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by Hans Utermöhl

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The improvement of quantitative phytoplankton methodology

by Hans Utermöhl

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With 1 table and 15 figures in the text and on one plate.

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Synopsis

All the important questions in counting phytoplankton are reviewed. The methods described refer primarily to fresh water but are applicable to marine phytoplankton as well. No attempt has been made to review the whole of the volumnous literature on counting technique or describe its development. The main aim is to describe the counting-chamber method. The numerous difficulties encountered in quantitative plankton research are discussed and ways of avoiding them are described together with improvements of technique that save time. Among the equipment described are the filling-chamber (Fullkammer) and the combination-chamber (Verbundkammer). A mixture of potassium iodide in iodine and sodium acetate is used for the preservation of phytoplankton. A new sort of fractionated counting designed to overcome a certain degree of unavoidably uneven distribution of the plankton sediments is described.

/2

A. Introduction

The necessity for quantitative phytoplankton determination does not have have to be argued here; it is generally recognized in limnology. In limnology and oceanology, the development tends towards the increasing use of chemical and physical investigation methods which permit conclusions regarding the phytoplankton content, e.g. protein, chlorophyll and C_{14} determinations, etc. Despite considerable advantages, chemical-physical measurement methods also have drawbacks: Frequently, they do not differentiate between abioseston and genuine plankton. In addition, they only give summary values. Thus all details of the qualitative composition of the plankton are not detected; in contrast to direct plankton counts, the significance of the individual phytoplankton varieties as indicators of the classification of the waters is not evaluated at all. For this reason, direct plankton counts will always be valuable in limnology and oceanology, if they are carried out accurately.

What, then, is the situation regarding accuracy? Over the decades, a large number of so-called "quantitative methods" together with their modifications have been described and also used. Unfortunately, however, most of these methods do not stand up to objective criticism (see Utermöhl, 1936). Only the chamber method in its modern form may be considered sufficiently accurate. With regard to detection efficiency it compares favourably with the indirect chemical or chemical-physical methods. Its main advantages may be briefly summarized as follows:

(1) High accuracy: The method does not have any systematic errors, only incidental ones. The latter follows laws of statistics; thus, they are mathematically determinable. For this reason, the chamber method gives values which are absolutely comparable.

(2) Adaptability: Accuracy may be adopted to the requirements of the

problem in question; i.e. lower accuracy results in time saving.

(3) Simplicity: Anyone who has previously worked with other methods and quickly becomes a supporter of this technique.

In spite of this, phytoplankton counts using the chamber method are not /3
always simple. Accurate counting of the common forms such as Ceratium, Asterionella, Pediastrum, Volvox, Pandorina, etc. with the chamber method is simple. However, nanno- and ultraplankton occurs much more frequently and is ecologically considerably more important. These plankters make the numberical determination difficult and result in the failure of the old, so-called quantitative plankton methods. Most hydrologists are unaware of these difficulties in methodology. However, every limnologist (and every oceanologist) who carries out plankton counts must consider - and thus be aware of - these difficulties in order to avoid errors. For this reason it is absolutely essential not to conceal the major difficulties which every planktologist using quantitative methods will have to contend with sooner or later, but rather to deal with them in this study, and subsequently show ways of overcoming them.

B. Sampling

I. General aspects

The phytoplankton is not distributed in the water as evenly as was initially assumed. Vertical layers occur regularly, i.e. in times of stagnation they always develop and at other times traces of layers may occur. Additionally, there are often irregularities in the horizontal direction, especially in ponds but also in large, deep lakes. These distribuiton anomalies must be considered in sampling; otherwise the results obtained are not accurate. Depending on the problems to be solved, the sampling methods vary. If an accurate determination of the vertical layers is required, or if flagellata movements are to be investigated, etc., a number of sharply demareated samples must be taken from different depths in such a way that the layers of the forms to be examined are covered accurately and distinctly ("spot" sampling). Larger or smaller spaces exist between these "spots"; these spaces do not have to be sampled. On the other hand, layer details are less important if a number of lakes are to be compared with regard to their phytoplankton production, or if th4 fertilizing effect of waste water, drainage influx, etc., on the phytoplankton is to be determined accurately. The accurate determination of the total quantity of phytoplankton in the waters in question, or at least of all the abundant phytoplankters in these waters, however, is import- and in this case. For this purpose, spot determinations do not suffice; continuous vertical profiles ("sample columns") are required, at least for the complete trophogenic layer.

The samples are best obtained with suitably designed scoops which cannot /4 be described here because of lack of space. Rubber tubes have also been used successfully for extracting sample columns (Kofoid, 1897 a and b, Ohle, 1955).

However, plankton nets and plankton sieves are completely unsuitable, at least for the quantitative determination of floating plants.

II. Investigation of the vertical layers of the phytoplankton

Frequently, but not always, the vertical layers are distinctly developed in a narrow space, especially at the boundary surfaces of large thermal or chemcial gradients. When samples are taken, this must be taken into account as follows:

(1) Proper selection of samples; they must not be spaced too far apart, so that the layer maxima of the individual phytoplankters, which do not always lie at the same depth, can be determined as accurately as possible.

(2) The samples must not be too long in the vertical direction, since then the maxima become poorly defined.

(3) Formation of turbulences during the extraction of samples would destroy the layers; it must therefore be strictly avoided.

III. Investigation of the horizontal layers of the phytoplankton

It is virtually impossible to provide exact guidelines for this, since the execution of this investigation is dependent on the applications on the one hand, and on local peculiarities on the other hand. For production-biological investigations, the samples are taken with a suitable tube scoop, or perhaps a piece of hose, at various well-chosen locations fo the Lake or pond. Inflows, wind pressure and other factors effecting plankton distribution must be taken into consideration; in shallow waters, special attention must be given to the absence of underwater plants. All samples can of course be counted separately, thus drawing conclusions on the distribution of the plankton. However, with a large number of samples, this process is too time-consuming. Furthermore, such detailed analysis of the individual samples is

rarely required. For this reason it is sufficient to pour a number of samples into collecting vessels (e.g. large bottles or cans). The samples are carefully mixed and average samples are taken from the mixture; these suffice for many applications.

Of course, the extraction of vertical water columns is not always practical. Irregularities of the horizontal distribution may also influence the layers (see, e.g., Elster, 1956, p. 251; Utermöhl 1925, pp. 221, 225, 227/228). If such cases were only investigated with scoops, many details would remain unknown. "Spot samples", appropriately distributed over the water, give a clearer picture. Occasionally, a combination of "spot" and column samples /5
may also be advisable. Thus, it must be left to the judgement of the scientist where and how the samples are taken, and which implements are used for this purpose.

C. Treatment of samples prior to counting

I. Counting of Living or dead samples?

Some scientists believe that the chamber method requires preservation of the samples and reject it for this reason, since many plankters cannot be identified in the preserved state, and thus cannot be quantitatively determined. The author believes this opinion to be a mixture of fact and fiction, as may be seen from the following:

Granted, fixing and preservation cause some plankters to change their shape to such an extent that these no longer have any similarity with living plankters of the same vaiety. In spite of this, they are clearly recognizable in the preserved state, and can be accurately counted, as shall be shown later[1)].

Does phytoplankton always have to be fixed in order to be counted? Not at all! The specific gravity of some forms, such as diatoms and protococcales is so high that they sink to the bottom of the chamber; they remain passive there and can thus be counted alive. However, in addition to such readily sedimented and easily counted forms, almost every water sample contains other forms which are difficult to determine. On the one hand, these are Flagellata and flagellated bacteria; they usually swim so fast that their restless movements have to be artificailly slowed down before an accurate count can be made. On the other hand, the specific gravity of certain Cyanophyceae and bacteria, as well as Botryococcus, is so low that they will not sediment without artificial weighting, i.e. suitable fixation. They will not even sediment with the aid of a centrifuge (Lohmann, 1908, p 190; Pascher, 1912, p. 106). Both groups of forms, the Flagellate and the Syenophyceae, often - and in eutrophic lakes

1) This does not include swarming spores and similar forms, which cannot be systematically categorized, or which cannot be categorized on the basis of protracted cultures.

and ponds in most cases - form such a substantial part of the phytoplankton, that they must definitely be considered.

A few scientists have tried to avoid fixing of the Flagellata by a kind of anaosthatization. Kolkwitz suggested putting them into "heat-rigour", e.g. by careful heating of the water samples in a thermostat bath (Kolkwitz, 1912, p. 343). However, it is easily realized that a specific temperature causing heat-rigour for all Flagellata does not exist; theis temperature differes for every variety. If it is exceeded, the flagellata burst. This is particularly true for the many oligothermal winter and spring forms, and especially for the stenothermal forms among them. /6

Fixation of the water samples appears to be especially justified since many plankters die and disintegrate soon after the samples have been taken. This is due to the sensitive plankters being so abruptly exposed to changed conditions. An attempt may be made to keep these changes as small as possible in order to delay dying: This may be accomplished by keeping the storage containers scrupulously clean, keeping the original water temperatures constant (using thermos flasks, moist cloths, storage boxes with felt-lined interiors), avoidance of glaring light, consideration of the original gas content and the like; however, it is not possible to maintain the original living conditions for the phytoplankters enclosed in narrow bottles. For this reason, the above environmental changes may at best be retareded, but not completely eliminated. This is also shown by sensitive chemical analyses, where the phosphorus content in samples, for instance, significantly increases only a few hours after the samples were taken - an indication of the dying and decomposition processes of the plankton.

Another factor must be considered: While careful counting of only a few water samples takes a number of hours, the quantitative processing of complete

series takes days and even weeks. For this reason, processing of live samples in the unchanged state is impractical. Therefore, only two alternatives remain: either careless work which is of no use to research, or fixation.

II. Fixing the phytoplankton

In deliberate contrast to Romeis (1948) and other well-known authors who do not clearly differentiate between fixing and preserving, together with Moll (1908) the author of this paper understands by fixing "a rapid killing of the protoplasm, so that it does not have time to change its shape by contractions or otherwise." In the following, preserving shall mean the preservation of fixed samples over longer periods (at least a few years). The two are of course not identical.

To be useful for quantitative phytoplankton investigations, a fixative must meet the following requirements:

(1) It must take effect rapidly, before contractile phytoplankters are above to contract, thus becoming unrecognizable.

(2) It must retain the shape of the most delicate, otherwise easily dissolving forms.

(3) It must weight-down the fixed plankters, so that even those forms which are normally lighter than water (water blossom algae) completely sediment in the counting chambers. /7

(4) The added fixing agent should take affect in such minute quantity that the sample volume - and thus the plankton concentration - remains unchanged by its addition.

(5) The fixing process must be simple and

(6) its cost moderate

Is there such an ideal fixing agent? Strictly speaking, hardly. Most of the common fixing liquids and mixtures are excluded, since they do not

satisfy one or a number of the above 6 conditions. On the other hand, the caueous iodine in potassium iodide solution, introduced to planktological practice in 1921, proved to be useful. It kills so rapidly that e.g. the metabolic movements of the euglena stiffen without contraction, and the pseudo-podia of the Rizochrysidaceae remain fully extended. Even the most delicate pellicles of many Flagellata, such as Chryso- and Cryptomonads, do not dissolve; Chloromanads and some Gymnodiania, however, do swell up (see later). Furthermore, the flagella remain intact, and are more clearly visible than on live flagellata, as are the pyrenoids which are very important for the determination. Even such sensitive shapes as the throat of the Cryptomonads and the collars of the Craspedomonads are not destroyed. Also, the gelatine of some phytoplankters often becomes more apparent, at times it is directly visible, or else it may be recognized by the iodine-browned bacteria sitting on it. Even the discharge of food vacuoles of colourless flagellata (and Infusoria) solidifies in statu edendi and may thus be clearly recognized.

A major advantage of the iodine in potassium iodide solution is that it weights most floating plants - even water blossom algae - in such a way that they sediment in the counting chambers. While there are exceptions, they are easily eliminated (see further below). Furthermore, it application is extremely simple: Addition of two to three drops of concentrated aqueous iodine in potassium iodide solution to 100 ml sample water. With this small quantity, volume, and thus also the plankton concentration, remain virtually unchanged.

This simple fixing method is usually adequate. However, as already mentioned, there are a few phytoplankters which cannot be brought to sediment with this fixative, or which sediment only after an extended fixing time.

Botryococcus, which often, but not always has a brownish or reddish colour due to substantial accumulation of fat-like substances, should be mentioned first. With some attentaiveness, the colouration permits recognition of these relatively large algae with the naked eye, even if the water sample contains only a few colonies of them. They do not sediment in such a fatty state, not even with the addition of a large quantity of iodine in potassium iodine solution. However, sedimentation can be easily forced by dissolving out the /8
fatty substances from the algae. The tests carried out for this purpose by Volk (1901 and 1906) with ether-alcohal mixtures were not successful. The author's tests with CS_2 (carbon disulphide), however, proved successful. Approximately 1 ml of CS_2 is added to a water sample previously fixed with iodine in potassium iodide solution. The mixture is shaken periodically, and after a few hours, Botryococcus sediments.

A few individual varieties of blue aglae behave similarly to this Chlorophycea; they, too, are at times difficult to sediment using only iodine in potassium iodide. Their sedimentation is easily achieved by the addition of 1 ml of trichloroathylene ($CNCl:CCl_2$).

Once the algae have settled. they must be separated from CS_2 or $CHCl:CCl_2$, since these chemicals also deposit (in drop shape) at the bottom of the bottle due to their specific gravity. Separation is achieved without difficulty by renewed shaking, whereby the whole sediment is whirled up in the water. Due to their greater gravity, the CS_2 or $CHCl:CCl_2$ drops sink to the bottom of the bottle quicker than the phytoplankton which may now be easily separated from the chemicals by decanting.

For another reason as well, decanting should be carried out as soon as possible: it is known that CS_2 is not only a solvent for fats, but also for

plant pigments (chlorophylls, carotenoids, etc.). It therefore discolours the algae more or less quickly. If discolouration is to be avoided altogether the samples which are found to contain or believed to contain Botryococcus are halved: one half of the sample contains only iodine in potassium iodide solution, the other half contains CS_2 as well. The latter sample is only used for counting Botryococcus.

Thus we come to what is probably the only disadvantage of the iodine in potassium iodide fixation: it gives the plankton a brownish colour. The experienced planktologist is not disturbed by this, he soon gets used to it. The beginner, of course, has to acquire a double knowledge of the plankter shapes, so to speak: on the one hand of their appearance in the live condition, on the other hand occording to their iodine in potassium iodide colouration. This knowledge is obtained by initially identifying the varieties in the live condition; subsequently, they are continuously observed under the microscope while being killed with iodine in potassium iodide. In this way, a methodically correct classification of plankters discoloured by fixing becomes possible.

This method even permits recognition of forms which swell after fixing, such as the earlier mentioned Chloromonads and various Gymnodinia whose natural appearance, by the way, cannot be retained wtih any known fixative. Luckily, these forms only occur fairly rarely in plankton. Thus, in the fixed state they are artifacts, but of characteristic appearance and easily identified by comparison with live flagellata, and an accurate determination of the species is also possible. Thus, there is justification for the statement that almost all phytoplankton forms can be counted after fixing with iodine in

potassium iodide, provided that the determination is carried out on live reference samples. For this reason, the planktologist should always take a few live samples, at least from waters which are less frequently examined. These samples should be protected against temperature changes with moist cloths or the like (see page 9). Furthermore, since the Epi- and Hypolimnium, and often the Metalimnium as well, have a qualitatively different population, at least three live samples should be taken from each lake. /9

III. Preservation and storage of the phytoplankton

Iodine in potassium iodide, while being an excellent fixative, has only limited value as a preservative. The author has learned from many years of observations that plankton samples fixed with iodine in potassium iodide do indeed remain unchanged for days, and usually even longer, but this is by no means always the case. After fixing with iodine in potassium iodide, Cyanophyocce and Chlorococcoceae, and even very delicate Flagellata (Prymnosium, Cryptomonad and others) have been kept under control for many months, and sometimes for over a year. The dyes bleached out to a greater or lesser extent, and the pseudovacuoles also gradually disappeared; however, the different forms remained recognizable despite these changes. However, in other cases the plankters dissolved more or less, at times completely, among them even robust and armoured varieties, such as Peridinium, Ceratium, etc. More precise investigations showed that the dissolution phenomena have three causes:-

(1) Autolysis of the plankton

(2) Dissolving of alkali from the storage bottles in the water.

(3) Formation of bacteria and low fungi in the storage bottles.

For preservation to be successful, all three causes must be eliminated.

This is by no means easy, as shall be seen from the following.

The last-mentioned cause may be eliminated most easily, since low fungi and bacteria do not grow in iodine in potassium iodide fixed samples, as long as the samples are still coloured yellow by traces of iodine, which is an extremely powerful poison for lower organisms. Unfortunately, the iodine tends to volatilize from the plankton samples. Ordinary cork stoppers adsorb iodine, and are thus totally unsuitable as bottle stoppers for plankton samples. Cork stoppers coated with paraffin seal better; best of all, however, are storage bottles (preferably of Jena laboratory glass) with ground glass stoppers, which for the purpose of secure sealing are lightly coated with paraffin paste. To be on the safe side, it is advisable to check the yellow colouring of plankton samples designated for long storage, from time to time. If the samples have almost lost their yellow colour, two drops of iodine in potassium iodide solution shoudl be added.

The alkali dissolving from the plankton storage bottles is of course dependent on the type of glass. Cheap bottles made of base-rich glass are cettainly to be avoided, since many plankters are changed beyond recognition or dissolved altogether in them. Jena glass bottles protect at least for some time. Polyethylene ("Kautex") bottles do not release alkali at all; however, they have the disadvantage of adsorbing considerable quantities of iodine. This may be the reason for various researchers experiencing difficulties in storage of plankton fixed with iodine in plastic bottles. Whether in time, part of the plankton adheres to the walls of the "Kautex" bottles and is thus lost in the count should also be checked. To my knowlege, this is not the case with glass bottles and iodine in potassium iodide fixing. /10

The autolysis of plankton may at least be delayed for some time, if not prevented altogether, by protein-precipitating agents. Acetic acid is such an agent, and therefore is a major constituent of many fixatives and preservatives. Some limnologists mix it in with the iodine in potassium iodide solution and report successful results. This is no doubt true for many plankters. Unfortunately, as with other acetic acid mixtures, such an acetic iodine in potassium iodide solution dissovles the shells of calcium flagellata, thus making them unidentifiable (see Utermöhl 1936, p. 1894). Calcium flagellata, are a major part of the marine phytoplankton; certain forms such as Phacotus, are frequently found in large numbers in fresh water and brackish water as well. Their small size, and especially improper fixing and preservation may be responsible for the fact that they have up till now usually been overlooked.

In order to obtain, as far as possible, the entire phytoplankton including the calcium flaggellata, sodium acetate is recommended in lieu of acetic acid. Sodium acetate assures a weakly alkaline solution. Tests carried out on numerous flagellata (Chrysomonada, Cruptomonadelae and Volvocales) some of which were preserved with acetic iodine in potassium iodide solution, and some with iodine in potassium iodide plus sodium acetate, did not reveal any differences in the state of preservation. Due to the relatively short testing period, the durability of the latter could not be determined. Gradual bleaching of the colours and gradual dissolution fo the pseudovacuoles must be assumed. For this reason the samples should not be kept longer than absolutely necessary. It is of course preferable to count them soon after extraction. However, it is not advisable to fill cold samples into the chambers, since gas bubbles (C_2, CH_4, CO_2) separate during sedimentation; these bubbles may interfere with the counting process, especially in flat chambers. If the

samples have to be counted quickly, they should be heated to approximately 40-45°C, to release the gases. Heating should be carried out after fixing, but prior to filling the samples into the chambers. It is better, and also less disturbing to the plankton, to allow the samples to stand at room temperature for one or two days which usually suffices to release the gases. /11

The following instructions result from the above statements:

(1) Preparation of the iodine in potassium iodide-acetate mixture: 10 grams of potassium iodide neutrale pro analysi are dissolved in 20 ml of water and 5 grams of double sublimed iodine pro analysi are added to this solution. When fully dissolved, another 50 ml of water and 5 grams of CH_3-COCNa pro analysis are added. The mixture is stored in a 100 ml narrow-necked bottle of neutral glass (e.g. Jena soft soda glass) with well fitting, ground glass stopper.

(2) Storage of plankton samples: Samples must be kept in a dark place. If glass bottles are used they must be hydrolyically resistant (preferably Jena narrow necked bottles, soda-glass 20, with standard grind and standard ground glass stoppers; the latter are to be thinly coated with paraffin paste); if "Kautex" bottles are used- but see remarks at top of page 10 -, special attention should be given to sealing, using paraffin paste on the thread of the "Kautex" bottles as well as on the ring nut of the associated screw-on lid.

D. The modern chamber method

I. Principle

The chamber method was introduced in hydrobiology by Kolkwitz in 1905 (Kolkwitz 1907 and 1911). He applied the underlying principle of the erythrocyte and leucocyte count to the phytoplankton, by allowing the latter to settle at the bottom of glass chambers of specific size, where they are counted. The chambers he used were flat, as used in medicine, i.e. depth of sedimentation was smaller than chamber diameter. This resulted in low plankton density and a large scanning area relative to chamber volumn; the time required for scanning anc counting was correspondingly long.

With tube chambers (Utermöhl, 1932, p. 571 ff.) the depth of sedimentation is greater than the chamber diameter. This giver the following advantages:

(1) Increase in chamber volume to 100 ml; this allows identification of rarer forms.

(2) Smaller bottle-base areas result in quicker counting and thus great time savings (ten-fold and more). /12

(3) Considerably improved optical investigation conditions: increase in magnification and resolution up to the limits of alight microscope, however only if a special microscope, the so-called inverted microscope, is used (Utermöhl, 1931). The latter is required since the lens clearance of the standard microscope is too small to allow examination or counting of the plankton settled at the bottom of the long tube chambers. This is easily done with an inverted micoscope: the magnifying components (lenses and eye-piece) are beneath

the slide, condenser (and light source) above. Thus, with an inverted micoscope, the chamber floor and the settled plankton can be scanned from below; only the cover-glass then bottom disc separates the plankton from the front lens of the microscope, so that even the strongest magnifications can be used.

II. Inverted microscopes general description and details

The different microscope models produced by the companies mentioned in the Appendix vary considerably, in both mechanical and optical details. In addition to excellent instruments, there are some which are totally unsuitable for plankton investigations, despite sometimes carrying the name "Plankton microscope". Completely misleading information may even be found in the scientific optics literature. For example, in his otherwise excellent book "Das Arbeiten mit auffallendem Licht in der Mikroskopie", (Hauser 1956), the author writes: "They (i.e. the plankton microscopes) operate only with weak magnifications". Rather, there are inverted microscopes which only achieve small magnifications and are called "plankton microscopes"; however, accurate plankton investigations cannot be carried out with these. Such instruments should be avoided at any cost!

Phytoplankton investigations require a high class optical system. Some determination errors which have become known in literature are due to insufficient magnification or insufficient resolving power (numerical apparatus) of the lenses used. In addition, the modern counting chambers require highly specialized assembly of the stand and accessories (counting table, photographic equipment, etc.). Only a high class instrument can satisfy the various requirements of the modern quantitative phytoplankton count.

A few limnologists feel justified in using price as the major criterion in the selection of an inverted microscope. This is similar to a chemist cutting costs by using aBubosque-colorimeter for water analysis in this day and age. /13

Similarly one may photograph with a box camera, but the pictures will hardly satisfy scientific requirements.

The price of inverted microscopes is largely determined by accessories. Which ones are especially important and what must they be like?

(1) A "counting-strip eyepiece", which subdivides the field of vision and prevents omission of plankton (Utermöhl, 1927), is indispensible for accurate counting. Two parallel thin threads which may be moved closer together or separated further using a small external lever are incorporated in this. The space between the two threads which gives the counting strip in the field of vision, may thus be narrowed or widened. A third thread stretches excentrically and is fixed (see figure 12 and section "Operation of counting-strip eyepiece" on pages 42 and 43).

(2) In addition a first class mechanical stage is also indispensible. It must be without "play" (tolerance of screw drive in relation to spindle nut), ao that the two co-ordinate directions are exactly constant on moving. Stages with too much "play" jump into the opposite direction during change of direction of movement, i.e. the field of vision jumps sideways in a manner difficult to control, and counting errors result. It is also important that stages and counting chambers fit each other so exactly that the latter neither wobble nor shift, whereby errors would also result.

As will be shown later (p. 47 ff.), a lot of time may be saved in the

processing of quantitative plankton samples, if only partial counts of an area of 1 cm^2, or even fractions thereof, are made in flat chambers. For this purpose the required sections of 10 mm length must be strictly adhered to, since every erroneous measurement (a partial section too short or too long) results in incorrect calculations. While it is true that the scales attached to the stages allow reading of the beginning and end of these 10 mm long sections, the scientist is thereby distracted from the plankton to the scale, and counting is disturbed. A facility on the special stage of the Carl Zeiss company (Oberhochen) prevents this by automatically limiting the individual counting strips to a length of exactly 10 mm., so that the operator may give his full attention ot the plankton. In addition, this special stage has long flexible shafts for the operating knobs; their operation is less tiring than that of the standard stages (see Michel 1957, pages 290 and 291). /14

A photographic facility is useful not only for theoretical plankton investigations, but also for those with practical application if it can be conveniently connected to the microscope, for the following two reasons:

(1) Unknown or otherwise significant plankton forms, various stages of development, deformities etc. are often encountered while counting, and a photographic record of these is desirable.

(2) It is very impressive to photographically illustrate any differences of individual bodies of water (river sections, limnic water layers etc.) in plankton or abioseston content. If the plankton or the tripton is left to settle in long tube chambers, these differences become extremely distinctive in the sediment. If for example, a larger number of samples is taken during a river trip, the occur-

rance of pollution, the development of waste-water forms, beginning or progressing self-purification etc. may be convincingly demonstrated in the corresponding chamber sediments. The number of such /15
samples is often so large that there are insufficient chambers available for all characteristic individual cases. With a photographic facility this problem is easily solved: let us assume that 50 samples were taken, and that only 5 fairly high tube chambers suitablve for sedimentation are available. In this case these chambers

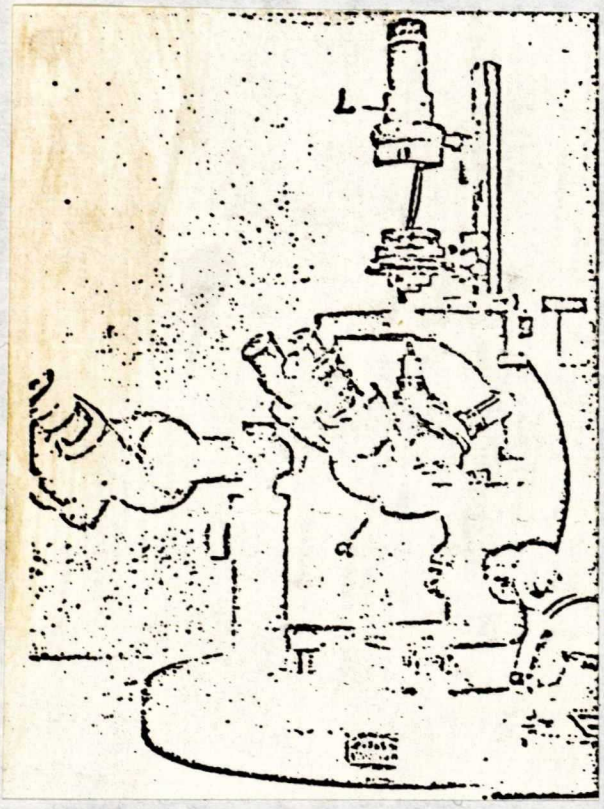

Figure 1. Inverted microscope with binocular tube, condenser and photographic facility; a flat chamber is shown on the counting stage.

are initially filled with the first five samples which are left to settle and the sediment is photographed. The chambers are subsequently cleaned and the same process is repeated with the next five samples, etc., until all 50 chamber sedimentations have been photographed. These photographs are compared and the 15 or 20 showing the best characteristic variations are selected. These photos are

of great documentary value. The water or plankton samples of these 15 or 20 photos - and only these - are now microscopically analyzed, and possibly counted. As simple as this principle is, it is difficult to perform with usual photographic accessories. A camera mounted on the inclined tube does not usually produce good pictures, since /16

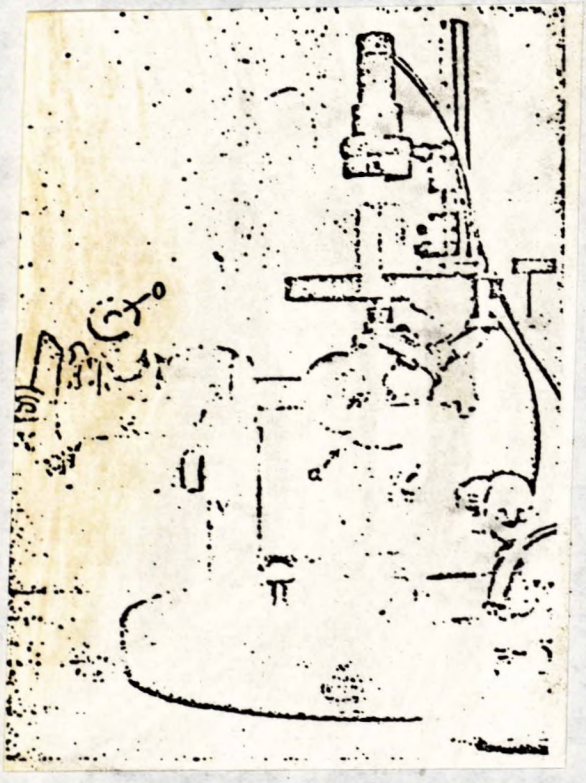

Figure 2. Inverted microscope with monocular tube, ready to take a photo; a tube chamber is shown on the counting stage. Note that 2 white spots at white arrow.

even the smallest vibrations, in cities for instances those caused by passing traffic, or even releasing the camera shutter, are disturbing and result in poor picture quality. On the other hand, taking pictures with the Carl Zeiss (Oberhochen) photo equipment with base plate is easy and time-saving. For such work it is best to have the inverted microscope rigidly attached to the base plate, as shown in figures 1 and 2. If the microscope only has amonocular tube, photographing is especially simple: the smallest

knurled head screw (a) is temporarily loosened and the tube set at 90° to the left as in figure 2; the correct position is conveniently located by two adjacent white spots (see white arrow), eliminating the need for repeated adjustments. Now the camera, with base plate (G) and adjusting ocular (O) is swung on the fixed vertical column (Y), the connector (V) is pushed over the tube and a lightproof seal is made with a sleave. The adjusting ocular (O) with graduated plate can be used on the one hand to set the picture boundary (possibly using the stage to make small adjustments), and on the other hand to check sharp focusing. Then the release button is pressed, the camera is pushed back on the swivel arm and the microscopic examination may continue until a new picture is required. Only one or two minutes are required to take such a picture.

Taking pictures is hardly more difficult or time-consuming if the microscope has a binocular: the latter is simply exchanged with the monocular tube by loosening the small screw (a), and the subsequent procedure is as described above.

III. Old and new tube chambers

In the past, all counting chambers were glass, the old Kolkwitz plankton chambers as well as the tube chambers. Glass is a particularly hard substance which is difficult to process. Plexiglas is a much more suitable material for counting chambers. Lund was probably the first scientist to make counting chambers from this material (Lund, 1951). Now most plankton chambers are made of plexigals. There is no reason why they cannot be made by the

researcher. Tube pieces of suitable dimensions (see table 1 further below) of plexiglas or similar plastic material are obtained and both ends are carefully ground to a smooth finish. A suitable circular cover slip is glued to one and with a good adhesive (e.g. Kraldit by Ciba, Basle). The other end is sealed with a loose 3 to 5 mm thick sealing plate of plexiglas or glass. The latter is preferred for its greater weight. The sealing plate can be either circular or square (see figures 5A, 8). The counting chambers could of course be any size or shape, but the following must be considered in deciding the dimensions:

(1) The chamber should not be made bigger or higher than absolutely necessary, since counting is otherwise too time-consuming /17

(2) The chamber size should be adapted to plankton quantity; e.g. small chambers suffice for samples rich in plankton. However, since plankton content eontinuously fluctuates, one chamber size is not sufficient, and a number of different sizes is required. Using standardized "chamber sets" is advisable (see table in subsection 4 below).

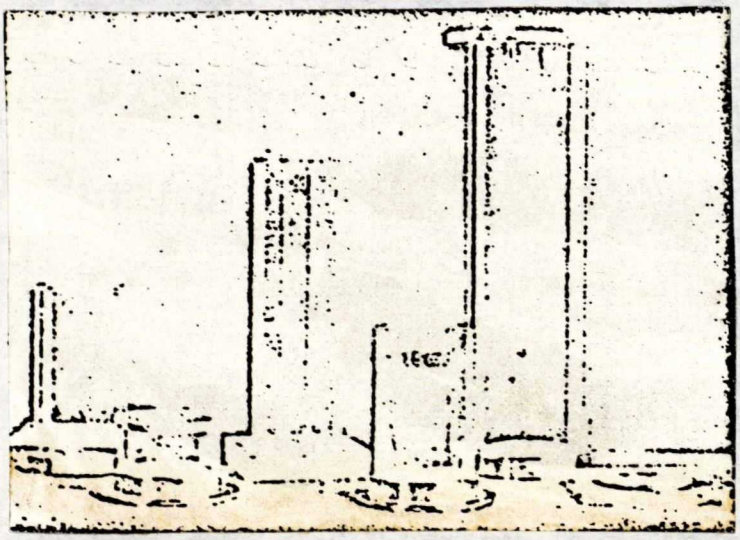

Figure 3. Various types of counting chambers.
From left to right:
Filling chamber
10 cc tube chamber
50 cc combination chamber
25 cc tube chamber
100 cc tube chamber
Flat chamber

(3) The chamber base area should usually be kept small to save time in counting, since counting is very time consuming, especially with strong magnifications. However, given the minuteness of many phytoplankters, high magnifications cannot be avoided. The counting of large areas would thus require much time. On the other hand, the tubes must not be too narrow either, since in this case too many plankters could stick to the walls.

(4) The volume of the tuve chambers must be suitable for easy conversion to 1 ml of the plankton data obtianed. The following chamber sizes, which form a "chamber set", have proved suitable:

Table 1 /18

Volume	Inner diameter	Area	Height
Volumen	**Innerer Durchmesser**	**Flächeninhalt**	**Höhe**
1 ml	**10 mm**	**80 mm²**	**25 mm**
5 ml	**16 mm**	**200 mm²**	**25 mm**
10 ml	**18 mm**	**255 mm²**	**40 mm**
20 ml	**20 mm**	**315 mm²**	**64 mm**
50 ml	**25 mm**	**500 mm²**	**100 mm**
100 ml	**34 mm**	**910 mm²**	**110 mm**

In this context brief reference should be made to the tube chambers of Carl Zeiss (Oberkochen), all of which have the same inner diameter (25 mm) but different lengths and volumes (see figure 3). This results in simplified conversion factors for partial counts (more on this on pages 51 and 52).

With the old glass chambers the base plates were gradually scratched with continued use. Also, increasing amounts of detritus deposited and were

difficult to remove by cleaning. Scratches and detritus, however, impair counting. These difficulties have been eliminated in modern chambers which have easily replaceable base plates. On commercial chambers, replacement is easily carried out using metal rings. Furthermore, in accordance with this author's suggestions, the plankton chambers of Carl Zeiss (Oberkochen) are assembled so that the base disc is recessed approximately 1 mm from the base of the tube; thus it no longer touches any surface and is thus less easily scratched or marked.

While the old plankton chambers (the Kolhwitz chambers as well as the tube chambers) still had relatively thick base plates, the modern chambers can have coverslip-thin base plates due to easy replacement. This substantially improves the optical picture for microscopic work and counting; the apertures of high-quality lenses can now be fully utilized which could not be done in the past. On the other hand, these thin glasses break more easily of course. For this reason a number of spare base plates should always be kept on hand. They may also be ordered from the supplier at any time.

In principle, the filling of the tube chambers is very simple. They are placed into a small glass (e.g. Petri) dish, a storage bottle with the plankton is shaken sufficiently to give even distribution and the tube chamber is filled to over-flowing. Then the appropriate cover disc is put on carefully to eliminate air bubbles, the chamber is dried, without moving it much, with a soft cloth or filter paper and is left to stand until all the plankton has settled (at least 24 hours).

The cleaning of the tube chambers is usually as simple: Half the content is poured out, the cover disc is replaced, the chamber is shaken vigor- /19

ously and the rest is poured out. Then the chamber is half filled with distilled water, shaken again and emptied, and the same process repeated twice. When cleaning is complete, the chamber base should be examined under the microscope for complete cleanliness. Some phytoplankters are very mucous and therefore stick easily to the chamber base, remaining there even after emptying the water. Such forms may cause errors when samples from other waters are examined. This may be avoided by careful cleaning fo the chamber base with a soft brush. Should the base plate still be insufficiently clean, it should be replaced (see above).

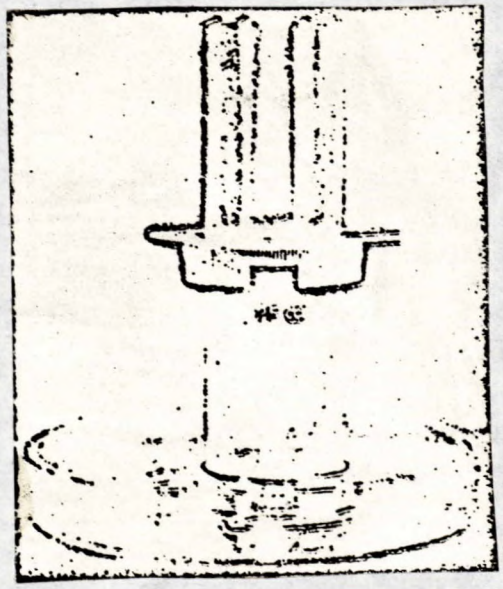

Figure 4. In the Petri dish: 25 cc tube chamber with filling chamber in position, ready for filling.

An evenly distributed plankton sediment cannot be achieved with the filling method for tube chambers described above. This does not matter if the whole chamber is counted. However, with partial counts the irregularities of distribution are further increased by the multiplication factor, resulting in substantial errors. For this reason, prior use of the filling chamber (see p. 37 ff. and figure 4) is recommended for partical counts; use of the filling chamber gives even distribution of the sediment.

IV. Combination chambers

Division chambers have been recommended by different people, at first by Kolkwitz (split chamber, Holkwitz, 1932), then by Lund (1951). The author's combination chambers which were first demonstrated at the German limnoligist' conference in 1952 in Schlitz, are also part of this group of sectioned chambers. They are manufactured by Carl Zeiss (Oberkochen) and have also been successfully used abroad. For this reason, they shall be discussed in greater detail below. /20

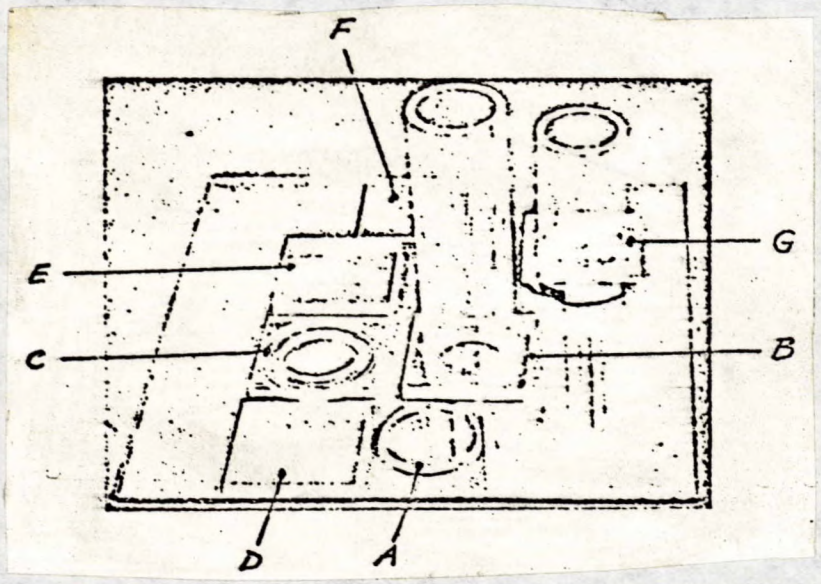

Figure 5. Combination chamber, stripped to its components:

A Cover disc
B Upper section
C Lower section (plate chamber)
D, E Guide plates
F Sliding plate (partly obscured by upper section
G Filling chamber

The combination chambers are made up of two major components: The lower "plate chamber" which may be considered a development of the Holkwitz chamber, and an upper section; the latter is comprized of a pleuigless tube,

a cover lid and a square plexiglass plate which has the same size and thickness (40 x 40 x 5 mm) as the above-mentioned "plate chamber". Two plexiglass plates (40 x 40 x 5 mm) and a glass plate (40 x 40 x 4 mm) complete the chamber equipment. All components are shown, neatly grouped, in figure 5: the plate chamber left centre; in front and behind, and narrowly separated from the plate chamber are the guide plates. The tube upper section is to the right of the plate chamber. In front of this lies the cover disc and behind the upper section, slightly offset to the left, the sliding plate. A filling chamber, which will be described in the next section, is on the extreme right, a little further back. Whilst the plexiglass tube upper section is open at both ends, the plate chamber (to its left) is sealed at the bottom by a cover-glass-thin base plate.

The combination chamber is filled as follows: First everything is laid /21
out similar to figure 5 on a large ground mirror-glass plate; the latter is to prevent overflowing water from running onto the work bench. Then the plankton samples are vigorously shaken to evenly distribute the plankton. Next the upper section is placed exactly above the plate chamber as shown in figure 6 [1] and the filling chamber placed on top of it. To prevent sliding of the two plexiglass plates, both components must be pressed firmly together from above, which also results in a waterproof seal. Now sample water is filled from the storage bottle into the filling chamber. It runs through its filter plate right down into the plate chamber, fills the plate chamber and then the tube upper section, eventually overflowing. Now the filling chamber is immediately removed and the upper section sealed with the cover disc

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1) Figures 6 - 9, see plate 1, pp. 60/61.

(figure 7). This sufficiently seals upper chamber and plate section, and eliminates any further need for hand pressure. This leaves the hands free for other duties such as filling more chambers. If the combination chamber is left unattended, plankton settles in it; the sedimentation period varies in accordance with size, specific gravity, etc., of the plankton, but is usually completed after 24 hours. Thus after this period almost all the plankton lies in the lower section of the combination chamber. Plate chamber and tube upper section have almost the same diameter: the latter 25 mm, the former 25.5 mm. This small difference is a planned safeguard against small lateral movements between upper and lower sections; thus even if this occurs, all the plankton reaches the plate chamber.

However, the upper and lower section of the combination chamber cannot be brought to a microscope for counting with such a loose connection. Thus, plate chamber and upper section of the combination chamber are to be separated without disturbing the settled plankton; in addition, the plate chamber (lower section) is to be suitably sealed for counting. This is easily accomplised as follows: The two guide plates are placed snugly to the left and right of the combination chamber, one of these plates is firmly held down and the sliding plate is placed on the other guide plate. The upper section is now slowly pushed onto the held guide plate (see figure 8). With care, this can easily be accomplished without loss of water from the upper section. Upon completion of transfer, the upper section rests on the guide plate, while the sliding plate now seals the plate chamber (see figure 9). The latter is thus ready for counting and can be placed without difficulty on the inverted microscope.

The transfer process takes only a few seconds and can be carried out without difficulty with a steady hand. However, the guide plates and the /22
plate chamber must be machined to an accuracy of only fractions of a millimetre. Failing this, the plate chamber will "hook" during displacement. But even such cases can be easily rectified, e.g. by slightly (!) bevelling the four edges of the guide plate with a triangular file. This can be done by the researcher, since plexiglass is readily workable. Then the plate chamber slides without "hooking" across the guide plate. It must be stressed again: Only minute bevels! If they are too large, water may escape from the tube upper section.

It must also be mentioned that the capacity stated on the tube upper section refers to the whole combination chamber, i.e. lower and upper section combined. In eutrophic lakes, the 50 cc combination chambers have proved to be most suitable.

The plate chamber may of course be used for counting without the upper section of the combination chamber, particularly with dense plankton. By itself, this plate chamber has a capacity of 2 cm^3 and a sedimentation height of 4 mm.

The following may be said regarding the relative merit of tube chambers and combination chambers: Simplicity of operation is the major advantage of tube chambers. As opposed to tube chambers, combination chambers have the following advantages:

(1) Lighter field of vision;

(2) a condenser can be used; this cannot be done with tube chambers,

at least not with long ones;

(3) with oil immersions the entire base chamber can be scanned; with tube chambers this cannot at present be done in the external fringe area;

(4) The plankton settled in the base chamber can be examined with a phase contrast facility (this is important for finer structures, e.g. with diatoms).

V. Magnitifcation and counting time

The great men of early plankton research, Hensen, Apatcin and others, recognized and proved, by comparison counts, that considerably stronger magnifications are required for counting than for identification of the plankters in question. This is no doubt due to the operation of the counting stage causing the plankton to slide through the field of vision. Not only inconspicuous forms are thereby overlooked, but also easily identifiable ones, which is somewhat surprising. The basic rule which result is that magnifications for counting must not be too small; this important rule is often broken, since the samples can be processed faster with smaller magnifications.

From a purely optical standpoint, the chamber area covered in the field of vision shrinks proportionately to the second power of linear magnification. In practice, however, conditions are much more unfavorable. With strong magnifications, not only the field of vision is decreased but also /23 depth of focus. The latter is of decisive importance for a quick identification of forms. Larger forms such as Ceratia, Asterionella, etc., may be recognized at a glance due to their usually characteristic shape, even if they are not in focus. The smaller the plankters become, the more difficult

it is, even with strong magnifications, to discern their distinguishing features - thin setae, etc., and their bodies become increasingly spherical, which also makes differentiation more difficult and increases counting time. Large Cyclotella for instance, or Stephanodisci, form distinct flat discs; the smallest forms of these two species (size 4 to 6μ) are also derived from this disc form but their diameter is approximately the same in all directions and in addition, their surfaces are curved so that they resemble minute spheres. Also, the detritue becomes more and more apparent with increasing magnification. It is not difficult to count Ceratia, Asterionella and Melosira in addition ot large quantities of detritus even with weak magnifications, and neither is the counting of 5 to 10μ large algae in water containing little detritus difficult with strong magnifications. Unfortunately, however, the water of all inland waters and many seas seems to be almost always tripton-rich with strong magnifications. But the more apparent the detritus becomes, especially by comparison with insignificant or minute algae, the more time is required to avoid overlooking the latter in counting. The additional time required is not constant: in addition to the detritus content and size of plankters it also varies with their colour. It is more difficult to recognize minute colourless monads than coloured flagellata. In short: In the author's experience, simple recognition of minute forms under such difficult conditions (accurate adjustment of micrometer screw, interference of detritus, etc.) takes at least three times as long as the recognition of more conspicuous forms, without taking magnification into account. A rough estimate shows that 3,000 times as much time is required for counting with magnification time 1,000 as for magnification x 30!

This being the case, one may decide to ignore the smallest forms (nanno- and ultraphankton) which require magnifications x 1,000, and thus immersion lenses, for counting. However, especially these plankters are particularly good indicators for nutrition content, pollution level, and other properties of the waters. Furthermore, they usually develop more numerously than the larger forms and almost always concurrently with them; for this reason they must not be omitted under any circumstances. Thus the following applies:

(1) The continuous presence of ecologically important minute phytoplankters requires application of the strongest magnifications.

(2) The time required for counting with strong magnifications is excessively long. /24

(3) The often very large quantities of phytoplankters - tens of thousands, even hundreds of thousands per ml of seawater, millions per ml of some ponds and pools - cause a further considerable lengthening of counting time.

With such nannoplankton quantities, a full week and more would be required for the quantitative processing of a single sample, if the whole content of a chamber were counted, even using the smallest chamber with a base area of only 80 mm^2 (see table 1, page 27). Of course, such a waste of time cannot be justified. By the way, Hensen (1887, p. 21) required at least a week for every plankton smaple. Time could probably be saved by diluting such plankton-rich samples with plankton-free water; however, it is almost impossible to find the correct dilution at the first attempt, since neither the qualitative nor the quantitative plankton composition of the sample is known. Furthermore, a number of dilutions would have to be made, since the

quantities of individual plankton species in the "catch" vary and cannot be predicted. The only solution to the problem of recording all phytoplankters of production-biological importance on the one hand, and keeping the counting time within reasonable limits on the other hand, is using an "elastic partial chamber count", i.e. rather than counting the whole sediment of a chamber, only as much is counted of every important species as required for the respective problem and the accuracy required. More on this in section VIII.

VI. Necessity for using a filling chamber

A reasonably even distribution of the plankters in the water sample to be examined as well as in the sediment at the base of the counting chamber is a requirement for the reliability of partial counts. Furthermore, partial counts are only permissible with large plankton quantities. In other words, the greater the plankton density, the smaller may be the chamber area, and the more even must be the distribution at the base of the chamber!

To achieve the latter, the plankton sample must be properly mixed in the storage bottle. Prolonged and vigorous shaking must be avoided, since it damages delicate forms. Thorough mixing, however, is important. It is accomplished by continuous shaking in opposite directions, i.e. to and fro, up and down, and clockwise and anticlockwise in a circular motion. Full sample bottles cannot be properly mixed. Best mixing results are obtained with bottles 3/4 to 4/5 filled.

Now to the filling of the counting chambers. It is very simple, as long as the sediment does not have to be evenly distributed. After adequate shaking, the plankton water sample is poured into the counting chamber, the /25

cover lid is put on and the chamber left standing until all the plankton has settled, which usually takes 24 hours or less. However, this filling method results in an uneven sediment: in some parts it is more dense, usually in a border zone, in other parts it is more dispersed (see p. 40, figure 11, right). Uncontrollable currents produced on pouring cause this. They are not easily eliminated. Their elimination is best achieved with a "filling chamber", described here for the first time. The chamber is based on the following principle: While with the usual pouring method a relatively thick liquid jet is flowing into the chamber and remains in motion for quite some time, the filling chamber divides the poured-in sample into seven thin water threads of correspondingly smaller force but stronger turbulence; these small individual turbulences megate each other, so that the liquid becomes motionless much sooner than with ordinary pouring, as may be observed on the suspended seston with a magnifying glass.

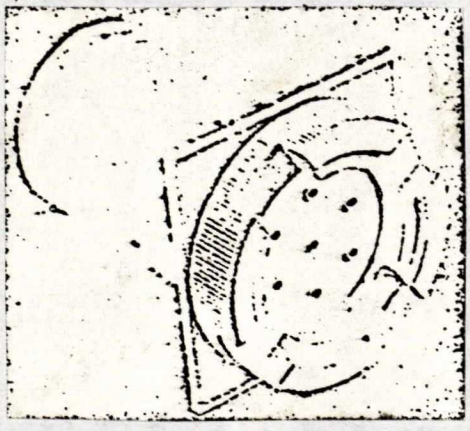

Figure 10. Filling chamber, viewed diagonally from below; the sieve plate with "collars" around each hole, the ring nut and 4 drainage slots can be seen.

The filling chamber was designed by the author and is manufactured by Zeiss (Oberkochen); as shown in figure 10, in lieu of a base it has an accurately machined sieve plate with 7 evenly space circular holes. Each one of these holes tapers off to a funnel-shaped "collar" designed to prevent the

individual water "threads" from flowing together in a single thick jet which would defeat the purpose of the chamber.

Use of the filling chamber is very simple: It is placed on top of a counting chamber (see figure 4 and 6) and the plankton containing water is poured into it after adequate shaking. The ring nut makes the filling chamber /26
fit centrically on the tube and combination chambers of Zeiss. The four drainage slots assure unimpeded draining off of surplus water. Nevertheless, in order to avoid additional currents, the quantity of water poured into the filling chamber should only slightly exceed the capacity of the respective counting chamber (tube chamber, combination chamber, flat chamber, as the case may be). Best of all, the quantity of sample water poured in should just be sufficient to form a convex head on top of the counting chamber, without any water draining off.

The seven collar holes have a diameter of 1.5 mm, sufficiently large to allow most phytoplankters to pass through. Only especially large Aphanizomenon flakes and Gloiotrichia might occasionally be caught in this sieve. In such exceptional cases, the samples are poured in without using the filling chamber.

Placement of the cover disc on the counting chamber is also very important for even sedimentation of the plankton. It must not be pushed on from the side or put on under an angle, as variously recommended by Kolkwitz and by Rylov; this, too, produces currents. Rather, it must be put on with the greatest care and without displacement. (In the photographs, the cover disc is sometimes shown resting on the chamber under an angle, but only to illustrate it better).

Using a filling chamber and careful placement of the cover plate, the currents in the plate chambers practically stopped within minutes of filling; even under the microscope they were barely detectable with magnification x 100. On a few occasions, especially sensitive tests could be carried out with a μ-alga (Heterococcale) occurring in large quantities: after 24 hours all μ-algae had completely settled with an almost improbable evenness never before observed. Currents and uneven sedimentation occurred in parallel tests with lateral application of the cover disc.

Fortunately, there is a simple and quick way of checking whether or not the chamber has been successfully filled: Some 5 minutes after filling the chamber is carefully placed on the microscope and scrutinized for currents, using weak magnification. If currents are practically absent, the chamber is removed and sedimentation may continue; the sediment will subsequently be evenly distributed. If currents are found, it is advisable to empty, rinse and refill the chamber. Having thus established the suitability of a sediment in advance, it would be pointless to continue sedimentation with unsuccessful fillings which may occur even after a lot of practice.

While it is possible to completely eliminate currents in flat chambers, this is not the case in tube chambers. The water quantity in the latter is probably too large. Nevertheless, much more even sedimentation may be attained when a filling chamber is used. /27

Cloudlike sediment accumulations have not been observed on the chamber base. In tube chambers, distribution is similar to that shown in figure 11. A concentrated zone stretches - usually more towards the edge - across a large section of the chamber, almost imperceptibly changing into a less

dense distribution. Use of a filling chamber substantially reduces these differences.

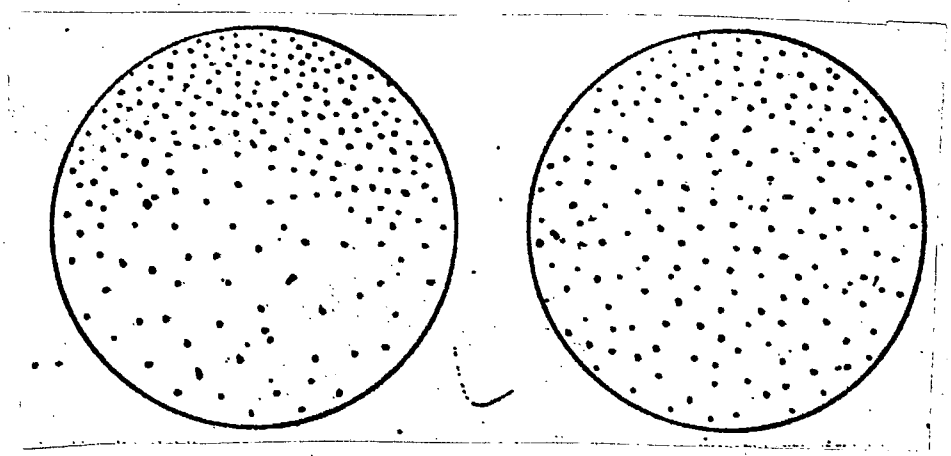

Figure 11. Plankton sediment in a tube or combination chamber. On the right without using a filling chamber on the left using a filling chamber.

The following conclusions are drawn from the fact that an even sediment can only be achieved in flat chambers, while a concentration strip, even though only weakly developed, cannot be completely eliminated:

(1) Different methods are to be used for partial counts with flat chambers on the one hand, and tube and combination chambers on the other hand.

(2) In partial counts with tube and combination chambers, plankton-rich and plankton-poor zones must be included proportionately as far as possible. This is best achieved by diametrical counting with a "counting strip eyepiece" in accordance with figure 15.

(3) With tube chambers it is not permissible to count only individual fields of vision and to distribute them arbitrarily across the chamber, as is done by some plankton researchers, since it is not possible to perceive prior to counting, which sections of the sediment are poorer, and which richer in plankton.

(4) Because of the even plankton distribution in flat chambers, such distribution and counting of fields of vision is permissible, but not very practical. Use of the counting strip eyepiece is more convenient, but /28
also more accurate for a number of reasons. Above all, the counting strip width may be adapted to the number of organisms which varies from sample to sample. The fact that every organism is located in the centre of the field of vision within a thread hair boundary at the moment when it is actually counted is greather advantage of the counting strip eyepiece. At the moment of counting the organism is in the optically most favourable position, and its position is localized at the same time. Consequently, it cannot be overlooked or counted twice. Furthermore, the evenness of distribution and the divergence of values can be easily checked with the counting strip eyepiece.

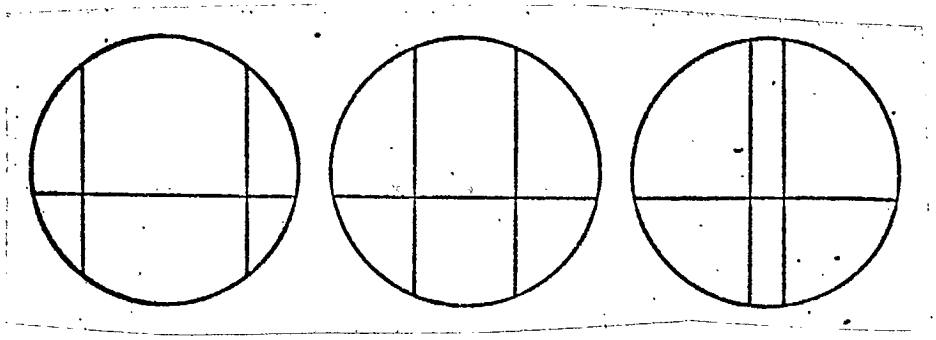

Figure 12. The field of vision of the counting strip eyepiece with wide, medium and narrow "counting strip".

VII. Operation of the counting strip eyepiece

This eyepiece must be inserted in the microscope in such a way that its parallel thread hairs run in one of the two main directions of adjustment of the mechanical stage. Thus, when the mechanical stage is operated, the plankton must glide through the field of vision parallel to these thread hairs. If the plankton should cross the parallel thread hairs, alignment is incorrect; in this case the counting strip eyepiece is rotated around its own

axis in the tube, with simultaneous operation of the mechanical stage, until the plankters no longer cross the parallel thread hairs.

The width of the counting strip may be varied as desired with the lever located on the counting eyepiece: medium (such as shown in figure 12, centre) for a sedimentation of normal density, narrow (figure 12, on right) for very dense sedimentation, and wide (figure 12, on left) for very sparse plankton.

The exact width of the counting strip must be known for the elimination of plankton density. It is either determined by measurement with an object micrometer, or - better - by shifting chamber with settled plankton a few times with width of the tentatively adjusted counting strip. The sum total of these shifting distances is then read from the vernier of the mechanical stage and divided by the nubmer of shifts. Example: 6 counting strip widths = 2 mm on the mechanical stage scale. Thus, one counting strip width = 1/3 mm. If, on the other hand, 6 strip wideths should add up to 2.3 mm, the counting strip would have to be slightly narrowed with the eyepiece lever. This procedure is repeated unitl an easily computable conversion factor is /29 obtained. The absolute value of the counting strip width depends of course also on the magnification; in general, it decreases with increasing magnification. With immersion lenses it is often only 0.1 to 0.05 mm, with very dense plankton sediment even less.

Everything between the parallel thread hairs is counted at the moment at which the plankton form in question crosses the centre section of the non-parallel thread hair during stage shifting. In addition, those plankters are counted which are half inside and half outside a counting strip.

(Duplicate counts must of course be avoided, and only the left or the right boundary thread hair of the counting strip is to be considered; after shifting of the counting strip by a counting strip width, this form is on the other side of the counting strip and must not be counted again.)

VIII. Theory and practice of counting

The task of counting plankton samples in chambers is often taken too lightly, along the following lines: One chamber is counted - with very large plankton quantities, counting is even limited to only a few fields of vision - and devergence is calculated from the counts obtained, using the known statistical formulae. If excessive deviation values are obtained, the counting of new chambers or other fields of vision is repeated until deviation has become sufficiently small. In reality, however, the situation is quite different. Every plankton smaple contains a number of, and often a great number of different species whose frequency differs by 1, 2, 3, 4, at times by 5 and even more decimal powers. How will counting be done and what will be counted? Maybe only the more frequent species?

This is not justifiable, since many of the less frequent forms are relatively large species which deserve to be considered for this reason as well as for their ecological significance. With small and minute plankters, on the other hand, their size is production-biologically more than compensated for by their larger numbers. It follows that all sizes and frequency classes of the phytoplankton must be considered in counting. However, the work required for this is greater than most limnologists anticipate. This is not due to the chamber method which is considerably simpler than other plankton methods; rather, it is due to the character of the plankton, which is a quali-

tatively and quantitatively extremely heterogenous collective of almost continuously changing composition. Adjusting investigation techniques to this intense, continuously changing heterogenaity, so as to provide for minimum labour with sufficient accuracy, is the most difficult problem in quantitative plankton methods. This problem can not be solved by counting a plankton sample in a single chamber size, as done by some limnologists. This would /30
be similar to considering one single lens sufficient for a microscope. Light-microscopic lenses differ in size by only 3 decimal powers; the frequency differences of phytoplankters of a single sample, however, may comprise 4 to 5, and sometimes even more decimal powers. Just as different magifications are used for microscopic investigations, so are a number of chamber sizes required for quantitative phytoplankton analyses.

Investigations could of course be confined to specific plankton sizes of frequency classes, as long as it is understood that only some facts are thereby recorded.

Since the author does not intend to deal with mathematical-statistical methods in this paper, it shall only be mentioned here that a sufficient number of individuals of every species should be counted to give adequate accuracy. The author had for a long time used the following rules of thumb:

1. If possible, approximately 100 (to 200) individuals should be counted of every important species of a sample. However, this can only be done approximately, since counting should not be stopped as soon as the above number is reached; this would make the calculation too complicated. It is better to finish the whole counting strip and to compute with slightly larger figures. Also, some species occur too infrequently in the samples.

2. Checks should be included in all counts (a description of how this is done follows later). Even if no mathematical-statistical evaluation of the check counts is made - this evaluation is advisable despite the extra work involved - they give already during counting a valuable survery of the degree of evenness of the plankton distribution in the sediment and thus of the size of dismersion. In the case of excessive deviations, further counts may be made.

3. The chamber must be the higher and larger, the more infrequently a species occurs in the plankton sample, so that this species can still be recorded. Thus, flat chambers suffice for species occurring in large numbers, while tube chambers should be used for those occurring less frequently. There is no definite borderline designating suitability of one chamber or the other. The following may serve as a rough guide:

> 10,000 phytoplankters per ml: flat chambers, e.g. plate chambers

< 10,000 phytoplankters per ml: tube and combination chambers.

It would of course be very convenient if the most suitable chamber size for the individual species, types of waters, etc., could be exactly specified for every particular case. However, this is impossible, since chamber size /31 is determined by the frequency of the forms in question, and since the latter constantly changes. Three chamber sizes are usually sufficient, sometimes even two. In the author's experience, the following chamber sizes are particularly suitable for eutrophic waters:

the 50 ml tube or combination chamber for rarer forms; the 10 ml tube or combination chamber for species of medium frequency;

the 1 ml tube chamber for more frequent species;

the flat chambers for species occurring in large numbers (see page

36, paragraph 3).

A combination of the last 3 chamber types is recommended for samples from strongly eutrophic waters; for samples from slightly eutrophic (and oligotrophic) lakes the first three chamber types are more practical. Consequently, however, other chamber since (see table on page 27) are to be preferred. For checking, at least 2, or better still 3 chambers of identical type and size are required for each frequency class. Thus, for plankton counts covering all frequency classes, 6 (to 9) chambers are required for each sample. For less accurate counts, the number of chambers can of course be reduced. However, more than one set of chambers is required for a series of tests, and this should be taken into account when the chambers are purchased.

Some readers may be shocked by the large number of chambers to be processed. Still, the associated counting is not as extensive as first appears, since partial counts are made in lieu of full counts as far as possible, i.e. for all small species with more than approximately 100 individuals per chamber. A lot of time is saved in this way, and the procedure is as follows:

In principle, because of the smaller numbers, the smallest chambers are used first, and the next chamber size is taken only after completed processing of the smallest chamber. Full counts with flat chambers would be much too time consuming because of their large base area. For this reason, only the 1 cm^2 square at the centre of the chamber base (figure 13) is counted; in the case of very dense phytoplankton only a fraction of this square needs to be counted. This is done in one of the following two ways:

(1) The number of counting strips is reduced to 5 or 6, in excep-

tional cases to 3 strips.

(2) Using an immersion, the width of the counting strips is reduced to, e.g., 0.2, 0.1, 0.05 mm, or a combined width of 1 mm is chosen for 12 counting strips, as in the example below. The size of the section to be counted is inversely proportional to the evenness of distribution (see page 37) and density of the plankton sediment, and this to the plankton quantity, provided that the first rule of thumb (see page 45) is not violated. /32

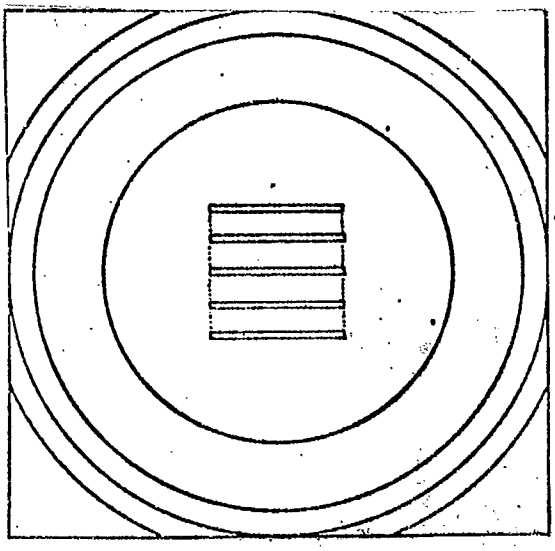

Figure 13. Partical count in a flat chamber; distribution of 5 counting strips over a central square of 10 x 10 mm.

To find out which forms are to be taken into consideration in the flat chamber, one counting strip is closely examined prior to counting, and all forms occurring at least 10 times are noted. In order to save time it is advisable to count those species occurring less frequently in the tube chamber.

The calculation of the plankton values is determined by the number and width of the counting strips on the one hand, and by the height of

sedimentation on the other hand. This may be illustrated by the following practical example. In addition to rarer forms, the following species were counted in a sample taken from the Grosser Plöner Sea on 14 April 1952:

Counting Strip	Stephanodiscus pusillus	Phodogonas minuta
1	52	25
2	48	19
3	50	15
4	49	16
5	58	20
6	50	17
Total	307	112

Chamber height 4 mm; 12 counting strips had a combined width of 1 mm, 6 strips therefore 0.5 mm.

Statement: $307 \times 2 \times 10 \times 2.5 = (15{,}350)$ 15,000/ml

$112 \times 50 = (5{,}600)$ 5,500/ml

When processing of the flat chambers has been completed, the tube or combination chambers are counted, again starting with the smallest chamber types. Full counts are simplest and most accurate with this chamber type. The count proceeds systematically from one side of the chamber to the other, as indicated in figure 14 (right). To save time only every second strip may /33 be counted with the more frequent forms (figure 14, left); the values obtained in this way must of course be doubled. Exactly the same counting methods are used for large tube chambers. The frequency of phytoplankton and the time available for counting are the only criteria determining chamber size; the following rule should be observed: The rarer the form, the larger the chamber.

Owing to their unsurpassed simplicity and reliability, tube chamber counts in this form have been generally adopted and may rightly be called standard methods. However, these counting techniques are still very time consuming, a problem which has been largely eliminated by diametric counts. Details of the latter are published here for the first time. The author has demonstrated this technique to Hamburg students and a few collegues for a number of years.

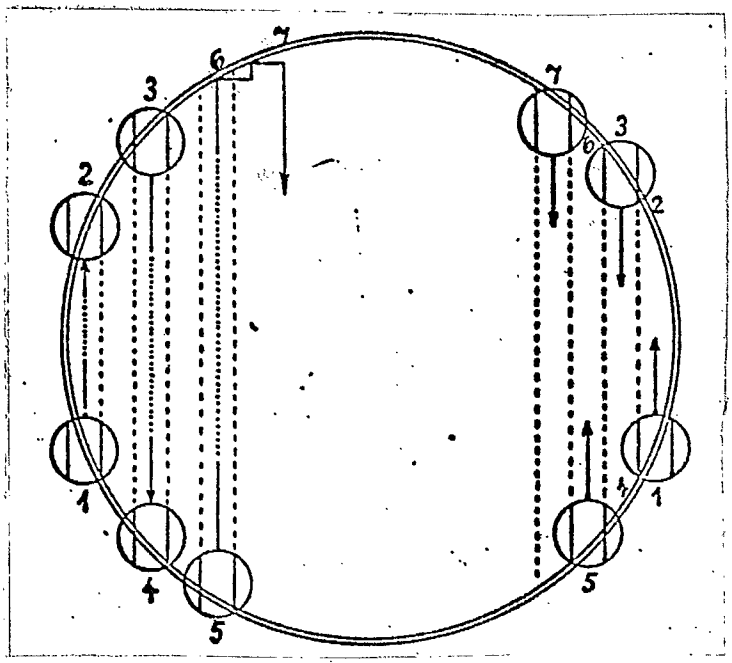

Figure 14. Apparent movement of the field of vision across a tube chamber: on right, counting every counting strip; on left, counting every second counting strip. In reality the chamber is sliding and the field of vision remains fixed.

Diametric counts are also partial counts, except that the counting strips are laid diagonally across the chamber, similar to diameters (see figure 15). This enables proportionate recording of plankton-poor and

plankton-richer zones in the tube or combination chambers (see pages 40 and 41). The individual diameters are at angles of approximately 45°. Since they are of identical length, they would also have to produce the same data /34
for an ideally even distribution of plankton, which of course is not the case. However, it is precisely these differences which give an indication of the magnitude of dispersion within the chamber; for this reason, they should also be recorded.

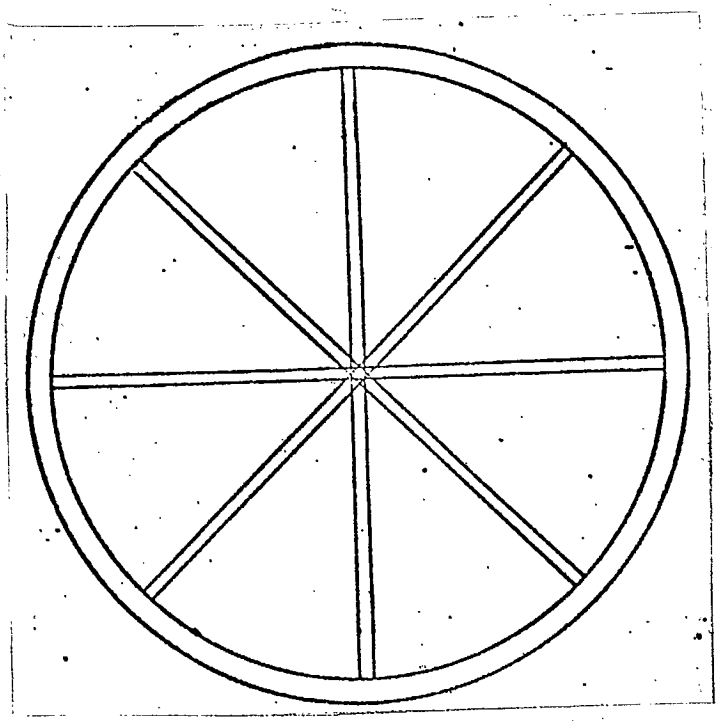

Figure 15. Diametric counting in a tube chamber.

Diametric counts are not intended to replace the present counting methods for tube chambers, but are rather intended to supplement them, especially for small and minute plankters. If they occur in sufficiently large quantities for flat chamber counts (in accordance with the description on

pp. 46-48), the latter are to be preferred. However, nanno- and ultraplankton rarely reach such high values. This being so, their counting was either too time consuming because of the strong magnification required (immerson lenses!), or, in tube chambers, not accurate enough. Diametric counting thus really closes a gap. It may also be used to advantage with slightly larger forms, e.g. in cases where the plankton sediment is too dense and where the conventional counting methods would produce excessively high values for the filled chambers. It is not always possible to select the correct chamber size prior to counting, and in such cases the time savings offered by diametric counting are appreciated.

Unfortunately, only the chamber sizes of 25 mm diameter are suitable for diametric counting, since only these give the rounded measurements required for the calculations: Base area 500 mm^2 (see table 1, page 27). /35
In addition, the combined length of the four diametrical strips in this chamber is exactly 100 mm, which gives a very convenient calculation factor. For any counting strip width, the area to be counted is easily determined and readily related to the chamber volume.

Example:

Counting strip width: 0.1 mm

counted area of 4 diameters: 10 mm^2

base area of 50 ml chamber: 500 mm^2

Conversion factor 50 gives 50 ml plankton value

Conversion factor 1 gives 1 ml plankton value

The author's 25 mm diameter recommendation to Zeiss-Oberkochen for

the tube and combination chambers was based on this convenient conversion factor. The various volume requirements are met by chambes in the height of the respective chambers of identical diameter. The author highly recommends these cahmber measurements and is convinced that diametric counting will soon be generally adopted.

IX. A few hints for counting, recording and checking with the the chamber method

Usually, the plankters are counted in the sequence in which they appear in the field of vision, and the count noted by strokes. This is not always practical. By concentrating on only a few specific forms, recognition is more certain and greatly facilitated. Thus, the time saving alone justifies a division of the counting task, whereby abundant varieties are counted separately.

It would furthermore be impractical with these abundant forms to record every individual by a separate stroke in the tally, as is generally done. Rather, it is advisable to count and record abundant forms in groups of 20; thus, the symbols used for single counts and groups of 20 must be distinctly different.

It has been pointed out in earlier sections of this paper that the evenness of plankton distribution in the chamber, and therefore the reliability of the counts can be accurately evaluated by partial counts. This, when partial counts are used as indicators for this purpose, this must be clearly and simply seen from the record. This can easily be done by separating the data obtained in each individual count by a dot. The table shown on page 49 would thus appear in a counting record as follows:

Stephanodiscus pusillus: 52 . 48 . 50 . 49 . 52 . 50

Rhodomonas minuta: 25 . 19 . 15 . 16 . 20 . 17

The musch more even distribution - due to the larger numbers - of the Stephanodisci, and therefore the much smaller deviation in their values is readily /36

apparent. In accordance with rule of thumb 1 (page 45) and instruction 1 (page 47), only 3 or 4 strips could have been counted without any appreciable loss of accuracy.

In conclusion a few lines on the question of plankters adhering to the chamber walls. In accordance with the author's observations, this source of error lies within the limits of accuracy of the counting method. However, this touches on statistical problems which, as already mentioned, lie outside the scope of this study. In any case, with combination chambers this source of error may be examined in detail; in addition, it may be completely eliminated in the following simple way:

The water sample contained in the upper section, which should be plankton-free after sedimentation (figure 9), is briefly shaken and filled into a second combination chamber. A few ml of distrilled water are added until the chamber is completely filled, and sedimentation is allowed to take place again. Any plankters deposited at the chamber base are then counted and the data obtained either added to the results of the first combination chamber count, or used to calculate the percentage of the forms which had adhered to the walls. The same procedure may of course be repeated.

Summary

Methods of collecting plankton for various purposes are discussed.

Certain species will not fall to the bottom of the counting-chamber unless treated in some way. A solution of iodine in potassium iodide approaches most nearly the ideal fixative, whose six features are listed. The addition of some other substance is necessary to make certain species sink. In containers of good glass with glass stoppers or waxed corks, the iodine in potassium iodide solution with sodium acetate is a satisfactory preservative.

The principle of the method is that algae are allowed or caused to fall to the bottom of a tube and are then counted from beneath with the aid of an inverted microscope (figs. 1 and 2). An essential adjunct is a first-class movable stage, and an eyepiece crossed by three lines, one fixed, the other two at right-angles to it and capable of being brought closer together or moved apart (fig. 12). A camera that can be fitted easily is useful (fig. 2). The counting-chambers consist of perspex tubes with very thin glass bottoms; they can be made by sticking cover slips to simple tubes. Table 1 shows suitable dimensions for different volumes of water, the latter varying according to the density of plankton, which subject is discussed more fully in section D VIII. If the whole catch is to be counted a simple tube can be used, but if only part is to be counted even distribution of algae over the bottom must be ensured by use of a filling-tube (fig. 5 G) which sits on top of the main tube (fig. 4), and is separated from it by a plate pierced with seven holes (fig. 1). If it is desired to use a condenser (fig. 10), the sample must be contained in a flat chamber (fig. 5 C). A cylinder with a square collar at the base (fig. 5 B) is superimposed upon it and a filling-chamber fitted to the top (fig. 6). After use, the filling-chamber is replaced by a cover (fig. 7, fig. 5 A) and, after 24 hours or so have been allowed for sedimentation, the cylinder is gently pushed off the flat chamber onto a square of exactly the same height (figs. 8 and 9 and fig. 5 D and E).

The conflicting demands of saving time and being accurate are discussed. Statistical theory is omitted but it is recommended as a rule of thumb that there should not be less than 100 specimens of the important species in any count. A duplicate of each sample should be examined. Methods of counting a fraction of a sample are described (figs. 13, 14 and 15).

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(Translation of titles only)

1. Introduction to microscopic investigation methods
2. The methodology of plankton research.
3. Working with incident light in microscopy.
4. The determination of plankton or plant and animal matter drifting in the sea.
6. a. Instruments for sampling and observation used in biological water investigations.
   b. The chamber plankton of fresh water and the oceans.
   c. Plankton and seston.
   d. The plankton chamber, using strong lenses.
7. Studies for determination of the complete plankton content of the sea.
9. Micro-photography = volume 10 of the series "Scientific and applied photography".
11. Discussion comment (contribuiton to a debate).
12. Tests on the methodics of centrifuge in plankton extraction.
13. Microscopic technique.
14. a. Limnological phytoplankton studies.
   b. Deficiencies in the present graduations of the microscop field of vision and their elimination by the counting strip eyepiece.
   c. The inverted microscope.
   d. New approaches to the quantitative determination of plankton.
   e. Quantitative methods for the investigation of nannoplankton.
15. a. Methods for the quantitative determination of plankton used in the Hamburg Elbe studies.

b. Studies of the effect of the draught of summer 1904 on the biological conditions of the river Elbe near Hamburg.

Appendix

Directory of companies

manufacturing inverted microscopes with

accessories and plankton chambers.

Feinmechanischer Cerätabau, formerly Schmeder,

Kiel-Wik, Wismarer Strasse 14:

Inverted microscope, mechanical stage, tube chambers of different diameters and heights.

E. Leitz, Wetzlar:

Inverted microscope, mechanical stage.

W.R. Prior and Co., 28a Devonshire Street, London, W.I.

Inverted microscope with mechanical stage.

VEB Carl Zeiss, Jena:

Inverted microscope, mechanical stage (see Appelt, 1955, page 137

Carl Zeiss, Oberkochen:

Inverted microscope, counting strip eyepiece, special mechanical stage, photographic facility, tube chambers (standardized 25 mm diameter, various heights),

combination chambers, filling chambers.

Plate 1

Filling and preparation of a combination chamber for counting

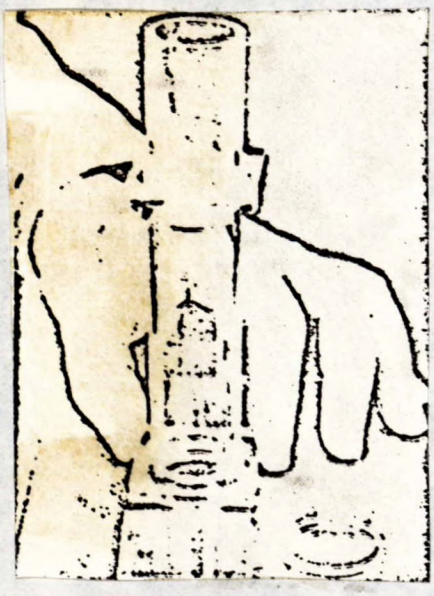

Figure 6. The upper section of the combination chamber is placed on the plate chamber, the filling chamber on top of the upper section. The (left) hand presses the 2 parts of the chamber together. The chamber can now be filled

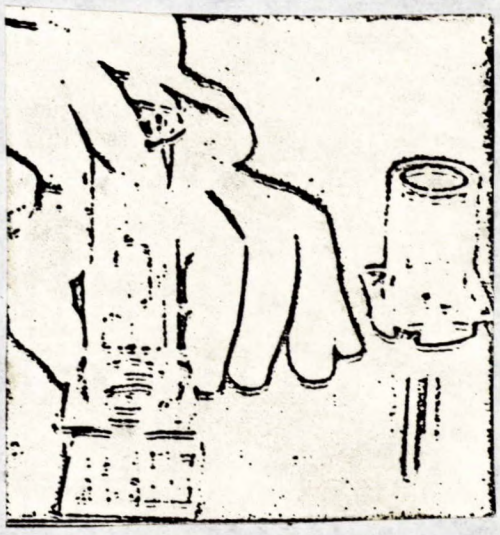

Figure 7. The combination chamber is filled, the filling chamber removed. The (left) hand still presses the parts of the combination chamber together. The cover disc is put on. Sedimentation can now begin.

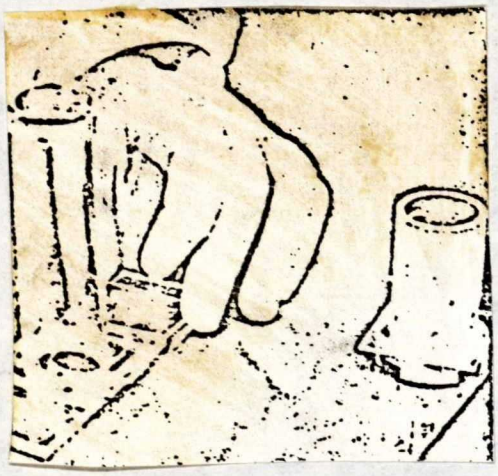

Figure 8. Sedimentation is completed. The 2 guide plates lie to the left and right of the plate chamber and immediately next to it. The sliding plate slides across one guide plate, pushing the upper section of the combination chamber onto the other guide plate.

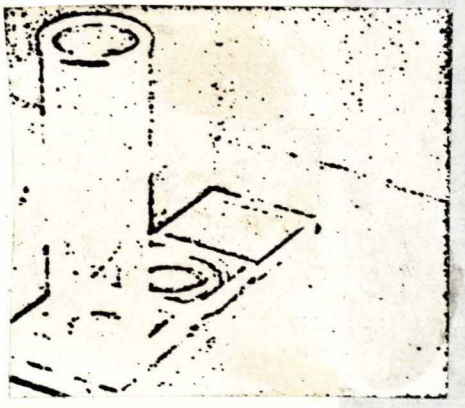

Figure 9. The lateral movement of the upper section has been completed and the plate chamber in the centre has been sealed with the sliding plate. The plate chamber is not ready for counting.