use of standard method on pooled water sample (North Sea Center Expt D and Dana Expt 9)**

| LAB | DPM | | |
|---|---|---|---|
| | MEAN | STD | N |
| 4 | 1362.50 | 33.23 | 2.00 |
| 8 | 1049.00 | 8.49 | 2.00 |
| 10 | 484.80 | 186.01 | 5.00 |
| 14 | 1914.14 | 678.39 | 7.00 |
| 15 | 1864.80 | 70.73 | 5.00 |
| 17 | 1204.50 | 4.95 | 2.00 |
| 18 | 1321.50 | 61.52 | 2.00 |
| 20 | 1310.00 | 204.70 | 6.00 |
| 22 | 1466.00 | 62.18 | 6.00 |
| 23 | 1577.50 | 208.60 | 2.00 |
| 24 | 911.83 | 243.17 | 6.00 |

For all three experiments, there was a significant difference between the results produced by the different laboratories (Experiments C and D/9 $p<.001$; Experiment 2 $p<.0016$). The Coefficient of Variation (%) reported for the Experiments C, 1 and D/9, were 25, 38, and 40%, respectively. It was suggested during the exercise that a major source of the variation reported may have been the fact that the laboratories had not previously employed the "cookbook" method. However, as the variation in the results reported the second time the laboratories attempted the "cookbook" method (Expt D/9) was not less than after the first attempt, lack of practice in the method would seem unlikely to be a major source of error.

5.2 Incubator effect in standard method

Since the "cookbook" method employed is based on incubations at fixed photon flux densities, the three laboratories with "simulated in situ" incubators working onboard Dana were unable to use their own incubators for this part of the Intercomparison Exercise. These laboratories were asked to conduct the experiment using their own filtration equipment, etc. but using the incubator permanently placed onboard Dana (standard incubator supplied by The International Agency for $^{14}C$ Carbon Determination, Hørsholm, Denmark - temperature controlled water bath, warm white fluorescent tubes, incubation bottles fixed to a rotating wheel). Thus, these three laboratories together which the laboratory already assigned to Dana's incubator used the same incubator for experiments 1 and 9.

Examination of results obtained from these four laboratories (Tables 5.4 and 5.5) indicates a coefficient of variation (%) of 14% for Expt 1 and 12% for Expt 9 and no significant difference between the results reported by the different laboratories could be demonstrated. This suggests that the "incubator effect" in the use of a "standard" method may be quite significant. However, more comprehensive studies in which a larger number of laboratories carry out incubations in both their own and a common incubator are necessary in order to quantify the incubator effect.

**Table 5.4 Dana Experiment 1**

| LAB | DPM | | |
|---|---|---|---|
| | MEAN | STD | N |
| 4 | 1880.00 | 302.64 | 2.00 |
| 17 | 2394.00 | 390.32 | 2.00 |
| 18 | 2247.00 | | 1.00 |
| 23 | 2265.00 | 120.21 | 2.00 |

**Table 5.5 Dana Experiment 9**

| LAB | DPM | | |
|---|---|---|---|
| | MEAN | STD | N |
| 4 | 1362.50 | 33.23 | 2.00 |
| 17 | 1204.50 | 4.95 | 2.00 |
| 18 | 1321.50 | 61.52 | 2.00 |
| 23 | 1577.50 | 208.60 | 2.00 |

5.3 Reproducibility of measurements made following the standard method

When conducting the standard method, the labortories working at the North Sea Center carried out between 4-7 replicates which gives the opportunity to examine reproducibility in the results produced. The number of replicates carried out by and the resulting coefficient of variation (CV%) for the respective laboratories is shown below.

**Table 5.6. Number of replicates (N) and coefficients of variation (CN%) for results obtained by different laboratories when employing the standard method.**

| | LAB | N | CV% |
|---|---|---|---|
| Expt C | 10 | 4 | 6.4 |
| Expt D | 10 | 5 | 2.8 |
| Expt C | 14 | 7 | 5.0 |
| Expt D | 14 | 7 | 3.5 |
| Expt C | 15 | 5 | 1.9 |
| Expt D | 15 | 5 | 3.8 |
| Expt C | 22 | 5 | 7.9 |
| Expt D | 22 | 6 | 4.2 |
| Expt C | 24 | 7 | 6.2 |
| Expt D | 24 | 6 | 26.7 |

In addition, laboratory 20 (working onboard Dana), carried out 6 replicates when conducting the standard method for the second time (Experiments 8 and 9 in Table 3.2). The coefficient of variation recorded for these results was 15.6%

5.4 Effect of $^{14}C$ source on primary production measurements

Examination of the residual radioactivity remaining after acid

bubbling (Expt 6) in the five different $^{14}CO_2$ stocks[1] onboard Dana (supplied by laboratories 4, 8, 17, 18 and 23) indicated a range from ca. 5 to 178 DPM per $\mu$Ci added.

When a single operator conducted the standard method (incubating all samples in the same incubator), adding the $^{14}CO_2$ supplied by the 7 different laboratories to water samples (Expt 4), no significant difference between the DPMS fixed per $\mu$Ci added at Pmax or in darkness (Table 5.7) could be demonstrated. Two replicates were carried out at Pmax and in darkness with all isotope solutions.

**Table 5.7. DPMs fixed per $\mu$Ci originally added to incubation bottles for incubations taking place at Pmax and in darkness. Lab is the laboratory which supplied the $^{14}CO_2$**

| | DPM fixed | |
|---|---|---|
| LAB | Pmax | Dark |
| 20 | 279.3 | 22.3 |
| 20 | 337.1 | 25.3 |
| 18 | 236.6 | 26.7 |
| 18 | 277.8 | 29.9 |
| 8 | 262.5 | 41.4 |
| 8 | 287.3 | 30.0 |
| 9 | 307.9 | 20.6 |
| 9 | 320.7 | 16.9 |
| 17 | 313.2 | 28.4 |
| 17 | 294.6 | 53.4 |
| 23 | 234.8 | 18.6 |
| 23 | 244.9 | 16.4 |
| 4 | 289.6 | 27.4 |
| 4 | 310.3 | 22.8 |

[1] 7 laboratories actually worked onboard Dana. However, some of them used different batches of $^{14}CO_2$ from the same source. Residual activity (Expt 6) was only determined in one $^{14}CO_2$ coming from different sources. In Experiment 4, the $^{14}C$ supplied by all laboratories was used.

## 5.5 Pmax and daily primary production determined using own method on a pooled sample

When laboratories employ their own methods with varying sample size, $^{14}C$ addition, incubation length, etc., it is no longer possible to directly compare incorporated DPMS between laboratories. Therefore, in this and Section 5.7 which deals with own methods including water sample collection, it is calculated hourly production at Pmax (mg C $m^{-3}hr^{-1}$) and, where possible, calculated daily production (mg C $m^{-2}d^{-1}$) that are compared. Onboard Dana, where laboratories 17 and 18 made simulated *in situ* incubations throughout the whole daylight period, the calculation of an hourly Pmax is meaningless. Therefore, these two laboratories have only reported Daily Primary Production. Of the laboratories using artificial light incubators, laboratories 8 and 23 used the P vs I curves generated together with the measured light extraction coefficient in the water column to estimate the Daily Primary Production. Results from the Dana experiment are shown in Table 5.8.

**Table 5.8. Hourly production at Pmax and daily primary production determined by applying own method to a pooled water sample (Expt 2a)**

| Laboratory | Pmax mg C $m^{-3}h^{-1}$ | Daily prod. mg C $m^{-2}d^{-1}$ | |
|---|---|---|---|
| 8 | 2.55 | 168.2 | ** |
| 20 | 2.02 | | |
| 18 | | 223 | ** |
| 23 | 2.45 | 313.5 | |
| 17 | | 347 | ** |
| 9 | 3.35 | | |
| 4 | 1.78-2.03*** | | |
| | see A and B below | see C below | |

| | A (using the shortest incubation time of lab. 4) | B (using the longest incubation time of Lab. 4) | C |
|---|---|---|---|
| X = | 2.43 | 2.48 | 262.93 |
| STD = | 0.60 | 0.54 | 82.04 |
| CV = | 24.79% | 21.87% | 31.20% |

** Simulated *in situ* light incubator
*** 4 incubation times (0.7; 1.1; 2.2; 4.1 hours)

At the North Sea Center, where all laboratories used articifial light incubators, all laboratories calculated hourly production (mg C fixed $h^{-1}$ at Pmax). The results from the North Sea Center are shown in Table 5.9.

**Table 5.9. Hourly production at Pmax determined by applying own method to a pooled water sample (North Sea Center A)**

| Laboratory | Pmax mg C $m^{-3}h^{-1}$ |
|---|---|
| 10 | 1.75 |
| 14 | 2.52 |
| 15 | 2.26 |
| 22 | 3.03 |
| 24 | 2.58 |
| X = | 2.43 |
| STD = | 0.47 |
| CV = | 19% |

The data base for these two experiments is too small to allow detailed statistical analysis. However, both at the North Sea Center and onboard Dana, estimates of hourly production varied by a factor of ca. 1.8.

The estimates of daily production carried out onboard Dana varied by over a factor of 2. This greater discrepency in daily production estimates compared to hourly presumably results from the application of different models used to convert P vs I curves to daily water column production (see Section 6.1) and/or problems associated with light measurement (Section 6.3).

The potential influence of post-incubation treatment (filtration, removal of excess $^{14}CO_2$, placement of filters in scintillation vials) when employing "own method" is examined in the next section.

## 5.6 Effect of post-incubation procedures on Pmax determinations using own method

In North Sea Center Experiment B, a single operator performed all procedures in the standard method on 20 replicates using a pooled sample. The 20 bottles were incubated in laboratory 22's incubator (see Appendix II). Following the incubation, 4 bottles were randomly selected and given to the 5 laboratories working at the North Sea Center. These labortories were asked to complete the procedures associated with primary production measurement (filtration, removal of incorporated $^{14}CO_2$ and placing of filters in scintillation vials) as they normally would when employing their own methods. As for all other experiments, the scintillation vials were then flown to the International Agency

for $^{14}C$ Determination where scintillation fluid was added and LSC counting carried out.

There was a highly significant (P<.001) difference demonstrated in the reported results (Table 5.10) and the coefficient of variation was 22%. This suggests that post-incubation procedures may contribute significantly to the differences in the results reported when different laboratories carry out primary production measurements using their own methods.

**Table 5.10 Effect of Post-incubation treatment on recorded Pmax**

| LAB | DPM | | | |
|---|---|---|---|---|
| | MEAN | STD | N | CV% |
| 10 | 535.00 | 48.61 | 4.00 | 9.1 |
| 14 | 685.50 | 22.16 | 4.00 | 3.2 |
| 15 | 883.00 | 154.79 | 4.00 | 6.5 |
| 22 | 623.00 | 42.23 | 4.00 | 6.1 |
| 24 | 588.25 | 86.79 | 4.00 | 14.7 |

The coefficient of variation recorded for the results from the different laboratories is of the same order of magnitude as that observed during the trials with the standard method (Section 5.3).

## 5.7 Hourly production at Pmax and Daily Primary Production determined using own method including water sample collection

Onboard Dana, it was possible to ask the participating laboratories to collect water samples at depths that they normally would select when conducting primary production measurements. To help in the selection process, all participants had access to light, CTD and fluorescence profiles made on the station. When the individual participants had selected the depths where they wanted samples, they were grouped together so that water collection could be accomplished with three water casts (Table 5.9).

Following water collection, all laboratories conducted primary production determinations (hourly and/or daily production) after their own methods (Dana Expt 2b). The results from these determinations are presented in Table 5.11. Note that Laboratories 17 and 18 used different incubation times. Therefore, the results from this experiment are not included in the analysis of the effect of spectral quality on the

determination on $\alpha$ (Section 6.4).

**Table 5.11. Hourly and/or Daily Primary Production determined using own method including water sample collection (Dana Expt 2b)**

| Laboratory | Water cast | Pmax (mg C $m^{-3}h^{-1}$) | Pmax mg chlorophyll_a $m^{-3}$ | Daily production mg C $m^{-2}d^{-1}$ |
|---|---|---|---|---|
| 8 | 1 | 2.84 | 4.5 | 264.5 |
| 17 | 1 | | | 290 |
| 18 | 1 | | | 286 (305)* |
| 4 ( 3 m) | 2 | 0.91-1.18** | 1.4 | |
| 23 ( 3 m | 2 | 1.33 | 1.6 | |
| 9 ( 5 m | 2 | 2.96 | 3.9 | 417 |
| 4 ( 8 m | 2 | 3.32-4.34** | 6.8 | |
| 23 ( 8 m | 2 | 4.99 | 7.8 | |
| 9 (15 m) | 2 | 0.41 | 1.4 | |
| 20 | 3 | 2.35 | 4.2 | |
| | X = | 2.47 | 3.95 | |
| | STD = | 1.52 | 2.44 | |
| | CV = | 62 | 62% | |

* (including DOM activity)
** 4 incubation times (0.4; 0.8; 1.1; 2.2 h)

Watercast 1: Water taken at 1,2,4,7,13 and 20 m and incubated at photon flux densities corresponding to those where samples were taken

Watercast 2: Water taken at 3,5,8 and 15 m and incubated at different photon flux densities

Watercast 3: Water taken 0,2,5,10,15 and 20 m samples incubated in full incubator in full incubator light

The hourly production at Pmax reported by the different laboratories varied from 0.41-4.99 mg C $m^{-3}$. Of course, owing to differences in chlorophyll concentration through the water column, it is not strictly fair to compare Pmax hourly production from different depths. It has been done here in an attempt to ascertain what effect letting individual researchers select the depth where primary production determinations should be made would have on the accuracy of the Pmax data reported to a hypothetical data bank. It should be noted from the Table, however, that normalizing the hourly production at Pmax to the chlorophyll concentration at the given depth does not, in this case, lessen the variability in the reported results.

The daily production measurements carried out by Laboratories 8 (using Lab 18's incubator), 17 and 18 appear to agree well with one another. However, on two other occasions (Expt 3 and Expt 5) when laboratories 17 and 18 compared daily production rates (after applying the same incubation time and on pooled samples), the agreement between the two laboratories was not nearly as good as for this experiment:

**Table 5.12**

| Expt | LAB | Daily Production mg C $m^{-2}d^{-1}$ 1) |
|---|---|---|
| 3 | 17 | 328 |
| | 18 | 186 |
| 5 | 17 | 347 |
| | 18 | 223 |

Thus, the reproducibility of daily primary production measurements carried out using simulated in situ incubators and long (½ or whole light period) incubations may not be as good as the results presented in Table 5.9 suggest.

## 6. The variability associated with the Calculation of Daily Primary Production Rates from $^{14}C$ Incorporation Data

For most purposes, primary production is expressed in units of g C fixed $m^{-2}d^{-1}$ or $y^{-1}$. Determinations of primary production are, however, usually made over a period of one to several hours as "bottle effects" can interfere with incubations made over a longer period. As a rule, these short-term incubations are made at different photon flux densities so that the photosynthetic response of the phytoplankton under different light regimes can be plotted. The time of day at which samples are taken and incubated will affect the shape of the photosynthesis (P) vs light (I) curve and some workers attempt to correct for this problem by always taking their samples at the same time of day. Another strategy for avoiding the influence of diurnal effects is to incubate large volumes in natural light over the entire light period. The time constraints imposed on this inter-comparison exercise did not allow detailed consideration of diurnal variations in the P vs I curve and as this potential source of error in production estimates has been well studied, the reader is referred elsewhere for a discussion of the topic (i.e. Neale and Richerson, 1987; Glover et al. 1985; Kana et al. 1985; Putt and Prézelin 1985; Harding et al. 1981; Doehler and Rosslenbroich 1979; Gargas et al. 1979).

1) For production profiles through the water column for these two experiments, see Figures 6.3 and 6.4.

Once a P vs I curve has been established, if the light attenuation coefficient through the water column and the incident light throughout the day are known to apply the known photosynthetic characteristics to the light climate at various depths in the water column over the entire day. A number of different models for converting P vs I curves to estimates of total daily production have been developed.

In the following section, the results obtained by applying different models to a given P vs I curve are compared.

## 6.1 Application of different models for estimating daily production to a common data set

Sixteen laboratories reported the estimated daily production calculated from the distributed P vs I data (Table 3.1). Different methods of calculating primary production were used. Six laboratories used the "Baltic method" (Ærtebjerg Nielsen and Bresta, 1984). Four laboratories used methods closely related to the method recommended by ICES (Anon., 1987). Among the rest of the laboratories, four used only the P vs I dataset given and not the "Pmax" or chlorophyll data given for other depths. Two laboratories used special corrections: Lab. 8 subtracted the positive intercept of the PI-curve (5%), and lab. 17 subtracted 7%.

Laboratories 17 and 18 estimated from the PI-dataset, the in situ production per square meter for the period 12.00-14.00 hours and estimated the daily production by multiplying by the ratio between the whole day irradiance and the irradiance within the period 12.00-14.00 hours. All other laboratories estimated the irradiance and production at different depths and hours of the day and finally integrated over time and depth.

The results of the estimation of daily production from a common dataset are shown in Fig. 6.1. No statistically significant difference could be demonstrated between the productivity results submitted by the various laboratories. However, the coefficient of variation exhibited in the results was ca. 10%. The results obtained by laboratories using the "Baltic", ICES, and "other" methods of calculating daily primary production have also been pooled and plotted separately (Fig. 6.2). The Baltic method appears to give a somewhat higher (ca. 15%) daily production than the ICES (and all other) methods.

Although primary production is usually expressed in units of g $m^{-2}d^{-1}$, the variability demonstrated here to be associated with calculating daily production from incubation data would argue for submitting raw incubation data to an eventual data bank for primary productivity. This would allow potential users of the data to select and apply the model they find most appropriate for calculating daily production and eliminate the primary production model employed as a source of error in the reported data.

**Fig. 6.1**

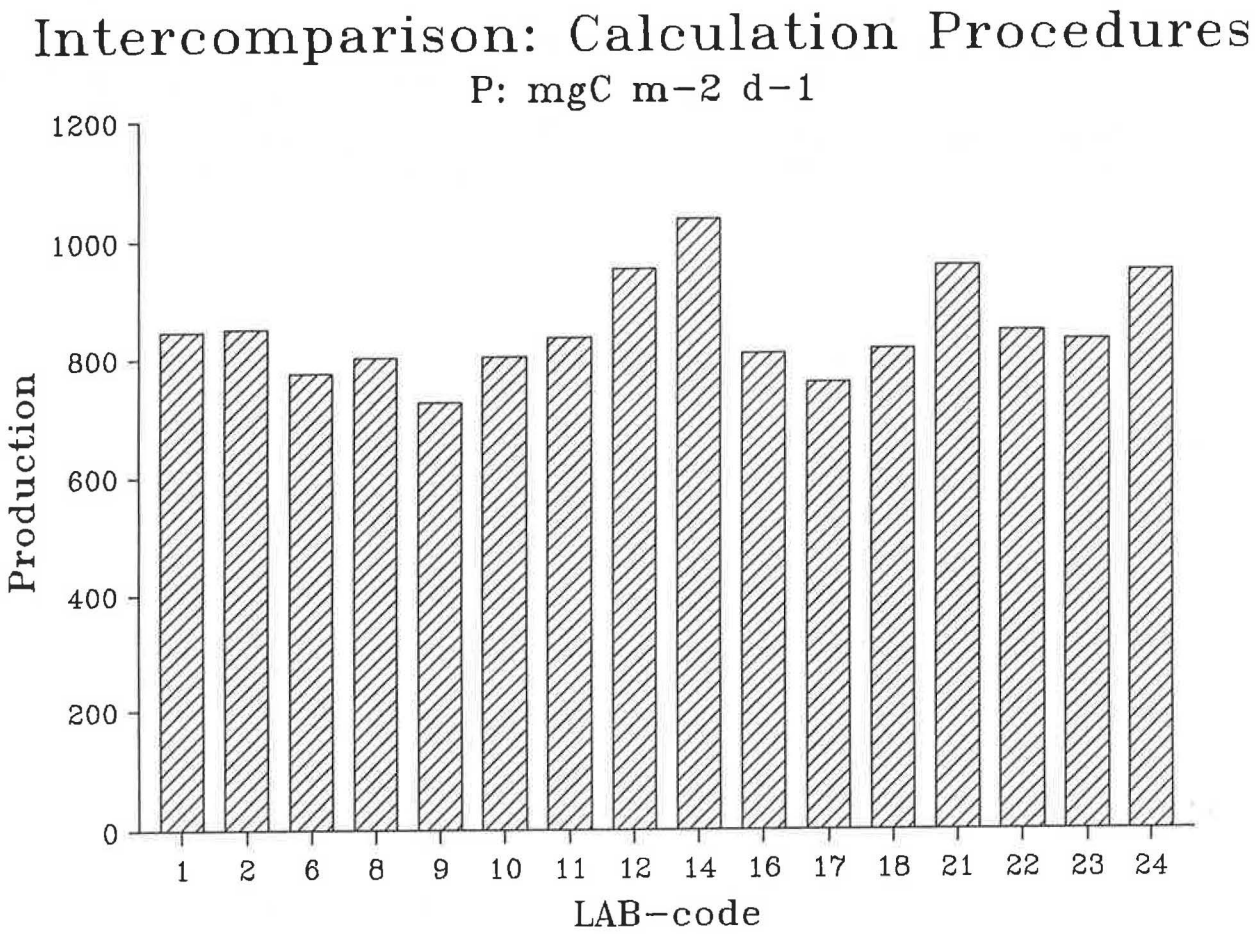
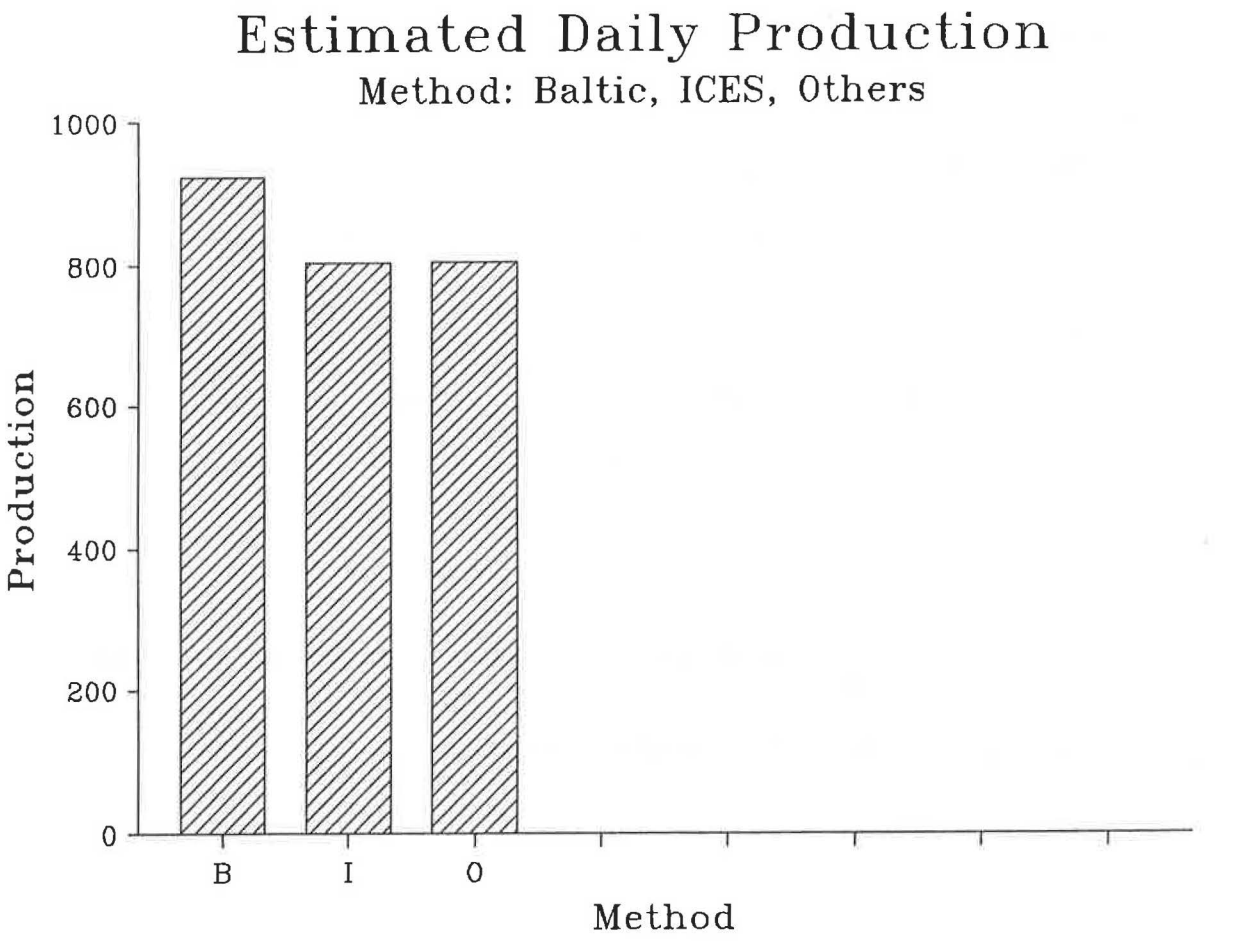

Intercomparison: Calculation Procedures
P: mgC m-2 d-1
Production
1200
1000
800
600
400
200
0
1 2 6 8 9 10 11 12 14 16 17 18 21 22 23 24
LAB-code

**Fig. 6.2**


Estimated Daily Production
Method: Baltic, ICES, Others
Production
1000
800
600
400
200
0
B I O
Method

## 6.2 Variability in α as compared to Pmax

In order to compare the variability associated with $\alpha^*_1$ and $Pmax^*_2$, P vs I curves have been generated after the method described by Gargas and Hare (1986) for data collected in North Sea Center experiments A, C and D taken together with Dana experiments 8 and 9. The results of this comparison are shown in the Table below:

Table 6.1

| | Expt. A | | Expt. C | | Expt. D and 9 | |
|---|---|---|---|---|---|---|
| LAB | Pmax | $\alpha \times 10^{-2}$ | Pmax | $\alpha \times 10^{-2}$ | Pmax | $\alpha \times 10^{-2}$ |
| 4 | | | | | 1.68 | |
| 8 | | | | | 1.49 | |
| 10 | 1.75 | 0.82 | 3.34 | 1.66 | 0.99 | 0.59 |
| 14 | 2.52 | 1.74 | 5.33 | 3.10 | 2.86 | 1.85 |
| 15 | 2.26 | | 5.43 | | 3.03 | |
| 17 | | | | | 1.58 | 1.70 |
| 18 | | | | | 1.88 | 0.54 |
| 20 | | | | | 1.83 | 1.40 |
| 22 | 3.03 | 3.36 | 3.71 | 3.87 | 2.52 | 1.31 |
| 23 | | | | | 2.24 | |
| 24 | 2.58 | 1.09 | 3.34 | 1.37 | 1.56 | 0.61 |
| N | 5 | 4 | 4 | 4 | 11 | 7 |
| X | 2.43 | 1.75 | 4.23 | 2.50 | 1.97 | 1.14 |
| SD | 0.47 | 1.14 | 1.06 | 1.19 | 0.63 | 0.56 |
| CV% | 19 | 65 | 25 | 48 | 32 | 49 |

$*_1$ Slope of the P vs I curve in the region when phytosynthesis is not light saturated

$*_2$ Rate of light saturated photosynthesis

The variability associated with determining $\alpha$ is, in all cases, greater than that associated with the determination of Pmax. For the experiments reported here, the variability in the determination of Pmax was ca. 25% while for $\alpha$, it was around 50%.

As the rate of photosynthesis at non-saturating photon flux densities is an important component of models designed to estimate total daily production, the variability associated with the determination of $\alpha$ must contribute significantly to the error associated with transforming incubation data to an estimate of total daily production. Thus, the estimate of total daily production is affected not only by the choice of model applied but also by the problems associated with the determination of the slope ($\alpha$) of the P vs I curve. This observation again argues for reporting incubation data rather than estimates of daily primary production to an eventual data bank. It also suggests that, for monitoring purposes, where the goal is to identify changes in a given region over time, that a comparison of Pmax may statistically speaking give a better basis to work with than estimates of total daily production.

In the following sections, possible sources of error in the determination of $\alpha$ are considered.

## 6.3 Light measurement

In establishing a P vs I curve, the accurate measurement of light is as important as the measurement of photosynthetic rate. Therefore, although the major purpose of this exercise was not to compare light meters, the group working on Dana took the opportunity to compare light measurements. The comparison was in two phases (Experiments 11 and 12). In the first, laboratories were asked to determine the 50, 25, 15, 5, 3 and 1% light level in the water column on both a sunny (2 June) and an overcast (3 June) day. The rationale for this part of the exercise was that many primary production methods call for the collection of water samples at specific depths in the water column based upon light penetration. In the second phase of the comparison, simultaneous measurements were made in air using all available light meters.

The results of these comparisons are shown in the Tables below:

Table 6.2. COMPARISON OF ESTIMATES OF % LIGHT DEPTHS

JUNE 2, 1987, 57°37'N 10°54'E

| COUNTRY CODE | | SENSOR | METHOD | TIME LOCAL | 50% | 25% | 15% | 10% | 5% | 3% | 1% |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 1 | LICOR COSINE | A | 1730- | 2.2 | 4.5 | 6.1 | 7.4 | 10.0 | 11.3 | 15.0 |
| 9 | 1 | LICOR COSINE | B | 1455-1520 | 2.2 | 4,3 | 6.1 | 7.7 | 11.4 | 14.1 | 21.1 |
| 18 | 2 | LICOR COSINE | C | 1605-1610 | 2.4 | 4.5 | 6.5 | 8.4 | 12.2 | 15.6 | 22.8 |
| 23A | 2 | Q-INST COSINE | D | | 3.6 | 7.1 | 9.8 | 11.9 | 15.4 | 18.1 | 23.7 |
| 23B | 2 | Q-INST COSINE | E | | 2.5 | 5.0 | 7.3 | 9.5 | 12.5 | 15.5 | 22.0 |
| MEAN = | | | | | 2.6 | 5.1 | 7.2 | 9.0 | 12.3 | 14.9 | 20.9 |
| COEFFICIENT OF VARIATION % = | | | | | 23 | 23 | 22 | 20 | 16 | 17 | 16 |

*NOTES:*

*Method A, 1 underwater sensor, light-depths computed from single extinction coefficient based on Beers-Lambert formula*

*Method B, 1 underwater sensor, semi-log graph paper used to determine % light-depths*

*Method C, Ratio of underwater: above water sensors, semi-log graph paper used to determine % light-depths*

*Method D, Ratio of underwater: above water sensors, attenuation from 10-15 m used to determine k which was assumed to be the same throughout the water column*

*Method E, Ratio of underwater: above water sensors, semi-log graph paper used to determine & light-depths*

Table 6.3. COMPARISON OF ESTIMATES OF % LIGHT DEPTHS

JUNE 3, 1987, 57°37'N 10°54'E

| COUNTRY CODE | | SENSOR | TIME LOCAL | 50% | 25% | 15% | 10% | 5% | 3% | 1% |
|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 1 | LICOR COSINE | 1115- | 2.7 | 5.3 | 7.3 | 8.9 | 11.5 | 13.5 | 17.7 |
| 9 | 1 | LICOR COSINE | 1040-1055 | 2.2 | 4.6 | 6.4 | 8.5 | 12.6 | 15.4 | 23.7 |
| 18 | 2 | LICOR COSIN | 1119-1123 | 2.5 | 4.9 | 6.7 | 8.0 | 10.3 | 11.8 | 15.8 |
| 23A | 2 | Q-INST COSINE | 1125 1130 | 3.3 | 6.6 | 9.0 | 11.0 | 14.3 | 16.7 | 21.9 |
| 23B | 2 | Q-INST COSINE | 1125-1130 | 2.5 | 4.5 | 6.2 | 8.3 | 12.0 | 14.8 | 20.3 |
| MEAN = | | | | 2.7 | 5.2 | 7.1 | 8.9 | 12.1 | 14.4 | 19.9 |
| COEFFICIENT OF VARIATION % = | | | | 13 | 16 | 16 | 13 | 12 | 13 | 16 |

*NOTES:*

*Method A, 1 underwater sensor, light-depths computed from single extinction coefficient based on Beers-Lambert formula*

*Method B, 1 underwater sensor, semi-log graph paper used to determine % light-depths*

*Method C, Ratio of underwater: above water sensors, semi-log graph paper used to determine % light-depths*

*Method D, Ratio of underwater: above water sensors, attenuation from 10-15 m used to determine k which was assumed to be the same throughout the water column*

*Method E, Ratio of underwater: above water sensors, semi-log graph paper used to determine & light-depths*

Table 6.4. COMPARISON OF INCIDENT PHOTOSYNTHETICALLY ACTIVE RADIATION[1]

| COUNTRY CODE | SENSOR TYPE | SENSOR # | TEST #1 | TEST #2 | TEST #3 | TEST #4 |
|---|---|---|---|---|---|---|
| 4 | COSINE | UWQ-3307 | | 583 | | 629 |
| 4 | COSINE | Q-7978 | | | 590 | 630 |
| 9 | COSINE | | | 410 | 413 | 482 |
| 23 | COSINE | | | 550 | 570 | |
| 23 | SPHERICAL | | 1160 | | | |
| 18 | SPHERCAL | SPQA-631 | 1120 | | | |
| 18 | COSINE | UWQ-233 | | 550 | 567 | 577 |
| 18 | COSINE | UWQ-234 | | 551 | 568 | 576 |
| MEAN | | | | 529 | 541 | 579 |
| CV | | | | 13 | 12 | 10 |

[1] ALL UNITS ARE $\mu$ moles photons $m^{-2}s^{-1}$

*NOTES:*

*Comparisons took place on lower Deck of RV DANA at 1120 local time, June 4, 1987. Overcast conditions with some occasional sun through clouds. Readings were made contemporaneously during intervals of constant incident light. All sensors were LICOR quantum-response sensors.*

Considerable variation (~ 20%) was observed in the determination of percent light depths through the water column. One source of error here is likely to be the fact that some workers continuously monitor light at the surface and compare that with in situ water column measurements in the determination of the attenuation coefficient. Those workers who do not have two light meters are, of course, not able to make simultaneous surface and water column measurements. The influence of passing clouds, etc., on the determined percent light depths will be greatest when surface and water column measurements are not made simultaneously.

There was relatively good agreement (CV ca. 10%) in the measurements obtained in air by the different light meters (Table 6.4). However, as all instruments were of the same make (LICOR), this small sample is probably not representative of the variability associated with light measurement used in connection

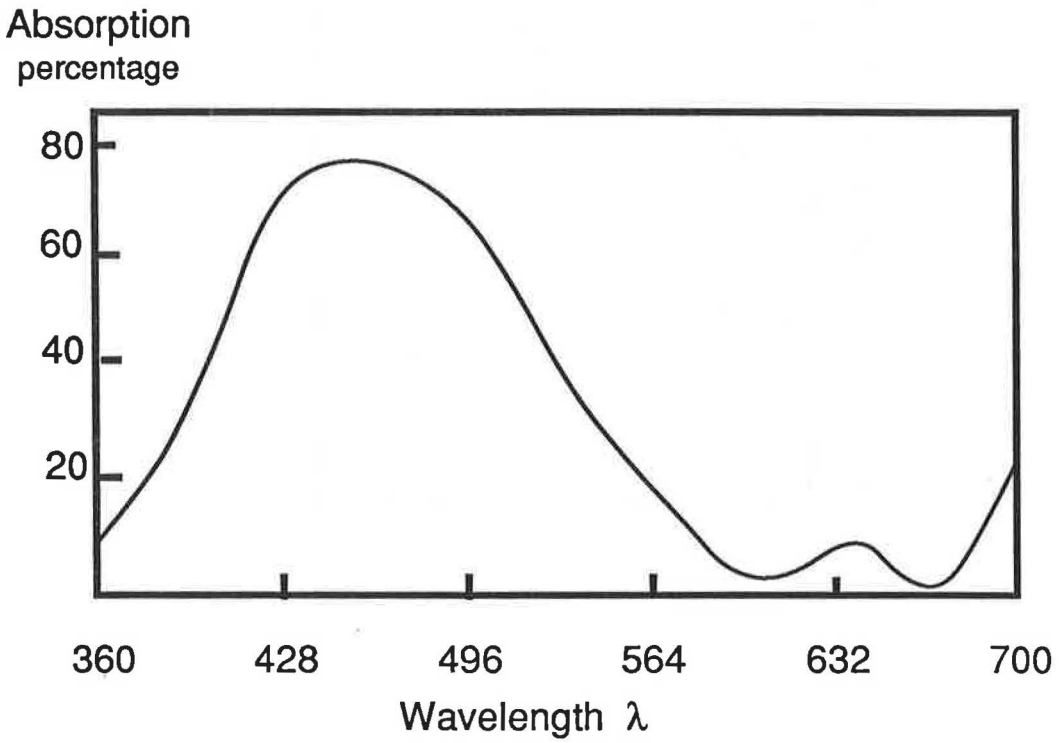
with primary production determinations made by the larger scientific community. A similar study carried out at a GAP Workshop in 1982 demonstrated a much larger variation in measurements obtained using different light meters (Anon,1982).

The conclusion from the comparisons carried out here on light measurement suggest that the measurement of light can contribute significantly to error in reported P vs I curves. On the basis of the limited studies carried out here, it is not possible to quantify the error associated wiht light measurement and different light meters. However, the problems associated with the in situ water column determination of light quantity would argue against recommending that samples be taken from specific light penetration depths in a "standard" method of determining primary production.

6.4 Influence of spectral quality on the determination of $\alpha$

The deck incubators brought to Hirtshals and used on board DANA by laboratories 17 and 18 were identical in every respect except that laboratory 17 used blue filters (absorbtion characteristics shown in Fig. 6.3) to attenuate incident light while laboratory 18 used neutral density filters. In experiments 3 and 5, these two laboratories carried out identical incubations on pooled material, thus allowing analysis of the effect of light quality on the productivity determinations.

**Fig. 6.3 Absorption characteristics of filters used in Laboratory 17's simulated in situ incubator**



Both of these laboratories employed a method whereby they collected samples from selected depths throughout the water column at daybreak. These samples were incubated on deck at photonflux densities approximately corresponding to those found at the collection depth for either ½ or the entire light period of the day. Thus, their results are best expressed as carbon incorporated per day at each depth. The results from experiments 3 and 5 are presented in this manner in Fig. 6.4a and b. Except at the very surface, consistently more carbon was incorporated in samples incubated in blue light than under neutral density filters.

**Fig. 6.4a. Daily Primary Production through the water column measured by Laboratories 17 and 18 (Expt. 3)**

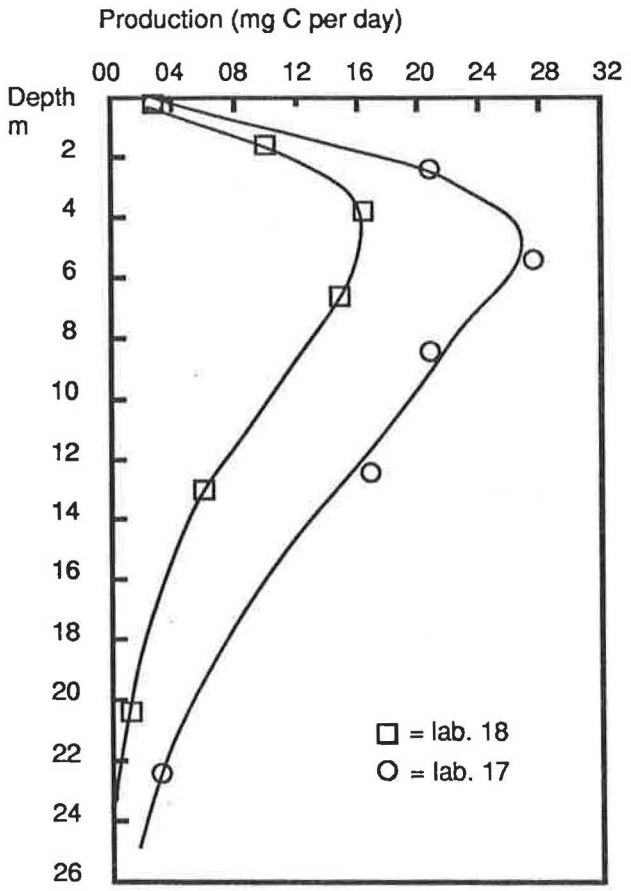
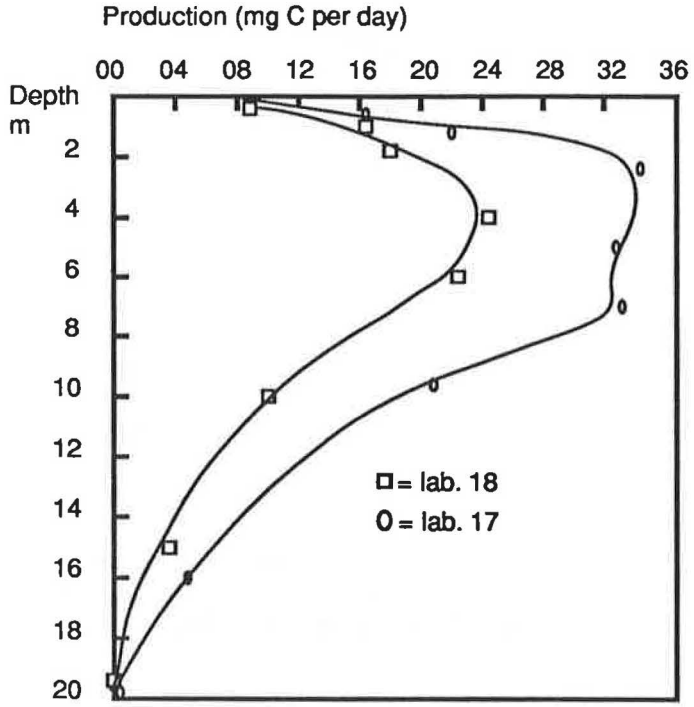


**Fig. 6.4b. Daily Production through the water column measured by Laboratories 17 and 18 (Expt. 5)**




This observation suggests that spectral quality (i.e. the filters used in attenuating light under incubations) can significantly affect the P vs I curve generated in primary production measurements. It is not possible, from the data presented here, to identify the relative effect of spectral quality on $\alpha$ and Pmax. However, M.J. Perry (Univ. of Washington) reported to the Working Group on Primary Productivity at its 1988 meeting that she had found significant differences in $\alpha$ when incubating phytoplankton samples under dark purple, dark blue, blue-green, green, orange, red and light blue light during the BIOWATT cruise in March, 1988. Spectral quality, then, can be expected to have a significant effect on the P vs I curves generated in primary production measurements.

6.5 Influence of incubation length on the determination of $\alpha$

Laboratory 4 routinely incubated over a number of different periods when applying its own method. In addition, an experiment (7) designed to examine effect of incubation length on photosynthetic characteristics was conducted by this laboratory. The results obtained by Laboratory 4 are presented below:

**Table 6.5. Effect of incubation length on photosynthetic rate measurements**

| Expt. | Depth of sample | Incubation length (h) | $\alpha$ | Pmax |
|---|---|---|---|---|
| 2 | mixed layer 3 m | .33 | 0.008 | not determined |
| | | .70 | 0.009 | " |
| | | 1.00 | 0.008 | " |
| | | 2.10 | 0.009 | " |
| 2 | pycnocline 7 m | .38 | .013 | 7.8 |
| | | .75 | .016 | 6.8 |
| | | 2.25 | .017 | 9.7 |
| 7 | mixed layer 3 m | 0.70 | .009 | 3.8 |
| | | 1.12 | .010 | 3.4 |
| | | 2.20 | .010 | 2.8 |
| | | 4.12 | .010 | 2.0 |

Alpha did not, in these experiments, appear to be greatly affected by incubation length. However, Pmax showed an increasing trend with increased incubation time in Expt 2 (pycnocline) and a decreasing trend in Expt 7.

The increase in Pmax (and slight increase in $\alpha$) observed in Expt 2 may be a result of acclimation of algae taken from low light (pycnocline) and incubated at high photon flux densities (36-1236 $\mu$mol $m^{-2}s^{-1}$).

The decrease in Pmax observed by Laboratory 4 in Expt 7 was also recorded by Laboratory 20 (Pmax = 3.0 after 1 hr incubation; 2.7 after 5 hr incubation) and Laboratory 23 (Pmax = 2.5 after 2 hr incubation; 2.2 after 4 hr).

As noted earlier, incubation length may be expected to be especially important under conditions where a large algal biomass is present. Chlorophyll concentrations in the present study were low (< 1 $\mu$g $l^{-1}$). Thus, the differences in Pmax and $\alpha$ recorded here may be expected to be somewhat less than if more chlorophyll had been present in the sample. In any event, length of incubation must be considered to be a potential source of error in the creation of P vs I curves.

## 7. Conclusions from the Exercise

Part I

The results from the studies in which filters onto which $^{14}C$ containing Isochrysis had been filtered were distributed to participating laboratories (Section 4) indicate that a significant source of variation in the primary productivity results from different laboratories may be the actual counting procedures used by the laboratories. The coefficient of variation between the means of the individual laboratories' results in this part of the exercise was about 15% for the three filter types which were studied (GF/F, membrane, membrane + quenching material).

It seems likely that some of the laboratories participating in this part of the exercise have introduced an error into their procedure for calculating DPM from CPM. Filter type and the presence/absence of a quenching agent (in this case chlorophyll) also had a significant effect of the reported results, albeit small in comparison to the laboratory effect. The filter and quenching effect was, however, not consistent from laboratory to laboratory. Thus, differences in measuring $^{14}C$ from laboratory to laboratory cannot be corrected for by distributing standards to the various laboratories unless filter type and quench correction procedures are also standardized.

When a common data set was distributed and participating laboratories asked to estimate daily primary production using their own calculation procedures, estimates of daily production again varied by about 15% (Section 6.1). In terms of the eventual establishment of a primary production data bank, this is one source of error that can be eliminated. It is not actually daily production that is being determined in most cases. Thus, although primary production data is usually converted to daily production before it is used, it would be most correct (and most accurate) to report data in the rawest form possible. In this manner, potential users of the data who require estimates of daily production can calculate it using their own methods and ensure that all data is handled in the same manner. In addition to the raw incorporation data, an eventual data bank should include the light attenuation coefficient through the water column, and possibly, daily irradiation at the sampling site.

Part II

This exercise has demonstrated that irregardless of whether or not laboratories are employing their own or standard methods, very significant differences are recorded in their estimates of Pmax (maximum rate of photosynthesis) and/or daily primary production (see Sections 5.1, 5.5 and 5.7).

There are indications (Section 5.2) that the type of artificial light incubator used and post-incubation procedures (filtration, removal of non-incorporated $^{14}C$ placing in scintillation vial: see Section 5.6) may be significant sources of error when

carrying out primary production determinations. On the other hand, the different $^{14}CO_2$ stocks used by the different laboratories did not appear to contribute significantly to the differences reported for Pmax by the different laboratories.

The reproducibility in the results from individual laboratories was about 10% irregardless of whether they were using their own method or the common method.

Primary production determinations generally require the creation of a P (Photosynthesis) vs I (Light) curve; where $\alpha$ represents the slope of the curve for that range of photon flux densities where photosynthesis is not saturated. This study has shown greater variability in the determination of $\alpha$ than in the determination of the maximum rate of photosynthesis (Pmax) (Section 6.2). The measurement of light and the spectral quality of the light used for incubations have been identified as potential sources of error in the determination of $\alpha$.

In view of the variability associated with the calculation of $\alpha$, and in measuring light in the water column as well as the potential introduction of error in converting P vs I curves made over short time periods to daily production estimates, it was suggested at the 1988 Meeting of the WG on Primary Productivity that, when using primary production measurements as a monitoring tool there is the need for the development of a new method. This method should be aimed at producing values for phytoplankton activity that would be comparable irregardless of the operator. The goal of such measurements would not be to calculate "primary production" in g C $m^{-2}d^{-1}$ but rather to measure $^{14}C$ incorporated in phytoplankton from a fixed depth during a fixed incubation period and in a well defined artificial light incubator. In this way, errors introduced to primary production estimates via light measurements in the water column, calculation procedures, different types of incubators etc. would be eliminated. Such a method, if developed, could not be used for physiological studies. Nevertheless, the Group felt that the lack of physiological meaning in such a measurement would be outweighed by the potential for creating time series of data relating to photosynthetic activity to which a number of laboratories could contribute.

In view of the apparent effect of post-incubation procedures on estimates of Pmax, a goal in the development of a standard monitoring method must be to reduce the number of handling steps. Thus, it might be an advantage to eliminate the filtration step entirely and adopt a method whereby incubations are carried out in scintillation vials and excess $^{14}CO_2$ removed by acidification. An added advantage to such a method is that the total (i.e. particulate and dissolved) incorporated $^{14}C$ is determined.

While the suggestion to develop a standard method for measuring photosynthetic activity for monitoring purposes met with general approval in the Working Group, many of the members present still felt that primary production determinations made by more traditional methods also ought to be included in an eventual data

bank. These members argued that, despite the large errors associated with primary production measurements carried out by different laboratories (see Section 5.7), these data may still be useful for some purposes. This question was not resolved by the Working Group although it was agreed that if data collected using different methods were to be included in an eventual data bank, it would be necessary to develop a standard code for the different methods used (i.e. simulated in situ; artificial light incubator, etc.). This code should be reported together with data so that potential users could evaluate the suitability of the data for their own purposes.

As the Working Group had not been directly asked to design a primary production data bank, no final conclusions as to the feasibility of such a data bank were reached. Many primary production determinations are, however, made annually by the member countries of ICES and there are many cases in which a comparison of the measurements made would be desirable. The purpose of the Intercomparison Exercise was to address the question of whether or not primary production measurements carried out by different laboratories can be directly compared. In view of the large differences recorded here between results from different laboratories, it must be concluded that primary production measurements carried out by different laboratories are not directly comparable, and when such comparison is carried out it can only be done using extreme caution.

## 8. Acknowledgements

The Working Group would like to thank the officers and crew of the RV Dana and the staff of the Danish Institute for Fisheries and Marine Research at the North Sea Center for their assistance in solving the logistical problems associated with making the equipment from 9 different laboratories (with instructions written in 7 languages!) function at the same time and place! Financial support for the Intercomparison Exercise was given by the Danish Department of the Environment and the International Agency for $^{14}C$ Determination, Hørsholm, Denmark. Niels Axel Nielsen deserves special thanks for having taken time from his busy schedule to try and sort out the statistics associated with Section 4.

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## 10. Appendices

I. Common method employed by participating laboratories (from Guidelines for the Baltic Monitoring Programme.

II. Detailed description of incubators and materials used in North Sea Center Experiments. A similar description for the Dana incubators was, unfortunately, not made.

Appendix I

## 8.2.1. PHYTOPLANKTON PRIMARY PRODUCTION

Obligatory measurements

### 8.2.1.1. Production measurements

Sampling depths

Sampling depths are selected to give an adequate vertical production curve. The standard sampling depths are: 1 m, (2 m), (3 m), 5 m, 10 m, (15 m), and 20 m (non-obligatory depths in brackets).

In water bodies with a thermocline and/or a halocline, a higher concentration of phytoplankton is often observed in the discontinuity layer than above or below this layer. If a pycnocline is found within the euphotic zone and does not correspond to one of the standard sampling depths, it is recommended to collect an additional sample in the discontinuity layer.

Instead of standard depths, samples may be collected at the light depths where the irradiance immediately below the water surface, $E_d$ (z=0), is reduced to: (95 %), 75 %, (50 %), 25 %, 10 %, (3 %), and 1 %.

Sampling time

Water samples for production measurements should preferably be sampled between 8 a.m. and 3 p.m. Central European Time.

Samplers

Non-transparent and non-toxic sampling devices must be used.

Experimental bottles

25 $cm^3$ bottles made of high quality laboratory glass and with standard grinding and glass stoppers are recommended.

The experimental bottles must be thoroughly cleaned before every experiment in order to avoid bacteria film or adsorption of toxic substances on the inside of the bottles. The bottles must be cleaned with a 10 % HCl solution, then rinsed in tapwater and in distilled water. If possible, the bottles should be dried at $170^0$ C. Before use, the bottles must be washed with water from the respective samples.

All handling of samples before and after the incubation experiment must take place in dimmed light.

The incubator experiment must be carried out as soon as possible after sampling.

From each sampling depth, 3 clear experimental bottles are filled with water. Additionally, 3 dark experimental bottles are filled with water from 1 m depth. If a pycnocline is found within the euphotic zone, additionally 7 clear experimental bottles are filled with water from 20 m depth.

The experimental bottles must not be filled totally, but space for the $^{14}C$-solution and a little air bubble should be left.

$^{14}C$-solution

The $^{14}C$-ampoules for use in production studies can be purchased from different manufacturers. These ampoules must fulfill the following specifications:

- alkalinity 1.5 $mM/dm^3$
- specific activity 4-20 $\mu Ci/cm^3$

Standardization of $^{14}C$-solution

Liquid scintillation counting shall be used as the basis for determination of the absolute activity.

Concentration of $^{14}C$-solution

The $^{14}C$-solution should be added to the experimental bottles in such concentrations that statistically sufficient estimations of the radioactivity fixed by photosynthesis in the sample can be obtained. However, it is also important not to disturb the $CO_2$ equilibrium in the water sample by adding too much $NaH^{14}CO_3$ solution.

Concentrations corresponding to a 1 $cm^3$ $^{14}C$-solution with a radioactivity of 1-4 $\mu Ci/cm^3$ per 25 $cm^3$ sample have been shown to be applicable for primary production studies in the Baltic.

Dark fixation of carbon

As the dark fixation of carbon is not directly related to photosynthetic production, it has to be regarded as blank and subtracted from the latter.

Incubator

The incubator used must have the following specifications:

- thermostatically controllable
- irradiance conditions ensuring photosynthetic saturation: at least $250 \cdot 10^{18}$ quanta $m^{-2} \cdot s^{-1}$ (400-700 nm) or 100 joules $m^{-2} \cdot s^{-1}$ (400-700 nm). (Philips TLD 18 W/33 meets these demands.)

Measurements of irradiance in the incubator

A calibrated irradiance meter (quanta meter, 400-700 nm) shall be used.

The irradiance meter is placed in the water-filled incubator facing the light source of the incubator and at the same distance from the light source as the experimental bottles during experiments.

The irradiances are measured in 5 different positions: At the center of the bottle-wheel of the incubator and at the outermost positions of the experimental bottles on each side and above and below the center of the bottle-wheel.

The measured irradiances are corrected for the immersion effect by multiplying by the immersion factor of the irradiance meter used.

Immersion factor: the ratio between the sensitivity of the irradiance meter in air and in water.

The mean of the 4 outermost measurements is calculated, and the irradiance in the incubator is expressed as the mean of the center measurement and the calculated mean of the 4 outermost measurements.

Incubation temperature

The temperature in the incubator during the experiment has to be adjusted to the mean temperature of the euphotic zone, or to the mean temperature found above an eventual pycnocline, if a pycnocline is present within the euphotic zone.

Incubation

The experimental bottles are placed at the bottle-wheel of the incubator in such a manner that only clear experimental bottles face the irradiance source.

Five of the experimental bottles in each of the series for determination of the PI-curves are covered with neutral filters of known different transmissions, e.g., 5 %, 10 %, 15 %, 25 % and 50 %. In addition, the production is determined at 100 % incubator irradiance by the sixth bottle of the series and at 175 % incubator irradiance by the seventh bottle of the series. The 175 % incubator irradiance is obtained by applying an aluminium foil coating as reflector behind the experimental bottles.

Incubation period

The incubation period is 120 minutes.

Filtration of samples

The samples should be filtered immediately after the production experiment is stopped, in order to avoid loss of $^{14}C$ due to respiration.

Filters with an even distribution of pore size and good solubility with respect to scintillation liquids are preferred. The pore size should not exceed 0.45 µm. The filters should be wet before the filtration starts.

The suction pressure should not exceed $0.3 \cdot 10^5$ $Nm^{-2}$ (0.3 atm).

The whole filtration procedure should not exceed 0.5 hour for the entire series of bottles. It is possible to comply with this requirement by arranging a series of filtration units. In case it is impossible to filter the whole contents of a single sample, a sub-sample may be filtered. The sub-sample shall be at least 15 $cm^3$ in volume. If filtering a sub-sample, both the volume of the sub-sample and the whole volume of the experimental bottle has to be measured. In the case of filtering the whole volume of an experimental bottle, such measurements are not necessary.

The filters should not be washed but, whenever bottles and filtration funnels need to be rinsed, this should occur at the end of the filtration procedure, but before the last $cm^3$ has passed through the filter.

Preparation of filters

In order to stop all biodegradation, and thus losses of radioactivity, and to remove possible $^{14}C$ precipitates extracellularly, the filters are transferred to a desiccator and exposed to vapours of fuming HCl for 5 minutes.

The filters can now be placed at the bottom of the empty scintillation vials.

When Geiger counting is used, the filters must be dried in a desiccator with freshly dried silica-gel and with soda lime, the latter for removal of excess HCl fumes.

Radioactivity measurements

Various counting techniques are available, ranging from Geiger counting and proportional counting to liquid scintillation counting and combustion.

| Exp. | Lab code no. | Incubators: Light tubes | Attenuation filters | µE $m^2S$ | Temp. °C | Bottle vol(ml) | Prehandling: Act. dpm x $10^6$ | t h | Filter: Type | Pore µm | Pore mm | Pres. kp-$cm^2$ | Filter handling: Wash found | GFC filter | Dry filter | Remove inorg. $^{14}C$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 10 | Radium NL18/25 | Blackish net-formed clothes | 383 | 13.3 | 25 | (4.610) 9.220 | 1.9 | A | 0.45 | 25 | 0.3 | + | + | - | HCl fumed |
| | 14 | Philip TLD 20/33 | Glass neutral filter | 280 | 13.0 | 30 | 4.396 | 2 | A | 0.45 | 25 | 0.3 | + | sample left | - | Exp. B HCl fumed few drops of 0.5N HCl |
| | 15 | Airam Daylight de lux | - | 200 | 20.0 | 100 | 5.468 | 24 | Millipore Polyester | 0.45 | 29 | 0.33 | + | + | - | Exp.B HCl fumed few drops of 0.5N HCl |
| | 22 | With reflector in the tube Philips TL 18/33 | Metal screens | 300 | 12.0 | 50 | 3.925 | 4 | Sartorius Cellnitrat | 0.2 | 35 | 0.3 | - | - | + | HCl fumed Exp. B - |
| | 24 | Philip LTD 20/33 | Glass neutral filter | 440 | 13.4 | 25 | 3.848 | 2 | Sartorius cellacetat | 0.45 | 25 | 0.3 | + | + | - | HCl fumed |
| C | 10 | ditto A | ditto A | ditto A | 12.9 | ditto A | 7.696 | 4 | A | ditto A | 25 | ditto A | ditto A | - | ditto A | HCl fumed |
| | 14 | ditto A | ditto A | ditto A | 13.0 | ditto | 7.696 | 4 | ditto A | ditto A | 25 | ditto A | ditto A | - | ditto A | ditto A |
| | 15 | ditto A | ditto A | ditto A | 13.0 | ditto | 7.696 | 4 | N | 0.2 | 29 | ditto A | ditto A | - | ditto A | ditto A |
| | 22 | ditto A | ditto A | ditto A | 12.5 | ditto | 7.696 | ditto A | A | 0.45 | 25 | ditto A | ditto A | ditto A | - | ditto A |
| | 24 | ditto A | ditto A | ditto A | 12.7 | ditto A | 7.696 | 4 | A | ditto A | 25 | ditto A | ditto A | - | ditto A | ditto A |
| | 10 | Philips TLM30/33 | ditto A | ditto A | 12.3 | ditto A | 7.696 | 2 | A | ditto A | 25 | ditto A | ditto A | - | ditto A | ditto A |
| | 14 | ditto A | ditto A | ditto A | 13.0 | 25 | 7.696 | 2 | ditto A | ditto A | 25 | ditto A | ditto A | - | ditto A | ditto A |
| | 15 | ditto A | ditto A | ditto A | 12.0 | 25 | 7.696 | 2 | N | 0.2 | 29 | ditto A | ditto A | - | ditto A | ditto A |
| | 22 | ditto A | ditto A | ditto A | 11.5 | 25 | 7.696 | 2 | A | 0.45 | 25 | ditto A | ditto A | ditto A | - | ditto A |
| | 24 | ditto A | ditto A | ditto A | 12.3 | ditto A | 7.696 | 2 | A | ditto A | 25 | ditto A | ditto A | - | ditto A | ditto A |

## Indication of spine colours

| | |
|---|---|
| Reports of the Advisory Committee on Fishery Management ..... | Red |
| Reports of the Advisory Committee on Marine Pollution ..... | Yellow |
| Fish Assessment Reports ..... | Grey |
| Pollution Studies ..... | Green |
| Others ..... | Black |

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