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Note on the Variability of Heterotrophic Activity Measurements by the ^{14}C Method in Sea Water

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Abstract

Twenty five measurements in triplicate of heterotrophic activity in sea water showed good reproducibility. The mean coefficient of variation was 6.2%, which is lower than the coefficient of variation obtained with a similar method for primary production measurements. This result indirectly confirms that most heterotrophic activity can be attributed to a great number of small cells, i.e., bacteria which, if not free-swimming, are fixed to particles of smaller size than the average phytoplankton. The good reproducibility obtained also suggests that there was no extraneous contamination despite non-sterile working conditions.

Introduction

In the sea, the measurement of heterotrophic activity (defined as the utilization of dissolved organic matter) and bacterial biomass still presents some difficult problems. Plate counts have notoriously little value in assessing this activity (Sorokin, 1971a), since the conditions of growth in enriched media are different from those of the natural environment, and the species developing in such media are not necessarily those which would be most active in the sea. Further, only colony-forming species are counted, and the determination of microbial biomass is inaccurate (Seki, 1971). Although direct optical counting may give good results in assessment of biomass (Sorokin, 1971b), the method is considered to be unsatisfactory since it is time-consuming and liable to sampling errors due to the small volumes studied. Methods based upon radioactive tracers (Parsons and Strickland, 1961; Vaccaro and Jannasch, 1966; Wright and Hobbie, 1966; Allen, 1969; Hamilton and Preslan, 1970) are in many respects more satisfactory for estimating heterotrophic activity since they involve low concentrations of added substrate and cause minimal perturbation of the incubated sample. In addition, since the sample volume is greater the statistical errors associated with the sampling process are expected to be reduced. The tracer can be added in concentrations low enough to modify neither the original activity nor the turnover time of the substrate initially present in the sample (Williams and Askew, 1968).

The purpose of this paper is to study the reproducibility of the ^{14}C method in determining heterotrophic activity of a natural population in sea water, and to compare this reproducibility with that of a similar ^{14}C method for primary production evaluation (Steemann Nielsen, 1952).

Three experiments were made: the first in March, 1973, in the Senegal-Mauritania upwelling waters, off Mauritania (north-west Africa), and the second and third in April, 1974, in coastal waters off the Ivory Coast.

Materials and Methods

Experiment 1

Sampling was performed with PVC bottles (5 l, General Oceanics). For each depth sampled, three 35 ml glass flasks, clean but not sterile, were filled. The tracer was a solution of ^{14}C-labelled amino acids (glutamic acid, 15%; aspartic acid, 18%; glycine, 11%; serine, 29%; lysine, 27%; by weight;) 0.25 ml of this solu-

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Table 1. ^{14}C counts for heterotrophic activity (x 10^3 cpm) in Experiment 1. dash: no data

Location	Depth (m)	Sample 1	Sample 2	Sample 3	Mean	Standard deviation, Sd	Coefficient of variation, Cv
18°42'N;	5	416.7	380.2	333.3	376.7	34.3	9.1
16°19'W	10	308.7	284.1	344.8	312.5	25.0	8.0
	20	409.8	446.2	390.6	415.5	23.3	5.6
	35	293.2	253.1	180.6	242.3	47.0	19.4
	5 (control)	2.0	2.3	2.5	2.3		
18°28'N;	5	187.8	201.9	187.4	192.4	6.8	3.52
16°19'W	9	223.7	232.5	218.2	224.8	5.9	2.65
	13	251.3	333.3	293.2	292.6	33.9	11.6
	35	193.5	214.4	231.7	213.2	15.8	7.4
	5 (control)	3.2	3.3	2.7	3.1		
18°06'N;	0	245.2	357.1	364.9	322.4	55.4	17.2
16°15'W	11	344.8	434.8	413.2	397.6	38.6	9.7
	0 (control)	3.8	4.5	-	4.1		
17°56'N1;	0	518.1	467.3	429.2	471.5	36.8	7.8
16°18'W	4	411.5	411.5	373.1	398.7	18.5	4.64
	8	552.5	510.2	510.2	524.3	20.0	3.82
	12	446.4	471.7	460.8	459.6	10.4	2.27

Table 2. ^{14}C counts for heterotrophic activity (x 10^3 cpm) in Experiments 2 and 3

Experiment 2 (incubation time = 10 h)		Sample			Mean	Control	Standard deviation, Sd	Coefficient of variation, Cv
Untreated sea water (ml)	Irradiated, filtered sea water (ml)	1	2	3				
20	15	21.6	24.6	26.1	24.1	0.19	2.30	9.55
25	10	23.8	25.7	24.4	24.6	0.14	0.94	3.86
30	5	22.7	25.7	23.1	23.8	0.14	1.61	6.78
32	3	22.5	23.5	25.7	23.9	0.13	1.63	6.85
33	2	22.4	22.8	22.4	22.5	0.17	0.23	1.03
34	1	23.0	22.4	21.6	22.3	0.15	0.69	3.12
Experiment 3 (incubation time = 6 h)		Sample			Mean	Control	Sd	Cv
Untreated sea water (ml)	Time of UV irradiation (h)	1	2	3				
25	2	36.7	35.9	39.2	37.3	0.12	1.73	4.66
25	4	37.2	36.4	37.2	37.0	0.17	0.45	1.22
25	8	37.3	35.9	37.4	38.9	0.14	0.88	2.27
25	16	36.9	36.8	36.0	36.6	0.17	0.46	1.26
35	-	35.0	34.3	35.8	35.0	0.15	0.75	2.16

tion, corresponding to 0.5 µCi and 0.445 µg C, was injected into each flask.

Incubation was carried out in the dark, at constant temperature (+16°C ± 0.5C°) for 24 h, and was stopped by the addition of 0.5 ml of 20% formalin. The samples were then filtered on 0.45 µ membrane filters (HA Millipore Corp.). The radioactivity taken up by the microorganisms was determined in a liquid scintillation counter (Intertechnique SL 30; for further details see Herbland and Bois, 1974). Controls were processed in the same manner except that the formalin was added before starting incubation.

Experiment 2

This experiment was designed to study the influence of ultraviolet-irradiated sea water added to the samples. Increasing volumes of irradiated and filtered surface sea water were added to untreated surface sea water samples. The incubation procedure was the same as in Experiment 1, except that the temperature was 24°C (approximate sea-surface temperature). The tracer was a solution of ^{14}C-labelled glucose; 0.2 ml was injected into 35 ml flasks, corresponding to 4.5 x 10^{-2} µg C and 3.0 x 10^{-2} µCi. The filters used for collecting microorganisms were of fiber glass (Gelman type A), and a very low suction pressure (50 mmHg) was used to avoid damage to the cells (Herbland, 1974).

Experiment 3

This experiment was made under the same general conditions as Experiment 2. However, in this case the volume of irradiated sea water was the same whereas the time of ultraviolet irradiation was different for each sample (Table 2), and the incubation time was reduced to 6 h.

Results

In the three experiments, controls showed very low ^{14}C values compared to the experimental samples (Tables 1 and 3). This indicates that most of the variations between samples were due to biological activity rather than methodological background.

In Experiment 1, nearly all coefficients of variation (11 out of 14) are less than 10%, the average coefficient being 8% (Table 1). In Experiment 2, the coefficients of variation are very low, between 1 and 9.5%, the mean being 5.2% (Table 2). In Experiment 3, the coefficients of variation are the lowest: between 1.3 and 4.7%, with a mean value of 2.3%. There appears to be no effect of irradiated sea water nor irradiation time (Table 2).

Table 3. Comparison between coefficient of variation (CF) with ^{14}C technique applied to bacteria and phytoplankton activities

Population	Average CF (%)	Source
Bacteria		
Natural populations		
Experiment 1	8.0	Present study
Experiment 2	5.2	Present study
Experiment 3	2.3	Present study
Phytoplankton		
Laboratory culture	9.5	Cassie (1962)
Natural population	22.2	Cassie (1962)
Laboratory culture	10.0	Platt and Filion (1973)
Natural population	17.4	Dufour (personal communication)
Natural population	14.0	Herbland (1974)

Discussion

In these experiments, errors may be partitioned into two components: one attributable to variations from the technique itself (uncertainty about the volumes of sea water and labelled solution in the flasks, errors due to filtration, counting of radioactivity); the other to biological variations (spatial distribution of cells, functional variations during the experiment).

The reproducibility of the ^{14}C method for primary production measurement has been studied by several authors (Cassie, 1962; Platt and Filion, 1973; Dufour, personal communication), who have all reached the same conclusion: it seems impossible to reduce the coefficient of variation of the ^{14}C technique below 10% (Cassie, 1962; and Table 3 of present paper).

All the above-mentioned authors agree that the purely physico-chemical aspects of the technique, if applied to inanimate material, could hardly be expected to produce so high an error. The two techniques for autotrophy and heterotrophy are identical except that the nature of labelled compounds and organisms which grow are different. The coefficients of variation obtained for the heterotrophy measurements are lower.

A possible explanation of this difference is that the density of organisms which grow upon dissolved organic matter was statistically the same in each of the triplicates we used, whereas for organisms which grow upon CO_2 in primary production studies, in an equivalent sample size the probability of having the same number of organisms decreases with increasing size of the organisms used (Sheldon *et al.*, 1973).

This suggests that heterotrophic activity is primarily due to bacteria, which are smaller than phytoplankton cells and more numerous in any given sample.

We may also infer that most heterotrophic organisms, if not free-swimming, are fixed to smaller-sized particles than the average phytoplankton. This conclusion, obtained by an indirect method, agrees well with that of Williams (1970) who found that 70% of heterotrophic microorganisms are smaller than 3 µ and 50% are smaller than 1 µ. Azam and Holm-Hansen (1973) reported for a 10 m seawater sample, that at least 86% of the heterotrophic activity could be attributed to cells smaller than 3 µ.

The good reproducibility of the measurements leads us to believe that extraneous contamination was negligible, despite non-sterile working conditions. This observation is valuable insofar as bacterial activity therefore can be measured from the same samples that are used for other physical and chemical analyses. Furthermore, the reproducibility of the method enables the use of only a single measurement for each depth and thus the sampling of more depths with the same amount of effort.

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