SUGGESTIONS FOR COLLECTING AND PREPARING DIATOMS.

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The study of the diatoms has taken on a much greater importance than formerly, because of the now generally recognized fact that the rôle they play in aquatic life, especially in the food supply of fishes, is a very great one. In their fossil state also new uses for diatom earth have been discovered and the amount of this material now supplied to the various industries is enormous. I have been asked to give some simple suggestions on the best methods for collecting these organisms and preparing them for microscopic study.

The living forms, inhabiting all waters, fresh and marine, will be first taken up. The presence of diatoms in any considerable quantity can be detected in quiet waters by the rich brown or yellowish-brown color of the film composed of diatoms, which coats the bottom of such places or clothes the submerged stones, sticks, and other bodies. By carefully removing this pellicle, for which a bent piece of tin or a clamshell are good instruments, the collector secures a rich gathering, very slightly mixed with dirt, sand, or other matter.

But there are few such places met with, especially in the collecting of marine diatoms. Two very distinct methods of securing material must be used, because we have to do with two unusually different habits of life of the diatoms. Some of these are plankton, that is to say, they live suspended in the water. The presence of the plankton forms is generally unsuspected, because they are not readily visible in the water, although generally present, and that, too, at times in immense quantities. The plankton diatoms are secured by means of a plankton net composed of silk bolting cloth with a mesh of about No. 18. This net is attached to a wire ring having a diameter of about 15 to 18 inches and is conical in shape, but should be as far as possible without folds, having a length of 2½ to 3 feet, and with the lower end or tip slightly rounded. This absence of folds can be secured by cutting the bolting cloth and carefully sewing up the seams with a double line of stitches so as to avoid any flaps. Attached to a stout cord by three lines tied to the wire rim at equidistant points, the

net is drawn through the water slowly, preferably back of a rowboat, until the desired quantity of plankton diatoms has been secured. This is washed into a jar, partly filled with water, by reversing the net. A small quantity of commercial formalin is then added to the jar to kill all the organisms and allow them to settle. The time required for settling depends upon the height of the water in the jar, but should be given an hour in a jar containing 10 to 12 inches of water, as the diatoms, which are characteristically delicate in plankton forms, settle slowly. When settling is complete the excess of water is very carefully poured away and the diatom sediment is bottled.

The method used for cleaning and preparing the more robust diatoms collected from the bottom of lakes, rivers, or the ocean, is not well adapted to these delicate plankton species and special methods are therefore necessary to prepare them for microscopic study. In general the material in the bottom of the bottle can be microscopically examined under a cover glass without further preparation; but as the markings upon many of the species are difficult to see in water, it is preferable to use a mounting medium of high refractive index. Several methods can be suggested. By transferring some of the material from the collecting bottle to a small homeopathic vial and gradually replacing it by alcohol, one can then mount the material in so-called gum thus, also known as frankincense. This is obtainable at most druggists. It is dissolved in pure alcohol to the consistency of thick syrup. After the diatoms have been transferred to a glass slide, and a drop of gum thus placed upon them and covered with a cover glass, the mount is ready for examination and has the advantage of being practically permanent, at least for several years. This mounting medium will bring out the markings of most of the plankton forms. There is, however, a better medium which, because of its very much higher refractive index, shows the more delicate structure which is hard to see in gum thus. This medium, a solution of barium mercuric iodide, is made as follows:

To a saturated aqueous solution to barium iodide is added red mercuric iodide until a slight excess of the latter remains undissolved. A drop or two of the saturated solution of barium iodide is then added, in order to effect the solution of this excess of the mercuric iodide. The perfectly clear yellow solution is now ready for use and can be kept indefinitely in a well-stoppered bottle. A minute quantity of the diatom sediment is placed by a pipette on the slide. This is taken from the collecting bottle where the diatoms are in weak formalin. The excess of water is drawn away from the material on the slide by means of a triangular piece of thin blotting paper or filter paper. A drop or two of the barium mercuric iodide is then added, the diatoms gently stirred in with the needle, and

covered with a cover glass. If it is desired to preserve this mount, any excess of the liquid is first removed from under the cover glass by means of another triangular piece of blotting paper; the mount is then sealed with a ring of hot paraffin, followed by one or two rings of some good cement.

It is also well to clean up a small portion of each sample of plankton material by the acid methods to be described under the next heading; as there are always a number of forms in most plankton gatherings sufficiently robust to stand this, and these forms are much better studied after such treatment than in the uncleaned condition of the mounts prepared by the foregoing methods. This is due to the removal of all organic matter from these denser diatoms and consequently the better view that is obtained of their sculpture. By using these two methods with plankton material, the student secures all the advantages of each, and has no difficulty in identifying all the species present.

We now turn to the collection and preparing of diatoms inhabiting the bottom of rivers, lakes, bays, etc. Aside from the places where diatoms can be collected in shallow waters, where they are seen by means of their color, as has been previously pointed out, the collector needs some form of dredge to obtain samples in the deeper water. The best dredge known to the writer is fortunately a very simple one, and one involving a very small outlay of money. This is made of about 15 inches of cast-iron drain pipe with a diameter of about $4\frac{1}{2}$ inches. One end of this is closed, either by a shallow wooden plug or by brazing a circular piece of metal to the end. At the other end a stout wire bail is attached by means of two holes drilled through the pipe about an inch apart and as close to the edge as possible. The side of the rim opposite to this bail is sharpened on the inner side with a round file, so as to make a cutting lip.

The advantages of this dredge are several—its cheapness and the ability to have one made even at remote places; its indestructibility when used on stony bottoms; and the fact that it always secures a satisfactory amount of material, about a liter of the surface of sand or mud. It should be drawn very slowly over the bottom, either back of a rowboat, or from the beach by casting the dredge out and drawing it slowly ashore. It should be remembered that approximately five times the depth of the water should represent the length of line when dredging, otherwise the dredge may be tilted at the front end and fail to scrape up the bottom. When the dredge is near the boat and is to be lifted it should be done gently, otherwise its contents will be spilled. It should also be raised gently to the boat, so that the rush of water does not sweep out the material. If the bottom is muddy, the material must be put in a jar or bottle, and the mud separated in the laboratory. If the bottom is sandy, the

dredge should be dumped into a pail, about a pint of water added, and the whole violently stirred with a stick. After the stirring and as soon as the sand is thought to have settled, perhaps 30 seconds, the muddy water is poured into a battery jar, another pint of water is added and the process repeated. This washes the diatoms out of the heavy sand and concentrates the material. Formalin is now added to the jar, as previously directed, and after the material has thoroughly settled the water is carefully poured away and the sediment is bottled.

These two kinds of bottom samples are to be treated differently at the laboratory. The muddy material is separated as far as possible by decantation from its clay and other very fine material in the gathering. Muddy gatherings are also liable to contain particles of decayed sticks, leaves, etc., which would be difficult to destroy by acids and should first be removed by means of a piece of wire gauze, having a mesh of perhaps about one-half mm. By pouring the muddy gathering, well diluted with water, through this gauze, these larger particles will be removed and the process of cleaning now to be described will be rendered much easier. A small amount of the mud is placed in a battery jar, about 50 cc. to a jar holding a liter, and water added. It is best to add the water from the faucet with a powerful stream, so as to mix up the contents thoroughly, or the whole may be stirred with a rod or stick. When such a jar has stood for 45 minutes, the muddy water is poured away, fresh water is added, and the process is repeated until the water after 45 minutes is almost clear. From this point the samples from sandy and from muddy bottoms are treated the same way. The water is poured off and the material transferred to a beaker and about ten times its volume of commercial hydrochloric acid is added. This is to partially bring into solution the organic matter, but especially to remove all lime, such as shells, corals, etc. The material is boiled in the acid for about 20 minutes. The acid is then poured away, after the contents have thoroughly settled, fresh acid is added and again brought to a boil. Commercial nitric acid is now added to the boiling beaker, drop by drop, care being taken that the contents do not foam up over the beaker. This adding of the nitric acid to the boiling solution brings about the rapid oxidation of the organic matter in the sample and leaves as a residue only the diatoms, such sand as is present, sponge spicules, and other bodies composed of silica. The adding of nitric acid should be continued until the red fumes of nitrous acid produced by the oxidation process are no longer given off. The beaker is now filled with water and the sediment washed free of acids by repeated additions of water and decantation after settling. The material is now examined under the microscope, and in most cases it will be found to be clean, the diatoms present being bril-

liantly clear. But where the organic matter is very high, as it is in very muddy material, the washed residue must be passed through the sulphuric acid process. If this is needed, the water is very carefully poured away so as to remove as much of it as possible. The sediment is transferred to a small distilling flask, or if this is not available, to a porcelain evaporating dish. Sulphuric acid is carefully added to the wet material and the residue in the beaker is washed over into the distilling flask with sulphuric acid. The heat generated by the union of sulphuric acid and the material is high and care must be taken to avoid explosion. The mass is now boiled over a sand bath. It generally becomes perfectly black or a dark brown. The boiling should continue an hour or two, as the sulphuric acid evaporates very slowly in the evaporating dish and not at all in the distilling flask. There is now cautiously added to the boiling mass minute particles of sodium nitrate. This is accompanied by violent sputtering, oxygen is liberated, the organic matter is entirely oxidized and destroyed, and the mass becomes perfectly white or pale straw color. After it has become cold it is poured into a large beaker with a capacity of at least one liter, half filled with water, and water then added to fill the beaker. Battery jars will not do for this step, because the heat generated by the union of the sulphuric acid with the water is liable to crack the jar. The material is now freed from acid by settling and decantation.

Whether the double or three-fold acid process has been used, the matter now reaches its final treatment to free it from undesirable material. This will consist principally of sand and perhaps large radiolaria. A little of the clean material is put in a porcelain evaporating dish with a diameter of about 4½ inches, water poured in so as to stir up the mass and the whole rotated in such a way as to give a slight whirling motion to the contents. It is difficult to describe this process, though easy to demonstate it. It may help to render clear the idea of the motion desired by saying that if we imagine an ink spot to be placed on the under side of the evaporating dish exactly in its center, the motion consists in rotating this ink spot around a tiny spot on the table in such a way that the circle of rotation will have a diameter of about a quarter of an inch. The effect of this peculiar motion is to roll the rounded particles of sand and the radiolaria into a little mound at the bottom of the dish, while the diatoms, which are mostly angular or flat, spread out in the water. After the rotation has continued for about a minute, the contents are quickly poured into a clean beaker, taking care that the sand, etc., remain in the dish. Fresh water is now added and the process is repeated until it is found that the residue in the dish is free from diatoms. This sand separation is not always necessary, in fact is never necessary unless

the investigator wishes to have his diatoms as free as possible from extraneous matter.

The thoroughly washed and cleaned material is now put up in bottles in 35 per cent alcohol and properly labeled.

No attempt will here be made to describe the subsequent mounting of clean diatoms. This must be left to the wishes of the investigator, dependent upon whether his purpose is to make strewings of the diatoms or to pick the individuals out and mount them separately. If the latter and of course the better method is used, it may be well to add one or two suggestions. The writer uses special microscope slides for picking out diatoms. The slides selected are thick, about 2 mm. Eleven lines are drawn across the slide near the middle, about 3 mm. apart with a writing diamond, and a median line is drawn across these 11 lines bisecting them, the lines thereby producing 20 spaces. Figures from 1 to 10 are written near the margin of the slide, numbering the 10 cross divisions. One has thereby 20 spaces, upper and lower No. 1, upper and lower No. 2, etc., and in picking diatoms he can proceed in an orderly way from one side to the other, back and forth through the spaces marked by the cross lines; and when a diatom is found and removed for mounting, he is able with this slide to go back to the exact place where he left off by remembering the part of the subdivision where the selection was made. He therefore avoids going over the same ground twice or missing part of the material on the slide.

In making a strewing from which to pick individual diatoms a couple of drops of the clean material well shaken up is placed upon the slide, spread out over the lines by tilting the slide, and the water and alcohol evaporated over a spirit lamp. The diatoms should lie evenly strewn on the slide and be perfectly dry. If the cleaning has been properly done and distilled water and absolute alcohol have been used for the 35 per cent solution, the diatoms should be loose upon the glass and can be picked up with the greatest ease. The only wholly satisfactory way of accomplishing this selecting and mounting of individual diatoms is by means of the apparatus known as the mechanical finger. The method of using this delicate instrument can not be taught by description. It must be demonstrated, and is even then a part of diatom technic which requires long practice. But the result obtained is so superior to diatom specimens in a strewn slide that the learning of this process is strongly recommended. A strewn slide bearing the name of any specific diatom contains many others, and some may be very similar in general appearance. How is the student to pick out the true type from this mass of material, when he probably is looking for an example of the type in order to know how it looks? But if each diatom is mounted separately, it becomes

an ideal herbarium specimen and its value for purposes of identification is great.

There remains the subject of the preparation of fossil diatoms. So-called diatomaceous earth is generally free from organic matter and is only subjected to acid treatment when it contains iron or other substances that can be dissolved out by acids, or especially when it contains calcareous matter. Diatomaceous earth of this last kind is easily disintegrated by treatment with hydrochloric acid; as the lime is thereby dissolved and the mass falls into a powder. But most diatomaceous earth is not so easily handled, being composed entirely of silica remains and often hardened into a stony condition. The breaking up of such samples can not be done by pulverizing, as this would shatter nearly all of the diatoms. The mass must be gently brought into a powdery condition. The best way of accomplishing this is to first break the material up into small pieces about the size of a pea, using for this purpose not a hammer, but a stout needle, which cracks off small particles without breaking many of the diatoms. The pieces having thus been reduced in size are boiled in a beaker with a weak solution of some mild alkali, like sodium carbonate. A solution of borax also sometimes works satisfactorily. The material is boiled until the liquid begins to look milky by the slow breaking away of the diatoms from the lumps. The liquid is then poured into a larger beaker, fresh alkali water added to the lumps, and again boiled. The process is kept up until by this gentle method the lumps are slowly broken down. After the combined boilings poured together have been allowed to settle, the liquid is poured off and the sediment washed by decantation until all trace of the alkali is removed.

In cases of extremely resistent fossil material, where neither hydrochloric acid nor long boiling in weak alkaline solutions breaks down the lumps, this disintegration may sometimes be effected by soaking the lumps in strong sodium carbonate, quickly replacing with hydrochloric acid, returning again to sodium carbonate, and so alternating until the violent chemical reactions set up *within* the lumps by these alternations have mechanically broken them down.

If the repeated washings necessary with any of the foregoing processes are properly timed they will also accomplish the removal of clay or minute broken particles of diatoms that are in the sediment. Where the fossil substance contains sand, the final process of rotating this in an evaporating dish will remove it, as in the case of the living diatom material previously described. The cleaned diatoms are then put up in bottles with 35 per cent alcohol, as in the case of the living material.

It sometimes happens with fossil material, more rarely with fresh, that a fine flocculent residue is mixed with the cleaned diatoms and

persistently resists separation by decanting. It can generally be removed by boiling in some thin colloidal solution, like soap solution, the flocculent matter being thereby held in suspension while the diatoms are settling. By one or two such boilings and careful decantations this silicious floc will be largely or wholly removed. Very careful washing is required after this soap treatment before the diatoms are put away in 35 per cent alcohol.

However the author may have tried to make plain the different steps in preparing diatoms for study, he recognizes that some confusion may exist in the minds of students in regard to some parts of this technic. A letter of inquiry, directed to him at the United States National Museum, Washington, District of Columbia, will receive an answer, in which the points in question will be more carefully explained.