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COMPARISON OF GEIGER AND LIQUID SCINTILLATION COUNTING EFFICIENCIES IN PRIMARY PRODUCTION STUDIES OFF WALTAIR, BAY OF BENGAL

D. V. SUBBA RAO*

Department of Zoology, Andhra University, Waltair, India

ABSTRACT

Geiger and liquid scintillation activities of C^{14}-labelled phytoplankton filters obtained from a tropical coastal station were compared. A decrease in the percent efficiency of GM/Sci with increasing primary production was observed and this is related to an increase in the thickness of the sample which is caused either by the productive algal blooms or by other particulate matter. If primary production is not to be underestimated in coastal turbid waters, it will be necessary to determine the absolute activities of the samples.

INTRODUCTION

THE carbon-14 technique (Steemann Nielsen, 1952) has been widely used for measuring primary production of both natural and culture populations. Geiger counters are generally used for determination of the C^{14} activities of the filters. It is usually assumed that the geometry of such filters is similar to that of the barium carbonate standard filters used in the determination of zero thickness activity, and that there are no counting losses due to back scattering and self absorption. However, this assumption has been shown to be untenable (Jitts and Scott, 1961; Wolfe and Schelske, 1967; Steemann Nielsen, 1965; Goldman, 1968). In particular, in eutrophic and turbid coastal waters where the ratio of phytoplankton to total particulate material is usually large, the determination of activities of filters containing C^{14} - labelled phytoplankton using a Geiger counter can lead to serious errors caused by self absorption and back scattering.

In this study, Geiger and liquid scintillation counting efficiencies are compared using filters with C^{14}-labelled phytoplankton obtained from a fixed coastal tropical station. The data presented here constitute the first seasonal study of this problem and cover a wide range of primary production values, in a succession of water types from oligotrophic to eutrophic.

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MATERIALS AND METHODS

Primary production measurements were carried out at a fixed station in Lawson's Bay, Waltair (17° 44'N and 83° 23' E) India, during 1962 at roughly weekly intervals. Pyrex bottles of 250 ml capacity were filled with surface sea water and the

*Present address: Marine Ecology Laboratory, Bedford Institute, Dartmouth, Nova Scotia, Canada.

contents of 1 ml ampoule containing 4μ c of carbon-14 were added. The bottles were stoppered and incubated at 1m in the sea for 6 hours. The samples were then filtered through membrane filters (0.8μ pore size using a gentle suction from a water pump). The filters were exposed to fuming hydrochloric acid for 20 min and then stored in a desiccator containing silica-jel. The activities of the filters were determined at the productivity laboratories of the C.S.I.R.O., Division of Fisheries and Oceanography, Cronulla, Australia. The Geiger activities were first measured with a windowless gas flow counter of 2π geometry resembling the Tracerlab, USA; SC-16 Counter. The Geiger gas used was tracerlab Q gas and the scaler was an EKCO N 530. The mean efficiency of this counting system, calculated from the Geiger and absolute activities of this C^{14} - labelled plastic films (Jitts and Scott, 1961) was 52.5%. Liquid scintillation counting of the filters was carried out following the procedure detailed by Jitts and Scott (1961). The filters were transferred to vials each containing 15 ml liquid phosphor and left to stand overnight. Each sample was counted 3 times for 10 min with EKCO type N 530 automatic scaler and EKCO type N 664 Universal scintillation counter. The mean efficiency of this counting system was 67.7%. Turbidity of the sea water was measured with a Hellige's turbidimeter. In a separate experiment data were collected on the variability of Geiger Muller counting system and liquid scintillation system. A culture of the diatom *Phaeodactylum tricornutum* was grown for 2 days in Woods Hole medium 'F' under a continuous light of 560 foot candles at 25°C. The culture had 750×10^6 cells/L and a total chlorophyll of 54.18 μg/L (38.70 μg chl *a*/L + 15.38 chl *c*/L).

The total inorganic carbon dioxide in the culture was 61.2 mg/L. Two sets, A and B each with 25 test tubes were used in sets of I to V (each set with one dark and four light). Ten ml of the diatom culture were pipetted into each of the test tubes and 100 μl of $NaC^{14}O_3$ were added, mixed, stoppered and incubated for 60 min over a bank of fluorescent lamps at 1040 foot candles and 25°C. The samples were then filtered through 0.45μ, 25 mm HAWP millipore filters (with a filtration area of 1.77 cm^2) at 10 inches suction pressure and known quantities of turbid solutions were added as detailed below.

Four levels of turbidity solutions were prepared by suspending 79.5; 159.0; 318.0 and 636.0 mg of infusorial earth each in a 200 ml of 0.45 μ millipore filtered seawater. When 10 ml of each of the slurry were diluted to 250 ml, the corresponding turbidities were 4, 8, 16, 32 APHA SiO_2 ppm. When 10 ml of the culture were diluted to 250 ml the sample had a turbidity of 1 APHA SiO_2 ppm. No turbidity solutions were added to set I, while sets II to V received each 10 ml of turbidity solutions 1 to 4 respectively. Thus the resulting turbidities were 1 APHA SiO_2 ppm for set I; 4 for set II; 8 for set III; 16 for set IV and 32 for set V. This simulates the measurements made in Bay of Bengal where 250 ml of seawater samples with a range of turbidities up to 25 APHA SiO_2 ppm were used.

The filters were washed with 10 ml of 0.45 μ millipore filtered culture medium and sucked dry and the 25 filters from set A were transferred to vials each containing 15 ml aquafluor, dissolved and used for liquid scintillation counting with a Packard Tri-Carb automatic spectrometer.

The 25 samples from set B were also filtered and identical quantities of slurry were added and washed (as in set A used in liquid scintillation counting). The filters from set B were mounted in planchettes, dried and stored over carbon dioxide free atmosphere for Geiger Muller counting.

A series of filters with known turbidities up to 32 APHA SiO_2 ppm were prepared, added to vials containing 15 ml aquafluor and known CPm were added and counted to determine whether any quench correction due to turbidity is necessary. Test for colour quench due to chlorophylls was also carried out by dissolving filters with known amount of chlorophylls up to 5.42 μg total pigment per sample.

RESULTS AND DISCUSSION

Figure 1 shows that the primary production estimates based on scintillation activities are higher than those based on Geiger activities. The regression equation describing the relation between primary production by scintillation counting (X) and ratio of GM/Sci(Y) for Bay of Bengal samples is:

$$\log Y = \log 216.2 - 0.39 \log X$$

The coefficient correlation of —0.65 (46 d.f.) is significant. A paired comparison t — test showed at the 95% confidence level the difference between the primary production rates determined by Geiger Muller and scintillation activities on Bay of Bengal samples was highly significant (t=2.65, 46 d.f.)

On the culture samples of *Phaeodactylum tricornutum* with different levels of turbidity, the regression between scintillation counting (X) and ratio of GM/Sci (Y) is:

$$\log Y = \log 0.000002 + 3.63 \log X$$

with a significant correlation coefficeient of 0.72 (19 d.f.). The difference between the GM and scintillation activities on paired samples was highly significant at the 95% confidence level (t = 8.78, 19 d. f.).

The results of analyses of variance are summarized in Table 1 which shows that the differences between the scintillation and Geiger activities at all turbidity levels are highly significant.

TABLE 1. *Analyses of variance on Scintillation and Geiger activities at different turbidity levels*

Turbidity APHA SiO_2 ppm	d.f	Mean square between groups	Mean square within groups	F	Significance
1	3	152.1	48.1	3.2	HS
4	3	145.4	19.5	7.4	HS
8	3	2,382.9	73.2	32.5	HS
16	3	3,654.6	13.6	267.5	HS
32	3	3,333.0	20.2	164.9	HS

HS—Highly significant : P. 1%

The relative magnitudes of the standard derivations within the different turbidity blocks for scintillation and Geiger activities were estimated and shown in Table 2. The ratio of the standard deviation within the blocks ($\sigma 0$) to the mean ($\bar{x}$) is taken as a measure of the precision of the measurement. For scintillation activities the ratio $\frac{\sigma 0}{\bar{x}} \times 100$ was not greater than 4.18 at any turbidity level, whereas for Geiger activities this ratio was not less than 6.0 at any turbidity level.

A plot of the AES counts per minute against percent efficiency, showed that the original 74% efficiency of the scintillation system remained unchanged up to a turbidity level of 4 APHA SiO_2 ppm but gradually decreased with increasing turbidity thus the efficiency was 71.4% at 8, 70.4 at 16; and 66.9% at 32 APHA SiO_2 ppm.

TABLE 2. *Comparison of the variability for scintillation and Geiger activities*

Turbidity APHA SiO_2 ppm	Scintillation			Geiger		
	$\bar{x}$	σO	$\frac{\sigma O}{\bar{x}} \times 100$	$\bar{x}$	σO	$\frac{\sigma O}{\bar{x}}$ 100
1	127.30	5.17	4.06	113.20	7.55	6.67
4	103.12	3.71	3.59	84.05	5.05	6.00
8	103.55	4.33	4.18	46.45	5.91	12.72
16	100.60	2.09	2.07	26.58	3.91	14.71
32	87.65	2.94	3.35	17.10	•5.75	33.63

σO is the standard derivation; $\bar{x}$ mean; 3 d.f. at each turbidity level

Colour quenching was 73.1% at 1.08 μg; 72.5 at 2.17; 65.8 at 4.34; and 65.6 at 5.42 μg total chlorophyll per sample.

I conclude that the decrease in the percent efficiency of GM/Sci with increasing primary production (Fig. 1) is related to the increase in the thickness of the

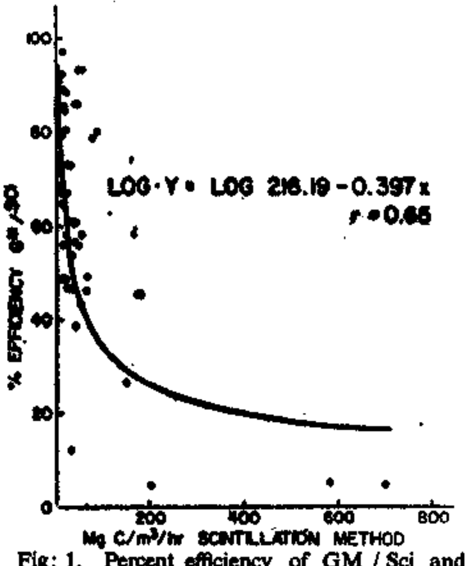

Fig: 1. Percent efficiency of GM / Sci and primary production estimates. Activities of the samples for primary production were determined by liquid scintillation.

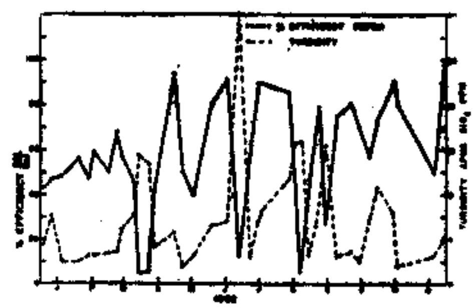

Fig. 2. Percent efficiency of GM / Sci and seasonal turbidity of sea water off Waltair.

sample on the planchette, either from the algae themselves, or from other particulate matter. Figure 2 shows that percent efficiency of GM/Sci and turbidity of the water are inversely related. The high turbidity of the water observed during

28 March-7 April is due to upwelling of turbid bottom waters and development of a major phytoplankton bloom; the two peaks of turbidity during June and August are caused by coastal dredging operations and consequent development of phytoplankton pulses; (Subba Rao, 1973) the third turbidity peak during September is due to run off from land (Subba Rao, 1965). During a bloom of a flagellate *Tairareasis* in the waters of La Parguera, Puerto Rico, a similar decrease in the percent efficiency of GM with increasing thickness of the sample was reported by Goldman (1968). In the scintillation counting system, quenching due to turbidity does not appear to be a significant problem. In our laboratory experiments the original efficiency of 74% decreased to 66.9% under maximum turbidity conditions and the results of Lind and Campbell (1969) show a decrease from about 69 to 61% when different volumes of lake water (turbidity—30 Jackson turbidity units,) from 25 to 200 ml were filtered and used. One definite advantage of the liquid scintillation counting system is that by the channels ratio method, quench may be detected and appropriate corrections for both turbidity and colour quenching to the sample count can be made. The present study also shows that for a smaller change in primary production, the corresponding change in the per cent efficiency of GM/Sci is greater in the region of low production than in the higher region (Fig. 1).

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