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Counting Methods for Determination of the Standing Crop of Phytoplankton

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The Role of Population Studies in Investigations of Organic Production.

Numerical estimates of the actual population of the seawater does not give any direct information concerning the primary production of a water mass at the time of sampling. Such data of the plant population, however, are essential for obtaining a closer insight into the dynamic aspects of production, the cause of the geographical and seasonal changes in the photosynthetic activity within the euphotic layer and in the formation of new protoplasts.

What stress should be laid upon inclusion of studies of populations in investigations concerned with the quantitative estimation of production may be subject to discussion.

The introduction of the ^{14}C-method has provided facilities for an extensive study of the potential production under a unit area of the sea at the moment of sampling. We are at present going through a period of collecting extensive material of such records, from various regions and different seasons. On the basis of these observations a correlation can be made using hydrographic data and observations on nutrients in order to draw up the broad lines of the interrelationship between the recorded production values and the environmental background. This can be done without making any observations of the organisms which are responsible for the photosynthetic activity.

After this first period the further exploration of the process of organic production in the sea will call for an intensification of the study of the productive organisms. The results which have been obtained on the biogeography, ecology and physiology of the phytoplankton, both in general and for various species, will then form a useful basis. A combination of these studies with observations by means of the ^{14}C-method should prove to be most fruitful.

Even during the first knowledge of the phytoplankton distribution and its annual cycle within a region would be most valuable for a rational planning of surveys of production.

In this work it is essential to have at hand a method for quantitative studies of the population which is as satisfactory and requires as little work as possible.

Available Methods.

The marine phytoplankton includes organisms of very different sizes, from the larger diatoms and dinoflagellates down to very small forms. This is the reason why it is difficult to develop methods which yield satisfactory data on the whole plant community, the larger forms being present in relatively small numbers as compared with the smaller forms, even at the times of their maximum abundance.

The bluegreen algae with a specific gravity lower than that of the seawater represent a special problem in quantitative plankton studies, but it will not be discussed here as this group is not of general importance in the sea.

The various methods for quantitative phytoplankton studies may be referred to the following categories:-

1) Net methods,
2) Centrifugation,
3) Filtration,
4) Sedimentation,
5) Direct counts,
6) Culture methods.

We may briefly consider the advantages and disadvamtages of each of these categories.

Net Methods.

The advantage of net hauls is the ease by which large volumes of water may be filtered so that organisms which are present in relatively small numbers may be sampled. The main disadvantage is the exclusion of the forms which pass through the meshes of the net and the distorted picture which is often obtained of the relative abundance of the larger forms due to clogging. Even in the case of the new nets which have smaller meshes than the old ones there are important groups too small to be retained.

Although various types of devices have been described for measuring the amount of water which is being filtered, in many cases uncertainly prevails as to the reliability of the values obtained.

In conclusion it may be stated that net methods should not be employed for quantitative phytoplankton studies.

Centrifugation.

Lohmann's (1908) centrifuge method was discarded for use in phytoplankton surveys when the sedimentation method was introduced. The objections raised against centrifugation by Allen (1919) and Steemann Nielsen (1933) are still unchallenged. The more favourable results obtained by centrifugation of live naked forms (Ballantine, 1953) may be due to the special adhesive nature of these objects, as indicated by Steemann Nielsen (l.c.) and can not be relied upon when the method is used for other forms, especially in a preserved state.

Centrifugation has the advantage that the volume of water examined may be varied easily within a wide range. This method, therefore, may be useful in culture work when a comparison with other methods has shown that it is satisfactory for the special object in question, such as with naked flagellates.

Filtration.

The older methods of filtration through sand and paper filters have been replaced by the more effective filtration through new types of filters. In the case of the molecular filters (Goldberg, Baker and Fox, 1952) they retain even the smallest phytoplankton organisms. In the latter form filtration has the advantage of having all size groups retained and ease in varying the volume of the sample filtered. The main disadvantage of this method are the long time required to prepare the sample for counting and difficulties in identification of the small as well as many of the larger

forms. This pertains to live as well as preserved material. The large forms cannot be turned over for closer inspection, while the small naked forms are often destroyed when filtered alive or subjected to great changes by preservation.

Sedimentation.

The sedimentation method of Utermöhl (1931) with the use of an inverted microscope has proved to be a useful method for estimating quantitatively the intermediate size groups of the phytoplankton. For the smallest forms Utermöhl has described a special procedure of sedimentation in Kolkwitz cell and examination of a minor fraction of the sediment at high magnification, a method which has been used with success by Rodhe (1955) for fresh-water samples. In both cases the disadvantage is that only preserved samples can be studied. As no preservative satisfactory for the small as well as some of the larger naked forms has yet been found for sea water, these are often inaccesible for identification. For the larger species the volumes which are commonly used are too small to give adequate representation.

The Haller Nielsen apparatus (1950) for a closer examination of forms which are not easily identified in the inverted microscope and the modification of the sedimentation cylinders so that sedimentation of larger samples is facilitated (Fraser, 1954) represent recent improvements of the method.

Direct Counts.

Only in exceptional cases can direct counts of the population give any adequate information on the total population. The method developed by Millar (Ballantine, 1953) using dark field inspection may give reliable figures for various size groups, but it cannot be recommended for general studies of phytoplankton populations as identification is not feasible.

Culture Methods.

Assessment of the number of autotrophic forms by means of dilution of the sea water sample in culture media and observation of the subsequent growth has been carried out and this method has been used for checking the results obtained by other methods (Allen, 1919, Ballantine, 1953). The method is too laborious to be adopted for general studies of phytoplankton populations.

The conclusions which may be drawn from this brief review of the various categories of methods now in use are:-

a) By means of a single counting operation it is not possible to have all the requirements satisfied which ought to be fulfilled if one should be fairly certain of obtaining an adequate record of all the autotrophic species occurring in the plankton.

b) Net haul methods or direct counts should not be employed.

c) The sedimentation method with the use of inverted microscope may be regarded as the most satisfactory standard method for obtaining quantitative records of the phytoplankton, except for the smallest groups. Attention, however, should be paid to the recent improvements of the method. Some large forms may not have been recorded in a satisfactory way when the largest volumes examined were 50 ml. or less.

d) The most obvious obstacle in enumerating the total phytoplankton population of sea water is the lack of a suitable preservative for general use and for small forms, like the naked chrysophyceans and other naked flagellates. In the future special regard should be paid to the smallest species, motile and non-motile μ-forms, which hitherto have beeb neglected in general studies of marine phytoplankton.

Which Procedure can be Recommended for Enumeration of the Total Population?

In view of the large amount of work involved in any enumeration of the total phytoplankton population of a water sample a research programme should be set up to consider the methods now in use in order to establish the least time-consuming and still satisfactory procedure and to find adequate preservatives.

For discussion during the Symposium the following procedure for enumeration of all size groups according to the principles laid down by Utermöhl (1931) may be suggested:-

1. The volume of the sample drawn from the water-bottle at sea should be increased to 300 ml.

2. Counting of the species belonging to the intermediate size groups, down to 5-10 μ and including the coccolithophorids, in sedimentation samples of 2(1)ml. and 50(25) ml. of preserved sea water by means of the inverted microscope (or by the modification introduced by Lund (1951)).

3. Counting of the largest diatoms and dinoflagellates in corresponding samples of 200 ml.

4. Counting of the smallest forms in Kolkwitz cell with the use of ordinary microscope and water immersion objective as used by Rodhe (l.c.). Only a small fraction of the field is examined.

This procedure would require 4 different counting operations and would be very laborious.

In case a somewhat more time-saving procedure has to be employed, it would seem better to reduce the volume of the samples examined than to exclude any of the counting operations.

It should be underlined that enumerations according to this complex procedure should only be employed in cases when hydrographic observations from the same points where the water samples for phytoplankton studies have been drawn are available and the whole plan for the survey is satisfactory from the point of view of a phytoplankton study. In many cases a cruder method which gives only a very rough idea of the population may be not only justified but appropriate.

The method for enumeration should in any case be chosen for the purpose of the special investigation, taking into account both the accuracy of the method and the time required for counting. Doubtless, this point has been disregarded in many cases of marine survey work because of the tendency to apply methods which are familiar to the person organising the survey.

When attempting to find a satisfactory preservative for sea water samples two points should be given preference: 1) to provide a satisfactory preservation of the naked forms without destroying the calcareous coccoliths of the coccolithophorids, 2) to find the concentrations needed for hindering bacterial activities even at fairly high temperature, so that samples can be stored for a longer period of time without being destroyed.

Concluding Remarks.

In view of the scarcity of specialists in marine phytoplankton it may seem as if the requirements for population studies in connexion with the study of primary production in the sea suggested above are too rigid. However, as so little is known of the relative importance of the various size groups for the joint photosynthetic activity in a water mass, it is at present important to take all size groups into account when population studies are undertaken for use in studies on production.

This does not in any way impede the value of botanical studies of /populations phytoplankton/by means of methods which from a production point of view would seem unsatisfactory as they pay regard to special fractions of the population only.

References.

Only a few of the numerous publications on methods for enumeration of phytoplankton populations have been included in the list below.

Allen, E.J.	1919	"A contribution to the quantitative study of plankton". Journ.Mar,Biol.Assoc.U.K.,XII,1.
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Lund, J.W.G.	1951	"A sedimentation technique for counting algae and other organisms". Hydrobiol.,III, 4.
Nielsen, P. Haller	1950	"An auxiliary apparatus for plankton studies by means of the sedimentation method". Journ.Cons.Int.Explor.Mer, XVI ,3.
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