

AUTORADIOGRAPHY AS A TOOL IN PRIMARY PRODUCTION RESEARCH*

by

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One of the difficulties most often encountered in phytoplankton productivity studies is the assessment of the phytoplankton standing crop.

Data on productivity obtained using dark and light bottle methods are not yet accompanied by sufficiently accurate methods of crop determination. The ways of assessing this have often been reviewed and their limitations emphasized (BALLANTINE, 1953; HOLMES & WIDRIG, 1956; LUND & TALLING, 1957; BRAARUD, 1958). One can consider two main approaches: chemical determination of a characteristic compound that remains sufficiently related to variations in the bulk of the phytoplankton community (*e.g.* photosynthetic pigments) or direct measurements of this bulk through weighing or counting. Counting remains one of the most successful methods. Recent mechanization of counting such as the use of a Coulter Counter (CUSHING & NICHOLSON, 1966) does not allow recognition of living cells from other particles of the same size, and thus gives variable results. Microscope counting, *e.g.* the sedimentation method (UTERMÖHL, 1936), yields very good results but is much more time-consuming than other methods. Moreover, it has its own limitation as very small nannoplankton may escape counting in many ways: destruction through inadequate fixation (BERNHARD, RAMPI & ZATTERA, 1967); confusion resulting from similarity between cells and detrital material; disappearance of cells behind bigger material which is especially true in heavily silted waters, as in estuaries (MOMMAERTS, 1969).

It is thus emphasized that both chemical and counting methods in common make inadequate distinction between dead and living pigments or between dead and living cells. This can easily lead to discrepancies between figures obtained by different methods.

The method suggested for bulk determination in this preliminary note is very simple indeed and theoretically yields absolute precision in

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the determination of the number of living phytoplankton cells in a water sample.

As in the STEEMANN NIELSEN (1952) method for primary productivity measurement, a sample is incubated with C-14. This is done under experimental conditions favouring the most vigorous penetration of labelled material (*in vitro* experiment). The sample is filtered and the filter dried as in the standard procedure for productivity measurement. The filter is then exposed for a suitable period to a photographic emulsion. The picture obtained through this technique is enlarged and dots counted as phytoplankton cells prints.

Autoradiography is a sensitive method even distinguishing intracellular organization. The simple requirement of getting prints of whole cells as mere spots allows the use of common photographic material (sheetfilm). The method may conveniently be used at the same time as C-14 productivity experiments, using a small aliquot of the incubated sample for separate filtration.

Counting in natural phytoplankton samples can be done with electronic scanning of autoradiographic pictures. Size distribution can be investigated in the same way.

The method requires a gentle handling of the samples so that the cells are not squashed onto the filter. Prefixation and low pressure filtration are recommended. The first results using a *Prorocentrum micans* culture (T 164 strain, Ekologie en Systematiek, V.U.B.) have given good pictures (Plate I) with an absolute coincidence of cells numbers counted by autoradiographic, and direct methods. Similar results are demonstrated with much smaller nanoplankton (paper in preparation).

SUMMARY

Autoradiography of C-14 labelled phytoplankton collected onto a millipore filter allows the determination of the number of photosynthetically active cells in a water sample and thus gives useful information on the standing crop in primary production studies.

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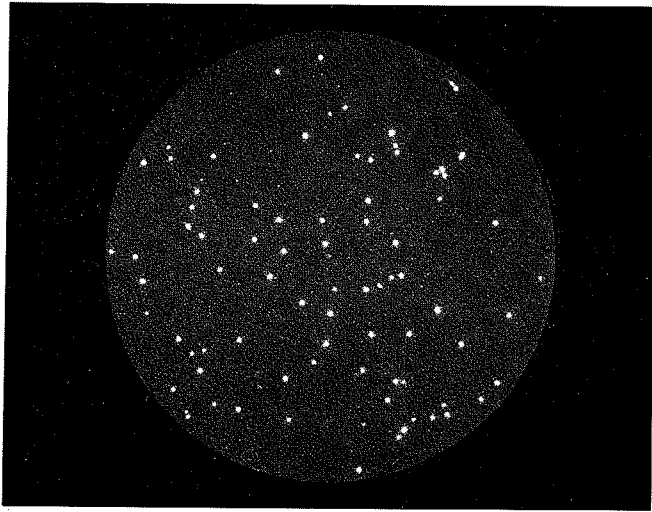


PLATE I

Autoradiograph of a *Prorocentrum micans* population ($\times 20$).

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