Techniques for Measuring Oceanic Primary Production Using Radioactive Carbon

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TECHNIQUES FOR MEASURING OCEANIC PRIMARY PRODUCTION USING RADIOACTIVE CARBON

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Summary

Changes made since 1957 in the ^{14}C technique are described. Descriptions are given of a modified sampler which automatically introduces ^{14}C, plastic filtration units for use with membrane filters, two constant light incubators, and a windowless gas-flow counter. Tests of sampling errors gave coefficients of variation between 10 and 30%; it is concluded that measurements which differ by more than 80% are significantly different.

I. INTRODUCTION

The ^{14}C method of measuring primary production of organic matter has been used by this Laboratory for extensive surveys in both the Indian and Pacific Oceans. Since the techniques used were described by Jitts (1957), modifications have been made both in the equipment and in the treatment of samples and data. These changes, and studies of the sampling errors involved in the ^{14}C method, are described in this report.

II. METHODS

(*a*) *Preparation of Ampoules*

$Na_2{}^{14}CO_3$ solution of high specific activity is obtained from the Radiochemical Centre, Amersham. It is diluted with CO_2-free distilled water, and NaCl and Na_2CO_3 added so that the solution contains 10 μc of ^{14}C, 30 mg NaCl, and 0·09 mg CO_2 in each millilitre. This solution is filtered through an HA Millipore filter.

One ml ($\pm$0·01 ml) aliquots of the solution are sealed in 2 ml glass ampoules and the ampoules are autoclaved at 15 lb/in^2 for 15 min. They are then immersed in a dye bath under a pressure of 75 lb/in^2, and inspected visually for leaks, volume, and particulate matter.

(*b*) *Collection and Treatment of Samples, 1957–61*

Until 1962, samples were taken with the twin 400 ml Perspex samplers (Fig. 1) described by Jitts (1957). These were incubated either *in situ* or in the upright position in an artificial light bath (Fig. 2) between two banks of three horizontal 20 W fluorescent tubes (Philips TLS 20 W/33), giving a light intensity of about 1100 f.c. For some experimental *in situ* measurements the twin 400 ml sampler was modified (Fig. 3) to allow the automatic introduction of ^{14}C without bringing the samples to the surface. As the sampler closed, a glass ampoule of the ^{14}C solution was broken in the sampler and a spring-loaded Perspex propeller stirred the contents.

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After incubation, the samples were filtered through 24 mm Millipore HA filters in the Perspex filtration units shown in Figure 4. In all other respects the treatment of samples was as described in the next section.

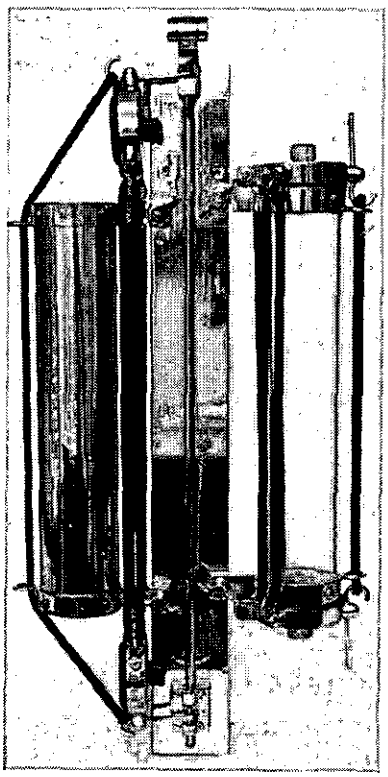

Fig. 1

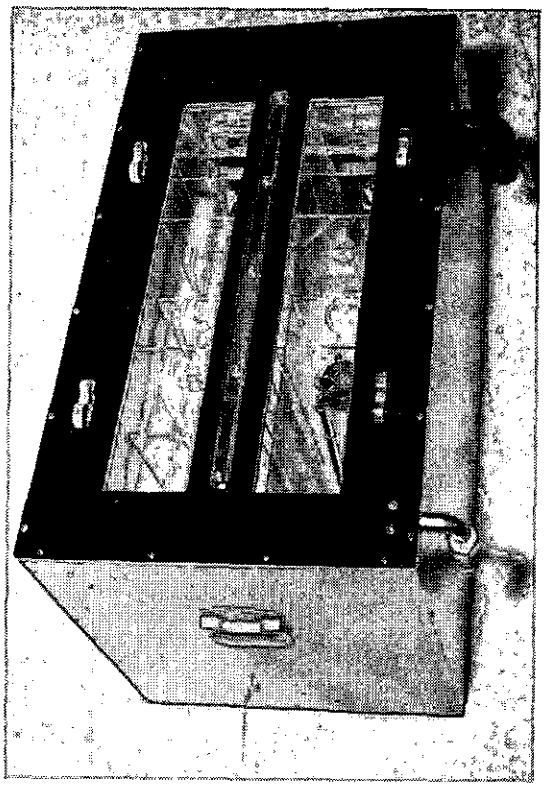

Fig. 2

Fig. 1.—A twin 400 ml light and dark plastic sampler for measuring primary production in sea-water without the need for further transfers of the samples. The light bottle is shown closed and the dark hooked open. The bottles can be detached from the frame for incubation in a light bath.

Fig. 2.—A constant artificial light incubator for use with the 400 ml samplers shown in Figure 1. The three banks of three horizontal fluorescent tubes (Philips TLS 20 W/33) give a light intensity of about 1100 f.c.

(c) *Collection and Treatment of Samples, 1962 onwards*

In 1962 the twin 400 ml Perspex samplers were superseded by a twin 6 l. sampler described by Jitts (1964). This sampler is used to enable samples for other work (phytoplankton, pigments, and particulate carbon) to be collected simultaneously.

The samples are collected from various predetermined depths, usually 0, 25, 50, 75, 100, and 150 m, or from depths determined by the "balance by depth" method of photometry described by Jitts (1963). Subsamples are drawn from these samplers into two 300 ml Pyrex glass bottles, one of which is clear and the other light-proofed

with black-painted adhesive bandage. Before incubation, the stoppers of the dark bottles are light-proofed by covering them with a sheet of aluminium foil.

The contents of a ^{14}C ampoule are added to the sample with a hypodermic syringe, the ampoule washed with 1 ml of the sample, and the washings added to the sample.

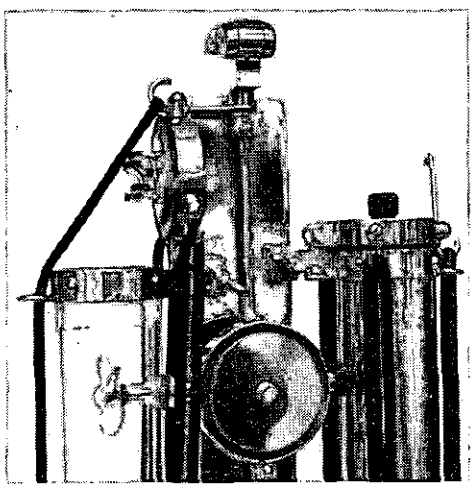

Fig. 3

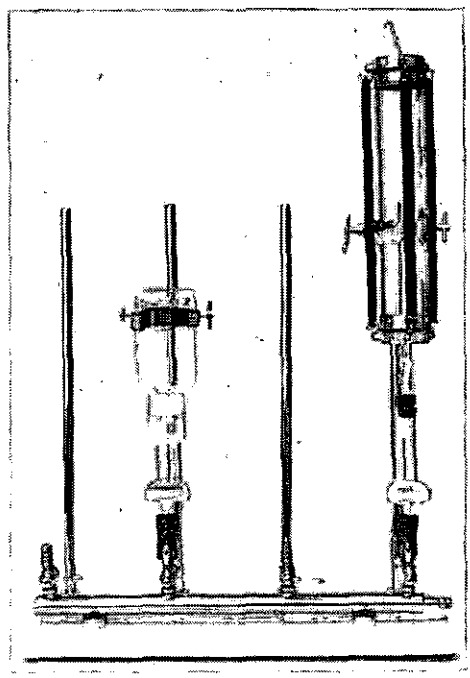

Fig. 4

Fig. 3.—A modification of the twin 400 ml plastic sampler shown in Figure 1; this allows the automatic introduction of ^{14}C into the sample when the sampler is closed. A glass ampoule containing the ^{14}C is inserted in the holder under the lid. When this closes, the ampoule is broken against the plastic rod across the top of the tube. At the same time the spring-loaded propeller is released and stirs the contents of the sampler.

Fig. 4.—Two types of plastic filtration units for use with 24 mm membrane filters. The one on the left is shown with a 300 ml bottle inverted into the unit. The unit on the right is shown connected to a 400 ml plastic sampler.

For measuring productivity under constant artificial light the bottles are placed in an incubator as shown in Figure 5. The samples are kept cool by surface sea-water passed through the Perspex tanks containing the bottles. This incubator is very similar to one described by Doty and Oguri (1959).

For measuring *in situ* production the bottles are placed either on wire clamps for *in situ* incubation or in the simulated *in situ* incubator described by Jitts (1963). The samples are incubated by either of these two methods for a half-day period, i.e. from sunrise to noon or, more frequently, from noon to sunset.

After incubation the bottles are inverted into the Perspex filter units (Fig. 4) and the sample is filtered through a 24 mm Millipore HA filter, using a vacuum of not more than 15 inHg. The vacuum is provided by a diaphragm or rotary oil pump via two 6 gal tanks connected in series. These act as a vacuum reservoir and container for the filtrate. After filtration the filters are washed with 10 ml 0·001N HCl in 3% NaCl, followed by 10 ml 3% NaCl to remove any inorganic ^{14}C remaining on the

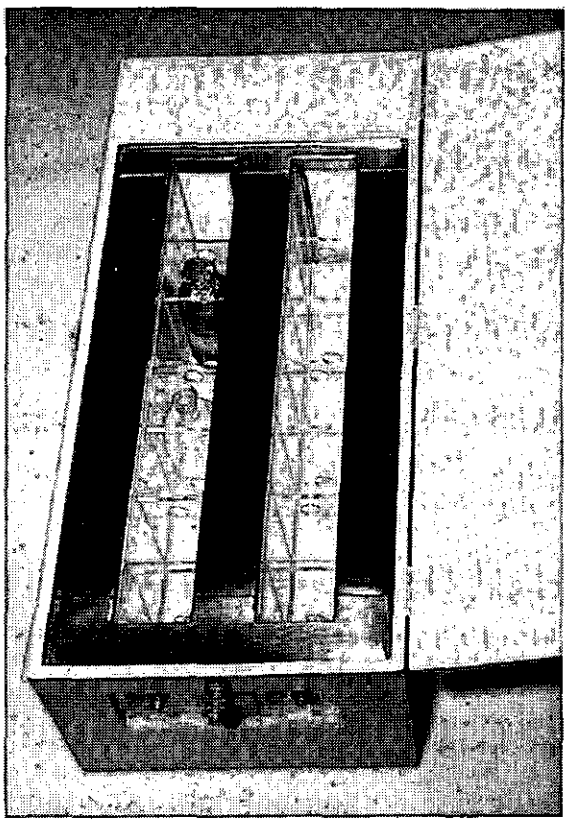

Fig. 5.—A constant artificial light incubator for use with 300 ml glass bottles. The three banks of two horizontal fluorescent tubes (Philips TLS 20 W/33) give a light intensity of about 1100 f.c.

filter. The filters are sucked dry and placed in numbered plastic holders, held by a smear of petroleum jelly. These are placed in a metal desiccator with silica gel and the filters dried for about two days before counting. Immediately before counting, the filters are numbered in the margin for identification.

After use, the glass bottles are washed with conc. HCl, then rinsed twice with fresh water and left to drain. The plastic samplers are rinsed with fresh water and drained dry after each use, but are also washed with conc. HCl and detergents before initial use and at about monthly intervals during cruises.

(*d*) *Counting*

A windowless gas-flow Geiger counter is used with an Ekco N530 scaler. The counter was made in this Laboratory. It has three planchet positions, a chromium-plated brass counting chamber, and a tungsten electrode; it is shielded by aluminium and lead (Fig. 6). This counter has a starting voltage of 1300 V and the Geiger plateau has a slope of 2% with no signs of continuous discharge below 1800 V. Using an operating voltage of 1450 V the counting efficiency is approximately 50% and the background is between 10 and 15 counts/min. The counter is used in an air-conditioned room to give greater stability.

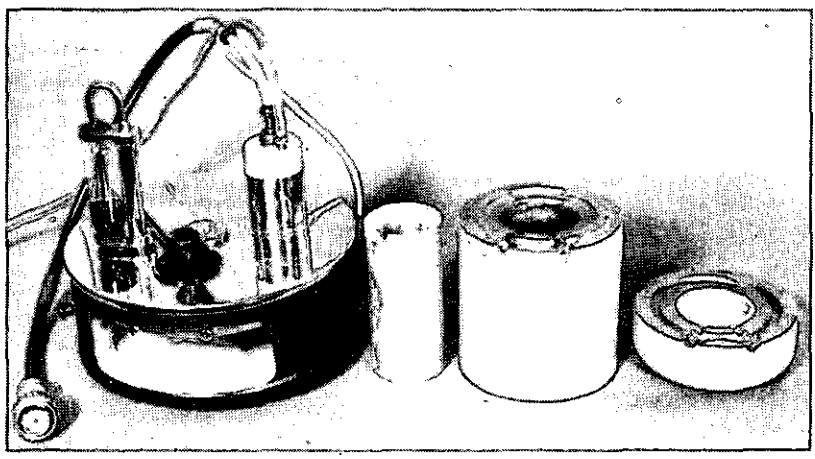

Fig. 6.—A windowless gas flow Geiger counter. Shown with the aluminium and lead shields removed from the chrome-plated brass counting chamber.

The filters, mounted on brass planchet holders, are preflushed with Tracerlab Q gas (helium with isobutanol) in the counter for 5 min at the rate of one bubble per second; the planchet carrier plate is then rotated and the sample counted for 5 min or 30,000 counts, whichever occurs first. After every second count, a ^{14}C standard is counted to correct for instability in the counter. This standard is a piece of poly (methyl-^{14}C) methacrylate (Amersham CFP 2) having the same geometry as the filtering area of the filters and a known activity of approximately 6000 counts/min. The counter background is determined by counting a blank filter for 1 hr on each day that counting is done.

The method of Jitts and Scott (1961) is used to determine the efficiency of the Geiger counter and the absolute and zero-thickness activities of the ampoules. These are standardized with a Hexadecane Reference Standard (Amersham, CFR 2) by liquid scintillation counting.

III. Calculation of Results

Raw data for samples collected at each station are recorded on the sheets shown in Table 1. Calculations are done by an electronic computer. The raw data are punched on cards and the results obtained printed out in the form shown in Table 2.

TABLE 1

LOG SHEET FOR RECORDING RAW DATA

CSIRO DIVISION OF FISHERIES AND OCEANOGRAPHY PRODUCTIVITY LOG SHEET NO. 2

Sheet No. 29 of 55

Vessel	Cruise	Station No.	Year	Month	Day	Time	TZ*	Latitude	NS	Longitude	EW	Observer
Diamantina	1	34	63	4	6	0930	−8	11° 06′	S	109° 57′	E	B. Scott

Sonic Depth	Dist. Coast	Incub. Meth.		Stock No.		Stock Activity	TE*	Bkgd†	CT*	OI*	Time In	Out	Period
4938		Bath	0	15		$9{\cdot}59\times10^{6}$		10	08		1000	1330	3·50

Depth	Temp.	Lt. Bottle		Dk. Bottle		Raw Count		St. Correction		DD*	CT*	OI*	Corrected Counts			Production	
		No.	Filter	No.	Filter	Light	Dark	Light	Dark				Light	Dark	Net	A	B
0		1	1641	7	1647	2106	422	0·96	1·00		80		395	74	321	0·26	
25		2	1642	8	1648	2258	279	0·99	0·98				437	45	392	0·29	
50		3	1643	9	1649	3244	265	0·99	0·98				633	42	591	0·43	
75		4	1644	10	1650	930	201	0·99	0·97				174	29	145	0·11	
100		5	1645	11	1651	392	201	0·99	0·97				67	29	38	0·03	
150		6	1646	12	1652	201	240	1·00	1·00				30	38	0	0·00	0·26

Remarks:

* Qualitative symbols used in the computer program.

† Bkgd is the background in counts/min.

TABLE 2

COMPUTER PRINT-OUT

SHIP	CRUISE	ST. NO.	YEAR	MONTH	DAY	TIME	LATITUDE	LONGITUDE
11	1	34	63	4	6	0930 H	11 06 S	109 57 E

SONIC DEPTH	DISTANCE FROM COAST	INCUBATION METHOD	STOCK NUMBER	STOCK ACTIVITY	BACKGROUND
4938	190	0	15	9·590	10

DEPTH	LIGHT COUNT	LIGHT COUNT	MEAN LIGHT	DARK COUNT	DARK USED	NET COUNT	INCUBATION PERIOD	PRODUCTION A	PRODUCTION B	LIGHT STANDARD	LIGHT STANDARD	DARK STANDARD
0	394	—	—	74	36	358	3·500	·26	·00	·96		1·00
25	437	—	—	45	45	392	3·500	·29	·07	·99		·98
50	633	—	—	42	42	591	3·500	·43	·16	·99		·98
75	174	—	—	29	29	145	3·500	·11	·23	·99		·97
100	67	—	—	29	29	38	3·500	·03	·25	·99		·97
150	30	—	—	38	38	−8	3·500	·00	·26	1·00		1·00

In the computer program, the raw count is reduced to counts/min, then corrected by the factor obtained from the ^{14}C standard count. From this the background is subtracted. The net activity is obtained by subtracting the dark bottle result from that of the light bottle. The rate of production in mg C/hr/m^3 (Production A) is calculated using the formula:

$$\text{Rate of Production} = \frac{\text{Net Activity}}{\text{Added Activity}} \times \frac{\text{Total } CO_2}{\text{Hours of Incubation}},$$

where the "Added Activity" is the zero-thickness Geiger activity in counts/min of the ^{14}C ampoules used and "Total CO_2" is assumed for oceanic waters to be constant at 24,500 mg C/m^3 (90 mg CO_2/l.).

In the case of *in situ* or simulated *in situ* incubations the rate of production in mg C/day/m^3 is calculated using the formula:

$$\text{Rate of Production} = \frac{\text{Net Activity}}{\text{Added Activity}} \times \frac{\text{Total } CO_2}{\text{Days of Incubation}},$$

where a "day" is assumed to be the period from sunrise to sunset.

The rate of production under 1 m^2 of the water column sampled in g C/day^2/m^2 (Production B) is obtained by integrating the results of all the samples in the column by the formula:

$$\text{Column Production} = \frac{f}{1000}\left[(d_1-d_0)\left(\frac{a+b}{2}\right)+(d_2-d_1)\left(\frac{b+c}{2}\right)+\ldots\right],$$

where d_0, d_1, d_2 are the depths sampled;

a, b, c are the respective production rates;

f is a factor for converting the units to production per day.

When incubation is *in situ* or simulated *in situ*, f is 1. When incubation is under a constant artificial light it is 10, because the daily rate is assumed to be 10 times the hourly rate.

The results of both Production A and Production B are rounded off to two decimal places. If duplicate light bottles are counted, the mean of the corrected counts is taken. If the dark count is missing or rejected as aberrant, i.e. it is greater than 50 counts/min and also greater than 10% of the mean light count, the mean dark count of all the non-aberrant values at the station is used. If all the dark counts are aberrant, an arbitrary figure of 20 counts/min is used.

IV. Errors in Estimation

The errors in estimation of production by the ^{14}C method described in the preceding sections were determined at: (i) two all-day stations carried out by F.R.V. *Derwent Hunter* on July 25 and 30, 1959, during which the ship was drifting about 50 miles off the coast near Sydney, (ii) seven stations by H.M.A.S. *Kimbla* from September 14 to 16, 1959, all at least 20 miles off the continental shelf between Coff's Harbour and Sydney, and (iii) a station in the Indian Ocean (19° 25′ S., 94° 27′ E.) by H.M.A.S. *Diamantina* on July 21, 1960 (Dm2/143/60).

In the first series, the errors due to the method were estimated by measuring the productivity of replicate subsamples from a large sample of well-mixed surface sea-water. Surface sea-water was collected with a plastic bucket and poured through Discovery N70 net into a 44 gal drum which had been lined with an epoxy resin. The water was stirred vigorously for 10 min with a wooden paddle, and subsamples dipped from it and poured through a plastic funnel into the clear plastic 400 ml samplers (Fig. 1). The productivity of each of these subsamples was then estimated as described earlier. The coefficients of variation of these measurements varied from 13 to 20% (Table 3).

TABLE 3

SAMPLING ERRORS IN THREE TESTS OF ^{14}C UPTAKE BY REPLICATE SAMPLES TAKEN FROM BARRELS OF SURFACE SEA-WATER

Ship Date Time	Mean Activity (counts/min)	No. of Samples	Standard Deviation	Coefficient of Variation
Derwent Hunter 25.vii.59 0530	329	8	43	13
Derwent Hunter 25.vii.59 1530	221	10	45	20
Kimbla 14.ix.59 1640	332	10	54	16
				Mean: 17

In the second series, several samples were taken at depths of 0, 25, and 50 m from a drifting ship. At each depth the samples were taken in succession within 20 min of each other with the 400 ml clear plastic samplers. The errors in this series (Table 4) include those due to the method and also to the variations of plankton distribution. The mean coefficients of variation at the three depths were 29, 12, and 22% respectively.

In the third series, 14 surface samples were taken at 5 min intervals with a plastic bucket from a ship (*Diamantina*, July 21, 1960) steaming at 2 knots along two lines bisecting each other at right angles. These were poured through plastic funnels into 300 ml glass bottles, and their productivity was measured. The mean productivity was 0·11 mg C/hr/m^3 with a coefficient of variation of 18%.

In the fourth series, the productivity of the column of sea-water under 1 m^2 was examined. The errors in this type of estimation were determined from casts taken with 400 ml plastic samplers at depths of 0, 25, and 50 m. Successive casts taken within 20 min of each other gave a mean coefficient of variation of 11% (Table 5).

The estimate of technique error in the first series of tests gave a mean coefficient of variation of 17%. This is somewhat higher than the 10% quoted by Doty and

TABLE 4

VARIATIONS BETWEEN SAMPLES TAKEN IN QUICK SUCCESSION

Ship Date Time	Depth (m)	Mean Activity (counts/min)	No. of Samples	Standard Deviation	Coefficient of Variation	Mean Coefficient at Each Depth
Derwent Hunter 30.vii.59 0500	0	655	12	218	33	29
Kimbla 15.ix.59 0630	0	656	12	168	26	
Derwent Hunter 30.vii.59 1200	25	1071	10	111	10	12
Kimbla 15.ix.59 1200	25	481	10	61	13	
Kimbla 15.ix.59 1800	50	510	10	112	22	22
						Mean: 21

TABLE 5

VARIATIONS IN SAMPLING A COLUMN OF WATER IN SUCCESSIVE CASTS FROM DEPTHS OF 0, 25, AND 50 M

Ship Date Name	Mean Activity/m^2	No. of Casts	Standard Deviation	Coefficient of Variation
Kimbla 16.ix.59 0630	2101	4	252	12
Kimbla 16.ix.59 1200	5336	4	546	10
Kimbla 16.ix.59 1700	2828	4	287	10
				Mean: 11

Oguri (1959) for a similar technique. On a culture of *Skeletonema*, Cassie (1962) found a range of from 9 to 15%, and pointed out that it is probable that sampling errors contribute a large part of this estimate of technique errors even in aliquots from a large well-mixed sample.

The results from the second, third, and fourth series show that the sampling plus method errors gave coefficients of variation varying from 10 to 33%. Doty and Oguri (1959) quote up to 25% for an experiment similar to the third series, whilst Cassie (1962) found 14 to 37% for sets of 25 samples taken simultaneously in close proximity to each other.

None of the tests reported here or those referred to showed a coefficient of variation of more than 40%, whereas most of them gave from 10 to 30%. This allows the conclusion that two measurements in which one is 80% greater than the other are significantly different. However, when considering measurements of productivity of samples incubated under constant light it should be remembered that the effects of diurnal variations (Doty and Oguri 1957) can cause considerably greater variations than 80% unless the samples were taken at the same time of day.

V. References

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