



# Interference of chlorophyll *a* in liquid scintillation counting of phytoplankton productivity samples by the $^{14}\text{C}$ technique

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## Abstract

The  $^{14}\text{C}$  tracer technique for phytoplankton productivity measurements is one of the most precise techniques used widely by phytoplankton ecologists. Most researchers overlook the possible interference of high concentrations of chlorophyll *a* during liquid scintillation counting, particularly in eutrophic ecosystems. Results from this study revealed that an optimal  $\text{H}_2\text{O}_2$  volume of 150  $\mu\text{l}$ , when added prior to scintillation counting, was sufficient to overcome an underestimation in phytoplankton productivity of up to 4.3%. © 2003 Elsevier Science B.V. All rights reserved.

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The advances in techniques for estimation of phytoplankton pigments and productivity have resulted in a major leap in aquatic ecological studies. Although relatively more research has been undertaken to modify and improve existing techniques for pigment estimation (Faust & Norris, 1985; Holm-Hansen, Lorenzen, Holmes, & Strickland, 1966; Konovalov & Bekasova, 1969; Lorenzen, 1966; Richards & Thompson, 1952; Venrick, 1987; Yentsch, 1957), the methodology for productivity measurements has not undergone much modification.

The use of  $^{14}\text{C}$  tracer techniques in phytoplankton productivity studies has been proven to yield precise productivity measurements, both in the field and laboratory (Pugh, 1973). The  $^{14}\text{C}$  tracer technique, originally proposed by Steemann Nielsen (1952), has seen little change over time, although there have been developments in the counters used for the radioactive assays. The Geiger Muller counters with low counting efficiencies, originally used by Steemann Nielsen in 1952 were replaced by gas flow counters, and in recent years

by the more efficient liquid scintillation counters. The literature on the techniques of measuring phytoplankton productivity using radioactive tracers, give an extensive coverage on the potential errors encountered in the technique (Arthur & Rigler, 1967; Cassie, 1962; Goldman & Mason, 1962; Lean & Burnison, 1979; McMahon, 1973; Nalewajko & Lean, 1972; Pugh, 1973; Wallen & Geen, 1968; Williams, Berman, & Holm-Hansen, 1972). The errors reported by these researchers include retention of unfixed radiotracer on the filter paper, passive adsorption of radioactivity by filters and/or seston, particulate and dissolved impurities in the commercially available bicarbonate, loss of tracer as dissolved organic carbon (DOC) fraction due to cell lysis during filtration, clogging of filters, and in certain cases, binding of the tracer to unknown substances in the sample, such as colloids. There are, however, no in-depth descriptions of the interference of phytoplankton pigments during scintillation counting of samples from eutrophic ecosystems. The few documented cases of probable interference of algal pigments in samples for scintillation counting failed to quantify the extent of interference in the form of an underestimation of productivity measurements (Parsons, Maita, & Lalli, 1989; Pugh, 1973; Wetzel & Likens, 2000). This study

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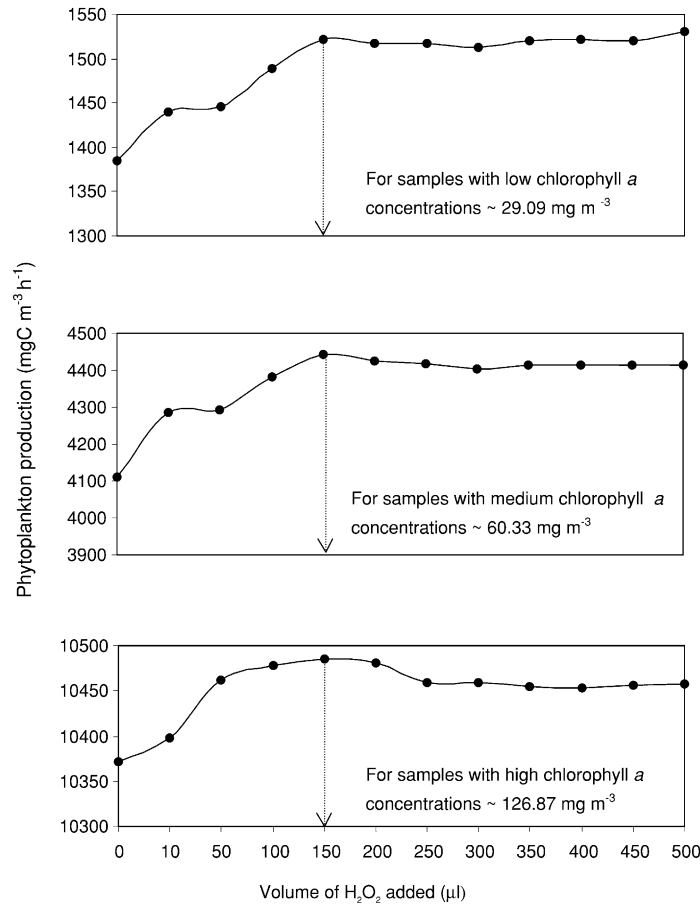


Fig. 1. Changes in phytoplankton productivity measurements with the addition of increasing volumes of H<sub>2</sub>O<sub>2</sub> to degrade the chlorophyll pigments contained in samples for scintillation counting (arrows in the graph coincide with the optimal volume of peroxide added).

was undertaken in a eutrophic tropical estuary to understand the extent of interference by chlorophyll pigments in the estimation of productivity, and to recommend possible measures to overcome this error.

Water samples were collected during high tide from Ponggol estuary (latitude: 01° 24' 87" N–01° 25' 45" N and longitude: 103° 52' 80" E–103° 54' 70" E), a eutrophic estuary located on the northeast coast of Singapore, from March to June 2000, at fortnightly intervals. Three sampling stations were chosen, with Station 1 located adjacent to a marina at the mouth of the estuary, Station 2 at mid-stream, and Station 3 at the up-stream region of the estuary. Surface (denoted as S) and subsurface (denoted as B) samples were collected from each station by filtering water through a 200 μm mesh using a portable battery-operated pump from an inflatable dinghy. Samples for chlorophyll *a* were transported to the laboratory under refrigerated conditions (+4 °C, transit time of 30 min). Known volumes of water samples (100–150 ml) were filtered through a Whatman 0.2 μm pore-size, 47 mm diameter cellulose acetate membrane filter under vacuum. A Shimadzu RF 1501 spectrofluorometer calibrated with Sigma chlo-

rophyll *a* standard was used to estimate the concentrations of chlorophyll *a* from the samples.

For phytoplankton productivity experiments, 100 ml of the water sample was dispensed into paired 100 ml dark and clear pyrex bottles and carried out in duplicates. Each bottle was spiked with 5 μCi NaH<sup>14</sup>CO<sub>3</sub> (NEN) and incubated in situ on rafts at the same depth where the original samples were collected, for an hour. At the end of incubation, the bottles were transported to the laboratory under dark and refrigerated conditions (+4 °C, transit time of 30 min). Samples were filtered onto Whatman 0.2 μm pore-size, 47 mm diameter cellulose acetate membrane filters under vacuum. Filters were repeatedly rinsed in pre-filtered water samples collected from the same site, to wash off any traces of unfixed radioactive tracer left on the filter paper. Filter papers were then folded and placed into 20 ml glass scintillation vials and 1 ml of 0.5 N HCl was added to each vial, to remove inorganic carbon. The vials were left open in a clean fume hood for 24 h, after which 10 ml of the scintillation cocktail Universol (ICN) was dispensed into each vial, and capped tightly. A Wallac 1414 liquid scintillation counter, calibrated using Wallac

$^{14}\text{C}$  unquenched standards, was used to assay the radioactivity of the filters. For the peroxide addition experiment, phytoplankton productivity samples from a specific day, from three stations, representative of low-est (average:  $29.09 \text{ mg m}^{-3}$ ), medial ( $60.33 \text{ mg m}^{-3}$ ) and highest ( $126.87 \text{ mg m}^{-3}$ ) chlorophyll *a* concentrations, in duplicate, were chosen. Radioactive counts of the vials were taken before and after the addition of increasing volumes of commercially available Merck® 30% AR hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), to determine the optimal volume of  $\text{H}_2\text{O}_2$  to be used. The data were analysed by single factor ANOVA Minitab Ver. 13.2.

Pooled averages of phytoplankton productivity in the estuary averaged between  $81.1$  and  $2110 \text{ mgC m}^{-3} \text{ h}^{-1}$  and chlorophyll *a* concentration ranged from  $2.3$  to  $64.7 \text{ mg m}^{-3}$  during the study period, indicating eutrophic conditions. Significant spatial variations for both parameters resulted in an observed high deviation from the pooled means. Substantial contributions by autotrophic cyanobacteria and diatoms at certain stations may explain the differences observed in trends for phytoplankton productivity and for chlorophyll *a*. High chlorophyll *a* concentrations observed resulted in excessive colour quenching thereby interfering with the scintillation counting of productivity samples.

To reduce the excessive colour quenching developed from the leaching of pigments during preparation of samples for phytoplankton productivity, Pugh (1973) bleached the samples for 24 h under strong illumination prior to counting. In spite of this, the residual colour and chemical quenching of the fluor solutions persisted with the addition of bleaching agent benzoyl peroxide (Hansen & Bush, 1967), reducing the efficiency of counting, and thereby affecting the precision and accuracy of productivity measurements. The more recent manuals on the methodology (Parsons et al., 1989; Wetzel & Likens, 2000) suggested the use of  $\text{H}_2\text{O}_2$ , an effective bleach. To quantify this, productivity measurements made after the addition of increasing volumes of 30%  $\text{H}_2\text{O}_2$  (AR) to samples representative of low, medial and high chlorophyll *a* concentrations showed consistent trends for all treatments (Fig. 1). This indicated that  $150 \mu\text{l}$  of peroxide addition gave the best estimates, above which no further increase in productivity values for any of the treatments was observed with increased volumes of  $\text{H}_2\text{O}_2$  added. Therefore,  $150 \mu\text{l}$  was used for further comparisons.

A scatter-plot of productivity measurements obtained from samples from all stations before and after the addition of peroxide (Fig. 2) shows a good correlation. The data were subject to one-way ANOVA and results showed significant differences ( $p < 0.05$ ) between the productivity values before and after addition of  $\text{H}_2\text{O}_2$ .

The magnitude of underestimation of phytoplankton productivity had a strong correlation with the background level of chlorophyll *a*, which in turn quantified

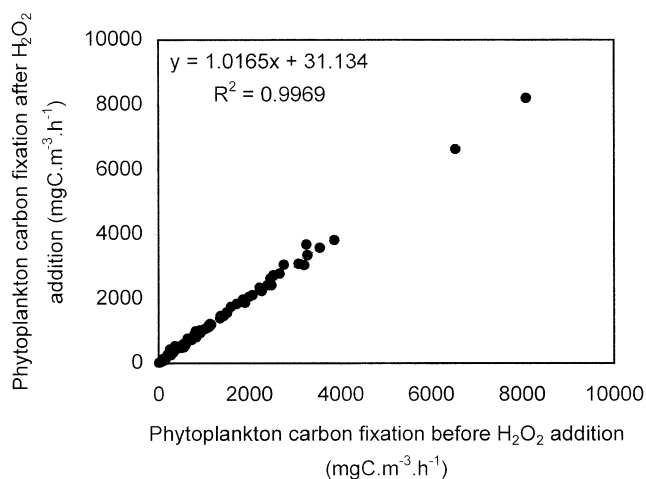


Fig. 2. Scatter-plot of phytoplankton carbon fixation before and after addition of  $\text{H}_2\text{O}_2$  ( $n = 95$ ).

the level of eutrophication (Fig. 3). When average chlorophyll *a* concentrations in this study ranged from  $30$  to  $35 \text{ mg m}^{-3}$ , the extent of underestimation ranged from  $4$  to  $4.2\%$ , in contrast to an underestimation of  $4.4$ – $4.6\%$  when chlorophyll *a* levels ranged from  $45$  to  $48 \text{ mg m}^{-3}$ .

In conclusion, the findings from this study point out that the interference of phytoplankton pigments during scintillation counting of  $^{14}\text{C}$  productivity samples is

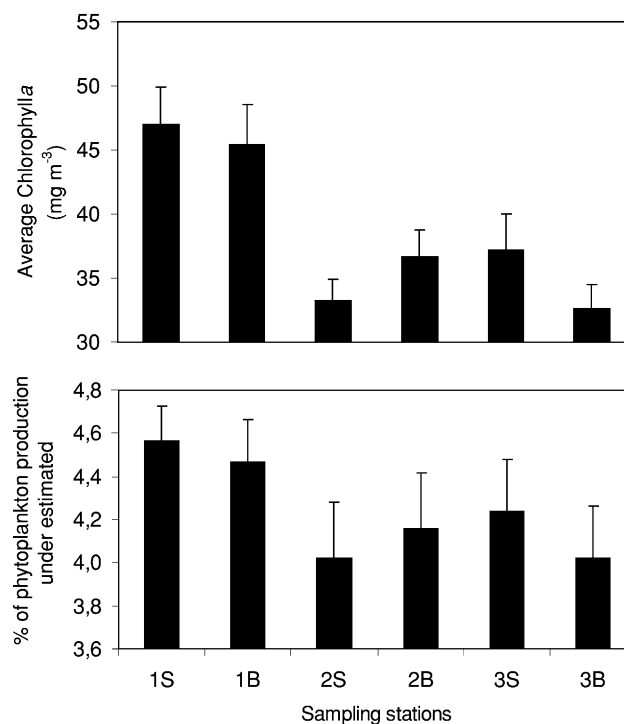


Fig. 3. Plot of average percentage primary productivity under-estimated without  $\text{H}_2\text{O}_2$  addition in comparison with the corresponding average chlorophyll *a* concentrations recorded at different stations, in duplicates for 8 sampling days.

more pronounced in eutrophic waters. This problem can be alleviated partially with the addition of 150  $\mu\text{l}$  of  $\text{H}_2\text{O}_2$  to the liquid scintillation cocktail prior to counting, based on the present findings.

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