COMMONWEALTH OF AUSTRALIA

Commonwealth Scientific and Industrial Research Organization

Division of Fisheries and Oceanography

REPORT 8

THE ^{14}C METHOD FOR MEASURING CO_2 UPTAKE IN MARINE PRODUCTIVITY STUDIES

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1957

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SUMMARY

The techniques and equipment at present used by the C.S.I.R.O. Division of Fisheries and Oceanography, for the measurement of CO_2 uptake by the ^{14}C method are described in detail. This method was used in the recent Equapac expedition in conjunction with the Institut Francais d'Océanie, Noumea, on the "Orsom III." A new sampler consisting of twin "light" and "dark" perspex tubes is described. This permits incubation without transferring or agitating the sample.

In reporting measurements of CO_2 uptake by the ^{14}C method, no corrections are made for discrimination against ^{14}C or for respiration. It is felt that presentation of direct experimental results under specified conditions is preferable to that of results transformed by the use of questionable empirical formulas. This permits more direct comparison with data from other sources.

A review of the evidence available suggests that the ^{14}C method measures net production during photosynthesis, and can be used as such as a rather crude index of the relative productivity of different waters.

In citing this report, abbreviate as follows:

C.S.I.R.O. Aust. Div. Fish. Oceanogr. Rep. No. 8.

THE ^{14}C METHOD FOR MEASURING CO_2 UPTAKE IN MARINE PRODUCTIVITY STUDIES

By H. R. Jitts

I. INTRODUCTION

Many empirical attempts have been made in the past to assess oceanic production of organic matter. Measurements have been made of the variations of such quantities as plankton volumes, nutrient content of the sea-water, pH, oxygen content, and many other quantities connected with the production of organic matter. Perhaps the most useful work on organic production has been done in attempts to measure directly the rate of photosynthesis in sea-water. The "light and dark bottle" method of Gaarder and Gran (1927) for the measurement of photosynthesis by oxygen evolution has been successfully used by several workers (Riley 1941; Riley et al. 1949). The more recently introduced method of Steemann Nielsen (1952) which measures photosynthesis by the rate of uptake of CO_2 using ^{14}C has given a needed impetus to these studies. An important feature of this method is that it can be more efficiently and conveniently carried out in research vessels at sea. Whilst the relative merits of the two methods of Gran and Steemann Nielsen might still be an open question, without doubt the ^{14}C method has certain distinct advantages over the O_2 evolution method. It is more practicable as a routine method. It also permits the measurement of photosynthesis in waters where this is low. As there is evidence that a low rate of photosynthesis is characteristic of a major part of the oceans, this latter advantage is of great importance. The work of Ryther and Vaccaro (1954) suggests that for experiments over short periods the two methods give comparable results.

From the results of the cruise of the "Galathea", Steemann Nielsen (1952) has made an estimate of global oceanic production which differs considerably from a like estimate (Rabinowitch 1945) made on results obtained by Riley. Steemann Nielsen's estimate has been criticized on various grounds. The main criticism is directed at his adjustment of the CO_2 uptake, as determined by the ^{14}C method, for losses by respiration. This adjusted figure was taken as a measure of "gross production" or organic matter. Ryther (1954, 1956b) has presented evidence that it is more a

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measure of "net photosynthesis" though this has been denied by Steemann Nielsen (1955). A more important criticism, which can also be levelled with equal justification at other estimates, is that his is based on insufficient data. More information is needed on the levels and seasonal variations of the CO_2 uptake of the various water masses, on the depths of the euphotic zone, and on the depth variations of photosynthesis. Until these are available, estimates of global oceanic production must be only of incidental interest.

Whilst one must agree with Ryther (1956b) that the physiological significance of the ^{14}C method requires further investigation, there are good reasons for believing that even in its present form the measurement of CO_2 uptake by this method can give reliable measures of the relative productivity of oceanic waters. With this in mind, as the method becomes more widely used, it is important that the results obtained by different workers be as directly comparable as possible. This could perhaps best be done by the universal adoption of standard techniques and equipment, but as this is not feasible at present it is essential whenever this method is used, that precise details of technique and equipment be given.

The methods used by this Laboratory, as detailed below, were adapted from those of Doty (1956). They differ mainly in the methods of sampling and incubation. They have been developed for the measurement of CO_2 uptake in coastal waters off Sydney and were also used (in conjunction with the Institut Francais d'Oceanie, Noumea) on the "Orsom III" in a study of equatorial waters during the Equapac joint expedition. Results obtained will be published elsewhere.

II. METHODS AND EQUIPMENT

(a) Collection and Treatment of Samples

Sea-water samples are taken with the samplers described below. Into each duplicate "light" and "dark" sample is injected the contents of an ampoule containing a minimal amount of $NaHCO_3$ labelled with a known quantity of ^{14}C. The samples are exposed to light for a measured period of time and are then filtered through a Millipore filter. The filters are washed with 10 ml of 0.001 N HCl in a 3 per cent. solution of NaCl, dried by sucking air through them, and preserved in a desiccator over silica gel. The activity of each filter is measured subsequently. The total CO_2 content of each sample is measured on a separate sample of the same sea-water. The rate of CO_2 uptake is determined as detailed below.

Exposure to light of the labelled samples is done either

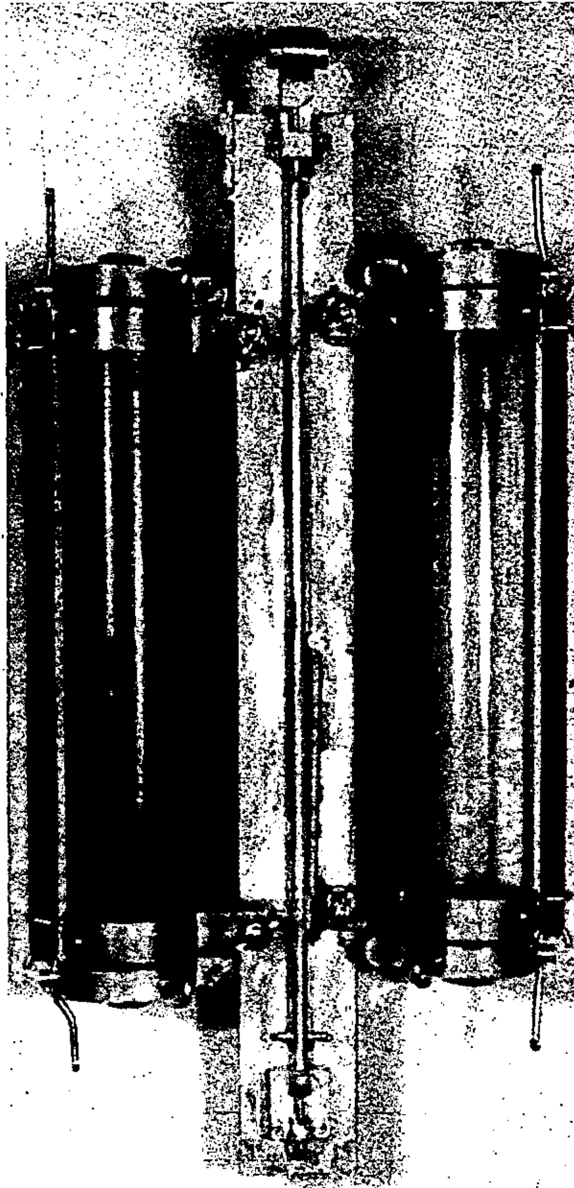

Fig. 1a.- Twin "light" and "dark" sampling bottle assembly.
Showing the two perspex tubes which are detachable from the central clamp and tripping mechanism.

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Fig. 1b.- Close-up of the twin sampler in the open position. Showing the perspex end-flaps with neoprene O-ring seals, and a central plug for introducing ^{14}C and for emptying the sampler.

by resuspending the samplers in the ocean at the depths from which they were taken, or by placing them in a light bath at a fixed temperature and light intensity. In the first case the samples are suspended in the ocean from sunrise to noon (local time) or from noon to sunset. The resultant uptake of CO_2 by the sample is multiplied by two. This gives the CO_2 uptake per unit volume of sea water per day at the depth sampled and under the conditions of light and temperature prevailing at that depth on the day of sampling.

When the light bath method is used, the samples are exposed for about four hours. In this case the resultant CO_2 uptake is divided by the number of hours of exposure. This gives the CO_2 uptake per unit volume per hour of the sea-water under the conditions of light and temperature of the light bath.

Doty and Oguri (1957) have presented some evidence that there is a diurnal cycle in the rate of photosynthesis of marine phytoplankton in Pacific Equatorial waters. Whilst the form and magnitude of this cycle have yet to be determined in other localities, the magnitude of variation they have shown is so great that it cannot be neglected. For this reason the time of sampling must always be recorded and whenever possible should be fixed at 0800 hours local time. This time has been chosen arbitrarily to conform with the method of Doty and also because his results suggest that photosynthesis is then at a maximum.

(b) Sampling Bottles

A special sampling bottle (Fig. 1) has been designed and constructed for the measurement of CO_2 uptake by sea-water. It consists of two perspex tubes each of 400 ml capacity, one clear and the other painted black. The ends of the tubes can be closed by means of hinged perspex end-flaps with neoprene O-ring seals. The end-flaps close under tension from three external rubber bands. The two tubes are attached by wing nuts to a tripping mechanism which can be clamped to normal hydrological cable. This mechanism holds the end-flaps open until actuated by a messenger. Several samplers can be clamped one above the other on the same cable to permit simultaneous sampling at several depths. All metal parts are made of nickel-plated brass.

The end-flaps are provided at top and bottom with threaded perspex plugs. Removal of the top plug permits the introduction of the tracer carbonate by means of a long-needled hypodermic syringe. For incubation, the closed tubes are detached from the tripping mechanism and placed in a light

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bath. Alternatively the sampler is reattached to the ca and lowered into the sea to the required depth for incuba under natural light conditions. After incubation the to is replaced by a threaded perspex nipple to which is atta a rubber tube with a suitable clamp. The tube is then i verted and the other plug removed to allow entry of air. The sample can then be run directly into the filtration apparatus for collecting the now radioactive phytoplankto

These samplers present several advantages over other methods of collection.-

(1) There is no transferring of the water sample, which is disturbed as little as possible.

(2) The water sample comes into contact mainly with perspex and to a very small extent, with neoprene.

(3) The twin "light" and "dark" sampling tubes permi simultaneous sampling at any depth and facilitate resuspe ion of the samples in the sea for incubation under natura conditions.

With very little alteration, the design of these sam would permit the automatic introduction of the tracer carbonate into the sample when the sampler is tripped. Th would avoid any possible effects of change in pressure an light intensities when the sampler is brought to the surf for the injection of the tracer carbonate.

(c) <u>Incubation Light-bath</u>

During the Equapac cruise of the "Orsom III" an incubation light-bath as shown in Figure 2 was used. This bath was built by the Institut Français d'Océanie, Noumea. It consisted of a square-sectioned metal-framed glass tank illuminated by eight 20 watt "daylight" type fluorescent tubes. This was contained in a lightproof box, and all the interior parts were painted white. Sea-water was pu continuously into the bottom of the tank and overflowed at the outlet pipe shown near the top. This kept the temper ature in the tank within 1°C of the surface temperature of the water through which the ship was sailing. The illumi ation in the tank was 1200 foot candles. This incubator took four of the sampling tubes described above: two "lig and two "dark". The "light" and "dark" tubes were placed in alternate corners so as to obtain as even a distributio of light as possible.

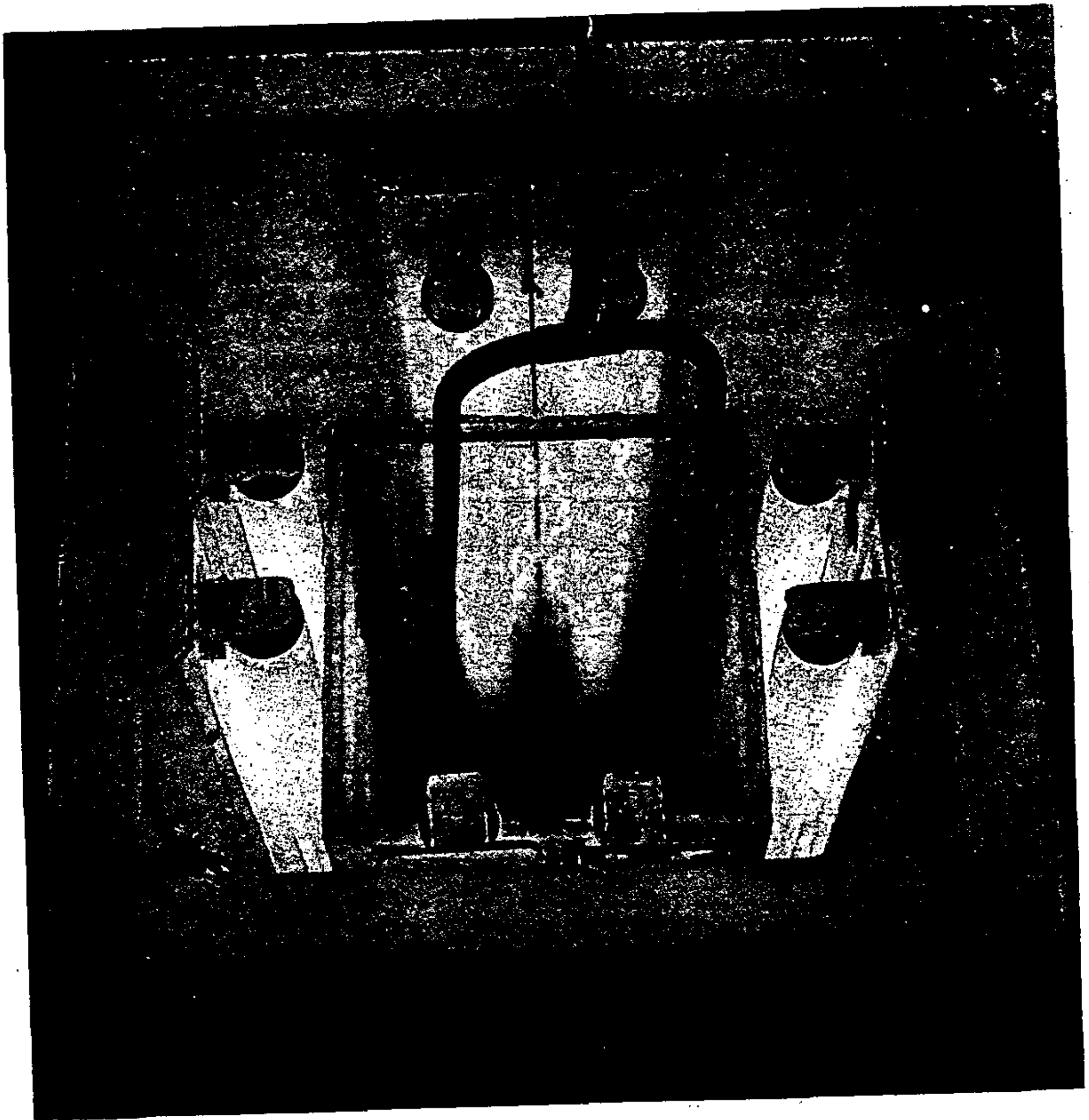

Fig. 2.- Incubation light bath. Used aboard the "Orsom III". Showing the glass tank surrounded by eight 20 watt fluorescent tubes. Sea-water is pumped continuously into the bottom of the tank and overflows through the central pipe shown.

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(d) Filtration and Counting of Samples

After incubation the samples are filtered with a Millipore Filtration Apparatus, Type XX10/100/00, and a rotary high-vacuum pump. Millipore membrane filters, Type HA (15/16th inch diameter), are used. These are rated to retain all particle sizes above 0.5 micron in diameter. The filtering surface area obtained with this arrangement is 2.54 cm^2. Filtration is very rapid and a 400 ml oceanic sample is normally passed through in less than five minutes. The filters are washed with 10 ml of 0.001 N HCl in 3 per cent. NaCl to remove excess labelled carbonate unassimilated by the phytoplankton. The filters are then dried by further suction and stored in a desiccator over silica gel pending subsequent counting.

All counting of the activity of the filters is done ashore in this Laboratory at Cronulla. It is carried out with EHM2 (G.E.C.) mica end window counters shielded with 1½ inches of lead. The scaler used is an "Austronic" SC3/100 with a resolving time of 20 µsec. The overall counting efficiency of this equipment is 7.5 per cent. of the theoretical number of disintegrations. This is considered unsatisfactory, and it is intended to replace the end window counter with a windowless gas flow counter in the near future. This should increase the efficiency by a factor of more than five.

Each sample is counted for at least 10 minutes. With the activities used, this gives a probable error of less than 5 per cent. for the majority of the samples. All counts are corrected for background.

(e) The Preparation of Ampoules of ^{14}C and Estimation of their Activity

The ^{14}C used is obtained in 1 millicurie ampoules from the Radiochemical Centre, Amersham, England. It is in the form of sodium bicarbonate, 14 mg $NaHCO_3$ in 0.83 ml of solution. This is diluted with 3 per cent. NaCl to give a solution of 30 microcuries (µC) per ml. The dilute 30µC/ml solution is dispensed in 1 ml aliquots into clean 2 ml ampoules by means of a tuberculin syringe. The ampoules are sealed and then autoclaved at 10 lb/in^2 for 40 minutes in a dye solution (Steemann Nielsen, personal communication). This ensures that the ampoules are properly sealed; they can then be kept indefinitely. The ampoules thus contain 30 µC of ^{14}C in the form of $NaHCO_3$ in 1 ml of 3 per cent. NaCl. The contents are emptied, with subsequent washing, into the samples for CO_2 uptake measurements.

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Whilst the activity contained in each ampoule is nominally 30 µC, it is necessary to measure its actual count rate under the same conditions of counter efficiency etc. as is used for the counting of sample filters. This permits the direct comparison of these two activities to be used in the estimation of CO_2 uptake in the method described in the next section.

In the measurement of sample filter activities it is assumed that there is no self absorption of the ^{14}C β particles, as the number of organisms on the filter is comparatively small and their density is low. However when measuring the activity contained in the ampoules the tracer carbonate is precipitated as an appreciable quantity of barium carbonate and the activity must be corrected to zero thickness of sample. This is carried out in the following manner.

The contents of an ampoule are transferred with washing into a solution containing 136 mg Na_2CO_3/l and made up to 1 l with the same solution. 10 ml aliquots of this active solution are treated with 1 ml of 0.1N $Ba(OH)_2$ and the $BaCO_3$ precipitate is filtered off with a Millipore membrane, using the same equipment as in the CO_2 uptake measurements. The filter area of 2.54 cm^2 gives a precipitate thickness of 1 mg $BaCO_3$ per cm^2.

To other 10 ml aliquots of the active solution are added a further 10 ml of the tracer free solution of 136 mg Na_2CO_3/l. Precipitation of these with 2 ml of 0.1N $Ba(OH)_2$ and filtration gives filters with precipitate thicknesses of 2 mg $BaCO_3/cm^2$. In the same way, by the addition of suitably larger volumes of the Na_2CO_3 solution and $Ba(OH)_2$, filters are obtained with precipitate thicknesses of 5 mg and 10 mg $BaCO_3/cm^2$. Although these filters are of varying thicknesses of $BaCO_3$, they all contain the same amount of activity, i.e. 1/100th of the activity in an ampoule.

The activities of the various filters obtained above are measured in the same way and with the same equipment as are the activities of the samples for CO_2 uptake measurements. The logarithms of these activities are plotted against the precipitate thickness. If the absorption of β particles by $BaCO_3$ is assumed to fall exponentially with the thickness of the precipitate, then extrapolation to zero thickness of the straight line connecting these results gives the activity of 1/100th of an ampoule.

This estimation of the zero thickness activity of the contents of the ampoules has been made and the results are

given in Figure 3. The extrapolation to zero thickness of the line of best fit, determined by the method of least-squares, gives an activity of 25×10^3 counts per minute (c/min). Thus when the contents of an ampoule are added to a sample the added activity is 25×10^5 c/min.

The above estimation can also be used for the calculation of the efficiency of the counting system used. Each ampoule is known to contain 30 μC of activity. With end-window counting a maximum of only half the total number of disintegrations can be counted. Thus the maximum count rate measurable from the 30 μC would be.-

$$\frac{1}{2} \times \frac{30}{1000} \times 3.7 \times 60 \times 10^7 \text{ c/min}$$

$$\text{i.e. } \underline{33.3 \times 10^6 \text{ c/min}}$$

With the counting system used in this Laboratory, the 30 μC of activity gives a count rate of 25×10^5 c/min. The efficiency of the counting system is therefore

$$\frac{25 \times 10^5}{33.3 \times 10^6} \times 10^2 \%$$

$$\text{i.e. } \underline{7.5\%}$$

(f) The Calculation of CO_2 Uptake

When the measured activity of the filter from the "dark" tube of the sampler is subtracted from that of the "light" tube, the "net activity" thus obtained is proportional solely to the amount of CO_2 taken up by the organisms in the sample during the period of incubation. The "dark" tube corrects for any CO_2 uptake by the filter or the organisms other than by photosynthesis.

The apparent specific activity of the CO_2 in the sample can be determined from the known "added activity" and CO_2 content of the sample. Thus the rate of photosynthetic uptake of CO_2 by the organisms, under the conditions of light etc. used, is calculated by means of the following formula, as given by Doty (1956).-

Rate of Photosynthetic uptake of CO_2

$$= \frac{\text{"Net Activity"}}{\text{"Added Activity"}} \times \frac{\text{Total } CO_2}{\text{Hours of incubation}} \times \frac{12}{44} \times 1000 \text{ mgC/hr/m}^3$$

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"Net Activity" and "Added Activity" are as described previously and are in units of counts per minute. "Total CO_2" is the measured content of CO_2 in all forms in the sample in mg CO_2/l. The factor $\frac{12}{44}$ x 1000 converts the CO_2 content to its equivalent in mg C/m3.

The results obtained by the use of the above formula are subject to corrections due to metabolic discrimination against ^{14}C as compared to normal ^{12}C and also due to loss of part of the ^{14}C assimilated due to respiration by the organisms during photosynthesis. Unfortunately the magnitudes of these two factors are still disputed. Rather tha apply questionable empirical corrections, none are used.

III. DISCUSSION

(a) Comparison of Incubation under Natural Conditions and in a Light Bath

In the previous section two methods were described for the incubation of the samples. The first was to resuspend the samples in the ocean for half the daylight period at the depths from which they were taken, the second to expose ther for a measured period in a light bath. As a measure of CO_2 uptake by a particular water mass, the first is certainl preferable. It measures the actual uptake of CO_2 by the water under study on the day of sampling. How representative this measure is depends only on the day to day variation of the CO_2 uptake itself and of conditions in the water mass. The only condition likely to vary considerably is the light intensity. Steemann Nielsen (1954) has sugges ed that even this would not have any great influence in tropical and subtropical waters. Two criticisms of the method are that it entails incubation of the sample for a longer period and that multiplication of the resultant CO_2 uptake by two to obtain the daily uptake may not be valid. The period of incubation of the sample must be kept as short as possible in order to minimise the effects of enclosure of the water sample. The suggestion by Doty and Oguri (1957) of a diurnal variation of the rate of photosynthesis would mean that CO_2 uptake during the first half of the daylight period would differ from the second. This could be answerec by extending the incubation to the whole daylight period. Unfortunately, the main disadvantage of this method is that it entails keeping the research ship hove to for extended periods which is often not feasible and is certainly costly.

The alternative method of incubation of the sample in a light bath is much simpler and less time consuming than the first method, but the results obtained can not be so

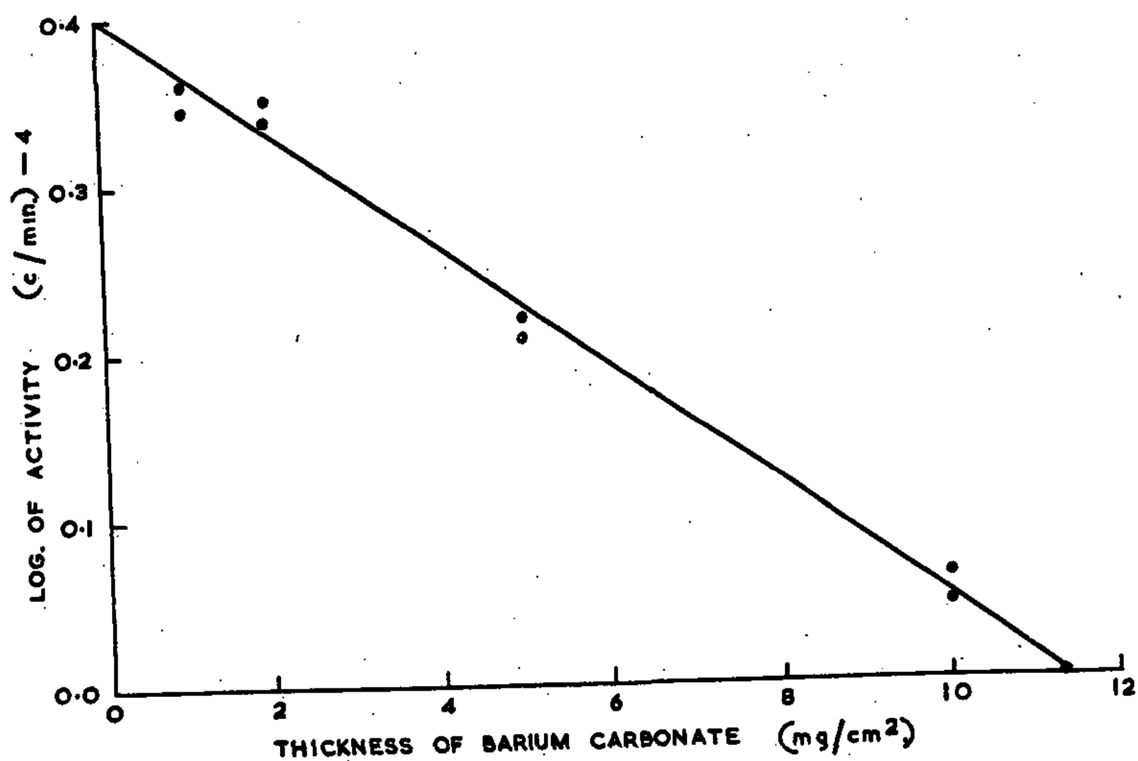

Fig. 3.- Determination of zero thickness activity of ^{14}C.
The log of the activity of several samples of $BaCO_3$, all containing the same amount of ^{14}C, is plotted against the thickness of the samples. The line of best fit is extrapolated to zero thickness of $BaCO_3$.

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readily used. In this method all samples are subjected to the same conditions of light intensity and temperature. The method measures the ability of the samples to photosynthesise under the specific conditions of light and temperature of the light bath and can be used as such for rather crude comparisons of the relative productivities of different water masses. If it is to be used to estimate the actual rate of photosynthesis of a water mass it would be necessary to know both light intensity and temperature at the position of sampling and their diurnal variations. It is also necessary to know the variation of the rate of photosynthesis of the sample with light intensity and temperature. The effect of temperature variations is largely eliminated by the system of keeping the light bath at the temperature of the sea surface waters. In oceanic waters, temperatures within the upper part of the euphotic zone, and those encountered at the surface during six hours steaming, do not differ usually by more than 1 or 2°C. The diurnal variation of light intensity at the depth from which the sample is taken can be estimated if one measures the extinction coefficient of the water concerned (see Ryther 1956a). However, in the case of the effect of light variations on photosynthesis much work is still needed. Steemann Nielsen (1952) has presented a relationship between light intensity and photosynthesis for tropical surface plankton and Ryther (1956a and b), one for pure cultures of various marine phytoplankton. There is little evidence that a general relationship can be used.

The above considerations suggest that, at the present stage, the method of incubation under natural conditions in the sea should be used whenever possible; preferably the two methods should be used simultaneously. When more information is obtainable, it should be possible to relate the results obtained by the two methods. This would permit the use of the second method of light bath incubation for easier collection of supplementary data.

(b) The Significance of CO_2 Uptake as Measured by the ^{14}C Method

From theoretical considerations, Steemann Nielsen (1952) applied a correction of + 6 per cent. to his results to account for metabolic discrimination against ^{14}C. Van Norman and Brown (1952) estimated this discrimination and obtained a figure of 14 per cent. Steemann Nielsen (1955) has criticized this figure, and using some of their data has recalculated it as 5 per cent. However his recalculation is based on certain assumptions concerning dark fixation and the relation of photosynthesis to respiration. In view of

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the evidence of Ryther (1956b and c) and Brown (1953) these assumptions cannot be accepted. Further work is required before any figure for discrimination against ^{14}C can be use with reasonable assurance.

Another correction applied by Steemann Nielsen to his CO_2 uptake measurements in order to obtain an estimate of gross production was + 4 per cent. to allow for ^{14}C lost during the experiment as a result of respiration. No reasons were given for the choice of this figure. In a later publication Steemann Nielsen (1955) stated that the correction for loss due to respiration would vary between 6 per cent. and 13 per cent. for respiration rates between 10 per cent. and 25 per cent. of the rate of photosynthesis. He suggests further that the latter quotient of 25 per cent. occurs only rarely in nature when light intensities are high It is evident however that the use of the ^{14}C method for the measurement of gross production is possible only if the quotient of respiration to photosynthesis is known. Whilst this quotient may well be below 25 per cent. at high light intensities, it will obviously increase with falling light intensities towards the bottom of the euphotic zone.

Ryther (1956 b and c) has presented evidence to show that the ^{14}C method measures the rate of net photosynthesis, i.e. the rate of CO_2 uptake due to photosynthesis less the simultaneous loss of CO_2 due to respiration. He suggests that this may be due to the preferential use of respiratory CO_2 for photosynthesis. He points out (1956b) that this is a measure of net production during photosynthesis only, as the method can give no estimate of the loss due to respiration at night.

This evidence of Ryther suggests that the ^{14}C method should be used as a direct measure of net production during photosynthesis, and that an arbitrary positive correction in order to obtain gross production is neither valid nor warranted. Basically, in a study of the productivity of the oceans information is required on two subjects; the size of the populations and the rate at which these change, i.e., the standing crop and the net production. Estimates of gross production can give only indirect information. In its present form the ^{14}C method can give a measure of net production during photosynthesis. To measure total net production, information is still necessary on the rate of phyto-plankton respiration. If, however, the method is used in conjunction with some other method which could give a

measure of standing crop of photosynthetic organisms, e.g., the measurement of chlorophyll content, a close approximation to a measure of oceanic productivity could be obtained. Alone, the ^{14}C method measures one of the vital factors in productivity, and as such can be used, in a more limited way, as a relative index of productivity.

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