METHODS OF PLANKTON INVESTIGATION IN THEIR RELATION TO PRACTICAL PROBLEMS.

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In this country the fisherman as a rule continues to fish in any locality until fishing in that locality has become unprofitable. He then moves his operations to new waters until these in turn are exhausted. He is apt to look upon each new body of water as inexhaustible, and rarely has occasion to ask himself whether it is possible to determine in advance the amount of fish that he may annually take from the water without soon depleting it.

On the other hand, the fish-culturist is apt to plant his fry in waters that are quite unsuited to them or to plant them in numbers far in excess of what the water can support.

The fisherman proceeds as a farmer might who imagined that he could continually reap without either sowing or fertilizing; while the fish-culturist proceeds often as if convinced that seed might grow on barren soil or that two seeds might be made to grow in place of one.

In some regions the public is beginning, through the machinery of the State, to insist that its interest in the fisheries be guarded; that neither fishing nor planting of fish should be carried on in excess; and the time is fast approaching when the State will everywhere exert its authority to control the fisheries. It will then become necessary to determine, at least approximately, the productive capacity of any body of water.

It is the purpose of the present paper to discuss the method by which it has been proposed to determine the relative productive capacities of bodies of water. This method, for there is really but one, was first proposed by Hensen[1] in the sea, and is based upon two principles. It is known that the many species of plants and animals which inhabit a body of water are interdependent. In the final analysis all the fishes are dependent, directly or indirectly, on the minute floating plants and animals which, taken together, we call the plankton. The total mass of plankton is, in most bodies of water, so great that, in comparison with it, it is customary to neglect the fixed plants along the shore and the animals that they harbor. That the plankton lies at the base of all life in the water is, then, the first principle.

The second principle is that the plankton, considered as a whole, is uniformly distributed. There is no longer any doubt that some constituents of the plankton, e. g., the crustacea, may not be distributed uniformly.[2] Wherever measurements have been

[1] Hensen, Victor. Ueber die Bestimmung des Planktons. Kiel, 1887.
[2] Marsh. On the Limnetic Crustacea of Green Lake. Transactions Wisconsin Academy of Science, Arts, and Letters, vol. 11, 1897, pp. 179–224.

made of the total plankton it has, on the other hand, been found[1 2 3] that this is so distributed that nearly the same volume of it occurs under each square yard of the surface at equal depths.

From these two principles Hensen concluded that a determination of the amount of plankton under a unit of area of any part of the sea would afford a measure of the productive capacity of that part.

It remained to find some means of making such determination. After much labor Hensen finally adopted the method of drawing a net vertically from the bottom to the surface. Such a net strains out the plankton contained in a vertical column of water and catches the whole amount of plankton under an area of the surface equal to the net opening. From the plankton so obtained the total plankton of the water under consideration may be calculated and the results expressed in volumes or by weight or by enumerating the contained individuals. The productive capacity of a body of water, as expressed in its plankton production, may thus be compared to that of other bodies of water and so may be made of practical use.

The method which Hensen used in the sea was later extended by Apstein, his pupil, to fresh water. Apstein's results were published in various special papers and finally collected into a single very useful volume.[1] This method, with some slight modifications, has since been used in this country by Reighard,[2] Ward,[3] and others.

The great advantage which this method enjoys over others is that the water from which the net strains the plankton is a vertical column extending from bottom to surface, and is thus a representative sample of all the water from all depths in the lake examined. This column of water bears the same relation to the whole body of water that a sample removed from a sheet of metal by a punch bears to the whole sheet. There is no other method applicable to all conditions which has been shown to have this advantage.

There are, however, certain difficulties in the use of this method. These were known to Hensen and he attempted to obviate them. The net does not, as a matter of fact, filter the whole of a column of water through which it passes. A part of the water is pushed aside and a part filtered. By an elaborate set of experiments Hensen tried to determine what part of the water was pushed aside. This depends upon the form of the net and upon the material of which it is made. If the net filters half of the column of water, then in order to know the amount of plankton actually in the column it is necessary to multiply the amount of plankton taken by two. The number by which one must thus multiply is known as the coefficient of the net. The coefficient of the net was assumed by Hensen to remain practically constant. There are, however, two factors which may cause a change in the net coefficient—clogging of the net by foreign particles and shrinkage of the net cloth so as to diminish the size of the openings in it. This change in net coefficient is the first difficulty in the use of Hensen's method. If the pores of the cloth (No. 20 bolting-cloth) used for such nets become clogged the net will filter less water than before, i. e., its coefficient will become greater. If the net coefficient thus changes, the results obtained with a given net at different times, or by different observers with different nets, can not be accurately compared, and a large part of the advantage of the method is lost. It is

[1] Apstein, C. Das Süsswasserplankton. Kiel, 1896.
[2] Reighard, Jacob. A biological examination of Lake St. Clair. Bulletin of the Michigan Fish Commission, No. 4.
[3] Ward, H. B. A biological examination of Lake Michigan. Bulletin of Michigan Fish Commission, No. 6.

customary in order to prevent clogging to wash the net at the end of each haul with a stream from a hose. It was further suggested by Hensen,[1] who recognized the effect of clogging on the net coefficient, that the net be more thoroughly washed at the end of each day's work. Hensen[2] and Frenzel[3] have more recently suggested other methods of cleaning the net.

The change in the net due to shrinkage of the cloth and consequent narrowing of the pores does not seem to have been noted by Hensen. It was first pointed out by Reighard.[4] Both causes of change in the net coefficient have been since studied by Kofoid.[5] He finds that owing to clogging of the net "the coefficient of the net varies with the amount and constitution of the plankton from 1.5 to 5.7," and that "from 84 per cent to 96 per cent of the 30-meter catch is taken in the first 15 meters of the (horizontal) haul." Kofoid finds further that from the shrinkage of the net "the total area of the openings in a square centimeter . . . decreases over 50 per cent."

The first difficulty in using Hensen's method, that arising from change in net coefficient, owing to clogging and shrinkage, seems at first sight to be sufficiently serious. The second difficulty is that the openings in the cloth, although very minute, are still so large that some of the organisms of the plankton pass through them and are lost. After correcting the "catch" by multiplying by the net coefficient, the result still does not express the total amount of plankton present in the column of water through which the net was drawn. This source of error was known to Hensen,[6] but he does not appear to have determined the extent to which the smaller plankton organisms pass through the net. Kofoid[5] has now called attention to this subject and has determined for certain forms the percentage of loss from this source. He finds that "of Codonella as many as twenty-one individuals may escape to one retained" and that there is a great loss of other small organisms. Kofoid adds, referring to his predecessors, that, "the leakage of the plankton through the silk has been minimized or ignored and without tests of the extent to which it occurs." An active purpose on the part of plankton workers, such as is implied in the phrase "minimized or ignored," is nowhere evident in the literature. The truth is rather that Kofoid's predecessors have omitted to investigate this source of error quantitatively.

Though neither the variation in the coefficient of the plankton net nor its penetrability to the smaller plankton organisms were discovered by Kofoid, he has rendered important service in pointing out their extent.

It remains to consider to what degree the errors due to the above causes detract from the value of the results hitherto obtained by the Hensen method. The plankton catches thus far made by this method (as by others) have been utilized principally in two directions:

I. They have been measured in order to determine the volume of plankton present in the water. For this purpose the plankton is concentrated, either by allowing it to settle in a graduated cylinder or by the use of the centrifuge, and the volume is then read off. This method is not accurate; it is merely the best method hitherto devised for the purpose. The plankton, which is thus measured, consists of large and small organisms, and as it settles the smaller organisms are mostly packed between the

[1] Hensen. Bestimmung des Planktons, p. 13.
[2] Hensen. Bernerkungen zur Plankton Methodik. Bio. Centralblatt, XVII, 1897, p. 510–512.
[3] Frenzel. Zur Plankton Methodik. Bio. Centralblatt, XVII, 1897, p. 364–371.
[4] Reighard. Loc. cit., p. 59.
[5] Kofoid. On some important sources of error in the Plankton Method. Science, Dec. 3, 1897.
[6] Hensen. Die Bestimmung, etc., p. 10, sec. 3, and p. 75.

larger, but being lighter, are in part deposited in a thin layer on the top of the mass of larger organisms. We may consider separately the errors which are introduced into the volumetric method from the three sources above mentioned.

(*a*) *Errors due to clogging of the net.*—This depends principally upon the area of the filtering surface of the net as compared to the volume of plankton present in the water. If the net surface is large and the volume of plankton in the water filtered small, there is but little clogging. The net employed by Kofoid was 25 cm. in diameter at the base and 40 cm. on one side. The plankton appears to have been unusually abundant (Kofoid gives no data) and the conditions otherwise unsuited to the use of any sort of net. The net employed by Reighard and Ward in the work above referred to had a diameter of 60 cm. and a slant height of 100 cm. Its filtering surface was thus about six times that of the net used by Kofoid, while the plankton in the water in which it was used was very little. In the work done by Reighard not more than 4.5 c.c. of plankton was taken in the net at one time and in the work of Ward not more than 11.9 c.c. In a majority of the hauls not more than a fraction of these volumes was taken. The net used by Hensen was much larger (Hensen, loc. cit., p. 6), while that used by Apstein was about the size of Kofoid's net, but it was probably used under more favorable conditions. Clogging, then, does not seem to me to be an important factor with nets of the size used by Hensen, Reighard, and Ward. It becomes important only in case a small net, such as Kofoid's, is used under unsuitable conditions. Some measure of its extent is desirable.

(*b*) *Error due to shrinkage.*—This error is largely if not wholly eliminated by previous thorough shrinking of the net. The cloth used by Reighard and Ward was several times dampened and ironed before it was made up into the net and was thus presumably thoroughly shrunken. The net was also many times wet and dried before it was used for quantitative work. As may be seen from the table on page 57 of Reighard's report, the cloth of the net used by him and later by Ward differed but little after a summer's use from new cloth which had been once wetted and then dried; the cloth in the two cases being measured under as nearly as possible the same conditions. Whether the nets of other workers were similarly shrunken before use does not appear. I have not encountered any such enormous shrinkage as that recorded by Kofoid, in which the average size of net openings was reduced from .000024 to .00001 sq. cm. Everything here depends on a uniform method of measuring the cloth.

(*c*) *Errors due to permeability of the cloth.* A large number of the smaller plankton organisms escape through the pores of the cloth. According to Kofoid "the silk net retains from $\frac{1}{2}$ to $\frac{1}{4\,6}$ of the total solid contents of the water." "The amount escaping through the silk bears no constant relation to the amount retained." These statements are certainly very startling, but one must reserve final judgment concerning them until the conditions of the experiments upon which they rest are made known. This degree of leakage through the net may be due to the peculiar constitution of the plankton examined. The extent to which this source of error vitiates previous work can only be determined by tests of the nets used by previous workers in comparison with other methods and in the waters in which the nets were used. In volumetric determinations most of the smaller plankton organisms are packed between the larger organisms in such a way as not to affect the total volume of plankton in the measuring tube. Some of them, however, remain in suspension longer than the larger and heavier organisms, and when they settle lie at the top of the whole mass measured, and so increase its volume.

On the whole, one may say that where nets of sufficient size have been used under favorable conditions there is no good reason for assuming that the volumetric results obtained by Hensen's method are vitiated by the first two sources of error noted above. To what extent they are vitiated by the third source of error (leakage) remains to be determined. Since the organisms which escape are the smallest in the plankton, they may be volumetrically of little importance. Their importance depends upon their abundance, and this must be investigated by other methods. When the considerable variations in the volume of the plankton itself are taken into account it seems improbable that the error arising from leakage is sufficient to seriously vitiate volumetric determinations by the Hensen method or their use for practical purposes.

II. The catches made by the Hensen net have also been used for enumerating the number of organisms contained in them. Of the three sources of error above enumerated the first two affect this method to the same extent that they affect the volumetric method, so that by using suitable nets properly shrunken these two sources of error may be avoided here also. The third source of error, that arising from permeability of the net, is, however, fatal to the method of enumeration, in so far as it is applied to smaller organisms. In the tables of Apstein and Hensen, then, the enumerations of smaller organisms can not be accepted as final until it is shown that these organisms can not escape through the net in considerable numbers.

For determining the productive capacity of a body of water use has been made of the volumetric method only. Where the net used has sufficient filtering surface, and where it is not attempted to use the net in situations to which it is unsuited—i. e., among water-plants and in silt-laden waters—it seems to me that this method is not only practicable, but it is the only practicable method hitherto devised, since it is the only method by which the plankton may be obtained from a representative sample of the entire body of water. It should be noted in this connection that the variations in the plankton itself are far greater than the errors of the method.

We may now consider the substitutes that have been offered for the Hensen method. By this method the plankton is removed from a measured quantity of water which remains in position in the lake. We may analyze this procedure into two processes—the measuring of the water and the obtaining of the plankton from the water. For each of these processes, as carried out by the Hensen method, one or more substitutes have been proposed.

Owing to the inconstancy of the net coefficient due to clogging and shrinkage, it may be a matter of uncertainty as to how much water the net actually strains. To obviate this difficulty it has been proposed by Kofoid (loc. cit.) and by Frenzel[1] that the water to be examined should be pumped through a hose. Water from any desired depth may thus be brought aboard the boat and plankton then removed from it by the Hensen net or other means. It is obvious that by this method the quantity of water obtained may be known with exactness, so the difficulty connected with net coefficient vanishes. By the Hensen method the column of water from which the plankton is obtained extends vertically from the bottom to the surface. This column includes equal volumes of water from all depths and is representative of the whole lake. It does not seem to me possible to obtain a representative sample of the water of the lake in any other form than that of a vertical column extending from

[1] Frenzel, Joh. Zur Plankton Methodik, I, Die Planktonpumpe. Bio. Centralblatt, XVII, 1897, pp. 190–198.

bottom to surface. If it is possible to obtain by the pump such a column of water, then the pump may very well replace the net so far as this part of the process is concerned. I do not say that this is not possible, but we should not assume that the water drawn in by a pump through the submerged end of a hose, which is being slowly moved from top to bottom, or vice versa, is a vertical column of water. Before the pump can replace the Hensen net there must be sufficient evidence that this is so, and such evidence is not yet forthcoming.

Having obtained the water by use of the pump, it is necessary to separate the plankton from it. To accomplish this, the second process into which we have analyzed the Hensen procedure, various means have been proposed. Frenzel, and at first Kofoid,[1] made use of the Hensen net to strain the water pumped. In order to avoid the loss of plankton due to the permeability of the net to small organisms, Kofoid later tried various other methods of separating the plankton from the water. These were the sand filter, the filter paper, the centrifuge, and the Berkefeld filter. By each of these methods a greater number of plankton organisms is retained than by the Hensen net. (Nothing is said of volumes.) In some cases as much as 98 per cent of the total number of organisms present is retained. By none of these methods is it possible to obtain the plankton from a large volume of water in a short time, and each has besides other disadvantages which are enumerated by Kofoid. In the case of the Berkefeld filter, which was found to be the most efficient method, it was necessary to remove the catch from the surface of the filter with a "stiff brush." The surface of the filter, which is composed of infusorial earth, was thereby disintegrated and the plankton contaminated by the fragments. It is to be hoped that the disintegration is confined to the filter. The large form of the Berkefeld filter (army filter) filters about 2 liters of water per minute. This is a very slow rate of filtration if one has to deal, as is sometimes desirable in plankton work, with a column of water several hundred feet long and perhaps 10 inches in diameter.

The methods which it has been proposed to substitute for the Hensen method are thus seen to be deficient in two ways. For obtaining the water the pumping method is (so far as yet shown) defective in that the source of the water pumped is uncertain. It is not known that the pump can be made to deliver with accuracy the contents of a vertical column of water. For filtering the water the methods proposed, although they remove the plankton organisms more perfectly than the Hensen net, are yet inferior to it in that they are incapable of handling large volumes of water. Is it possible to so modify the Hensen method or to so combine it with other methods as to correct its errors and at the same time retain its good points? Its errors are the variation in net coefficient, due to clogging and shrinkage, and the permeability of the net for small plankton organisms. Its advantages are that it filters a representative vertical column of water, and that it filters rapidly very large volumes of water. Now, if it is possible to measure the volume of water that passes through the net at each haul the difficulties of clogging, shrinkage, and net coefficient at once vanish. I have not made any attempts in this direction, but I see no reason why a small current meter can not be placed within the opening of the plankton net, so as to register the rate of the current of water passing through the opening during each haul. If this rate were known the volume of water passing through the net could be calculated,

[1] Bulletin Illinois State Laboratory of Natural History, vol. v, article 1.

and the plankton taken would be that found in this volume of water. No further calculations of any sort would then be necessary.

If it is possible to thus meet the difficulty arising from clogging and shrinkage there still remains the further difficulty due to the leakage of small organisms through the net. The net will have collected the larger organisms from a representative column of water. In order to obtain these large organisms it is desirable that the net should filter a very large volume of water, in some cases many cubic meters. In order to obtain the smaller organisms it is, however, not necessary to filter so large a volume of water; a few liters would probably suffice. Water for this purpose might be obtained by the pumping method or perhaps quite as satisfactorily by the well-known method of using flasks so arranged that they can be filled after being lowered to desired depths. It would be necessary to take small samples of water from several different depths and to remove the plankton from them by some one of the methods described by Kofoid as retaining the smaller organisms. The objection to this double method is that while it is entirely accurate for the large organisms taken by the net from a vertical column of water, it does not give us the smaller organisms from the whole of this vertical column of water, but rather from isolated samples of water from different levels. It seems to me, however, that if we know the large organisms in a vertical column of water, and if we know also the ratio of the larger to the smaller for certain parts of the column, we may readily calculate the volume or number of small organisms in the whole column. This volume may then be added to that obtained by the net and the total volume thus obtained.

In conclusion, it seems to me that the errors of the Hensen method, the extent of which Kofoid has pointed out, are probably greatly exaggerated by the condition under which he has used the method. This Kofoid himself suggests. The originator of the method probably never intended that it should be used among water-plants and in silt-laden waters. For such waters, which are shallow, the pumping and filtering methods described by Kofoid are undoubtedly best adapted. On the other hand, these methods are by no means so well adapted to deeper and larger bodies of water. For these it seems to me the Hensen method must still be retained, and if it can be modified as suggested above, it may be of value in such waters as those of central Illinois. Whether or not it can be modified in the way suggested, it can at least be supplemented by a method by which the smaller organisms may be more perfectly obtained.

Even in its present form the method is probably sufficiently accurate under most circumstances for the purpose of making rough determinations of the relative productive capacities of different bodies of water. It must be remembered that the method as used for this purpose is at best rough, but it must also be remembered that the variations in volume of plankton are considerable, so that the errors in method are probably within the variations in the material upon which it is used.

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