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Primary production: Guidelines for measurement by ^{14}C incorporation

Prepared by the ICES Working Group on Primary Production

Edited by

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EDITOR'S NOTE

In the years intervening between the preparation and publication of this document, at least two other manuals for use in making primary production determinations have been introduced (Nielsen and Bresta, 1984; O'Reilly and Thomas, 1983). The emphasis in these three manuals is different enough to warrant their coexistence in the scientific literature. However, the actual methods outlined in these manuals do differ considerably on some points.

Most workers concerned with primary production determinations will find more than one of these manuals coming to rest on their bookshelves. Thus, the flurry of activity in recent years aimed at clarifying and standardizing methods of making primary production estimates may, in some cases, have actually had the opposite effect. While some workers may lament the lack of emergence of a "cookbook" recipe for making primary production measurements, the lack of such a recipe can also be interpreted as a positive step towards the goal of producing more reliable measurements of photosynthesis in aquatic environments. Owing to the different facilities available, the precise details of the primary production measurements made will always differ from laboratory to laboratory. To ensure that reliable results are produced, those responsible for designing the procedures employed by different groups must have a thorough understanding of the processes being measured and an appreciation of the limitations of the method being used. Furthermore, in order to enhance the comparability between results collected by different workers, a number of routine controls must be observed and specific information supplied when reporting results.

The purpose of this manual is to provide a reference for workers designing programmes incorporating primary production measurements to aid them in producing results which can be used not only in their own research/monitoring programmes but also in the compilation of a database suited to describing the temporal and spatial distribution of primary production in the ICES area. In places where this manual refers to methods or procedures that have been more thoroughly described in another primary production manual, I have taken the editorial liberty of adding references.

Katherine Richardson

June 1985

1 INTRODUCTION

These guidelines aim to standardize, in the ICES area, the methodology of primary production measurements made by determination of ^{14}C incorporation, taking into account, as far as possible, the diversity of techniques in use at the time of writing. As well as dealing with purely technical aspects of the method, new concepts have been incorporated which represent considerable modifications to the current approach to primary production determination and which may alter the calculated daily production estimates.

In order to collect reliable primary production data, it is important to consider the numerous processes assumed to take place in the sea which are relevant to primary production experiments. These processes include:

uptake phenomena: gross photosynthesis and dark uptake (anaplerotic reactions, e.g., Wood-Werkman);

losses: phytoplanktonic respiration and photorespiration, excretion of dissolved organic matter (DOM) by phytoplankton, "natural" mortality of phytoplankton, grazing mortality of phytoplankton, and recycling processes: bacterial consumption of produced DOM and bacterial dark uptake of ^{14}C.

Respiration and internal recycling processes and the effect they may have on measured ^{14}C incorporation are problems which perhaps deserve more attention than they have hitherto received. Indeed, the problem of net *versus* gross photosynthesis estimates is central to an operational definition of primary productivity and it is still unclear to what degree the ^{14}C method of estimating primary production relates to net or gross photosynthesis (Colÿn *et al.*, 1983; Gieskes and Kraay, 1984; Peterson, 1980).

One recent laboratory study suggests that the relationship between ^{14}C incorporation and net photosynthesis may vary depending on the respiration rate of the phytoplankton and that the ^{14}C method can significantly under- or over-estimate net photosynthesis, depending upon the sampling conditions (Richardson *et al.*, 1984).

Attempts to relate ^{14}C incorporation to net production occurring in the field are complicated by the lack of an absolute standard to use as a reference. Those workers who have tried to address the question of how ^{14}C incorporation relates to naturally occurring production have produced conflicting results. Some report that ^{14}C incorporation over-estimates, others that it under-estimates or agrees well with net production (see Peterson, 1980). Whether these disparate claims may, in part, result from changes in the relationship of ^{14}C incorporation to net photosynthesis is still unknown. In any event, it is clear that work aimed at elucidating our understanding of what the ^{14}C incorporation method of measuring primary production actually measures must continue.

In spite of the uncertainties which surround the interpretation of results obtained when using this method, the ^{14}C method of determin-

ing primary productivity is the most widely accepted and valuable technique currently available for estimating rates of photosynthesis in aquatic environments. The purpose of this manual is to provide guidelines for the collection of ^{14}C incorporation data which should maximize the reliability and comparability of primary productivity determinations made by different workers.

In view of the uncertainty surrounding the interpretation of ^{14}C incorporation data, it was the opinion of the Working Group that results should be presented in a more objective manner and should provide ^{14}C incorporation rates; rather than net or gross production rates. It is important to consider these incorporation rates as an index of primary production rather than as an absolute measurement of aquatic photosynthesis.

Essential information relating to primary production can be gained from examining the behaviour of such biologically significant parameters as the rate of change in photosynthetic rates. Therefore, it was agreed that knowledge of the photosynthesis - light relationship (the P I curve) is required for the compilation of integrated incorporation rates over depth and light-day periods.[1]

The first section of these guidelines is devoted to concepts, incubation strategies, and calculations related to the P I curve. The second section is devoted to more technical aspects of the ^{14}C method. With respect to the latter, the Working Group decided that it was not possible to effect a complete standardization of methods, since each area might require modification and resources differ from one laboratory to another. The exact details of the method used can be left to the intelligence and experience of the operator. Various points which are thought to require judgement are identified in the final section of this manual and references given.

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[1] To date, field ecologists have seldom considered the underlying photochemical reactions when measuring photosynthesis at sea: the parameterization of the P I curve is the best that has been achieved in this respect.

Recently, however, several authors have been taking a closer look at the light-harvesting apparatus in phytoplankton. Hence, the concept of photosynthetic unit (PSU), which is the ratio of light-harvesting pigments to P700 (reaction-center chlorophyll of Photosystem I) has emerged.

The size of PSU has been shown to vary in response to fluctuations of environmental factors (Perry et al., 1981; Prézelin, 1981). Fasham and Platt (1983) have developed a new theoretical representation for the relationship between photosynthesis and light in phytoplankton, based on a simple model of processes associated with electron flow through Photosystem II, in which the size of the PSU of PS II is an important parameter. The quality of fit to various typical P I curves for natural assemblages of marine phytoplankton is unprecedented.

This new approach to primary production assessment seems promising and deserves attention in the future.

2 ^{14}C INCORPORATION AND THE PHOTOSYNTHESIS - LIGHT (P I) RELATIONSHIP

2.1 Concepts and strategies for sampling and incubation

Sampling and incubation strategies for measuring photosynthetic carbon incorporation rates must often take into account conflicting objectives, i.e., monitoring needs (frequently implying survey of very large areas within a short time period) and precision needs (implying a more detailed description of the production profile whenever local conditions justify it). Thus, there are two basic types of experiments which may be considered.

(a) An experiment which allows a complete characterization of the photosynthesis - light relationship (the P I curve) for each of the relevant depths at a given station.

The P I curve is meant to be used as a calculating tool for extrapolation to whole-day (i.e., light-day) inorganic carbon incorporation. Moreover, characteristic parameters such as initial slope (rate of incorporation per unit light at low photon flux densities) and saturated incorporation rate are valuable physiological and environmental indices when normalized to chlorophyll content. Several incubation techniques can be used to derive a P I curve: in situ incubations and "simulated" in situ either under natural daylight or artificial light. The simulated in situ techniques ought to be calibrated against in situ incubations.

(b) An experiment which allows interpolation between stations where type 1 experiments are carried out. - Adopting this approach, it is assumed that the P I curve, normalized to phytoplankton standing stock, will not change significantly for a given area over a given time period during the survey cruise.

Phytoplankton standing stock, or a related parameter, can then be measured at the recommended depths (see section 3.6) instead of undertaking a full primary production exercise. The parameter measured could be chlorophyll a or, alternatively, one of the two characteristic parameters of the non-normalized P I curve (either the rate of change of incorporation per unit light or the saturated rate of carbon incorporation; in the latter case, samples are incubated at a single light intensity using the light incubator).

NB There are definite advantages in using the parameters of the P I curve since the sensitivity is high and the values measured also have environmental meaning, especially when expressed per unit chlorophyll a. There are, however, considerable risks in using these parameters in a survey since diel variation - especially that of the saturated rate - is an established fact (Gargas and Hare, 1976; McCaull and Platt, 1977). This ought to be taken into account in the calculations of integral production (e.g., Gargas and Hare, 1976; Mommaerts, 1982).

In all cases, the incubation time should be the same for in situ, deck, and artificial light incubations; that is between 2 and 4 hours.

2.2 Calculation of light-day carbon incorporation (particulate and dissolved) at a given station, using the P I curve (see also example given at the end of this section)

The incorporation rate (mg C m^{-3} h^{-1}) versus light curves from a given station or from the nearest reference station in the area should be normalized to the chosen index of standing stock (chlorophyll, saturation incorporation rate, or rate change per unit light) and plotted.

The light field (depth versus time) for the entire day should be calculated using the light penetration profile and the day course of 100 % irradiance and tabulated. Similarly, the standing stock field (depth versus time) should be tabulated. Although the latter might reduce to a single profile in the simplest cases, provision should be made here for a possible diel variation of the parameter chosen to serve as an index of standing stock.

The normalized P I curve should be used to convert all the light values of the depth-time field into incorporation values normalized to standing stock. Finally, these values are transformed into total incorporation values (mg C m^{-3} h^{-1}) by multiplying them by the standing stock values from the corresponding depths and times.

The calculation of daily incorporation rate (mg C m^{-2} day^{-1}) can be made in several ways once the table of time versus depth incorporation values has been established. This is largely a matter of more or less empirical integration techniques.

(a) These operations - especially those mentioned in the preceding two paragraphs - are lengthy and are most easily managed with the help of a small computer (e.g., the desk-top type).

(b) The experimental P I profile can be simulated by a variety of mathematical functions. Hence, the computing work is greatly facilitated. The most appealing formulae are those that are precisely parameterized by the maximum incorporation rate and the change of rate per unit light at low photon flux densities (Mommaerts, 1982; Platt et al., 1977).

2.3 Results to be reported

Primary production, chlorophyll, and light data need to be reported. There are basically three levels of primary production data to be considered.

(a) **First-level data** refer to light bottle, dark bottle, and zero-time incorporation rates, as calculated in section 3.12, for particulate and dissolved production from the different incubations. Each ought to be reported separately, together with the corresponding photon flux density.

(b) **Second-level data** refer to more synthetic information which stems from the P I curve. The figures which should be reported are:

the saturated rate of incorporation (p_{max}) from the best fitted curve (mg C m^{-3} h^{-1}),

the corresponding photon flux density, I_{max},

the rate (α) of change of the incorporation per unit light in the proportional range, from the best fitted curve (mg C m^{-3} h^{-1} I^{-1}),

the rate of apparent loss (r) measured at photon flux density zero (extrapolation of the tangent to the y-axis)(mg C m^{-3} h^{-1}).

NB From this stage on, the rates refer to total incorporation (particulate and dissolved) and are corrected for dark uptake.

(c) **Third-level data** refer to incorporation integrated over the light-day and the water column (mg C m^{-2} day^{-1}).

Chlorophyll profiles, light penetration profiles, and day courses of 100 % irradiance need to be reported along with details of incubation procedures, including length of incubation, water collection methods, etc.

2.4 Detailed example of calculation (based on fictitious results from an in situ incubation around 10.00 a.m.)

Table 1. Incorporation profile.

Depth (m)	Light bottle (mg C m^{-3} h^{-1})	Dark bottle (mg C m^{-3} h^{-1})	Difference (mg C m^{-3} h^{-1})
0	12.06	-	11.20
0.65	15.68	-	14.82
1.50	18.62	-	17.76
3.00	15.94	0.86	15.08
5.00	7.16	-	6.30
10.00	1.52	-	0.66

Table 2. Chlorophyll a profile and interpolation.

Depth (m)	Chl a measured (mg m^{-3})	Depth (m)	Chl interpolated (mg m^{-3})
0	4	0	4
0.65	3.9	1	3.85
1.50	3.7	2	3.45
3.00	2.9	3	2.90
5.00	1.5	4	2.15
10.00	1.1	5	1.50
		6	1.30
		7	1.20
		8	1.15
		9	1.1
		10	1.1

Table 3. Light penetration profile (calculated from an attenuation coefficient $\eta = 0.45\ m^{-1}$)

Depth (m)	Light intensity (%) at selected sampling depth	Depth (m)	Light intensity (%) in the 10-m profile
0	100	0	100
0.65	75	1	63
1.50	50	2	40
3.00	25	3	25
5.00	10	4	16
10.00	1	5	10
		6	6
		7	4
		8	3
		9	2
		10	1

Table 4. Actual photon flux density during the incubation (based on average value of surface irradiance $I_o = 6$).

Depth (m)	Light intensity (arbitrary units)
0	6
0.65	4.5
1.50	3
3.00	1.5
5.00	0.6
10.00	0.06

Table 5. Surface light climate during the day (read from, e.g., continuous recording).

Time (h)	Light intensity (= irradiance at 100 % = I_o) (arbitrary units)
6	0
7	1.7
8	1.4
9	3.5
10	6.0
11	7.3
12	3.6
13	4.0
14	4.0
15	2.0
16	2.5
17	1.0
18	0

Table 6. Table of depth versus time light field (computed from the daily course of I_o and the formula

$$I_d = I_o \exp(-\eta d) \text{ (with } \eta = 0.45)$$

or combining the daily course of I_o with the actual light penetration (%) profile if the Lambert law is not obeyed).

Depth (m)	Time (h)												
	6	7	8	9	10	11	12	13	14	15	16	17	18
0	0	1.70	1.40	3.50	6.00	7.30	3.60	4.00	4.00	2.00	2.50	1.00	0
1	0	1.07	0.88	2.21	3.79	4.61	2.27	2.53	2.53	1.26	1.58	0.63	0
2	0	0.68	0.56	1.39	2.39	2.91	1.43	1.59	1.59	0.80	1.00	0.40	0
3	0	0.43	0.35	0.88	1.51	1.84	0.91	1.01	1.01	0.50	0.63	0.25	0
4	0	0.27	0.22	0.56	0.95	1.16	0.57	0.64	0.64	0.32	0.40	0.16	0
5	0	0.17	0.14	0.35	0.60	0.73	0.36	0.40	0.40	0.20	0.25	0.10	0
6	0	0.11	0.09	0.22	0.38	0.46	0.23	0.25	0.25	0.13	0.16	0.06	0
7	0	0.07	0.06	0.14	0.24	0.29	0.14	0.16	0.16	0.08	0.10	0.04	0
8	0	0.04	0.04	0.09	0.15	0.18	0.09	0.10	0.10	0.05	0.06	0.03	0
9	0	0.03	0.02	0.06	0.10	0.12	0.06	0.06	0.06	0.03	0.04	0.02	0
10	0	0.02	0.01	0.04	0.06	0.07	0.04	0.04	0.04	0.02	0.03	0.01	0

Table 7. Normalization of the incorporation profile to chlorophyll a.

Light (I)	Incorporation	Chlorophyll a	$\frac{\text{Incorporation}}{\text{Chlorophyll a}} = k$
6	11.20	4	2.8
4.5	14.82	3.9	3.8
3	17.76	3.7	4.8
1.5	15.08	2.9	5.2
0.6	6.30	1.5	4.2
0.06	0.66	1.1	0.6

Plotting k as a function of I generates the P I curve (Fig. 1) which will serve as an extrapolating tool for the calculation of daily integrated carbon incorporation.

Therefore, values of k are read on the curve for each value of I_d,[2] hence creating Table 8.

[2] As this is particularly tedious, a mathematical function adjusted to the experimental P I curve will help to compute k's instead of reading them.

Such a function utilizes the parameters k_{max} and α measured on the experimental P I curve (see Fig. 1). The fitted mathematical function is also shown in this figure. A perfect agreement between both experimental and mathematical curves is seldom achieved.

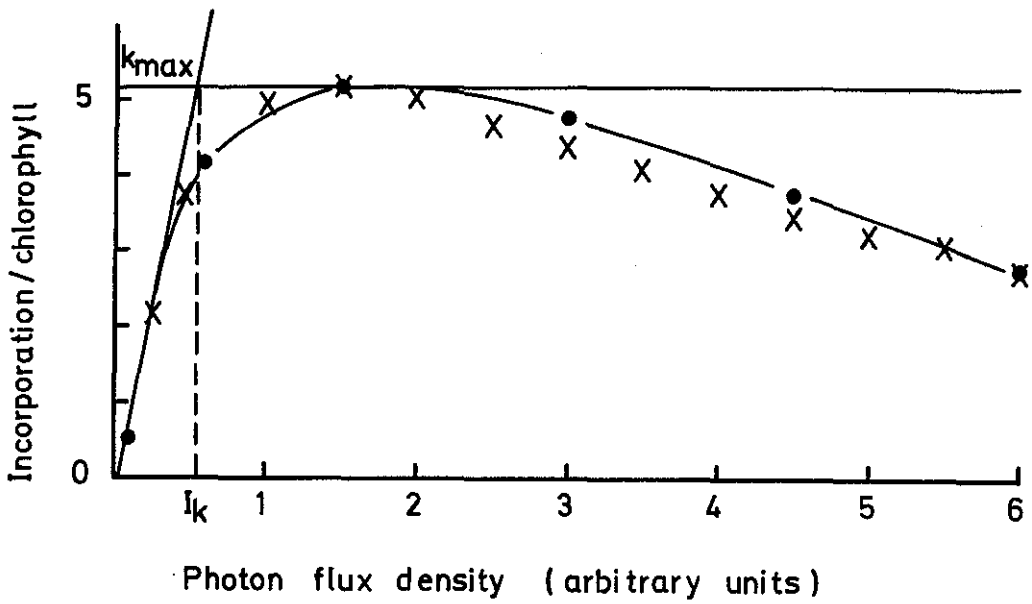

Figure 1. Photosynthesis/chlorophyll versus I plot. Characteristic parameters: $k_{max} = 5.2$; $I_k = 0.55$; $\alpha = k_{max}/I_k = 9.45$. o, experimental; x, model fitting (additional parameters: b = 1.22, $\alpha = 0.22$, and n = 1 (Mommaerts, 1982)).

Table 8. Table of k's in the time-depth field, calculated from the P I curve and time-depth light field.

Depth (m)	Time (h)												
	6	7	8	9	10	11	12	13	14	15	16	17	18
0	0	5.15	5.20	4.09	2.86	2.44	4.03	3.79	3.79	5.03	4.74	5.01	0
1	0	5.07	4.85	4.92	3.92	3.46	4.88	4.72	4.72	5.18	5.18	4.25	0
2	0	4.41	4.00	5.20	4.81	4.47	5.20	5.18	5.18	4.70	5.01	3.22	0
3	0	3.39	2.91	4.85	5.20	5.10	4.89	5.02	5.02	3.74	4.25	2.21	0
4	0	2.36	1.97	4.00	4.95	5.13	4.04	4.29	4.29	2.72	3.22	1.47	0
5	0	1.56	1.29	2.91	4.15	4.54	2.98	3.22	3.22	1.81	2.21	0.93	0
6	0	1.03	0.84	1.97	3.10	3.55	2.05	2.21	2.21	1.21	1.47	0.56	0
7	0	0.66	0.56	1.29	2.13	2.51	1.29	1.47	1.47	0.75	0.93	0.38	0
8	0	0.38	0.38	0.84	1.38	1.64	0.84	0.93	0.93	0.47	0.56	0.28	0
9	0	0.28	0.19	0.56	0.93	1.12	0.56	0.56	0.56	0.28	0.38	0.19	0
10	0	0.19	0.09	0.38	0.56	0.66	0.38	0.38	0.38	0.19	0.28	0.09	0

Table 9. Table of primary production (carbon incorporation) values in the time-depth field, calculated from the values of k (see Table 8) and the chlorophyll a profile (see Table 2). Values in the core of the table are in mg C m^{-3} h^{-1}. The sums of the rows are mg C m^{-3} day^{-1} and the sums of the columns are mg C m^{-2} h^{-1}. The total sum is, of course, the daily integrated incorporation value in mg C m^{-2} day^{-1}.

Depth (m)	Time (h)													
	6	7	8	9	10	11	12	13	14	15	16	17	18	Total
0	0	20.60	20.80	16.36	11.44	9.76	16.12	15.16	15.16	20.12	18.96	20.04	0	184.52
1	0	19.52	18.67	18.94	15.09	13.32	18.79	18.17	18.17	19.94	19.94	16.36	0	196.91
2	0	15.21	13.80	17.94	16.59	15.42	17.94	17.87	17.87	16.22	17.28	11.11	0	177.25
3	0	9.83	8.44	14.07	15.08	14.79	14.18	14.56	14.56	10.85	12.33	6.41	0	135.10
4	0	5.07	4.24	8.60	10.64	11.03	8.69	9.22	9.22	5.85	6.92	3.16	0	82.64
5	0	2.34	1.94	4.37	6.23	6.81	4.47	4.83	4.83	2.72	3.32	1.40	0	43.26
6	0	1.34	1.09	2.56	4.03	4.62	2.67	2.87	2.87	1.57	1.91	0.73	0	26.26
7	0	0.79	0.67	1.55	2.56	3.01	1.55	1.76	1.76	0.90	1.12	0.46	0	16.15
8	0	0.44	0.44	0.97	1.59	1.89	0.97	1.07	1.07	0.54	0.64	0.32	0	9.94
9	0	0.31	0.21	0.62	1.02	1.23	0.62	0.62	0.62	0.31	0.42	0.21	0	6.19
10	0	0.21	0.10	0.42	0.62	0.73	0.42	0.42	0.42	0.21	0.31	0.10	0	3.96
Total	0	75.66	70.40	86.40	84.89	82.61	86.42	86.55	96.55	79.23	83.15	60.30	0	882.16

NB A matrix with a time step = 1 hour and a depth step = 1 metre provides a fairly good approximation of the true integral incorporation. However, other techniques are equally valid and perhaps less time consuming, especially if there is no computer available. Other detailed examples can be found in e.g., Nielsen and Bresta (1984); Danish Standards Association, Standard No. DS 293 (1983); and Mommaerts (1982).

3 TECHNICAL ASPECTS OF THE ^{14}C METHOD

3.1 Preparation of ^{14}C solution

Ampoules can be obtained from a number of sources including the International Agency for ^{14}C Determination in Denmark. Alternatively, a solution of $^{14}CO_2$ can be prepared from $Ba^{14}CO_3$ transferred to $NaH^{14}CO_3$ in a closed evacuated system by acidification of the $Ba^{14}CO_3$ and absorption of the evolved $^{14}CO_2$ in a NaOH solution (Steemann Nielsen, 1952). A detailed description of the preparation of a $^{14}CO_2$ solution can be found in O'Reilly and Thomas (1983).

(1) The active solution should be diluted with freshly prepared double quartz glass distilled water. (2) The pH of the solution should be adjusted to 9.5-10.0. The pH range is chosen to minimize loss of ^{14}C during storage and handling of the solution and should not affect either the partial pressure of CO_2 or the photosynthesis of algae in sea water. (3) Only high-grade (p.a.) chemicals should be used for preparation of the ^{14}C solution.

3.2 Standardization of ^{14}C solution in the ampoules

Liquid scintillation counting can conveniently be used as a basis for computation of the absolute radioactivity. However, care should be taken to ensure that $^{14}CO_2$ is not "lost" upon the addition of scintillation fluid (Iverson et al., 1976). For the purpose of intercalibration, ampoules (preferably 10 chosen randomly from the batch) should be sent to the International Agency for ^{14}C Determination. Experience has shown that this procedure should be repeated each time a new source of $Ba^{14}CO_3$ is used and each time the preparatory technique is changed.

3.3 Samplers and bottles

Non-transparent, non-toxic sampling devices must be used. Experimental bottles should be thoroughly cleaned to meet standards similar to those required for culture flasks. Special care should be taken in the cleaning of sampling and incubation bottles to minimize contamination by trace metals (Fitzwater et al., 1982).

For practical purposes, bottle size can range between 25 and 100 cm^3 whenever simulated in situ or artificial light incubation is considered. Larger vessels may be required for in situ incubations in specific situations (Gieskes and Kraay, 1979). The bottles should be made of high-quality hard glass (e.g., Jena) or polycarbonate.

Before the start of an experiment, the bottles should be rinsed with water from the appropriate sample. The bottles should be filled up to the neck, leaving an air bubble. The stoppers of the bottle should always be tightly inserted in order to avoid loss of ^{14}C during the experiment.

3.4 The ^{14}C solution should be added to the experimental bottles in such concentration that statistically sufficient estimations of the radioactivity fixed by photosynthesis in the different fractions of the sample (dissolved and particulate) 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.

The concentration of $^{14}CO_2$ in the experimental vessels should be recorded by removing an aliquot (~ 50 µl) from replicate vials prior to the incubation. This aliquot should be added to a suitable volume of a strong base (e.g., NaOH or phenylethylamine) and the associated radioactivity determined (Iverson et al., 1976). This check is important to ensure comparability between results obtained in different water masses and/or by different workers, as handling procedures and water characteristics (especially pH) can affect the percentage of radioactive CO_2 initially present in an ampoule/or working solution which is transferred to water samples.

3.5 Dark fixation and non-biological fixation of carbon

Fixation measured in the dark can result from biological and non-biological processes. The biological processes are associated with the tricarboxylic cycle (e.g., the Wood-Werkman reaction) and, hence, also occur in bacteria and zooplankton. Non-biological phenomena are related to adsorption, contamination, and to background sensu stricto.

Dark fixation of carbon should be reported separately from the light bottle fixation.

Zero-time incorporation is thought to measure the non-biological processes. This should be determined whenever possible.

At each station, at least one dark bottle should be used. If vertical inhomogeneity of dark fixation is suspected (for example, if the chlorophyll is inhomogeneously distributed), more dark incubations should be performed at the relevant depths.

3.6 Sampling depths

Sampling depths should be selected so as to give adequate coverage of the photosynthesis-depth profile. It is, therefore, recommended that sampling depths be selected from those standard oceanographic depths which are as close as possible to depths at which, e.g., 100 %, 75 %, 50 %, 30 %, 10%, 3 %, and 1 % of the sub-surface levels are found.

3.7 Irradiance and light penetration profile

Irradiance should be expressed in quanta or other energy units. Whenever possible, measurements should be performed with a quantameter operating in the range 350/400 to 700 nm. Other types of instruments (e.g., pyranometers) may be used, provided an appropriate calibration with the quantameter has taken place. The time course of irradiance during the whole light-day should be recorded on each occasion. The

following approximate conversion factors for the spectral region 400 to 700 nm can be used:

$$1\ \mu E\ m^{-2}\ s^{-1} \{6.02 \times 10^{17}\ \text{quanta}\ m^{-2}\ s^{-1}\}$$
$$\approx 0.2174\ W\ m^{-2}$$
$$\approx 3.12 \times 10^{-4}\ ly\ min^{-1}$$
$$\approx 55\ lux.$$

Light penetration profiles ranging from sub-surface irradiance (taken as 100 %) to 1 % irradiance should be established with a quantameter operating in the range 350/400 to 700 nm whenever possible. Other devices might be used (e.g., submarine photometer equipped with a green filter and a diffusing filter; Secchi disc), provided an appropriate calibration with a quantameter has been carried out. It must be noted that the relation of log (1 %) to depth is not always linear, especially in the 60 % to 100 % irradiance range.

3.8 Incubation techniques

3.8.1 In situ

In this technique, samples are resuspended at standard depths (see 3.6) after ^{14}C inoculation. In doing this, special care must be taken to avoid excess light. This method has been described in many papers (for a review see O'Reilly and Thomas, 1983; Strickland, 1960, 1965; Strickland and Parsons, 1968).

3.8.2 Simulated in situ (deck incubator)

Samples are placed in an incubator, cooled by surface sea water, and exposed to daylight. Light levels in the incubator are controlled by means of neutral filters (e.g., black gauze) or coloured filters simulating light quality at the various depths. Some precautions must be taken in order to eliminate UV radiation (either using special glass plates or maintaining a sufficiently thick water layer above the incubation bottles) and avoid shadows (either from the incubator walls or ship superstructures). The attenuation coefficients of the filters should be determined with a quantameter in the operating incubator (i.e., with the cooling water) (O'Reilly and Thomas, 1983).

3.8.3 Artificial light incubators

Incubators in which it is possible to expose phytoplankton samples to constant photon flux densities have been described by several authors (e.g., Steemann Nielsen, 1963). Philips TLD 33 fluorescent tubes are usually used and the maximum irradiance normally allows saturation rates to be measured. Lower light levels are managed with the aid of neutral density filters. The bottles are usually fixed on a rotating wheel that provides adequate agitation. Cooling is provided either by circulating surface sea water or with a refrigeration system. Recently, a system which allows incubation of samples under varying light conditions has been described (Gallegos and Platt, 1982). Such systems may

be valuable in the study of, for example, upwelling regions. However, results obtained with such incubators cannot be compared with those obtained from more traditional incubators.

3.9 Filter and filtrate procedure

3.9.1 Filter and filtrate collection

Water samples should be filtered immediately after the incubation has been completed, in order to avoid loss of ^{14}C owing to respiration. Filters with even distribution of pore size and good solubility with respect to scintillation liquids are preferred. Pore size should not exceed 0.5 µm (Ursin, 1979). Filters should be wetted before filtration starts. The suction pressure should not exceed 0.3 kp cm^{-2}. The whole filtration procedure should not exceed 0.5 h for the entire series of bottles. If this is unavoidable, subsampling is recommended since this will also prevent self-absorption or quenching at the counting stage. The filters normally do not need to be washed. However, 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.

For practical reasons, filtrates will often be subsampled. In that case, the ratio of the subsample volume to initial volume must be known.

3.9.2 Preservation of filters and filtrate samples

When scintillation counting is used, the filters can be introduced to the empty scintillation vials and then deep frozen.

When Geiger counting is used, filters are dried in the presence of freshly dried silica gel in order to ensure rapid desiccation.

If these procedures are not possible, the filters should be exposed to formalin vapors to stop all biodegradation mechanisms.

Filtrate subsamples should either be deep frozen or acidified (pH = 2). The scintillation vials are adequate containers.

3.9.3 Preparation of filters and filtrate samples

The filters should be exposed to HCl fumes for 1 to 10 minutes prior to Geiger counting or further preparation for scintillation counting (Nielsen and Bresta, 1984; O'Reilly and Thomas, 1983).

The filtrate should be acidified to pH 2 to 3 and bubbled in the scintillation vial until all inorganic labelled ^{14}C has been eliminated (no more variation in the radioactivity level) (Schindler *et al.*, 1972; Theodórson and Bjarnson, 1975). The bubbling time will depend on the experimental set-up and should, therefore, be determined on a test sample.

3.9.4 Total sample method

It is possible to acidify and bubble incubated water samples in the same way filtrates are treated, hence eliminating the filtration stage (Schindler et al., 1972). Results are expressed as total ^{14}C incorporation. This simplified method might prove useful when speed is a more important factor in the sampling strategy than the completeness of information. It is useful to intercalibrate this technique with the filter and filtrate technique (O'Reilly and Thomas, 1983).

3.10 Counting

Two different methods are in use today for determining ^{14}C associated with samples: liquid scintillation counters (LSC) and end-window gas counters (Geiger or proportional counters). For determination of filter activity, both methods give similar accuracy.

Liquid scintillation counters are the more versatile as they can be used to measure the absolute activity of ampoules, filters, and filtrates and the total activity of acidified and bubbled samples. The counting efficiency of the various samples is found by measuring a standard of known activity under conditions practically identical to those of the samples. The counting efficiency of the LSC is 80 to 90 %.

End-window gas counters can be used for determining the activity of filters. The counting efficiency is found by measuring subsequently the absolute activity of a number of filters with an LSC. The counting efficiency is 10 to 20 %.

For both methods, proper care must be taken with various details in order to avoid errors.

With Geiger counting: (1) self-absorption caused by penetration of labelled matter into the filters, and (2) geometry. The former can be corrected for by counting the filters from both sides (Theodórsson, 1984).

With LSC: (1) solubility of sample in scintillator, (2) quenching, (3) initial chemoluminescence (causing a delay before counting can start), and (4) use of glass counting vials if long storage times are expected.

It is recommended that specialized publications be consulted for further details (Theodórsson, 1984; Ursin, 1979; Ursin and Bresta, 1980).

3.11 Total CO_2 concentration

Carbon dioxide concentration can be calculated from carbonate alkalinity, temperature, pH, and chlorinity. The relation between these variables is well established for the Baltic and the North Sea (Buch et al., 1932; Buch, 1945, 1951).

The carbonate alkalinity - total CO_2 conversion factor can be read either from the original graphs of Buch or from tables published in standard books (e.g., Strickland and Parsons, 1968).

Carbonate alkalinity is calculated from total alkalinity. This concerns a correction for the presence of boric acid, usually assuming that the free boric acid is always present in sea water at a constant ratio to chlorinity. In the Baltic area, the concentration of H_2BO_3 is about 1 % of total alkalinity.

The ratio of total alkalinity to chlorinity is well known for the Baltic area (Buch 1945; Gargas and Hare, 1976). On the other hand, it is known to be fairly constant in most sea areas (= 0.123). In practice, alkalinity needs to be experimentally determined only in coastal areas with marked land drainage and at great depths.

3.12 Calculation of carbon uptake

Carbon incorporation is calculated separately for each fraction using the following equation:

$$\frac{\text{incorporated radioactivity (DPM)}}{\text{added radioactivity (DPM)}} = \frac{\text{incorporated carbon}}{\text{available carbon}} .$$

"Incorporated radioactivity" refers to disintegrations per minute (DPM). Therefore, counts per minutes (CPM) must be converted into DPM, using the efficiency of the counting technique as the conversion factor. If a subsample is counted, this radioactivity is, of course, multiplied by the ratio of the total incubated volume to the subsampled volume. Historically, there has been widespread use of an isotope discrimination factor; a number of standard descriptions of the ^{14}C method for measuring photosynthesis recommend that "incorporated radioactivity" be multiplied by an isotope discrimination factor of 1.05. In view of the uncertainty as to what the ^{14}C method measures (see page 4), the validity of introducing this factor may be questioned. Inasmuch as this manual deals with actual "incorporation rates", the factor is not included here. Whether or not an isotope discrimination factor has been employed in calculating "primary production" rates should always be reported, so that different data sets may be adjusted for accurate comparisons.

"Added radioactivity" also refers to DPM. The absolute radioactivity of an ampoule can be determined by liquid scintillation counting or can be standardized at the International Agency for ^{14}C Determination. In addition, the amount of ^{14}C (DPMs) added to samples can also be determined (see 3.4) and used as "added radioactivity".

"Available carbon" refers to total CO_2 concentration in the experimental water, in the same units as incorporated carbon (mg C m^{-3}).

It is recommended that further correction factors (e.g., respiration) not be introduced at the present stage.

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5 MEETINGS OF AND PARTICIPANTS IN THE WORKING GROUP ON PRIMARY PRODUCTION

Texel, Netherlands, 23-25 June 1980

J.P. Mommaerts (Chairman)	Belgium
G. Berge	Norway
J. Davis	United Kingdom
W. Gieskes (Rapporteur)	Netherlands
M. Gillbricht	Federal Republic of Germany
K. Vagn Hansen	Denmark
P. Lehmusluoto	Finland
O. Lindahl	Sweden
J.E. O'Reilly	USA
T. Thórdardóttir	Iceland
J. Throndsen	Norway
T. Wyatt	United Kingdom

Tvärminne, Finland, 1-5 June 1981

J.P. Mommaerts (Chairman)	Belgium
G. Berge	Norway
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