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# STANDARDIZATION OF $C^{14}$ STOCK SOLUTION AND FILTER EFFICIENCY IN THE COMPARISON OF PRIMARY PRODUCTIVITY MEASUREMENTS

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## INTRODUCTION

DURING the intercalibration trials on primary production conducted at the University of Hawaii, Honolulu, in September 1961, in which one of the authors (R. R. P.) also participated, factors influencing the variability of productivity measurements like sampling error, differential toxicity of samplers, sample treatment before incubation, inoculation, incubation, planchet preparation and counting were considered. Of these some of the factors such as sampling error and inoculation were made uniform by each group of scientists sub-sampling one and the same sample and all participants using the same $C^{14}$ stock (C.S.I.R.O., Australia, No. 9). Different techniques were used only for incubation and two techniques were used for counting (for details *see* Doty, 1961).

The method for the measurement of primary production, followed in this Institute till recently, was on the lines marked out by the International Agency for $C^{14}$ Determination at Charlottenlund, Denmark, including the computation of photosynthetic rates (*see* Steemann Nielsen, 1958). Since all the equipments used in this technique were not available at the time of the intercalibration tests at Hawaii, techniques used by participants from Australia, Japan, U.S.A. and U.S.S.R. only were compared. But later during July 1962 to June 1963 the first two authors conducted a series of parallel *in situ* experiments in inshore waters using the same C.S.I.R.O. stock of $C^{14}$ (8 $\mu$c), filters and filtering device as well as those of the International Agency (4 $\mu$c). The counting of the planchets was done by the C.S.I.R.O. and the International Agency respectively.

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## RESULTS

Twenty-eight *in situ* experiments were conducted mostly in the inshore waters of Palk Bay using replicate samples containing natural population and under identical conditions. The results are given in Table I.

In the final values of primary production a 10% correction has been applied by the Agency for isotopic discrimination and respiration of $C^{14}$, whereas for values from C.S.I.R.O. counting this correction has not been applied. Though any similarity in the activities of the two sets of planchets is not to be expected because of the difference in the strength of the stocks as well as the efficiencies of the counting systems, there should have been greater agreement in the ultimate values especially in view of the high sensitivity of the technique. The almost consistently lower rates in the C.S.I.R.O. values cannot be due to the higher added activity since the strength of $C^{14}$ is of no importance. Nevertheless, experiments were conducted with dilutions of 1, 2, 4, 5 and 10 $\mu$c from a Nuclear Chicago 0·5 millicurie stock solution of sodium carbonate and these gave proportional recovery of activity (173, 181, 410, 461 and 920 c.p.m. respectively) in a gas flow proportional counter. It may be mentioned here that the respective figures of 'added activity' at zero-thickness used in the computation of photosynthetic rates have been obtained by two different techniques (exponential extrapolation in the Agency technique and calculated from absolute activity in the C.S.I.R.O. technique). According to Jitts and Scott (1961) the value obtained by exponential extrapolation can be low by 26% (subsequently corrected to 20% by Jitts, 1961) as compared to the latter method. The application of a lower value of added activity brings an overestimation in the production values. Hence it is obvious that the difference in approach in the standardization of stock is partly responsible for the observed disparity in the production values obtained by the two techniques.

Various methods have been used for the measurement of added activity, such as drying small aliquot of the stock solution on a planchet or converting to gaseous $C^{14}O_2$ and determining in a gas counter and the more common method of determining indirectly from self-absorption curves of $BaCO_3$ planchets of varying thickness (*ref.* Jitts, 1961). While discussing the merits and demerits of these methods Jitts (*op. cit.*) has remarked that though the extrapolation of self-absorption curves can be made to be highly reproducible

TABLE I

*Comparison of values of* in situ *experiments in Palk Bay obtained by the International Agency and C.S.I.R.O. techniques*

| Expt. No. | Date | Station | | International Agency | | C.S.I.R.O. Australia | |
|---|---|---|---|---|---|---|---|
| | | | | c.p.m.* | mg. C/m.$^3$/hour | c.p.m.† | mg. C/m.$^3$/hour |
| 1 | 26–6–1962 | Off Mandapam | S | 10,921 | 60·175 | 36,360 | 22·875 |
| 2 | ,, | ,, | B | 1,576 | 8·684 | 7,412 | 4·663 |
| 3 | 4–7–1962 | ,, | S | 32,122 | 132·760 | 24,915 | 11·756 |
| 4 | ,, | ,, | M | 7,476 | 30·898 | 43,568 | 20·557 |
| 5 | ,, | ,, | B | 720 | 2·975 | 3,663 | 1·728 |
| 6 | 9–7–1962 | ,, | S | 18,863 | 77·961 | 76,564 | 36·126 |
| 7 | ,, | ,, | M | 731 | 3·021 | 2,881 | 1·359 |
| 8 | ,, | ,, | B | 514 | 2·124 | 2,102 | 0·992 |
| 9 | 11–7–1962 | Off Thangachimadam | S | 10,620 | 43·892 | 27,897 | 13·163 |
| 10 | ,, | ,, | M | 2,019 | 8·344 | 10,294 | 4·857 |
| 11 | ,, | ,, | B | 122·6 | 0·507 | 907 | 0·428 |
| 12 | ,, | ,, | S (D) | 93·6 | 0·258 | 783 | 0·246 |
| 13 | ,, | ,, | B (D) | 71·6 | 0·197 | 726 | 0·228 |
| 14 | 18–7–1962 | Off Athangarai | S | 5,553 | 30·791 | 27,832 | 15·759 |
| 15 | ,, | ,, | M | 2,565 | 14·223 | 13,809 | 7·819 |
| 16 | ,, | ,, | B | 396 | 2·196 | 1,432 | 0·811 |
| 17 | ,, | ,, | S (D) | 122·6 | 0·338 | 434 | 0·137 |
| 18 | ,, | ,, | B (D) | 37·1 | 0·102 | 470 | 0·148 |
| 19 | 4–9–1962 | Off Vizhingam | S | 1,984 | 7·55 | 12,652 | 5·97 |
| 20 | ,, | ,, | 15 m. | 299 | 1·14 | 1,358 | 0·64 |
| 21 | ,, | ,, | 30 m. | 67·5 | 0·26 | 296 | 0·13 |
| 22 | 11–6–1963 | Off Mandapam | S | 1,291 | 7·31 | 10,692 | 5·05 |
| 23 | ,, | , | M | 1,043 | 5·90 | 10,018 | 4·73 |
| 24 | ,, | ,, | B | 50·2 | 0·28 | 483 | 0·23 |
| 25 | 17–6–1963 | ,, | S | 2,067 | 11·70 | 16,493 | 7·78 |
| 26 | ,, | ,, | 4 m. | 3,235 | 18·31 | 23,738 | 11·20 |
| 27 | ,, | ,, | 8 m. | 1,498 | 8·48 | 15,002 | 7·08 |
| 28 | ,, | ,, | 10 m. | 626 | 3·54 | 6,863 | 3·24 |

* Added activity at zero-thickness $1·033 \times 10^6$ c.p.m.

† Added activity at zero-thickness $8·67 \times 10^6$ c.p.m.

S, Surface; B, Bottom; M, Middlelayer; D, Dark bottle

TABLE II

*Comparative retention of activity on 3 types of filters of varying pore sizes*

| Experiment No. and Date | Type of filter and pore size | | |
|---|---|---|---|
| | Membranfilter Göttingen (0·50 μ) | Millipore Type HA (0·45 μ) | Gelman Membrane Type AM-7 (0·30 μ) |
| 1. 7–8–1963 | | | |
| Activity (c.p.m.) .. .. | 2,445 | 2,574 | 2,964 |
| mg. C/m $^3$/hour .. .. | 3·8 | 4·0 | 4·6 |
| Daily rate .. .. .. | 45·6 | 48·0 | 55·2 |
| % lost .. .. .. | 17·3 | 13·0 | .. |
| 2. 8–8–1963 | | | |
| Activity (c.p.m.) .. .. | 8,303 | 8,434 | (8,672) |
| mg. C/m.$^3$/hour .. .. | 12·92 | 13·13 | (13·50) |
| Daily rate .. .. .. | 155·04 | 157·56 | (162·00) |
| % lost .. .. .. | 4·3 | 2·8 | .. |
| Recovered activity (c.p.m.) .. | .. | 238 | .. |
| 3. 12–8–1963 | | | |
| Activity (c.p.m.) .. .. | 7,408 | 8,087 | 8,553 |
| mg. C/m.$^3$/hour .. .. | 11·53 | 12·59 | 13·31 |
| Daily rate .. .. .. | 138·36 | 151·08 | 159·72 |
| % lost .. .. .. | 13·4 | 5·4 | .. |
| Recovered activity (c.p.m.) .. | 100 | 67 | .. |
| 4. 17–8–1963 | | | |
| Activity (c.p.m.) .. .. | 51,649 | 56,900 | 47,326 |
| mg. C/m.$^3$/hour .. .. | 83·74 | 92·25 | 76·73 |
| Daily rate .. .. .. | 1,004·88 | 1,107·00 | 920·76 |
| % lost .. .. .. | 9·2 | .. | 16·8 |
| Recovered activity (c.p.m.) .. | .. | 158 | .. |
| 5. 17–8–1963 | | | |
| Activity (c.p.m.) .. .. | 12,882 | 13,839 | 15,985 |
| mg. C/m.$^3$/hour .. .. | 20·89 | 22·44 | 25·92 |
| Daily rate .. .. .. | 250·68 | 269·28 | 311·04 |
| % lost .. .. .. | 19·4 | 13·4 | .. |
| Recovered activity (c.p.m.) .. | 70 | 86 | .. |

The figures in brackets in experiment 2 were obtained by filtering the filtrate from Millipore on Gelman filter after the original filtration on the latter went wrong.

and can be used for relative measurements of primary production it can introduce considerable errors when measurements using different counters are compared. The above series of experiments clearly illustrate this fact and stress the need for the adoption of a uniform method of standardization of the stock to make the results of various workers comparable. It is felt that the method suggested recently by Jitts and Scott (1961) which consists of determining the absolute activity of the $C^{14}$ added and the efficiency of the counter at zero-thickness and thereby the added activity could offset much of the difficulty and make comparison of values more feasible.

Another important aspect in productivity measurements, which has not received much attention, is the comparative efficiency of the filters commonly used by different workers in the retention of the activity. Lasker and Holmes (1957) studied the variability in retention of marine phytoplankton labelled with $C^{14}$ and concluded that it is advisable to employ filters with a porosity of at least $0{\cdot}45\,\mu$ and with relatively rapid flow characteristics. Recently Thomas (1961) has rightly drawn attention to the need of obtaining more knowledge of just how fine a filter is necessary to retain all or most of the radioactivity from $C^{14}$ labelled populations and also what negative pressure should be used for filtrations. During the intercalibration trials Millipore filters AA grade (pore size $0{\cdot}8\,\mu$) were used throughout. In the experiments mentiond above the authors used Millipore AA filters for the C.S.I.R.O. stock and Göttingen Membranfilter ($0{\cdot}5\,\mu$) for the Agency stock. AA filters retain less activity than HA filters ($0{\cdot}45\,\mu$) and considering the significance of the contribution of nanno-plankton in the primary production of these waters HA filters have been used for routine work in this Institute. Steemann Nielsen also has pointed out in a recent personal communication that for plankton algae like naked flagellates the filtration efficiency is an important factor.

In order to assess the relative efficiency of filters few experiments were conducted in August 1963 when the third author was here under the U.S. Program of Biology in connection with the International Indian Ocean Expedition. Natural populations were usedland the retention of activity was measured on Millipore HA filters, Göttingen Membranfilter supplied along with the International Agency stock and also Gelman Membrane filters Type AM-7 having a smaller pore size ($0{\cdot}30\,\mu$).

Table II gives the results of these experiments.

It may be seen that excepting in experiment 4 when phytoplankton was very abundant in the sample, Gelman filters showed a higher retention of

activity than the other filters. The decrease in retention on Gelman filter in experiment 4 may, in all probability, be due to rupturing of the cells during filtration as stated by Thomas (*op. cit.*) after the initial deposition of cells and further reduction of porosity of the filter, as phytoplankton was abundant in this sample. The higher values obtained by HA filters over Göttingen filters are also not altogether insignificant. This may be either due to the slight difference in pore size or due to the difference in the filtration techniques (vacuum for HA and pressure for the latter). The higher retention of activity of the Gelman filters was also indicated, when the filtrate from Millipore HA and Göttingen filters were refiltered at random through Gelman filters (*see* Table II, recovered activity). Hence it is felt that for plankton-rich waters, filters of Millipore HA type (0·45 $\mu$) and for plankton-sparse waters, filters with smaller pore size like Gelman Membrane type AM-7 (pore size 0·30 $\mu$) and less would be suitable yielding more comparable results.

The results of these observations reveal that the adoption of a uniform method of standardization of stock solution and careful selection of the type of filters are necessary more than anything else if values obtained by different workers are to be compared with reasonable accuracy in view of the high sensitivity and wide popularity which the Carbon-14 technique commands.

## SUMMARY

Parallel experiments using natural populations were conducted to find out the variability of values of organic production obtained by two different $C^{14}$ stock solutions, standardization techniques and counting systems as well as the relative efficiency of three types of filters. The results indicate that a uniform method of standardization of stock solution and a careful selection of filters are necessary more than anything else if values of organic production obtained by different workers are to be compared.

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