Laboratorium voor Ekologie en Systematiek - V.U.B.

AUTORADIOGRAPHY AS A TOOL IN PRIMARY PRODUCTION RESEARCH

by J.P. MOMMAERTS

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 succesful 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 use make in adequate 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 the determination of the number of living phytoplankton cells in a water sample.

As in the Steeman 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).

In press in the Netherlands Journal of Sea Research. Vol. 5 (3) 1972

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 photgraphic emulsion. The picture obtained through this technique is enlarged and dots counted as phytoplankton cells prints.

Autoradiography is a sensitive method distinguishing even as far as 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 nannoplankton (paper in prepararation).

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.

REFERENCES

BALLANTINE D., 1953. Comparison of the different methods of estimating nannoplankton. - J. mar. biol. Ass. U.K. 32 : 129-132.

BERNHARD, M., L. RAMPI EN A. ZATTERA, 1967. A phytoplankton component not considered by the Utermöhl method. - Publ. Staz. Zool. Napoli. 35 : 170-214.

BRAARUD, T., 1958. Counting methods for determination of the standing crop of phytoplankton. - Rapp. p.-v. Réun. Cons. perm. int. Explor. Mer. 144 : 17-19.

BROCK, M.L. & T.D. BROCK, 1968. The application of micro-autoradiographic techniques to ecological studies. - Mitt. int. theor. angew. Verein. Limnol. 15 : 1-29.

CUSHING, D.H. & H.F. NICHOLSON, 1966. Methods of estimating algal production rates at sea. - Nature, London. 212 : 310-311.

HOLMES, R.W. & T.M. WIDRIG, 1956. The enumeration and collection of marine phytoplankton. - J. Cons. perm. int. Explor. Mer. 22 : 21-32.

LUND, J.G. & J.F. TALLING, 1957. Botanical limnological methods with special reference to the algae. - Bot. Rev. 23 : 489-583.

MOMMAERTS, J.P., 1969. On the distribution of major nutrients and phytoplankton in the Tamar estuary. - J.mar. biol. Ass. U.K. 49 : 749-766.

STEEMAN NIELSEN, E., 1952. The use of radioactive carbon ($.C^{14}$) for measuring organic production in the sea. - J. Cons. perm. int. Explor. Mer. 18 : 117-140.

UTERMÖHL, H., 1936. Quantitative Methoden zur Untersuchugen des Nannoplanktons. - Handb. biol. ArbMeth. 9 : 1879-1937.