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TABLE 1

Conditions of measurement of the quantity of primary production

Methods of Determination	Beginning of Exposure	Duration of Exposure	Conditions of Exposure
1. <u>In situ</u>	Dawn	Halfday, whole light day, or 24 hours.	In a water body at different horizons.
2. <u>Concentration Cfp</u>	Dawn or astronomical noon	Half light day	In a barrel on board.
K_T	Indifferent	Several hours	Indifferent, but under identical conditions of illumination
K_T	Dawn	Halfday, whole day or 24 hours	In a water body at different horizons.

During experiments in situ the time of exposure should envelop daily variations of natural illumination because of which half a light day is the shortest period of exposure. When conditions allow it the duration of exposure can be increased to a whole light day to one or even several 24 hour periods. A 24 hour exposure is the most desirable during such experiments. Under certain conditions (e.g. marked alkalization of the medium during mass development of algae in fresh water bodies and others) the exposure shortened to half a light day. With low water temperature and low photosynthesis the period of exposure can be increased to two to three 24 hour periods. It is desirable that samples from lower horizons be not exposed to bright daylight during the start and finish of the experiment. Therefore it is best to start the experiment at dawn and finish after sunset.

Photosynthesis at the water surface (Cfp) is main quantity for calculating the production by the indirect method. The period of exposure as in in situ experiments must envelop the entire range of natural illumination. At the same time it should be as short as possible since a number of side phenomena originate during exposure which lead to underestimating the results. Therefore, the standard period for these determinations is half a light day (either first or second). The samples for determining Cfp can be taken at any time and kept in a cool place until the isotope is added to them at dawn or noon. During the determination of Cfp the conditions of illumination should be as close to natural conditions at the sea surface as possible. Since this quantity is usually determined on moving vessels, one has to imitate natural conditions on board. This is achieved best in a large wooden tank (e.g. in a barrel) with circulating water. During exposure the flasks should be fixed directly under the surface so that they are not shaded by the tank walls. The side walls of the latter should be opaque and dark within. The use of glass aquaria is inadmissible because there is excess illuminations in them. The determination of Cfp should be done two or three times.

Coefficients K_T . Conditions and time of exposure of the flasks for determining K_T are indicated in all the conditions of measurement.

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ACADEMY OF SCIENCES U.S.S.R. INSTITUTE OF OCEANOLOGYMETHODOLOGICAL MANUAL

for determining primary production of organic matter
in water bodies by radiocarbon methods

CONTENTS

	Page
Main principles of the radio carbon method for measuring the amount of primary production	4
Schemes for calculating quantities of primary production and for carrying out measurements	5
Techniques of operations	11
Calculations	20
Accident prevention	24
List of main equipment	25
References	26

This manual was written during the conference on the primary production of water bodies organized by the Institute of Oceanology of the U.S.S.R. Academy of Science at the Black Sea station of the Institute in the summer of 1959. In compiling the manual the following took part:-

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The section on the determination of the initial activity from the self absorption curve of C^{14} was written by the research officer of the Laboratory of Biophysics and Isotopes of the Byelorussian Academy of Science, V.L. Kalin. The manual was discussed at the scientific conference on the primary production of water bodies held in Minsk in February 1960. The methods described were used and improved in expeditions carried out by the Institute of Oceanology mainly according to the plan of the I.C.I.

The manual is intended for persons with suitable biological, chemical, and radio-chemical training.

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The main principles of the radiocarbon method for measuring the quantity of primary production

The primary production of a water body is understood to be the quantity of organic matter synthesized per time unit from mineral substances by the autotrophic organisms which populate the water body of a certain size (area). The prominent part of the organic matter is created anew in the water body in the process of photosynthesis of phytoplankton. The photosynthesis of benthic plants plays usually a subordinate role, though in some water bodies, the shallow-water particularly, it might be of great importance.

The other autotrophic process, the bacterial chemosynthesis, is usually realized at the expense of the chemical energy of products of incomplete decomposition and oxidation of organic compounds formed in due time in the photosynthesis process (NH_3 , M_2 , and others). Therefore the chemosynthesis of bacteria, with some exceptions relative to a water body, is not, in its essence, an autotrophic process. Thus, the photosynthesis of phytoplankton is the main process, in the course of which new formation of organic compounds takes place in water bodies.

The organic matter of algae represents the initial source of nutrition for heterotrophic organisms inhabiting the water body. At the same time the release of oxygen during photosynthesis is the principal way to replenish the supply of this gas in the hydrosphere and the atmosphere. Oxygen is a necessary condition of the life activity of animals and, generally, of all aerobic organisms; it has a great influence of many physical and chemical properties of natural waters and is of great geochemical importance. Therefore, quantitative studies on photosynthesis of phytoplankton are very important in theory and practice.

At the present time, in order to determine the primary production of a water body, i.e. to measure the intensity of phytoplankton photosynthesis, the flask method is employed, in two modifications - the oxygen and the radio-carbon ones. The radio-carbon method proposed by the Danish scientist Steemann Nielsen (1952) is widespread in oceanographic investigations. In the Soviet Union, the radio-carbon method was used first by S.I. Kusnetsov (1955) in relation to fresh waters, and then by Yu. I. Sorokin (1956) who worked out an original scheme of experiments described below for determining the primary production. A number of works were carried out both in the sea and the fresh-water bodies with the use of this method (see Contributions to Plankton Symposium, 1957; Vinberg and Kaler, 1960).

Measurement of the primary production by the C^{14} method is carried out in the following way. A certain amount of carbon-labelled sodium bicarbonate, or sodium carbonate, is added into the flask filled with water taken from the water body, which contains some plankton in suspension. The flask is then exposed to light for a certain period. Under the effect of light photosynthesis is realized by the phytoplankton, in the course of which the carbon contained in the water (both radioactive and natural) is transformed by the algae into the organic substances of their body. On filtering the plankton on a membrane filter at the end of the exposure and on measuring its beta-activity, it is possible, taking also into account the initial $\text{C}^{14}/\text{C}^{12}$ ratio, to calculate the total amount of carbon assimilate in the process of photosynthesis.

The radio carbon method has a number of advantages and imperfections in comparison with the oxygen method. The main advantage of the radio carbon method consists in its higher sensitivity which exceeds that of the oxygen method by a factor of 100. Thus, the radio-carbon method makes it possible to measure photosynthesis of a very low intensity, or at short exposure periods. Besides this, the long half-life of C^{14} makes it possible to deal with the material long after its collection.

The main imperfection of the radio-active method is that there has been not as yet a sufficiently clear idea regarding under which conditions and to what extent the results of measurement of production are affected by the loss of C^{14} exhaled with carbon dioxide during respiration.

Procedure for calculating the quantities of primary production for carrying out the measurements

The calculation of the observation measurements obtained with the radio carbon method, is carried out by means of the formula

$$Q_p = \frac{C_k \cdot r}{R}$$

where Q_p is the quantity of photosynthesis during exposure, in mgC/l; C_k - the total quantity of carbon in all forms of carbon dioxide in water (CO_2 , HCO_3^- , CO_3^{2-}), in mgC/l; r - the radio activity acquired by the phytoplankton during exposure; R - the radio-activity introduced into the experimental flasks, measured under the same conditions as r , and expressed in the same units.

It is evident that the r/R ratio indicates what fraction of the added amount of C^{14} is assimilated by the plankton by the end of the exposure. Assuming that the same fraction of the total carbon content in the water is assimilated during the experiment, we obtain the above stated formula. Correct results of calculations are obtained when the following conditions are fulfilled:

(i) C^{14} is supplied to a cell during the process of photosynthesis only; (ii) C^{14} is assimilated by the algae at the same rate as C^{12} , i.e. the same fraction of the total amount of carbon (C_k) as that of the C^{14} isotope which is known from the r/R ratio is assimilated; (iii) the C^{14} assimilated in the process of photosynthesis is not concerned in other processes taking place simultaneously.

However, none of these conditions is completely fulfilled. Therefore Steemann Nielsen prepared a series of corrections in the calculation of production by C^{14} .

(1) The correction for "dark" fixation of CO_2 is associated with various biological and chemical processes. The magnitude as determined by various authors varies considerably. For instance, in most cases in open oceans it is small (one or a few per cent. of the magnitude measured) while in waters with large amounts of bacteria it is much larger. With very small quantities of production the dark fixation, though insignificant in its absolute value, comprises a considerable fraction of production.

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(2) The correction for the retarded assimilation of C^{14} in comparison with that of C^{12} was determined by Steemann Nielsen (1952) as equal to 5 per cent. of the photosynthetic production, and by Sorokin (1959) as 6.79 per cent. These figures cannot be regarded as final and are subject to further more accurate definition. It is known in particular that the isotopic effect increases with decreasing period of exposure.

(3) The correction for respiration (i.e. the liberation of CO_2 in light) is according to Steemann Nielsen 6 per cent. of photosynthetic production under optimum light conditions. It follows however from Ryther's experiments that C^{14} is practically not liberated in light.

Due to a lack of definite data, and the contradictoriness of data given by different authors, many research workers suggest the above corrections not be introduced for the time being, restricting themselves to the direct determination of the amount of radioactive carbon which entered the particles retained on the filters in darkness. It is recommended that samples in darkened flasks be exposed simultaneously with those in light flasks under the same conditions. This eliminates the necessity for introducing the correction for dark fixation of CO_2 . When publishing the results it is essential to indicate whether or not corrections were introduced and if they were to state which.

In order to obtain the quantity of primary production per unit of area of the water body one should know the distribution of the intensity of photosynthesis over depths which depend mainly on the vertical distribution of light, phytoplankton, and partially temperature.

It is possible to calculate the total quantity of photosynthesis or the production under a square meter of the surface of the water body by different methods. As was shown by comparative investigations the two methods which have been used so far give nearly identical results so that the procedure selected depends on operating conditions.

By the method of direct determination of the rate of photosynthesis (in situ) the latter is measured in samples of water taken from different depths and exposed at the same horizons after the addition of the isotope (Procedure H, Fig.1). This procedure makes it possible to take into account changes in the intensity of phytoplankton photosynthesis with depth in the most ideal way.

On measuring photosynthesis at each horizon a curve is plotted. The area included by the curve corresponds to the production in the water column.

To use the in situ method, prolonged exposures of the flasks in the water body are necessary which is far from being possible. Therefore particularly in ocean conditions one should frequently use the other method.

The method of indirect calculation of production at different depth is based on the determination of the quantity of photosynthesis in a sample from surface water layers and two correction coefficients which reflect the dependance of the rate of photosynthesis at different depths on the penetration of light (K_T) and on the vertical distribution of phytoplankton (K_p) (Sorokin 1956, 1958, 1959a).

The coefficient K_T can be determined in two ways. When the first way is used an in situ experiment is carried out in the water body (Procedure A, Fig.1). The results of measurements obtained at each depth are divided by the quantities obtained for parallel samples

i.e. the results of measurements are expressed in relative quantities, the quantity of photosynthesis measured under constant light (on board) being taken as unity. These relative quantities represent the coefficient K_p for each horizon. In practice when the same amount of C radioisotope is added to the flasks of identical volume and the activity is determined under identical conditions it is sufficient to take the results of measurements of the activity of corresponding filters for calculating the coefficients.

When the second way is used, a uniform sample of water is poured into a number of flasks (Procedure B, Fig.1) which are then exposed in the water body at the same horizons as in the case of the *in situ* experiments. The results are also expressed in relative units, i.e. in fractions of the photosynthesis at the surface. To increase the accuracy and to shorten the periods of exposure during the determination of K_p it is recommended that flasks be filled with water first enriched with phytoplankton. When the water is enriched with phytoplankton zooplankton can be eliminated by different methods, for instance by filtration through coarse meshed sieves. For oceanic plankton it is possible to hold the sample of thickened plankton for 15-20 minutes. During this period the oxygen content decreases, zooplankton settles to the bottom and it becomes possible to eliminate them by decantation.

Coefficients K_p are determined only at occasional prolonged stations with the specific optical characteristics of the water for a given region and season with relatively low variations from station to station. Therefore, as it has been shown by studies of the method, the results of determinations of K_p obtained at one station can be applied to neighbouring stations.

The above method of calculation gives approximate results only, because it does not take into account variations in the quantity of solar radiation from day to day. The coefficients K_p obtained under certain weather conditions are applied to calculations at other stations carried out under different conditions. Therefore, another method for obtaining the coefficient K_p that is derived from Ryther's papers, is briefly described below. According to this method, the experiments for finding the relationship between photosynthesis and light must be accompanied with the measurements which make it possible to calculate the total solar radiation for a day at different horizons, which is possible without lengthy stoppages of the vessel. If curves with subsurface maxima such as in Fig. 2A are obtained as the result of the measurement of photosynthesis of the uniform samples of phytoplankton placed at different depths, they can then be used for further calculations. Their components are expressed in relative units, the maximum value being taken as 100%. Substituting for depths the corresponding quantities of light energy it is easy to plot the curve for variation of photosynthesis with light (Fig. 2B), similar to those given by Steemann Nielsen (1959) and Ryther (1956-57) in their work. The difference consists in that these authors obtained their curves under conditions of artificial illumination but the method suggested makes it possible to obtain them under natural conditions. Preliminary comparisons showed that the behaviour of the curves for the phytoplankton which inhabit one and the same biogeographical type of water varies insignificantly. It is sufficient therefore to obtain one curve and it would be possible to use it within a given region and season. To obtain the coefficients K_p the values of the relative photosynthesis are taken from the curve, which corresponds to the total radiation recorded at different depths and the values obtained are expressed in per cent of surface photosynthesis.

The coefficients K_p vary much more from station to station than K_d and should be determined separately at each station.

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To determine coefficients K_f (Procedure C, Fig.1) the flasks are filled with water taken from different horizons and incubated with identical illumination (on board, in the laboratory etc.). The value of K_f is equal to the quotient of the division of the activity of the filter obtained by means of filtering the sample taken at a certain depth by the activity of the surface sample.

By multiplying the coefficients K_f and K_p , referring to one and same depth, the total correction coefficient K_0 is calculated.

$$K_0 = K_f \times K_p$$

The coefficient K_0 shows the factor of the intensity of photosynthesis at a given depth to that at the surface. Multiplying the intensity of photosynthesis at the surface by K_0 we obtain the photosynthesis at the depth to which the given magnitude of K_0 corresponds. Having determined it for a sufficient number of horizons it is possible to obtain the production under a square meter of surface of the water body. By calculating the intensity of photosynthesis (production) for each depth and plotting the corresponding graphor by plotting K_0 (see page 23) in the latter case the area restricted by the curve shows the ratio of photosynthesis in the water column to that at the surface. In order to obtain the absolute quantity of photosynthesis under one square meter this ratio should be multiplied by the photosynthesis at the surface, i.e. by Gfp. The area can be measured in different ways (planimetry, weighing, calculation).

TECHNIQUES OF OPERATIONS

Preparation of the initial solution containing radioactive carbon. Different quantities of radioactive carbon are added into experimental flasks depending on the abundance of phytoplankton in the water body. Usually the isotope ^{14}C is brought into a flask so that its activity is 2 to 80 C/1. This quantity is selected depending on season, abundance of phytoplankton and the duration of the experiment. For instance, less active initial solutions should be used in summer than in winter; in the case of strong development of phytoplankton in the water less active solutions should also be used than in the case of water with poorer phytoplankton.

The initial solution of ^{14}C is prepared from the preparation supplied by industry as follows. A certain quantity of distilled water freed from CO_2 by boiling is poured into well washed flasks with ground stoppers and 1% solution of KOH is added (2 ml of alkali per 1 l. of water). The alkaline medium prevents volatilization of the $^{14}\text{CO}_2$. The content of the ampoule of radioactive isotope is transferred into the same flask, fulfilling all the rules of accident prevention, after which the flask is closed and the solution shaken. In the case of high activity of ^{14}C preparation it is diluted repeatedly.

The solution must be kept in carefully closed flasks in darkness. The activity of the solution decreases with time due to exchange of ^{14}C with ^{12}C of the atmosphere. Besides this micro-organisms can develop in the solution. In connection with this before being introduced into the experimental flasks the working solution is filtered each time through a membrane filter No.1-2 to remove particles, mostly bacteria containing ^{14}C .

After filtering the working solution of radioactive carbon can be poured into ampoules in required concentrations and volumes calculated per flask or per the whole number of flasks for a single experiment. The ampoules should be sealed, sterilized in an autoclave, and used directly with the arrangement of the experiment.

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Sampling, filling of flasks, introduction of the isotopes. The flasks to be used in experiments should be washed with a chromium mixture or cleaned from bacteria by other known methods. It is preferable to use for sampling a water sampler made of polyethylene plastic, plexiglass, or glass (Van Dorn 1956, Jitts 1957) because metal oxides have toxic effects on algae. Water samplers made of plastics should be washed with hot fresh water. When a metallic water sampler is used its internal surface should be carefully cleaned and covered with a mixture of wax and paraffin (1-1) before the operation.

To determine the depth of sampling one should take into account the transparency of water and the vertical distribution of phytoplankton. When working in open seas standard horizons are ordinarily used (0, 10, 25, 50, 75, 100, 150, and 200 m). To obtain the curves for K_T and in in situ experiments one should take additional samples at depths of 5, 15, and 35 m, and for obtaining the curves for K_T at the horizons of phytoplankton accumulations which are frequently observed in the layer of water density discontinuity. A layer of phytoplankton accumulation can first be found optically by means of a transparency meter.

Samples of water taken by the water sampler are poured into light and dark flasks with ground stoppers. The volume of flasks should be equal to or more than 500 ml in oceanic operations, whilst in fresh water bodies it could be 60 to 200 ml. If the flasks are submerged to different depths the light flasks should first be placed in black bags or a case, filled with water and removed just before being submerged. Otherwise a burst of photosynthesis will occur in light and the production in depth will prove to be over-estimated. As well as placing the flasks in darkness one should also arrange a control for their exposure to light for a period equal to that for the start and finish of the experiment. For the period of exposure these control flasks are placed at a depth to which practically no light penetrates. For such a control 2 flasks are usually sufficient which are kept at a depth of say 100 or 150 m.

The working solution is introduced either with a Mohr pipette connected by a rubber tube with a syringe or a bulb, or an automatic pipette or a burette.

After the isotope is added the flasks are closed with stoppers and their content carefully shaken.

Conditions, time and duration of exposure. Conditions of exposure are different depending on the procedure of experiments chosen. When the primary production is determined by the direct method (in situ) (Procedure A, Fig.1). The flasks are exposed at the same depths from which water was sampled. When determining the production by the indirect (computation) method one should know the quantities of daily photosynthesis at the surface (Cfp) and the coefficients K_T and K_p . The exposure during determination of these quantities occurs under different conditions which are summed up in Table I.

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illumination and at identical temperatures and the duration of exposure is sufficient to obtain measurable results. However, it is most convenient that the exposure during the determination of K_r be of the same duration and under the same conditions as in the case of determining G_{fp} because the repeatability of the determination of photosynthesis of the surface sample is thereby increased.

Coefficient K_r Prolonged stations are used for determining K_r . Duration and conditions of exposure are associated with the same reasons as in the in situ experiments.

Completion of Experiments.

Immediately after the experiments are finished the contents of the flasks are fixed with neutralized formalin (10 ml 4% formalin + 1 l. of water). When working at sea where the amount of phytoplankton is low and samples filtered fairly quickly, it is possible not to fix the contents of the flasks.

The samples are filtered through a funnel with a membrane filter by a vacuum pump. The filters ordinarily used for filtering the samples are of the commercial type "Preliminary" or No. 5. Special experiments showed that the use of "Preliminary" filters during the determination of phytoplankton production can give an error because these filters happen to be of inferior quality and let pass the finest forms of algae (Peridinea, Coecolithophores, coloured flagellates, fine Protozoans and others) which may compose a considerable amount of the phytoplankton. In connection with this the "Preliminary" filters should be carefully selected by microscopic examination. The filters No. 5 retain practically the entire phytoplankton contained in the water sample but filtration through them takes much longer particularly in the case of fresh water bodies. When working at sea and oligotrophic fresh water in which the amount of phytoplankton is low and generally speaking always when possible, it is preferable to use filters No. 5. Before filtration the membrane filters should be boiled several times in distilled water until the odour disappears. One should observe that filtration takes place over the whole surface of the filter and that the deposit is uniformly distributed which is assisted by a plankton sieve, hose, or filter paper laid beneath the membrane filter. In this case the rate of filtration is slightly decreased.

The filtration of a sample should be carried out through a funnel with a smaller filtering area than that of the window of the end window counter used for measuring the radioactivity of the filters. When selecting the size of the funnel one should take into consideration that the smaller the diameter of the filtering area of the filter, the more accurate the results of the count of the activity. It is best to use funnels made of polished plexiglass, glass and in an extreme case of nickel-plated brass. It is convenient to use a number of funnels combined in one set (Angot, Doty and Oguri, 1956). The samples can be filtered completely or partially with subsequent calculation of the quantities of production per certain volume.

Treatment of Filters. After the filtration of a sample mineral particles containing the radioactive isotope can be found on the filters besides the plankton. They should be removed by means of hydro-chloric acid which can be done by one of three methods :

1. 1 ml of 2% HCl is passed through the filter.
2. The filters are transferred to a filter paper and wetted with a few drops of 2% HCl.
3. The filters with their contents are placed on the surface of 0.1N HCl poured into a Petri dish and left floating on the acid surface for 2 to 3 minutes.
(The surface of the filter with the plankton should face upwards)

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When working at sea the HCl is prepared from a solution of isotomic NaCl.

In all the three cases in order the remaining HCl the filters are washed with distilled or filtered sea-water in the same way as in the acid treatment.

When the first method is used it is necessary either to add an alkali into the filtration flask or to observe that the air containing $^{14}\text{CO}_2$ which passed through the vacuum pump is removed from the working area by means of a hose put on the outlet pipe. In such a form the first method is most convenient from the point of view of accident prevention.

After washing with water the filters are dried first in the air for which purpose they are placed onto a filter paper in a Petri dish and finally in a desiccator over CaCl_2 and soda-lime. The activity of the filters is counted by means of an end-window counter. To avoid curling of the filters when dried it is recommended that special holders be used which ensure the flat surface of the filters which is necessary for determining their activity. The holders should not touch the filtering surface.

When there is no possibility of determining the activity of the filters immediately after their treatment they should be stored in a desiccator over CaCl_2 and soda-lime. The latter is introduced in order to decrease the loss of activity of the filters due to isotopic exchange with the CO_2 of the air. During storage of the filters one should pay specific attention to ensuring conditions under which the upper surface of the filters have no contact with anything else.

To control the cleanliness of operations (the quality of the initial solution of isotopes, the adequate washing of the filters, etc.) one should perform from time to time a blank determination either with distilled water or with the water used in the experiments without exposure.

The Determination of the Initial Radioactivity

The determination of the initial radioactivity R_0 , i.e. the activity added to the experimental flasks is carried out under the

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same conditions as the determination of the activity of the experimental filters; the same filtering equipment (and therefore filters of the same area) is used, the same counter, the same counting geometry etc. In this case it is sufficient to determine the relative activity and to express the results of the experiment in the number of pulses per time unit.

To determine the initial activity of the solution which contains ^{14}C in carbonates, bicarbonates and free CO_2 , BaCl_2 is added to it and it is filtered through a membrane filter onto which a layer of the deposited BaCO_3 is so placed which contains the whole amount of ^{14}C in the solution.

If the layer of precipitate is fairly thin (less than 0.032 mg/cm^2) the activity of the precipitate measured corresponds to the initial activity. However, the presence of CO_2 in the reagents and the surrounding air result in that the precipitates obtained are usually thicker. The influence of two opposite effects, self-absorption and self-scattering begin to appear with increasing thickness of the precipitate, which makes the introduction of corrections necessary. The number of pulses measured decreases with increasing thickness of the preparation due to self-absorption. The self-scattering of the radiation within the preparation increases the number of pulses counted by the instrument. At first it rapidly increases with the thickness of the preparation, reaches a maximum at a still thin layer (tenths of a mg/cm^2) and then decreases.

An example of the relationship between the count rate and the thickness of the preparation is given in Fig.3. The magnitude of the maximum and the slope of the decrease observed on the curve when operating with end-window counters, depend on the counting geometry, the construction of the counter thickness of its window and the method by which the preparation was made. When working with internal counters it is possible to use published curves (Calvin, 1949, Steemann Nielsen, 1957). In all other cases each research worker should obtain a correction curve for the conditions used. It is possible to store

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prepared specimens with different thicknesses of precipitate and use them when counting conditions are changed.

In practice the self absorption curve is plotted as follows. A quantity of the initial radioactive solution suitably diluted to contain 0.1 to 0.15 C of the radioactive carbonate is placed in a number of wide test tubes washed immediately before the work. Then different quantities of a solution of sodium carbonate with the stable isotope of carbon are added into each test tube so that the weights of barium carbonate precipitates obtained were within 0.1 to 30 mg/cm². For a better coagulation of the precipitate 0.1 ml of 1% solution of Ni₂Cl are also added.

For precipitating, a mixture is prepared of 200 ml of 0.1N NaOH and 5 ml of 2N BaCl₂. The mixture is allowed to settle in a flask (for precipitating the carbonates which were in the alkali). After decantation, 3 ml of NaOH solution free of carbonates are added to each test tube with a burette, after which the test tubes closed with Bunsen valves and heated in a water bath for 30 minutes at 80°C. The precipitate thickens and settles to the bottom of the test tube. The content of the test tubes are cooled down to room temperature and transferred quantitatively to the filter No.5

(Fig.3 here)

The completeness of precipitation is checked as follows. 1 ml of the filtrate is evaporated on a disc of filter paper 1 cm in diameter after which the disc is placed under the counter to measure the activity. If the increase over the background is equal to or less than 3 counts per minute the precipitation can be regarded as complete. The precipitate on the filter is washed twice with fresh boiled cold water. The filters are dried in holders which prevent the filters from curling and the precipitates from cracking at room temperature. The drying is continued until the weight of the precipitate is constant and after that the radioactivity of the filters is determined.

The results obtained are plotted on a graph. The initial activity can be obtained in two ways. The method of extrapolating

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the left descending section of the curve into the region of "weightless" precipitates is more accurate. It is difficult however to obtain precipitates of low thickness and weight and very careful work is required under good laboratory conditions; therefore another method can be used. Precipitates of thicknesses of 10 to 40 mg/cm² are obtained and their activity measured. In this case logs of the activities measured at different thicknesses of the BaCl₂ layer on the filters are plotted on a graph. The graph represents a straight line whose point of intersection with the axis of the ordinates gives the log of the initial radio activity of the volume of the initial solution analysed (Fig. 3).

EXAMPLE

No. of the Flask	Weight of precipitate (mg/cm ²)	Activity measured Counts / min.	Log Activity
1	12.08	1087±11	3.0362
2	15.14	974±10	2.9886
3	22.22	736±13	2.8669
4	25.11	683±10	2.8344
5	30.23	479±7	2.6803
6	34.97	428±8	2.6314
7	40.08	317±7	2.5011

The extrapolated value of the log corresponding to the point of intersection of the straight line and the ordinate is 3.27, hence R (activity) = 1860 counts/min.

In the case when the time for determining R is limited or the conditions required for an accurate determination are absent, it is possible to operate for obtaining preliminary results in the following way. An initial solution with a specific radioactivity of about 10 C/ml is prepared, 0.01 ml of this solution is taken with a micropipette spread on a plastic target and dried rapidly. The dried preparation is counted under the end-window counter. Later on, on the completion of the expedition, the results so obtained are made more accurate. With this purpose the initial solution of the isotope is periodically sampled in a strictly measured quantity placed into an alkaline medium and in this form delivered to the laboratory in which its

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activity is accurately determined by the first method.

In the case when suitably prepared sterile ampoules are used with a solution containing $\frac{1}{L}$ C, its initial activity is determined only during preparation of ampoules and after a certain period of use (at the end of an investigation) for control.

COMPUTATIONS

The calculation of the total content of C in all forms of CO₂ in water.

To calculate the total amount of CO₂ compounds in sea-water it is necessary to know its pH, temperature, chlorinity and carbonate alkalinity. In oceanic waters the ratio of alkalinity to salinity is a constant magnitude equal to 0.123 therefore it is possible not to determine directly the alkalinity in this case. In estuarine waters the alkalinity/chlorinity coefficient varies greatly.

For sea-water the total content of carbonate carbon (C_k) can be determined with sufficient accuracy from the following slightly simplified formula (Harvey 1948)

$$C_k = \frac{A \times 12}{1 \times \frac{K_2}{a_n}} \left(1 + \frac{K_2}{a_n} \right)$$

where K_2 - the second apparent dissociation constant of CO₂ in sea-water at a given temperature and chlorinity; a_n - the concentration of active hydrogen ions;
A - the carbonate alkalinity in milliequivalents/l

The values of K_2 under the conditions of the measurement are found from Table 2. The values of a_n are easily calculated from the equation

$$pH = \log \frac{1}{a_n}$$

To simplify the calculations it is possible to use the auxiliary Table 3. Tenth and hundredth fractions of pH are given in the column pQ of this table, i.e. multiplied by 100 negative mantissas of logs and by corresponding values of antilogs (Q). Finding Q in the table and multiplying this value by 10 to the negative power equal to the numerical value of the pH in whole numbers, i.e. the characteristic of the log, we obtain a_n .

Taking an example of computation.

Taking pH = 8.28, A = 2.37, temperature of the water as 16°C,

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chlorinity 17‰. In these conditions K_2 equals 0.83×10^{-9} from table 2. From table 3 we find that a_n equals 0.562×10^{-8} .

Hence

$$C_k = \frac{2.37 \times 12}{1 + \frac{2 \times 0.83 \times 10^{-9}}{0.562 \times 10^{-8}}} \left(1 + \frac{0.83 \times 10^{-9}}{0.562 \times 10^{-8}} \right)$$

$$= 24.67 \text{ mgC/l}$$

In oceanic water the total content of CO_2 in its various forms varies in the range 20 to 30 mgC/l, therefore it is possible to take for approximate calculations that $C_k = 25 \text{ mgC/l}$.

TABLE 2

The second apparent dissociation constant of CO_2 (K_2) in seawater at different temperatures and chlorinities (after Buch 1933 taken from Harvey 194E)

Cl ‰

TABLE 3

Values of Q with $pQ = \log \frac{1}{Q}$

For fresh water the total content of carbonate carbon is found from the results of determination of total CO_2 (Alakin, 1944). For the waters of carbonate type it is usually possible to consider without a large error that the total content of CO_2 is equal to the alkalinity.

Example of the Calculation of Production.

The following results were obtained for example during the arrangement of the experiment.

$$R = 4.4 \times 10^6 \text{ counts/min}; r = 52 \text{ counts/min}; C_k = 25 \text{ mgC/l} = 25 \times 10^3 \text{ mgC/m}^3.$$

C_{fp} is calculated as has been shown above from the formula:

$$C_{fp} = \frac{r \cdot C_k}{R} = \frac{52 \times 25 \times 10^3}{4.4 \times 10^6} = 0.295 \text{ mgC/m}^3$$

Depth	K _r		K _r		K _s
m	Counts/min.	%	Counts/min	%	%

In order to calculate the production in a water column the curve K_s is plotted. It has the following form (Fig.4)/

(Fig. 4 here)

It is necessary to determine for the calculation the ratio of the areas ABCDEFG and ABHI. The ABHI area corresponds to the production of 1 m³ of the water. It is most expedient to cut out these figures from paper and to weigh them. In the case when the weight of the ABCDEFG area is 166 mg and the weight of the ABHI area 5.4 mg, the ratio of the values of these areas is 31. By multiplying C_{fp} by 31 we obtain the production in the water column under 1 m² of the water body surface. In our example it is

$$C_x = 0.295 \times 31 = 1.15 \text{ mgC/m}^2 = 0.009 \text{ gC/m}^2$$

In this example the result is expressed in weight units of carbon. In order to express the production in other units it is possible to use Table 4 (Vinberg, 1960 p.59)

TABLE 4

Coefficients for transforming the results of measurements of production into different ways of expression.

The coefficients included in table 4 are only suitable with the photosynthetic coefficient ¹ equal to one. In reality

1 The ratio of the volume of CO₂ consumed in the process of photosynthesis to the volume of O₂ given off is called the photosynthetic coefficient.

photosynthetic coefficients higher than one correspond to the elementary composition of phytoplankton according to the literature, they are about 1.25 therefore when calculating the primary production of water bodies the quantities marked with one asterisk should be multiplied by 1.25 and those with two asterisks divided by the same factor.

ACCIDENT PREVENTION

All operations with the radioactive isotope ¹⁴C must be carried out with strict observation of the accident prevention rules by the staff specially trained. It is necessary to work in special clothing: laboratory coat, cap, sleeve-guards, rubber gloves, and a long apron.

All the operations in diluting the isotope, filtering active liquids etc. should be carried out in enamelled basins covered with a thick layer of filter paper. The filtrates should be treated by HCl in a fume cupboard under a hood with barium absorber or under other conditions which are able to protect from gaseous ¹⁴CO₂ discharged during treatment. All the radioactive solutions in filtrates should be kept under lock. One should always use only

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special pincers and pipettes with syringes or bulbs adjusted to them.

In cases when radioactive solutions have been split they should be collected with filter paper and the contamination areas should be treated first with soda then with dilute HCl and washed many times with water.

Radioactive by-products on land should be disposed of according to the rules established by the Health Authorities. In the sea they can be simply thrown away but avoiding the use of the ship's drainage system and at a distance from the shore.

It is recommended to wash contaminated ware first either with soda solution or with a solution of the following composition : water 10 l conc. HNO_3 675 ml, oxalic acid (saturated solution) 1 l, common salt 200 gr., and then to wash them by usual methods.

LIST OF MAIN EQUIPMENT

1. Equipment B1 B-1 or "Tobol".
2. Vertical lead castle.
3. End-window counters, type MS-25 or MS-30 manufactured by the workshop of the Timiryasev Agricultural Academy.
4. A funnel or filtration set.
5. A pump, Komovskii or Shchipts, or a Forvacuum pump with an electric drive, type VN-461.
6. A sampler, preferably of plexiglass or polyethylene.
7. Flasks of colourless glass with ground stoppers or Jitta samplers.
8. A pipette with syringe for introducing the radioactive solution or a set of ampoules with the isotope measured into them. The latter can be broken directly in the flasks by means of a tube with notches on the end which is put on the ampoule end.
9. Analytical or torsion balance.
10. Basins, desiccators, drying cupboard, etc. as usual.

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TEXT FOR FIGURES

Fig.1 (p.8) The procedure of filling and exposing the flasks when measuring the primary production according to Yu.I. Sorokin's method :

- A - the "in situ" method of observation and an example of the curve for the variation of photosynthesis intensity with depth;
- B - determination of the values of the coeff K_P and K_T and example of the curve for the distribution over depths;
- C - determination of K_R over depths.

The experimental results of the photosynthetic intensity expressed in per cent. of the intensity of the photosynthesis in a surface sample, are given on the horizontal axis; the depth, in m, on the vertical axis.

Fig.2 (p.10) Variation of the photosynthetic intensity with the light conditions :

- A - the distribution of the photosynthetic intensity over depths from the results of measurements by in situ methods. On the abscissa photosynthesis in per cent. of the maximum on the ordinates depth in m, and the values of radiation energy corresponding to them in cal/cm²/day.
- B - the same data given in the form of a curve for the variation of photosynthetic intensity with the value of daily radiation energy in cal/cm²/day, or ordinates photosynthesis in per cent. of the maximum.

Fig.3 The self absorption curve of BaC¹⁴O₃, obtained with an end-window counter.

- B - variation of the activity to be measured with the thickness of the BaCO₃ layer.
- A - a part of the same data in the form of a semi-logarithmic graph which makes it possible to determine the value R. On abscissa the thickness of BaCO₃ layer in mg/cm², on ordinates the ratio of the activity measured at a

given thickness of the preparation (I) to the activity
at zero-thickness (10)

Fig.4 An example of the curve for the distribution of values of K_s over depth, which serves for calculating production under 1 m^2 . The sides of the squares are : 2 m of depth in the vertical and 10% of Sfp in the horizontal. Other explanations in the text.