

# INSTRUCTIONS FOR COLLECTING AND FIXING ROTIFERS IN BULK.<sup>1</sup>

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A glance at the published zoological results of scientific expeditions is sufficient to show that rotifers are conspicuous by their rarity, if not by their total absence. This is accounted for by the small interest generally accorded these animals, as well as by the fact that investigators, even when devoting their attention exclusively to the fresh-water fauna, rarely procure material in such condition as to be of any use for systematic work. Rotifers are very delicate and contractile, and in collections preserved in bulk in alcohol or formalin they become as a rule unrecognizable, with the exception of those species which have the body incased in a chitinous shell or lorica; in some cases this is sufficient for specific determination. In the case of the genera *Anuræa* and *Brachionus* this has become a positive misfortune, as it has resulted in an absolutely unjustifiable multiplication of species based on the excessively variable spiny prolongations of the lorica.

It may be objected that this void is of small importance, as it is generally conceded that rotifers are distributed with almost absolute uniformity all over the world.<sup>2</sup> But it is equally well known that there is a host of rare and local forms, often of the greatest interest to the morphologist, which can be discovered only by careful exploration. As an example, it is sufficient to mention the famous genus *Trochosphaera*, which although widely distributed appears to be confined to subtropical latitudes. Furthermore underlying this apparent uniformity there is a multitude of problems relating to the means whereby it is established and maintained, and they can be solved only by an exact knowledge of the faunal development under varying conditions and in different environments. As specially important

<sup>1</sup> Translated and adapted from Archives de zoologie expérimentale et générale, ser. 4, vol. 4, 1906, Notes et revue, pp. xxvii-xxxlii, by H. K. Haring, Bureau of Standards.

<sup>2</sup> See on this subject: C. T. Hudson, Journ. Roy. Micr. Soc., 1891, p. 6; V. G. Thorpe, Journ. Roy. Micr. Soc., 1896, p. 485; H. S. Jennings, Bull. U. S. Fish Comm., vol. 19 (1899), 1900, p. 67.

subjects for investigation may be mentioned lakes situated at great altitudes, isolated oceanic islands, unexplored and not yet colonized regions (man is probably one of the most active factors in this dispersion), and brackish waters of varying degrees of salinity.

The value of limnologic and hydrobiologic research in its relation to fish culture and hygiene is now universally admitted, quite apart from its theoretical interest, and every student of fresh-water biology knows that rotifers are frequently the predominant group, and always an important one, furnishing one of the most valuable characteristics. As an example may be cited Lauterborn's recent work on the fauna of the Rhine,<sup>1</sup> according to which a list of the rotifers from a certain body of water enables the specialist to decide whether it is pure and running, stagnant and full of vegetation, or polluted and putrid. But the determination of rotifers can not usually be made with certainty except from living or carefully prepared specimens, conditions demanding not only time and equipment usually not available on expeditions or limnologic campaigns, but an experience which few have the time to acquire.

As the methods suitable for Crustacea and Planarians are useless here, I have, in order to overcome these difficulties, tried to discover, and I believe have found, a method which, without being as simple as placing the whole catch in alcohol, will allow any careful worker and especially a resident naturalist to prepare rotifers in bulk in such condition that they may be useful for subsequent study. The method now used exclusively for the preparation of rotifers as objects for the microscope is due to Rousselet;<sup>2</sup> it consists in narcotizing the animals with a cocaine solution, followed by fixation with osmic acid and mounting in weak formalin solution. I have succeeded in reducing its application to animals in large quantities to an almost automatic process.

The necessary reagents are:

1. A concentrated narcotizing solution (about three times as strong as Rousselet's original formula):

Cocaine hydrochlorate.....	gram..	1
Pure methyl alcohol.....	cubic centimeter..	10
Distilled water.....	do.....do.....	10

Instead of cocaine one may use the same amount of stovaine, or  $\beta$ -eucaine hydrochlorate.

2. A solution of osmic acid of 1 per cent strength, to which is added 1 per cent chloro-platinic acid, commonly known as platinic chlorid, sold either in the form of crystals or as a 10 per cent solution. The latter is added to prevent reduction of the osmic acid, which in this way will keep almost indefinitely.

<sup>1</sup> Arb. aus dem kais. Gesundheitsamte, Berlin, vol. 22, 1905, pp. 630-652.

<sup>2</sup> Journ. Quekett Micr. Club, ser. 2, vol. 6, 1895, pp. 5-13; also Proc. 4th Int. Congr. Zool., Cambridge, 1898 (published 1899), p. 197.

Their use is, even though apparently complicated, in reality quite simple. It is necessary to distinguish, both in collecting and subsequent fixation, two classes of rotifers—the purely pelagic forms, which swim without ever anchoring themselves and belong to the true plankton, and the forms which are not good swimmers, but move about among detritus and aquatic vegetation in search of sustenance, not traveling far, and frequently fixing themselves by the toes. We will treat them separately.

The plankton of smaller bodies of water, ponds, pools, ditches, etc. ("Heleoplankton" of Zacharias), is periodic, appearing and disappearing in a few days or a couple of weeks. The rotifers in such places generally have two periods of especial abundance, one in the spring, the other in the autumn. April-May and September-October are for the neighborhood of Washington the best times to collect these animals. This is also the time for the appearance of the males. For their capture a small net of what is known as "china silk" is very suitable. The lower end of the net should be tied around the neck of a wide-mouthed bottle. In this way the surplus water is filtered off; and the animals remain in the bottle in a small quantity of water, which is poured into a suitable bottle for transportation. If procurable, an aluminum tube, closed at the bottom, is preferable to the wide-mouthed bottle for use with the net, as it does not break on accidental contact with stones or other hard objects. No stagnant pool should be neglected, no matter how small or apparently impure. A number of species accommodate themselves to these conditions. Water with abundant organic matter, as, for instance, farm-yard ponds, is the favorite resort of certain species like *Hydatina senta*, in fact, wherever microscopic algæ are abundant. The plankton of swamps, lakes, and rivers may be collected by the usual methods, although the apparatus can be much simpler. This applies also to marine rotifers occasionally found in great abundance in littoral plankton.

The fixation of the collected material may without detriment be deferred a few hours, according to convenience. By the aid of a strong magnifier it is ascertained whether the rotifers are present in sufficient numbers to warrant the treatment. It is well to concentrate the animals in the smallest possible quantity of water, as the reagents are rather expensive. This is accomplished quite easily by exposing the collection in a glass jar to a one-sided illumination for half an hour. The rotifers soon assemble in a small, whitish cloud, easily visible to the naked eye, on the illuminated side, near the surface, and with a pipette they are transferred to a tube of 2 to 10 cubic centimeters capacity for fixation.<sup>1</sup>

The narcotizing operation consists in adding to this tube solution No. 1 in small portions, mixing well each time. The animals at first

<sup>1</sup>If apothecaries' measure is used, it may be noted that 1 cc. equals  $\frac{1}{8}$  fluid dram.

suspended in the water gradually sink and finally fall to the bottom, leaving the liquid clear; a sign that their cilia have ceased to move. At this moment they should be fixed, in order to retain their form; if killed before completely narcotized, they will contract. The doses and time intervals are: Every five minutes add from 1 to 3 drops of the cocaine solution for each cubic centimeter of water in the tube. After repeating this three times the narcotizing is usually finished. To acquire practice, it is advisable to go through the operation two or three times with a microscope at hand. Other animals, mainly Entomostraca, that may be present do not suffer at all from the narcotization, as they are also affected by the cocaine and sink to the bottom about the same time.

For fixation add to the tube 1 drop of the osmic acid mixture for each cubic centimeter of its contents and mix rapidly. This amount should not be exceeded or the animals will be strongly blackened. When they have settled to the bottom of the tube, in 5 or 10 minutes at the most, the liquid is carefully decanted and the tube refilled with water. This is repeated two or three times in a few hours at intervals according to convenience, finally filling the tube with formalin solution, 1 part commercial formalin (40 per cent formaldehyde) to 9 parts distilled water. For transportation it is well to use quite a small tube. A slip of paper with necessary data should be inclosed in each.

To obtain the nonpelagic forms, aquatic plants (not temporarily submerged plants), especially such as have finely divided leaves, *Batrachium*, *Myriophyllum*, *Ceratophyllum*, and also floating plants, as *Lemna*, *Riccia*, etc., should be brought home in a jar or tin can. They should then be put into a glass jar with sufficient water to cover them and left standing for two or three hours. All the animals gradually come to the surface and collect on the illuminated side. Direct sunlight should be avoided, as it liberates oxygen from the plants and it is the lack of this that drives them to the surface.

Quite an extensive fauna is to be found in mosses, *Sphagnum*, etc., and as the only treatment necessary for this is simply to pick a few handfuls of it and allow it to dry naturally, it is one of the easiest to obtain. It should be collected from both wet and dry places. The *Bdelloida* are equally at home in both, and if Hepatics, such as *Jungermannia*, *Frullania*, and others can be obtained, so much the better. The surface layer of mud in dried-up pools should be scraped off, well dried, but not heated, and finally stored in paper bags, if possible sterilized, which, when once closed, should not again be opened until arrival at the point of destination in order to avoid contamination. The samples should be guarded against too high temperature, laboratory fumes, etc., which are usually the reasons why the animals fail to revive.

This method is useful not only for rotifers, but most of the accompanying forms, Oligochætes, Planarians, Entomostraca, and even the smaller Nematodes, are obtained in good condition, as well as the majority of Infusoria, unicellular Algæ, and Flagellates. If Protozoa are the main object, the narcotization may be dispensed with, as it is injurious to certain delicate species. Finally, it may be added that the method of Rousselet, that is, narcotization, followed by fixation with dilute osmic acid, and final mounting in weak formalin, is the only known method for the preservation of all the small transparent, vacuolate animals—in other words, the great majority of pelagic forms, marine as well as fresh water. It yields results not attainable by any other method. The usual dehydrating and clearing agents ruin nearly all these delicate animals.

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The United States National Museum will be glad to receive material preserved in this manner and will arrange for its determination.