Vol. 72: 189–201, 1991 | MARINE ECOLOGY PROGRESS SERIES
Mar. Ecol. Prog. Ser. | Published May 23

Comparison of ^{14}C primary production determinations made by different laboratories

Katherine Richardson

Danish Institute for Fisheries & Marine Research, Charlottenlund Castle, DK-2920 Charlottenlund, Denmark

ABSTRACT: The results of an intercomparison exercise of primary production determinations made by different laboratories are reported here. The exercise was carried out by a Working Group of the International Council for Exploration of the Seas (ICES) in 1987 and was divided into 2 phases. In the first, different types of filters onto which ^{14}C-containing algae had been filtered were distributed to 24 different laboratories from 15 countries. These laboratories counted the associated radioactivity using their usual methods. Significant differences were recorded in the results obtained by the different laboratories. Filter type and presence/absence of a quenching agent had a lesser but also significant effect on counting results. A data set describing ^{14}C incorporation as a function of photon flux density at different depths in the water column was also distributed to these laboratories and they were asked to calculate total daily primary production. Results from different laboratories varied by ca 15 %. The second phase was comprised of field studies where 12 laboratories representing 9 countries met and compared primary productivity measurements using 'own' and 'standard' methods on pooled and non-pooled samples. Very significant differences in results were recorded between laboratories even when using a 'standard method' on pooled samples. Incubator and filtering procedures have been identified as potential sources of error in ^{14}C incorporation studies.

INTRODUCTION

Quantifying the primary production occurring in the world's oceans is of crucial importance both for an understanding of the production processes relating to higher trophic levels and for assessing the potential capacity of the oceans as a sink for atmospheric CO_2. However, estimates of this fundamental process vary by a factor of about 10 (Platt et al. 1989).

Platt et al. (1989) argue that 'because the ocean is undersampled with respect to primary production measurements, we should attempt to assemble all relevant data into a single data base, after intercomparison of different techniques'. These authors go on to identify some of the problems associated with comparing 'in vitro' methods of primary production determination (in which ^{14}C assimilation, O_2 evolution or $^{15}NO_3$ assimilation are measured on samples taken from discrete depths) with methods based on changes in bulk properties of the water column (dissolved O_2, sedimentation rates, oxygen utilization rate, and the vertical flux of nitrate into the photic zone).

Because ^{14}C assimilation is the most commonly employed method of determining primary production in vitro, this method, has, by default, become the standard to which results obtained by all other methods are compared. This, however, is an unfortunate situation both because there is still considerable uncertainty as to what the ^{14}C assimilation method actually measures (see Peterson 1980, Li & Goldman 1981, Richardson et al. 1984) and because each laboratory employing the method has developed, over the years, its own particular procedures for carrying out the method. As a consequence of the latter, the comparability of results obtained by different laboratories is unknown. Thus, it would not be possible to place confidence limits on ^{14}C primary production data in the 'single data base' called for by Platt et al. (1989).

The purpose of this study was to compare results obtained by different laboratories using the ^{14}C primary production method. The exercise was carried out under the regie of the International Council for Exploration of the Seas (ICES) and was coordinated and organized by the Danish members of the Working Group on Primary Production (K. Richardson [Chairman], G. Ærtebjerg Nielsen and L. M. Jensen).

0171-8630/91/0072/0189/$ 03.00

MATERIALS AND METHODS

In the first part of the study, 2 types of filters onto which ^{14}C containing phytoplankton had been filtered were distributed to 24 laboratories from 15 countries. Laboratories were instructed to determine the amount of ^{14}C associated with the filters using the normal procedures employed by each laboratory. At the same time, ^{14}C incorporation 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 RV 'Dana' (Danish Fisheries Ministry) and in the North Sea Center Laboratories of the Danish Institute for Fisheries and Marine Research from 1 to 6 June 1987.

A list of the laboratories participating in the exercise is presented in Table 1. All laboratories receiving filters were allocated a code number to ensure anonymity. Thus, the order of presentation in Table 1 bears no relationship to the code numbers presented with the data. One laboratory, No. 5, did not respond and is therefore not included in Table 1. A detailed description of the 2 parts of the exercise follows.

Part I: Filter and data distribution

Intercomparison of ^{14}C determination by participating laboratories. Filters were prepared at the International Agency for ^{14}C Determination (Hørsholm, Denmark) 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 algal culture at the Danish Institute for Fisheries and Marine Research) onto Whatman GF/F and Sartorius cellulose nitrate membrane filters (0.2 µm). 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

'High quenching' was obtained by filtering non-radioactive *Isochrysis* onto the experimental filters until the total algal concentration was 10 to 15 times higher on the high quench filters than on the low. Filters were acid-fumed (5 min), dried (60 °C, 20 min) and packed in plastic containers (1 set filters/container) which were subsequently mailed to the participating laboratories. Counting of the filters from the various treatments carried out at the International Agency for ^{14}C Determination indicated a low (ca 2 %) coefficient of variation between the measurements obtained from the prepared filters. Two of the receiving laboratories rinsed the containers that the filters had been sent in with liquid scintillation fluid and examined the fluid for radioactivity. They were, however, unable to measure radioactivity levels above background in this fluid which suggests no or minimal loss of material from the filters during shipping and handling. All laboratories received filters from the same batch, except Laboratory 24.

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

Sixteen laboratories reported the estimated daily production calculated from the distributed P vs I data (Table 2). Different methods of calculating primary production were used. Six laboratories used the 'Baltic method' (Ærtebjerg Nielsen & Bresta 1984). Four laboratories used methods closely related to the method recommended by ICES (Richardson 1987). Among the rest of the laboratories, 4 used only the P vs I dataset given and not the 'P_{max}' or chlorophyll data given for other depths. Two laboratories used special corrections: Lab. 8 subtracted the positive intercept of the P-I curve (5 %), and Lab. 17 subtracted 7 %.

Laboratories 17 and 18 estimated, from the P-I dataset, the in situ production m^{-2} for the period 12:00 to 14:00 h and estimated the daily production by multiplying by the ratio between the whole day irradiance and the irradiance within the period 12:00 to 14:00 h. All other laboratories estimated the irradiance and production at different depths and hours of the day integrated over time and depth.

Part II: Intercomparison of experimental procedures

Due to the large number of participants it was not possible for all laboratories to work onboard the ship. Therefore, field studies designed to compare experimental procedures were carried out by 2 groups: one based on the ship, the other at the North Sea Center, Hirtshals.

Ship 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.

Table 1. List of laboratories participating in Intercomparison Exercise

Univ. Libre de Bruxelles*
Avenue F.D Roosevelt
CP 160
B-1050 Bruxelles
Belgium

Dept. of Marine Biology
Kenklaan 30
9751 NN Haren
The Netherlands

Netherlands Inst. for Sea Research*
PO Box 59
1790 AB Den Burg (Texel)
The Netherlands

Inst. Hydrografico
Rua des Trinas 49
P-1296 Lisboa
Portugal

Institut für Meereskunde*
Düsternbrooker Weg 20
W-2300 Kiel 1
Germany

Institut für Meereskunde
Academy of Science of GDR
Seestraße 15
O-2530 Rostock-Warnemünde
Germany

Bedford Inst. of Oceanography
PO Box 1006
Dartmouth
Nova Scotia
Canada

Finnish Inst. of Marine Research*
PO Box 33
SF-00931 Helsinki
Finland

National Board of Waters and Environment
PO Box 250
SF-00101 Helsinki
Finland

Finnish Center for Radiation and Nuclear Safety*
PO Box 268
SF-00101 Helsinki
Finland

Dept. of Agriculture and Fisheries for Scotland
Marine Laboratory
PO Box 101, Victoria Road
Torry, Aberdeen
Scotland

Inst. of Marine Research, Bergen*
PO Box 1870
N-5011 Nordnes
Norway

Norwegian Inst. for Water Research, NIVA
PO Box 333, Blindern
N-0314 Oslo 3
Norway

Swedish Meteorological and Hydrological Inst. (SMHI)*
Oceanographical Laboratory
PO Box 2212
S-403 14 Göteborg
Sweden

Univ. of Stockholm
Askö Laboratory
S-106 91 Stockholm
Sweden

Norrby Laboratory
'Färjeläget'
Norrbyn
S-910 20 Hörnefors
Sweden

US Dept. of Commerce, NOAA, NMFS*
Northeast Fisheries Center
Sandy Hook Laboratory
Highland, New Jersey 07732
USA

Marine Pollution Laboratory*
National Agency of Environmental Protection
Jægersborg Allé 1B
DK-2920 Charlottenlund
Denmark

Det Danske Hedeselskab
Klostermarken 12
DK-8800 Viborg
Denmark

Tubitak-Marmara Bilimsel ve
Endustriyel Arastirma Enstituso
PK 21 Gebze – Kocaeli
Turkey

Marine Research Inst.*
PO Box 390, Skulagata 4
IS-121 Reykjavik
Iceland

Danish Institute for Fisheries and Marine Research*
Charlottenlund Castle
DK-2920 Charlottenlund
Denmark

Tvärminne Zoological Station*
SF-10900 Hanko
Finland

* Participated in both parts of the Intercomparison Exercise

Surface salinity ranged from 21.80 to 22.19 ‰ during the study. Bottom salinity ranged from 32.88 to 33.44 ‰. Surface temperatures ranged from 11.65 to 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 (Lorenzen method; Strickland & Parsons 1972) were made by the host laboratory. Below the pycnocline, concentrations

Table 2. Data used in comparison of procedures used in calculating daily primary production

Notes provided to laboratories:

It should be possible from the data-set below to calculate the daily primary production per m^2 water surface (mg C m^{-2} d^{-1}) 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 *P-I* curve given can be regarded as an in situ (or simulated in situ) incubation of water from 2.5 m depth for 2 h from noon (12:00 h) to 14:00 h at the irradiances given, 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.

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) on 24 August 1986 and incubated for 2 h (12:00 to 14:00 h) 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 P-I 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 h incubation.

TCO_2 : 2.10 mM
pH : 8.14 (at all sampling depths)
^{14}C-activity added: 4442700 DPM (= 2.00 µCi = 74.05 kBq)
Irradiance in incubator: 500 µE m^{-2} s^{-1} (= 3.01×10^{20} quanta m^{-2} s^{-1})
Temperature in incubator: 16.8 °C

P-I curve

Irradiance µE m^{-2} s^{-1}	25	50	75	125	250	500	875
DPM (per 2 h)	61	287	569	1108	2582	4068	3958

Irradiance ($Id_{(z=0)}$ just below water surface (reflection subtracted) from noon to sunset*

Time	12–13	13–14	14–15	15–16	16–17	17–18	18–19
Irradiance (µmol m^{-2} s^{-1})	1126	1199	1057	788	533	269	89

Depth profiles

Depth (m)	Irradiance (%)	Temp. (°C)	Salinity (‰)	TCO_2 (mM)	Potential production DPM per 2 h	Chl *a* (mg m^{-3})
0.1	95	17.7	32.6	2.10	3822	1.29
0.8	75	17.7	32.6	2.10	2945	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

of 0.2 to 0.3 mg chl *a* m^{-3} were measured during the study period. Above the pycnocline, values ranged from 0.5 to 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.

In addition to carrying out comparisons of various aspects of the ^{14}C incubation method, the 4 laboratories who had brought equipment for the determination of in situ photon flux densities were asked to determine the depth of 1 % light penetration. The experiment was carried out once on a clear sunny day and once on an overcast day. All measurements were made within a 45 min period.

North Sea Center experiments. Samples were collected from an outdoor (92 m^3) experimental tank. The salinity was ca 33.2 ‰ and the temperature between 12 and 13 °C. The chlorophyll *a* concentration was between 0.5 and 1.2 mg m^{-3} during the study period. The phytoplankton population consisted mainly of dinoflagellates and small flagellates. Approximately 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 performed with artificial light incubators from the 5 different laboratories that participated in this part of the intercomparison exercise. No attempt was made to standardize light or other conditions in the incubators as the object of this exercise was to estimate the variance that can be anticipated when different laboratories independently measure primary production on a single water sample.

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. At the time of these determinations, the chlorophyll *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) where scintillation fluid was added and the incorporated radioactivity determined.

In the actual Intercomparison Exercise, a number of experiments were carried out that will not be presented here. A complete presentation of the experiments carried out and results obtained can be found in Anon. (1990). Field experiments which dealt with the following topics are discussed here:

(1) Comparison of ^{14}C incorporation (at what the individual laboratories defined as the maximum photosynthesis rate, P_{max}) using a 'standard' experimental procedure where the standard method used was taken from the Guidelines for the Baltic Monitoring Programme for the Second Stage, 1983.

Briefly, the method calls for 2 h incubations in 25 ml glass bottles following the addition of 4 µCi $^{14}CO_2$. The incubator should be a temperature-controlled artificial light incubator. All laboratories maintained that the photon flux densities in their incubators were saturating for photosynthesis. Filtration should be onto filters with a pore size of ≤ 0.45 µm and suction should not exceed 0.3×10^5 N m^{-2}. Excess CO_2 is removed by exposing filters to fumes from concentrated HCl for 5 min. The 'standard' method of carrying out primary production determinations was applied by the different laboratories on 3 different occasions during the course of the Intercomparison Exercise:

Expt I: With participation by all laboratories working at the North Sea Center;

Expt II: All laboratories working onboard 'Dana';

Expt III: All laboratories from North Sea Center and 'Dana' working on a sample collected by 'Dana' and transported to Hirtshals prior to the experiment.

Since the standard method employed is based on incubations at fixed photon flux densities, the 3 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 Determination – temperature-controlled water bath, warm white fluorescent tubes, incubation bottles fixed to a rotating wheel). Thus, these 3 laboratories together with the laboratory already assigned to 'Dana's' incubator used the same incubator for Expts II and III.

(2) Comparison of ^{14}C incorporation (at P_{max}) determined using own method on a pooled sample.

In addition to having each laboratory conduct their own routine methods including collection, incubation and filtration of samples, the potential influence of postincubation treatment (filtration, removal of excess $^{14}CO_2$, placement of filters in scintillation vials) when employing 'own method' was examined.

At the North Sea Center, an experiment was conducted in which 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. Following the incubation, 4 bottles were randomly selected and given to the 5 laboratories working at the North Sea Center. These laboratories were asked to complete the procedures associated with primary production measurement (filtration, removal of incorporated $^{14}CO_2$ and placing of filters in scientillation 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 counting carried out.

(3) Comparison of ^{14}C incorporation (at P_{max}) 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 at the station. When the individual participants had selected the depths at which they wanted samples, they were grouped together so that water collection could be accomplished with 2 water casts. For this experiment, data from Laboratory 4 which used a simulated in situ incubator (i.e. using natural illumination) are also included. Natural photon flux densities were, during the entire incubation period, sufficient to saturate photosynthesis.

Following water collection, all laboratories conducted hourly primary production determinations after their own methods.

(4) P vs I curves: A comparison of variability in experimentally determined α and P_{max}.

In order to compare the variability associated with α (slope of the P vs I curve in the region when photosynthesis is not light saturated) and P_{max} (rate of light-

saturated photosynthesis), P vs I curves were generated after the method described by Gargas & Hare (1976) for the 3 experiments in which the relationship between photon flux density and photosynthesis was determined.

RESULTS

Part I: ^{14}C counting precision

A total of 23 laboratories (representing 14 countries) returned data on filter counting. 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 DPM recorded for those filters onto which ^{14}C containing algae had been filtered.

These results of the filter counting by the different laboratories are plotted in Fig. 1. One laboratory made their determinations using a modified Geiger Counter; all others employed Liquid Scintillation Counting (LSC).

In the statistical analysis of these data, 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 (SAS 1988).

The results of the analysis (Table 3) indicate that laboratory, filter type and quenching are all significant factors in the results reported. However, the laboratory effect is large compared to both filter type and quenching.

The latter 2 factors show no consistent pattern between laboratories (Table 3). Thus, the differences recorded between laboratories can be corrected only by distributing standards for all laboratories with standardized filter type and quench correction procedures.

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 much more closely resem-

Table 3. Summary of Analysis of Variance conducted on filter counting data from 21 laboratories. Data from Labs. 7 and 24 were not included in the analysis (see text)

Source of Variation	Degrees of freedom	Sum of squares	F-value	$p > F$
Lab × Filter	18	0.1896	11.35	***
Lab × Quench	20	0.1388	7.48	***
Lab	20	1.3720	73.88	***
Filter	1	0.1286	138.52	***
Quench	1	0.0110	11.86	**

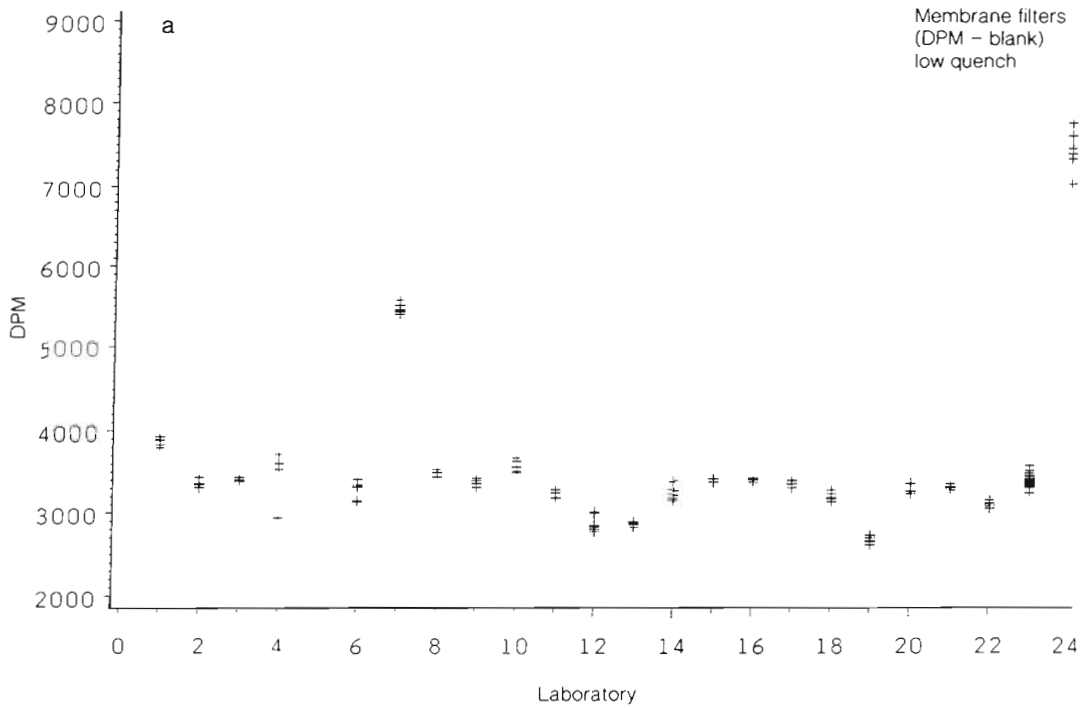
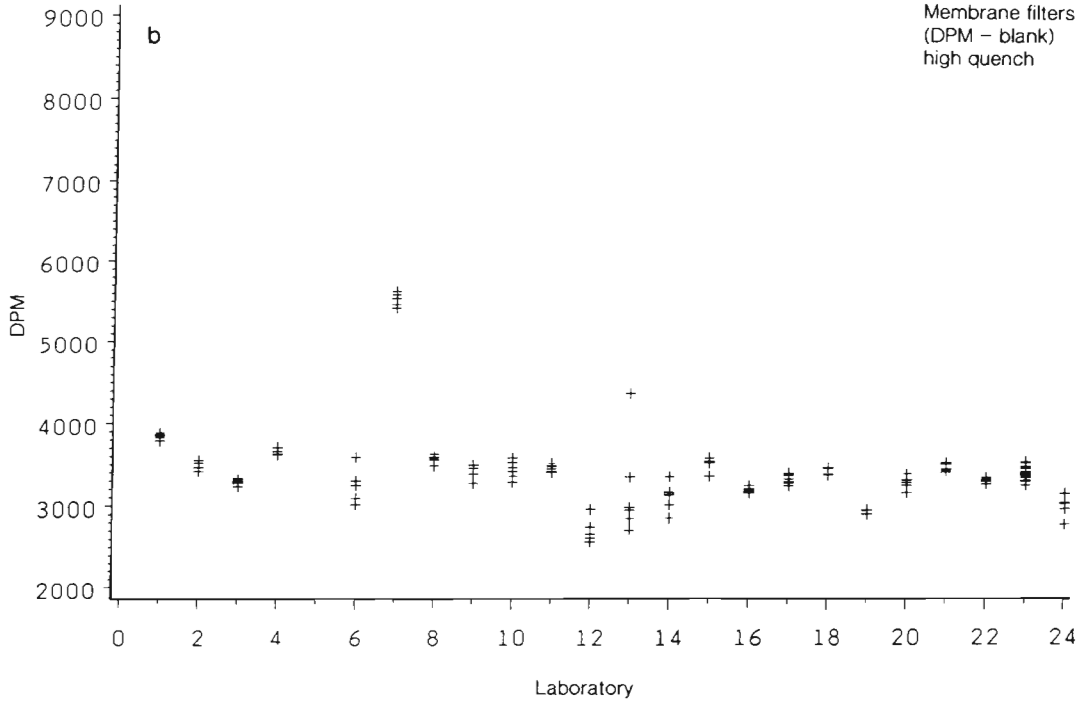
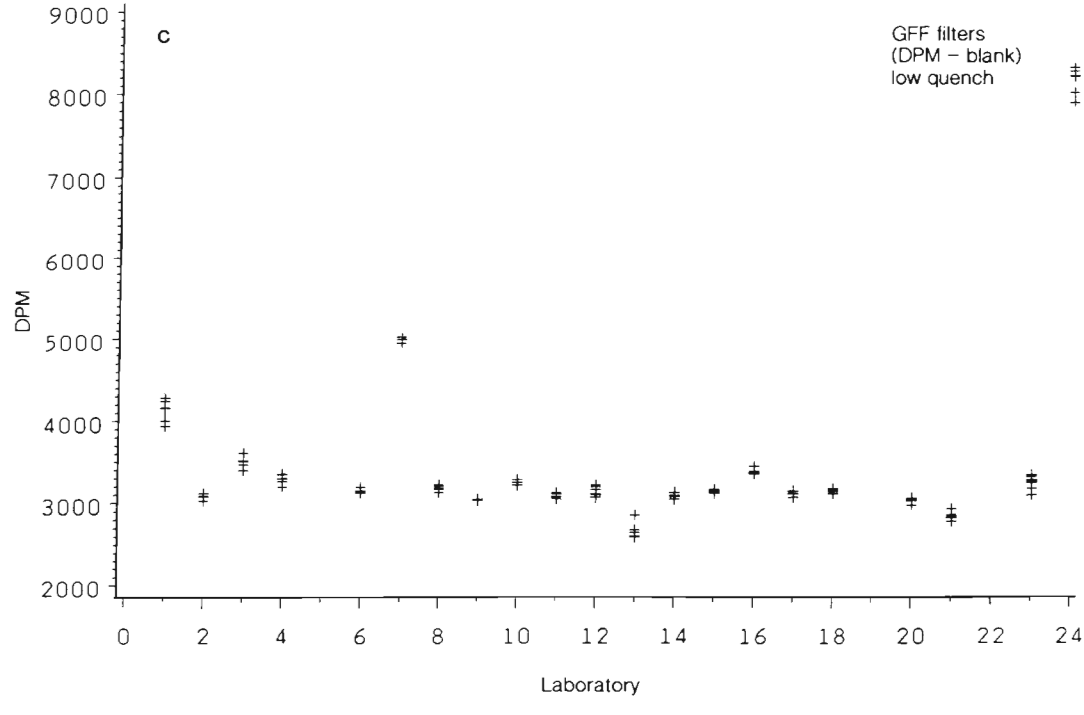


Fig. 1. (a) Radioactivity associated with membrane filters (DPM – blank; see text). Opposite: (b) Radioactivity associated with membrane filters in the presence of a quenching agent (DPM – blank; see text). (c) Radioactivity associated with GFF filters (DPM – blank; see text)

bled the results obtained by all other laboratories. However, in view of the fact the 'true' values had become known to many of the participants before the revised results were received from Laboratory 7, their original results are included in the figures. Laboratory 7's results are not included in the statistical analysis.

Laboratory 24 forwarded the filters it 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 statistical analysis. Their data are included in the figures to illustrate how dramatic the effect of the presence/absence of a





quenching agent can be on obtained results for some laboratories (compare Fig. 1a and b).

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

The results of the estimation of daily production from a common dataset are shown in Fig. 2. 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. 3). The Baltic method appears to give a somewhat higher (ca 15 %) daily production than the ICES (and all other) methods.

Part II: Intercomparison of experimental procedures

^{14}C incorporation recorded using a standard experimental procedure

The results from the experiments in which a standard method was applied to pooled samples are shown in Tables 4 to 6. Analysis of the data (DPMs recorded for the incubation bottles incubated at photon flux densities above which the individual laboratories maintained that photosynthesis was saturated [P_{max} achieved]) was carried out using SAS (GLM procedure). For all 3 experiments, there was a significant difference between the results produced by the different laboratories (Expts I and III $p < 0.0010$; Expt II $p < 0.0016$). The Coefficients of Variation (CV; %) reported for Expts I, II and III were 25, 38, and 40 %, respectively. A major source of the variation reported may have been the fact that the laboratories had not previously employed the 'standard' method. It should be noted, however, that no decrease in the variation in the results obtained was observed the second time that laboratories conducted the standard method (Expt III).

Examination of results from studies in which 4 laboratories employed the standard method but used the same incubator (Tables 7 & 8) indicates a CV of 14 % for Expt I and 12 % for Expt III. 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.

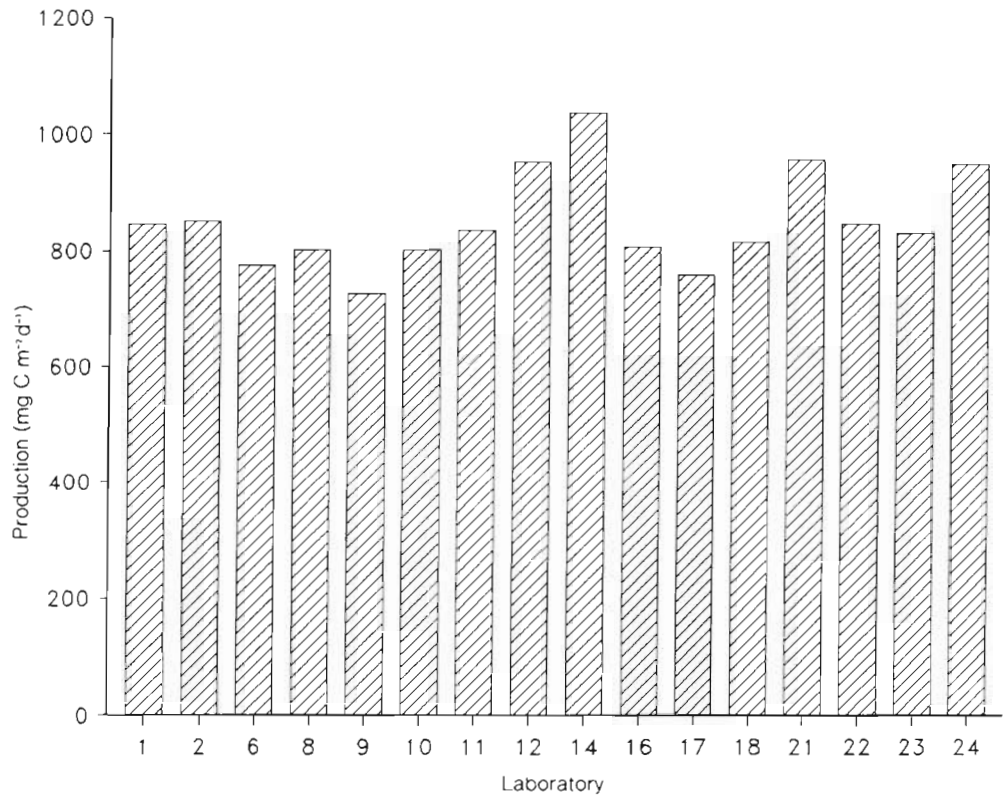


Fig. 2. Daily primary production (mg C m^{-2} d^{-1}) calculated by different laboratories from a common data set (Table 2)

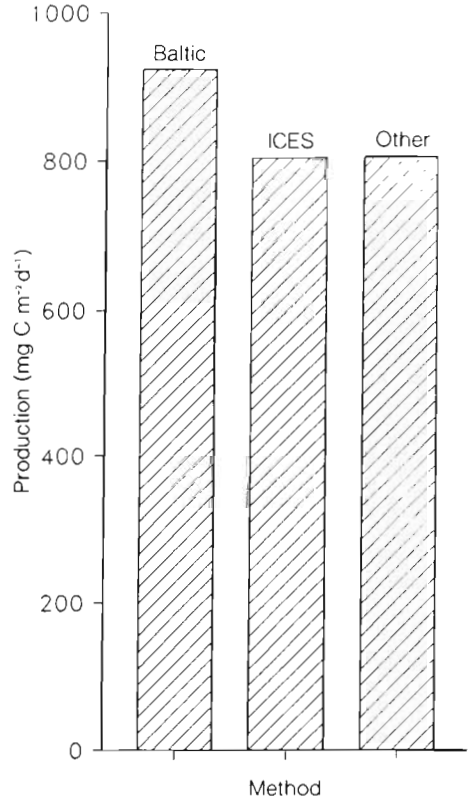


Fig. 3. Average daily primary production (mg C m^{-2} d^{-1}) calculated by different laboratories grouped according to the model applied: Baltic, ICES or 'other' (see text)

Table 4. Results from use of standard method on a pooled water sample – Expt I in which all laboratories at the North Sea Center participated

Lab.	DPM		
	Mean	SD	n
10	3763.5	649.0	4
14	6186.7	308.5	7
15	6681.2	127.1	5
22	4358.0	342.6	5
24	3944.1	245.6	7

Table 5. Results from use of standard method on a pooled water sample – Expt II in which all laboratories onboard 'Dana' participated

Lab.	DPM		
	Mean	SD	n
4	1880.0	302.6	2
8	1588.5	96.9	2
9	475.0	123.0	2
17	2394.0	390.3	2
18	2247.0		1
20	1523.5	113.8	2
23	2265.0	120.2	2

Table 5. Results from use of standard method on a pooled water sample – Expt III in which all laboratories from both 'Dana' and the North Sea Center participated

Lab.	DPM		
	Mean	SD	n
4	1362.5	33.2	2
8	1049.0	8.5	2
10	484.8	186.0	5
14	1914.1	678.4	7
15	1864.8	70.7	5
17	1204.5	4.9	2
18	1321.5	61.5	2
20	1310.0	204.7	6
22	14.660	62.2	6
23	1577.5	208.6	2
24	911.8	243.2	6

Table 7. Expt II. Results from laboratories using the same incubator

Lab.	DPM		
	Mean	SD	n
4	1880.0	302.6	2
17	2394.0	390.3	2
18	2247.0		1
23	2265.0	120.2	2

Table 8. Expt III. Results from laboratories using the same incubator

Lab.	DPM		
	Mean	SD	n
4	1362.5	33.2	2
17	1204.5	4.9	2
18	1321.5	61.5	2
23	1577.5	208.6	2

Reproducibility of measurements made following the standard method

When conducting the standard method, the laboratories working at the North Sea Center carried out between 4 and 7 replicates which gives the opportunity to examine reproducibility in their results (Table 9). With one exception, the CV for the data obtained by the different laboratories was under 10 %.

'P_{max}' 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 incor-

Table 9. Number of replicates (n) and coefficients of variation (CV; %) for results obtained by North Sea Center laboratories when employing the standard method

Expt	Lab.	n	CV (%)
I	10	4	6.4
III	10	5	2.8
I	14	7	5.0
III	14	7	3.5
I	15	5	1.9
III	15	5	3.8
I	22	5	7.9
III	22	6	4.2
I	24	7	6.2
III	24	6	26.7

porated DPMs between laboratories. Therefore, in the sections dealing with results in which the individual laboratories' methods were employed, calculated hourly production at what the individual laboratories define as P_{max} (mg C m^{-3} h^{-1}) is compared.

Results from the experiment carried out on 'Dana' in which each laboratory used their own method on a pooled water samples are shown in Table 10. Results from a similar experiment carried out by the group working at the North Sea Center are shown in Table 11. The data base for these 2 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 1.7.

Table 10. Hourly production at P_{max} determined by applying own method to a pooled water sample. The experiment was carried out onboard 'Dana'

Lab.	P_{max} (mg C m^{-3} h^{-1})
8[a]	2.55
20[a]	2.02
23[a]	2.45
9[a]	3.35
4[b]	1.78–2.03[c]
	See A and B below
A (using the lowest P_{max} obtained by Lab. 4	B (using the highest P_{max} obtained by Lab. 4
$\bar{x}$ 2.43	2.48
SD 0.60	0.54
CV 24.79 %	21.87 %

[a] Artificial light incubator
[b] Simulated in situ light incubator
[c] 4 incubation times (0.7; 1.1; 2.2; 4.1 h)

Table 11. Hourly production at P_{max} determined by applying own method to a pooled water sample (North Sea Center)

Lab.	P_{max} (mg C m^{-3} h^{-1})
10	1.75
14	2.52
15	2.26
22	3.03
24	2.58
$\bar{x}$ =	2.43
SD =	0.47
CV =	19 %

Effect of post-incubation procedures on P_{max} determinations using own method

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. There was a highly significant ($p < 0.001$) difference demonstrated in the reported results (Table 12) when the effect of post-incubation procedures on P_{max} determinations was examined. The CV in the results from this experiment was 22 %, i.e. of the same order of magnitude as that observed between the different laboratories during trials with the standard method.

Table 12. Effect of post-incubation treatment on recorded P_{max}

Lab.	DPM		n	CV (%)
	Mean	SD		
10	535.0	48.6	4	9.1
14	685.5	22.2	4	3.2
15	883.0	154.8	4	6.5
22	623.0	42.2	4	6.1
24	588.2	86.8	4	14.7

Hourly production at P_{max} determined using own method including water sample collection

Results from the 'Dana' experiments in which individual laboratories used their own methods (including selection of sampling depth and collection of samples) are shown in Table 13.

The hourly production at P_{max} from the different depths reported by the different laboratories varied from 0.41 to 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 P_{max} 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

Table 13. Hourly primary production determined using own method including water sample collection. Watercast 1: Water taken at 3, 5, 8 and 15 m and incubated at different photon flux densities; Watercast 2: Water taken 0, 2, 5, 10, 15 and 20 m, samples incubated in full incubator light

Lab.	Water cast	P_{max} (mg C m^{-3} h^{-1})	P_{max} (mg chl *a* m^{-3})
4 (3 m)	1	0.91–1.18[a]	1.4[b]
23 (3 m)	1	1.33	1.6
9 (5 m)	1	2.96	3.9
4 (8 m)	1	3.32–4.34[a]	6.8[b]
23 (8 m)	1	4.99	7.8
9 (15 m)	1	0.41	1.4
20	2	2.35	4.2
		$\bar{x}$ 2.42	3.87
		SD 1.60	2.63
		CV 66 %	68 %

[a] 4 incubation times (0.4; 0.8; 1.1; 2.2 h)
[b] Using highest P_{max}

accuracy of the P_{max} data reported to a hypothetical data bank. It should be noted from the table, however, that normalizing the hourly production at P_{max} to the chlorophyll concentration at the given depth does not, in this case, reduce the variability in the reported results.

Laboratories participating in the first water cast would, presumably, have weighted results obtained from their 2 study depths in some manner to estimate water column production. This process, however, requires a description of the photosynthesis vs photon flux density (P vs I) relationship. Therefore, the variability associated with the determination of the slope of the P vs I curve (α) was also examined.

Variability in α as compared to P_{max} in P vs I curves

The variability associated with determining α was, in all cases, greater than that associated with the determination of P_{max}. For the experiments reported here, the CV in the determination of P_{max} was ca 25 %, while for α 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 α must contribute significantly to the error associated with transforming incubation data to an estimate of total daily production.

Light measurement

Under clear sunny skies, estimates of the 1 % light penetration depth by the 4 laboratories participating in this exercise varied from 15.0 to 23.7 m ($\bar{x}$ = 20.6, SD = 3.9). Similar results were obtained the following day, under cloudy skies, when the 1 % depth estimates varied between 15.8 and 23.7 m ($\bar{x}$ = 19.8, SD = 3.7)

DISCUSSION

The Intercomparison Exercise described here was initiated in order to assess the feasibility of establishing a data bank for primary production data with contributions from different laboratories. In view of the variability in the results reported here, the author does not recommend the establishment of such a data bank at the present time. However, the results of this study can be used to indicate possible controls or conditions that could be considered in the eventual establishment of a primary production data bank.

There were significant differences recorded in the counting of the radioactivity associated with filters when similarly treated filters were distributed to different laboratories. This potential source of error in reported primary production results could be reduced by distributing counting standards to the laboratories

Table 14. P_{max} and α recorded by different laboratories for the 3 different experiments in which P vs I curves were determined

Lab.	Expt 1		Expt 2		Expt 3	
	P_{max}	$\alpha \times 10^{-2}$	P_{max}	$\alpha \times 10^{-2}$	P_{max}	$\alpha \times 10^{-2}$
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
17					1.58	1.70
18					1.58	0.54
20					1.83	1.40
22	3.03	3.36	3.71	3.87	2.52	1.31
24	2.58	1.09	3.34	1.37	1.56	0.61
n	4	4	4	4	7	7
$\bar{x}$	2.47	1.75	3.93	2.50	1.82	1.14
SD	0.53	1.14	0.95	1.19	0.52	0.56
CV (%)	21	65	24	48	28	49

contributing to a data bank. Filter type and quenching also had a significant (but much smaller than the laboratory) effect on counting data. A possible condition for submission of data to a data bank could also be that filter type and quench correction procedures be standardized.

The variability observed in the comparison of daily primary production estimated by different laboratories from a single data set suggests that an eventual data base should include raw data (i.e. incorporation data) rather than estimates of total daily production as the conversion of incubation data to estimates of total production introduces unnecessary error. It is, after all, incubation data that is collected. Therefore, it is this data that ought to be reported. Users of the data bank can, then, apply the model for estimating total primary production that they find most appropriate.

Further arguments for reporting incubation rather than total primary production data can be made on the basis of the variability reported here and elsewhere (Jewsen et al. 1984) in light measurements in situ and in the greater variability associated with α than P_{max} in the P vs I curves produced during the present study.

Both light determination in the water column and the slope of the P vs I curve are important elements in the calculation of total daily production. When actual incubation data is reported, the potential user can select the data to be used. Given the large differences observed in the determination of P_{max} in the water column when individual workers were given the opportunity to select the depths at which samples should be taken, using P_{max} for surface waters might be the most appropriate for many purposes. In this manner, one also reduces the likelihood of introduction of error from the determination of in situ light determination and/or α into the calculation of primary production.

The experiments carried out here using a standard method on pooled samples suggest that careful control and standardization of artificial light incubators are prerequisites for obtaining comparable ^{14}C incorporation results. No detailed examination of the effect of incubation length on results was conducted here. However, the results from Laboratory 4, which incubated for 4 different time periods in all experiments where 'own method' was employed, suggest that it may be necessary either to record the length of incubation or to standardize incubation length in an eventual primary production data bank.

The comparison of post-incubation procedures (filtration and removal of non-incorporated $^{14}CO_2$) indicates that these procedures can introduce significant error into the determination of ^{14}C incorporation. This observation argues for either standardizing or eliminating the filtration step. The increasing awareness of the importance of photosynthesis in the production of dissolved organic compounds is an excellent argument for designing a standard primary production method where the filtration step is eliminated. Via acid bubbling of samples, total (i.e. particulate plus dissolved organic) production can be determined (i.e. Schindler et al. 1972). It may be that application of this method rather than a method employing filtration would give more comparable results between laboratories, and future intercomparison exercises ought to include an examination of the errors associated when different laboratories employ this method or variations thereof.

This study has demonstrated that the variability in primary production data determined by different laboratories (even when a 'standard' method is applied to pooled samples) is such that a central data bank containing primary production data would, in the author's opinion, be of limited quantitative value. Nevertheless, it has been possible through the exercise to identify a number of potential sources of error in the determination of primary production that can be useful in the future establishment of a badly needed primary production data bank. Although difficult and time consuming to organize and carry out, such intercomparison exercises cannot be valued too highly in situations where there is a need to compare biological measurements made by different laboratories.

Acknowledgements. Much of the basic data treatment was carried out by the ICES Working Group on Primary Production. This Working Group thanks 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 12 different laboratories (with instructions in 9 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, as well as the participating laboratories. Niels Axel Nielsen deserves special thanks for sorting out the statistics associated with filter counting.

LITERATURE CITED

Ærtebjerg Nielsen, G., Bresta, A.-M. (1984). Guidelines for the measurement of phytoplankton primary production. Baltic Marine Biologists, No. 1 (2nd edn). Marine Pollution Laboratory, Charlottenlund

Anon (1990). Report of the ICES ^{14}C Primary Production Intercomparison Exercise. Cooperative Research Report No. 170, ICES, Copenhagen

Gargas, E., Hare, J. (1976). User's manual for estimating the daily phytoplankton production measured in an incubator. Contr. Water Qual. Inst., Hørsholm 2: 1–75

Jewsen, D. H., Talling, J. F., Dring, M. J., Tilzer, M. M., Heaney, S. I., Cunningham, C. (1984). Measurement of photosynthetically available radiation in freshwater: comparative tests of some current instruments used in studies of primary production. J. Plankton Res. 6 (2): 259–273

Li, W. K. W., Goldman, J. C. (1981). Problems in estimating growth rates of marine phytoplankton from short-term ^{14}C assays. Microbiol. Ecol. 7: 113–121

Peterson, B. J. (1980). Aquatic primary productivity and the $^{14}CO_2$ method: a history of the productivity problem. Ann. Rev. Ecol. Syst. 11: 369–385

Platt, T., Harrison, W. G., Lewis, M. R., Li, W. K. W., Sathtyendranath, S., Smith, R. E., Vezina, A. F. (1989). Biological production of the oceans: the case for a consensus. Mar Ecol. Prog. Ser. 52: 77–88

Richardson, K. (ed.) (1987). Primary production: guidelines for measurement by ^{14}C incorporation. Techniques in Marine Environmental Sciences No. 5. ICES, Copenhagen

This article was submitted to the editor

Richardson, K., Samuelsson, G., Hällgren, J.-E. (1984). The relationship between photosynthesis measured by ^{14}C incorporation and by uptake of inorganic carbon in unicellular algae. J. exp. mar. Biol. Ecol. 81: 241–250

SAS (1988). SAS/STAT^{TM} user's giude, Release 6.03 edn. SAS Institute Inc., Cary, N. Carolina

Shindler, D. W., Schmidt, R. V., Reid, R. A. (1972). Acidification and bubbling as an alternative to filtration in determining phytoplankton production by the ^{14}C method. J. Fish. Res. Bd, Can. 29: 1627–1631

Strickland, J. D. H., Parsons, T. R. (1972). A practical handbook of seawater analysis, 2nd edn. Bull. Fish. Res. Bd Can. 167

Manuscript first received: July 4, 1990
Revised version accepted: January 14, 1991