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R. O. Anderson
F. F. Hooper

INSTITUTE FOR FISHERIES RESEARCH
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UNIVERSITY OF MICHIGAN

GERALD P. COOPER PH.D.
DIRECTOR

ADDRESS
UNIVERSITY MUSEUMS ANNEX
ANN ARBOR, MICHIGAN

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A MODIFIED FLOTATION TECHNIQUE FOR SORTING BOTTOM FAUNA SAMPLES

by

Richard O. Anderson

A Modified Flotation Technique for Sorting Bottom Fauna Samples[1]

The removal of organisms from benthic samples that contain a large quantity of organic debris is well known as a tedious and time-consuming task. The difficulty is compounded when particularly small invertebrates are numerous. A flotation procedure utilizing a sugar solution has proved efficient for sorting benthic samples collected from a variety of substrates.

One of the first investigators to propose the use of a high-density solution for separating invertebrates from debris in samples was Ladell (1936). He utilized a solution of magnesium sulfate to remove insects and other arthropods from soil samples. This technique was adopted by Beak (1938) who devised an apparatus for sorting bottom fauna from samples collected in streams. Lyman (1943) described a "salting-out" procedure in which he used a saturated solution of sodium chloride. Caveness (1955) used a sugar solution for the separation of nematodes and their eggs from soils and plant tissues. Birkett (1957) used carbon tetrachloride for sorting living molluscs and invertebrates from debris consisting largely of shell.

The flotation principle has not been widely applied for sorting benthos samples except in situations where the substrate has been almost entirely inorganic. Moffett (1943) found the process practical for removing invertebrates from samples collected from a sandy, wave-swept shoal. Hunt (1953),

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in a study of the mayfly, Hexagenia limbata, reported that the "salting-out" procedure proved effective provided that residues were relatively heavier than the nymphs.

Specific gravity of organisms and debris.--Numerous tests have shown that the specific gravity of most organic debris (except fresh plant and algal material) in benthic samples is greater than 1.12. The specific gravity of most invertebrates is less than this value. When sample material is placed in a solution of this specific gravity, most of the invertebrates float to the surface and the bulk of organic material slowly sinks. This solution will float all organisms commonly encountered except insects with inorganic cases and molluscs.

Flotation time.--When using the flotation technique, the probability of recovering organisms is dependent, in part, on the length of time organisms remain at the surface. Animals shrink by fluid loss in any hypertonic solution and therefore increase in specific gravity. When the specific gravity of the invertebrates becomes greater than that of the solution, they sink unless retained by surface tension. The hypertonicity or osmotic pressure of different solutions at a given specific gravity is dependent on the molecular weight of the solute and the number of ions or molecules in solution. Therefore the flotation time should be longer in a sugar solution than in solutions of inorganic salts.

Tests were run to determine the difference in flotation time for various preserved organisms in solutions of calcium chloride (mol. wt. 111) and sucrose (mol. wt. 342). The specific gravity of each solution was 1.11. In order to prevent organisms from being retained by surface tension, a piece of clear plastic, bent to the shape of a U, was inverted into a finger bowl. In each test, the finger bowl was filled to a sufficient depth to submerge the plastic strip and 10 organisms of the group to be tested were then introduced into the solution underneath the plastic. The length of time that 50

per cent of the individuals remained floating up against the plastic was recorded as the flotation time. Tests were conducted with Tubificidae, the amphipod Hyalella azteca, larvae of the mayfly Caenis (2-3 mm long) and of the midges Tanytarsus juncundus (10-11 mm) and Polypedilum (3-4 mm).

The length of time that the different organisms floated in the different solutions varied widely (Table 1). Tubificid worms are very susceptible to shrinkage and therefore had a short flotation time, whereas the midges floated for an appreciable period. Flotation time in most instances was more than twice as long in the sugar solution than in calcium chloride.

The original specific gravity of organisms can be approximately reconstituted by soaking in water. This was demonstrated with a group of 10 Hyalella. In this test the 10 organisms were alternately placed in a sugar solution until all of them had sunk and then in water for 10 minutes. The length of time that 50 per cent of these individuals floated in the sugar solution in three successive trials was 8, 6, and 6 minutes.

Flotation of fresh versus preserved samples.--The flotation time for living organisms is longer than for preserved specimens. The advisability of sorting samples while the organisms are alive or after preservation is dependent upon the type of organisms present in the sample. Certain insect larvae (Trichoptera, Ephemeroptera, Odonata, and others) cling to debris when alive, and do not float at the surface. As a result, they may not be recovered quantitatively. Therefore when these organisms are common, samples should be preserved before sorting by flotation. However when aquatic Oligochaetes occur in large numbers, it is desirable to sort the samples while the organisms are still alive. (The flotation time for this group when preserved was the shortest of all forms encountered.) These

organisms remain alive for 10 to 15 minutes in the sugar solution and this increases the flotation time to approximately 20 minutes.

In one test, a sample, in which annelids were abundant, was floated-out alive. The organisms were counted, then put back with the debris, and the sample was preserved and floated-out again. The sample was soaked in water for 20 minutes between each flotation period. During the first live flotation, approximately 99 per cent of the annelids present in the sample were recovered (Table 2). After the sample was preserved, only 85 per cent of the worms could be recovered in three flotation periods. The shorter flotation time after preservation made efficient recovery difficult.

Efficiency of flotation.--In order to compare the efficiency of hand-sorting and flotation, two preserved samples were first floated-out, the organisms were counted, then put back with the debris. The samples were then sorted without the aid of flotation. In the two samples the total number of organisms recovered by hand-sorting amounted to 86 per cent and 64 per cent of the number removed during two flotation periods (Table 3). Each sample was sorted by flotation in less than one-fifth the time required for hand-sorting.

Flotation procedure adopted.--The following procedure has been adopted for sorting benthic samples. A sugar solution with a specific gravity of 1.12 is prepared (approximately 2.5 pounds of granulated sugar per gallon of solution). The specific gravity is determined with a hydrometer. The sample is drained as completely as possible through a sieve and placed in a 12- by 17-inch, white enamel dissecting pan. One-fourth of a quart to one quart of debris can be sorted at one time depending on the nature of the debris and the type and size of organisms in the sample. The material is flooded with 3 to 4 quarts of sugar solution and the debris is then

stirred and distributed evenly over the bottom of the pan. Organisms are removed from the surface with a fine-mesh wire scoop. When no more organisms can be found, the sample is stirred and all additional organisms which come to the surface are removed. The sugar solution is then decanted off through the sieve and the sample is covered with water. While the sample is in water, the material is carefully examined for molluscs, insects in cases, and individuals entangled in vegetation. A careful examination will ordinarily take at least 20 minutes. After this period the water is poured off through the sieve, the sugar solution is again added, and all additional organisms are removed. If a large number of organisms are found during the second flotation period, the sample is soaked and floated-out again.

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Table 1.--Flotation time in minutes for organisms in solutions of calcium chloride and sucrose with a specific gravity of 1.11

Organisms	Calcium chloride	Sucrose
Tubificidae	2	5
Hyalella azteca	4	9
Caenis sp.	20	30
Tanytarsus jucundus	17	45
Polypedilum sp.	23	90

Table 2.--Number of organisms removed from a bottom sample before and after preservation

	Organisms			Time required (minutes)
	Tubificidae	Tendipedidae	Others	
Alive				
Flotation 1	1,145	31	2	15
Flotation 2	15	1	0	10
Total	1,160	32	2	25
Preserved				
Flotation 1	570	29	1	10
Flotation 2	269	3	1	8
Flotation 3	145	0	0	7
Hand-sort	158	0	0	45
Total	1,142	32	2	70

Table 3.--Number of organisms removed from bottom samples by flotation in a sugar solution and by hand-sorting

	Organisms						Total organisms recovered	Time required (minutes)
	Hyalella azteca	Tendipedidae	Heleidae	Caenis	Trichoptera	Others		
Sample 1								
Flotation 1	270	93	14	24	19	8	428	20
Flotation 2	30	8	0	1	2	0	41	20
Total	300	101	14	25	21	8	469	40
Hand-sort	281	85	7	7	15	7	402	240
Sample 2								
Flotation 1	0	225	6	2	32	3	268	20
Flotation 2	1	28	6	0	3	1	39	20
Total	1	253	12	2	35	4	307	40
Hand-sort	1	176	5	0	7	7	196	300