ANNEX IV

REPORT OF SCOR WORKING GROUP 24
ON
ESTIMATION OF PRIMARY PRODUCTION UNDER SPECIAL CONDITIONS
(SCOR-IBP/PM)

REPORT OF MEETING IN SOUTHAMPTON, 30 JULY-1 AUGUST 1968

Members present:

T.R. Parsons (Canada), Chairman
S.Z. Qasim (India)
O.I. Koblentz-Mishke (USSR)
Shun-ei Ichimura (Japan)
P.D.V. Savage (UK), Rapporteur

Professor Raymont (UK) was also present at one meeting as an observer.

Preface

The following account of the meeting is primarily a set of working instructions for the group, rather than a final report on our conclusions. It is anticipated that when the necessary action has been taken in respect to this report, a final report of the working group will be prepared for circulation through our sponsors, the Scientific Committee on Oceanic Research (SCOR) and the International Biological Programme (IBP). This final report should be available following a second meeting of the group in approximately 18 months time.

Introduction

It was considered that the problems of this working group were so diverse that discussion should be divided into sections dealing primarily with (1) General problems concerning all environments and methods and (2) Specific problems dealing only with certain environments or methods.

1. General Problems

(1) In many environments, and especially in estuaries, eutrophic waters and polluted waters, the problem of obtaining a representative sample becomes very difficult. Where possible, therefore, it was recommended that a turbidity meter should be employed to locate patches of plankton and other suspended matter, and that samples from these patches should be collected along with samples taken at standard depths or light intensities.

(2) The integration of primary production values per unit volume per hour to units per m^2 per day could be carried out in a variety of ways to give different results. It was therefore recommended that all studies be carried out in situ (at least until such time as SCOR WG 15 could recommend the use of a standard incubator) and that the following procedure be used for integration, whenever possible:

Bottles should be placed at standard light intensity depths of 100, 60, 30, 10, 5 and 1% T[1] or at some fewer number of depths in cases where light attenuation was very rapid. Incubations should be carried out for half a day and multiplied by a factor of 2 to obtain the daily production.

1 A table of depths from Secchi disc measurements is to be given as an annex to the final report.

Integration of production values at different depths should be carried out by obtaining the sum of the average production between depths, times the depth interval.

In cases, where tidal range is large and where half day in situ incubations are not possible, 2 hour[2] in situ incubations should be carried out and the relative production with depth should be determined. The production for the water column should then be obtained from the absolute production of a surface sample incubated for half a day in a water cooled incubator exposed to natural light, and the relative production obtained from the 2 hour in situ incubation. For production estimates within intertidal areas, the final production figure ($mgC/m^2/day$) should be corrected to give the production for the average depth of water covering the area during the day.

In another special case where it is known that the mixed layer depth (Dm) is greater than the compensation depth (Dc), the final integrated value for primary production per m^2 per day should be multiplied by Dc/Dm to give the production for the water column (Cushing, 1962, J.Cons.Int.explor. Mer 27: 131-140). Finally in the absence of a turbidity meter (see item 1 of this section) it is recommended that samples be collected from immediately below, in the middle and above the mixed layer, in addition to those being collected at depths of standard light intensity.

(3) Although the ^{14}C-method has received wide use in measuring primary production, in certain circumstances it may not be the best method to employ. These circumstances will be dealt with in the section on "Specific Problems". However, carbon dioxide uptake is the only exact measure of primary production, as defined in Supplement 1. The use of derived methods (e.g. the oxygen technique, changes in standing stock) must therefore be considered, by definition, as being less accurate measures of photosynthesis except in such circumstances as may be described below.

(4) The working group recommended that in general the description of procedures given by Strickland and Parsons (A Handbook of Seawater Analysis, 3rd Edition, 1968) would be those to which any modification, or use referred to in the following text, would apply.

(5) While it might have been desirable for the group to review all published material connected with the special conditions, it was felt that this was impractical and instead it was decided that a list of supplementary references to certain aspects of the final report should be prepared. Members of the working group would send all their reference material to Dr. Parsons for the preparation of this bibliography.

(6) It was felt that readers should have adequate information on the availability of certain types of apparatus connected with this report and for this purpose a list of apparatus and suppliers will be prepared as an annex to the final report. Members of the working group would send all information which they had on apparatus to Dr. Savage who would compile this section of the report.

2. Specific Problems

I Use of the ^{14}C-technique for measuring primary production.

The working group noted that there already had been another WG concerned with the broad aspects of the ^{14}C-technique (SCOR WG No. 20). It was felt therefore that in general the recommendations of SCOR WG No. 20 should be adopted with the following additional comments on the general use of this method.

(i) Standardization of ampoules and counting.

It was agreed that either standardised ^{14}C ampoules, on which the activity is stated, should

2 A period of 2 hours is suggested here as being the most probable period at high water during which the bottles would not be subject to strong tidal currents. In some estuaries longer incubation may be possible.

be obtained from an agency or that ampoules prepared by an individual experimenter should be standardised using a scintillation counter. It was felt essential, however, that with each batch of ampoules, the standard deviation of the activity of each batch of ampoules should be given. In reporting results of counts it was further recommended that authors should always specify the type of counter used and give some value for the efficiency of their counting system.

(ii) Determination of total CO_2 in sea water.

For all truly oceanic environments, total carbonate estimation could be based on pH and chlorinity tables. For all other areas, however, the only satisfactory method to be recommended was direct manometric determination of carbon dioxide. Convenient instruments for this determination are the Van Slyke apparatus (North America and Europe) and the Productometer made by Nikko K.K. (Japan).

(iii) Loss of material on filters during filtration.

This effect was concluded to be highly variable but could in part be avoided by low suction pressures (1/3 - 1/2 vacuum). An approximate check on its magnitude can be made by filtering different volumes of the same sample and plotting the results (Arthur and Rigler, 1967, Limnol.Oceanogr. 12: 121-124).

(iv) Size of incubation bottles.

Participants were not in complete accord on the effect of bottle size on production. It was agreed, however, that experiments on this effect would be done both by Dr. Qasim and Dr. Savage on tropical and temperate environments respectively and the results communicated to Dr. Parsons so that they could be discussed at the next meeting.

II Use of the ^{14}C technique for measuring primary production in estuarine environments.

(i) Self-absorption.

In turbid inshore samples, self-absorption can present considerable problems in counting the activity of the filters. The amount of self- absorbing material on a filter can be reduced, however, by reducing the volume of sample filtered (e.g. for turbid waters containing 100 mg seston/l, the activity lost from a 40 ml sample on a 5 cm^2* filter will be approximately 4%). In eutrophic waters, however, subsampling from large (e.g. 250 ml) volumes of incubated samples was generally recommended, particularly when the ratio of plant to total particulate material was large. In oligotrophic waters it was recommended that large volumes of samples should be incubated, but if such waters happen to contain a large amount of non-photosynthetic material it was suggested that the activity should either be measured with a liquid scintillation counter or by total combustion and recovery of active CO_2 (Jenkins, 1965, J. Water Poll.Cont.Fed. 37: 1281-1288). Finally it was decided that the effect of different amounts of materials on the self-absorption of estuarine samples (both oligotrophic and eutrophic) should be found by experiments to be carried out by Drs. Qasim and Savage and reported at the next meeting.

(ii) Uptake of $^{14}CO_2$ in the dark.

Generally the uptake of CO_2 in the dark will be less than 5% of uptake in the light. Under turbid conditions, however, dark assimilation may amount to more than 25%, and up to 50% of the light bottle uptake. Under these conditions the assumption that the uptake of CO_2 in the dark is the same in both dark and light bottles may lead to erroneous results.

It was decided that certain experiments could be performed to test the effect of light on the chemosynthetic uptake of CO_2. These experiments would be carried out by Dr. Parsons.

* The actual surface area of the filter should match the area of the detecting head used for counting.

It was further recommended that in turbid estuarine waters it was necessary to have one dark bottle for each light bottle since variations in dark bottle uptake are generally quite large. It was reported that dark bottle uptake may vary with time and depth and the extent of these changes will be the subject of some experiments to be performed by Dr. Parsons.

The retention of carbonate by certain types of membrane filters was commented on and it was decided that this source of error could be readily checked.

(iii) Difficulties of carrying out ^{14}C in situ incubations in tidal areas.

Where the tidal range was small it was decided that in situ incubations for half a day should be carried out. In areas where estuaries are subject to strong tidal influence, incubations should be carried out for 2* hours at slack water and the results integrated, using a surface sample incubated on shore (see General Problems, item 2).

The possibility of using unmoored buoys which could float in and out with the water mass during a period of tidal exchange was also considered. In general, however, it was felt that these would constitute a navigational hazard in most estuarine areas.

(iv) Measuring of primary production of benthic algae in estuaries.

Much of the primary production in estuarine environments may be caused by attached macrophytes or algal "mats" on mud or sand surface. For the measurement of the primary production of these organisms it was decided that special techniques would be necessary such as had been described for macrophytes by Wetzel (Verh.Internat.Verein.Limnol. 15: 425-436, 1964) and for algae at the sediment surface by Brock and Brock (Limnol.Oceanogr. 12: 600-605, 1967) and Steele and Baird (Limnol.Oceanogr. 13: 14-25, 1968). It was recommended that some procedures for such measurements should be extracted from these references and further that the following people might be approached by correspondence in order to obtain further information on this subject:

Macrophytes	(Dr. Bellamy - U.K. to be contacted by Dr. Savage (Prof. Katada - Japan to be contacted by Dr. Ichimura
Algal mats	(Dr. Steele - U.K. to be contacted by Dr. Parsons (Dr. Marshall - U.S.A. to be contacted by Dr. Parsons (Dr. Round - U.K. to be contacted by Dr. Savage

As an ultimate result of this correspondence and by the use of published material it is hoped that recommended procedures for these conditions would be included as part of the working group's final report. Dr. Parsons would coordinate these efforts and circulate drafts of the recommended procedures.

III Use of the ^{14}C-technique for measuring primary production in polluted environments.

After some discussion of this problem it was decided that the effect of pollutants was generally highly specific and that particular methods might be formulated only when the particular pollutant was identified from among the very large number of substances discharged into marine environments. However, in cases where pollution resulted in eutrification of the environment, a few considerations given in the next section (IV), might be applicable.

IV Use of the ^{14}C-technique for measuring primary productivity in eutrophic environments.

(i) Two problems immediately associated with eutrophic environments were the tendency for filters to clog after small volumes had been filtered and self-absorption due to the quan-

* See footnote, page 15.

tity of material on the filters. Since both these problems are interconnected, it was decided that in general small aliquots of incubated samples should be taken for filtration, but that experiments would be done by Drs. Qasim and Savage to obtain some guidelines on the amount of material to be filtered, in order to avoid appreciable self-absorption and to give satisfactory filtering times (less than 5 minutes if possible). These experiments would be carried out primarily with different phytoplankton cultures.

(ii) In exceptionally eutrophic waters (Chl a 200-400 mg/m^3) it was recognized that carbon dioxide could become a limiting factor for growth and that an alternative technique to the addition of ^{14}C-carbonate should be employed*.

(iii) The possibility was noted that waters heavily eutrified with amine groups, might cause, through bacterial action, the production of ammonia which in turn could lead to local precipitation of carbonate (Sieburth, 1965. J.Gen.Microbiol. 41: XX). In such situations it was recommended that filters should be placed briefly in fumes of concentrated HCl before the commencement of counting.

V Use of the ^{14}C-technique for measuring primary production in oligotrophic waters.

(i) It was reported that in some oligotrophic areas where microscopic examination showed that the bulk of the primary production was due to blue-green algae, the measured ^{14}C-production was found to be considerably lower than was to be expected from visual examination of the standing stock. It was believed that this may have been caused either by the blue-green algae being exceptionally fragile to handling operations or that the inclusion of gas bubbles within the cells of these species causes a poor exchange of ^{14}C-carbonate with the cellular material. No immediate solution was suggested for these problems beyond the possible use of cell counts (either visual or electronic) as a method for measuring primary production.

(ii) It was recognized that in some oligotrophic environments the chemical precipitation of carbonate (as $CaCO_3$) might lead to erroneous results during the course of incubations. It was believed that brief treatment of filters in fumes of concentrated HCl would remove this possible source of error.

VI Use of the ^{14}C-technique for measuring primary production under the ice.

(i) Reports had been received from Dr. Kawamura (Japan) and Dr. Bunt (USA) on procedures for measuring primary production under ice. Members of the working group were most grateful to these persons for their assistance and it was decided that the 'précis' of Dr. Bunt's report should be included in this report and that further details of methodology should be requested from both the above mentioned authors and other workers in this field, as well as from reports that might be obtained from the literature (e.g. Fogg, G.E. (1967) "Observations on the snow algae of the South Orkney Islands:. Phil.Tran.Roy.Soc.Lond. B, Vol. 252, 279-287. Burkholder, P.B. and E.F. Mandelli (1965) "Productivity of microalgae in Antarctic Sea Ice". Science, 149, 872-874).

Précis of Dr. Bunt's report:

"In the ice 6-20 feet thick, holes are cut and metal liners are installed through the floor of sledge-mounted-huts. Immersion heaters are placed in these holes to slow down ice formation. A diver collects samples of ice in hand-held Van Dorn samplers. The ice samples thus collected are immediately transferred to the base camp laboratory for incubation, as in situ measurements are not possible. Generally the phytoplankton organisms are found in tiny spaces in between the ice crystals and therefore draining off the melted ice is not desirable. Sub-samples are taken from the partially melted ice for incubation, and these are placed in a specially designed incubator in which the source of illumination is from below. This is important as many of the cells are heavily silicified and soon settle to the bottom. The incubator is placed in a refrigerated bath which

* This problem is discussed further under the oxygen technique with respect to Trichodesmium blooms.

maintains a temperature of -2°C, as the organisms are very susceptible to an increase in temperature. The intensity of light is another important factor to be considered. The organisms are markedly shade adapted and therefore they should not be exposed to bright illumination. During incubation the use of neutral density-filters to bring the light source almost equal to natural illumination is extremely important.

In winter, as the environment becomes oligotrophic, a concentration of cells becomes necessary. In summer the water becomes highly eutrophic and therefore dilution (or incubation of small samples) is recommended. After incubation the ice crystals are allowed to thaw and the samples are filtered in a partially melted state."

Finally it was noted that the outline of a procedure submitted by Dr. Bunt included the use of an incubator which had not been considered for any other procedure in this report. In order to return to the concept of carrying out all primary production measurements in situ it was decided that the use of an oxygen electrode might be convenient in some circumstances and Dr. Koblentz-Mishke would look into this possibility.

VII Use of the oxygen technique for measuring primary production.

(i) It was noted that under certain circumstances it may be preferable to measure primary production by the oxygen technique. This included the fact that it was, in practice, the simplest way to measure primary production, particularly in such laboratories where facilities for other methods are not available. It was emphasized, however, that the oxygen technique would generally only be valid when the level of primary productivity was greater than 5 $mgC/m^3/hr$. In other circumstances it was noted that for macrophytes, under the ice (electrode), and in exceptionally eutrophic waters, the oxygen technique was probably as satisfactory as other methods, particularly when in situ measurements are sought.

(ii) In carrying out the oxygen technique it was recognised that there were certain precautions which could be added to the procedure already described in the Handbook of Seawater Analysis. These were as follows:

(a) For eutrophic waters and with certain species of algae (e.g. Trichodesmium) the possibility of bubble formation requires the use of a special incubation bottle having a bubble trap on or near the neck. This can be used to entrap any bubble when reagents are added at the end of the incubation period.

(b) Chemical oxidation may not be the same in light and dark bottles when samples contain large amounts of soluble organic substances. The role of photo-oxidation under these circumstances does not appear to have been well investigated and some experiments using organic pollutants will be performed by Drs. Qasim and Savage and will be reported on at the next meeting.

(c) Certain modifications may be necessary in the composition of the oxygen reagents added when dealing with eutrified and/or polluted waters. Published material on these modifications will be reviewed by Drs. Qasim and Savage and some experiments may be done in this respect.

VIII Other methods for the measurement of primary production.

A brief discussion was held on the use of other methods for the measurement of primary production. It was noted in particular that while dealing with problems related to marine food chains and trophodynamics, it would be more suitable to employ methodology which could be used to describe both an increase in plants by photosynthesis and their decrease as a result of grazing. In such situations the ^{14}C and oxygen techniques may be of limited use. In contrast, therefore, methods which directly measured an increase in standing stock, such as chlorophyll a determination or direct counts of particles (Sheldon and Parsons, 1967. Coulter Electronics (Canada) 66 p.)

would be preferable. In the former case it was noted that recent refinements in the methodology of distinguishing between living and dead chlorophyll (Lorenzen, 1967. Limnol.Oceanogr. 12: 343-346) and increasing the sensitivity of the method by the use of a fluorometer (Holm-Hansen et al., 1965. J.Cons.int.explor.Mer, 30: 3-15), would be of considerable help in determining small changes in the concentration of chlorophyll a. In the latter case it was noted that the size spectrum of particles gave more information on changes in the standing stock than estimates of total production (e.g. the ^{14}C-technique). However, it was concluded that in general these newer techniques had not become sufficiently well established to warrant their discussion in greater detail. Their inclusion for further discussion at the second meeting of the working group was recommended.

ANNEX IV
Supplement 1

Definitions (The following definitions are only intended for the purpose of clarifying terms used in the text of this report).

1. Primary production is the photosynthetic production of organic carbon in which carbon dioxide is the only source of carbon (units: $M.L.^{-3}\ T^{-1}$ or $M.L.^{-2}\ T^{-1}$).

2.

Highly Eutrophic	50-100 $mgC/m^3/hr$*
Eutrophic	10-50 $mgC/m^3/hr$
Oligotrophic	4-10 $mgC/m^3/hr$
Highly Oligotrophic	< 4 $mgC/m^3/hr$

3. Limit of sensitivity (approximate values)

	$mgC/m^3/hr$
^{14}C method	0.01
Oxygen	5.0
Chlorophyll a	5.0
Electronic particle counting	1.0

4a. Exact measurements of primary production:

Carbon dioxide uptake per unit time measured by the loss of dissolved CO_2, or the uptake of radioactive CO_2.

4b. Derived measurements of primary production:

Measurement of plant growth in which the property measured can be directly related to uptake of CO_2 by photosynthesis. Methods which may be included here are measurements of (I) oxygen evolved per unit time, and (II) changes in the standing stock per unit time (e.g. chlorophyll a.

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* There are wide differences in the definitions of these items and this appears to be in part related to the range of production values encountered by different investigators(e.g. coastal or oceanic). For Soviet scientists, however, the generally accepted classification is as follows:

$<$ 0.2 $mgC/m^3/hr$ oligotrophic
0.2 - 0.5 $mgC/m^3/hr$ transitional
0.5 - 1.0 $mgC/m^3/hr$ mesotrophic
1.0 - 10.0 $mgC/m^3/hr$ transitional
$>$ 10 $mgC/m^3/hr$ eutrophic