

**"SORTING BENTHOS  
USING  
FLOATATION MEDIA"**

by

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FISHERIES RESEARCH BOARD OF CANADA

**TECHNICAL REPORT NO. 354**

**1973**



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SORTING BENTHOS USING FLOATATION MEDIA

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This is the Thirtieth FRB Technical Report from the  
Fisheries Research Board of Canada  
Freshwater Institute  
Winnipeg, Manitoba

1973

### Abstract

A comparison of the efficiency of sucrose (S.G. 1.10, 1.13) magnesium sulphate (S.G. 1.20), D-mannitol (S.G. 1.05) calcium chloride (S.G. 1.24) and sodium chloride (S.G. 1.18) indicated that live invertebrates floated longer than preserved, that 10% formalin preserved invertebrates floated longer than 10% alcohol preserved invertebrates and that there was variation in the floatation time between the various taxa. Sucrose solutions proved to be the most efficient media, however, since some organism e.g. stone-cased caddis larvae and molluscs did not float at all, the technique is considered inappropriate for general quantitative samples. Attempts to rehydrate the specimens in both preservative and water suggested that return to the original buoyancy was not achieved. Toxicity tests indicated that the chloride solutions used are acutely toxic to Chironomus tentans larvae, the remaining four solutions being varyingly toxic to the larvae but having little or no sub-lethal effect.

## Introduction

Quantitative sampling of benthos requires that each step, towards final analyses of the animals collected, be within certain predetermined limits of accuracy. Previous research at the Freshwater Institute into the first step in this procedure, the actual collection of the animals, has elucidated many of the drawbacks of commonly used benthos samplers (Flannagan, 1970), and has produced two improved benthos samplers (Hamilton, Burton and Flannagan, 1970; Burton and Flannagan, in press). The next step in the sampling procedure is the separation of the animals from the organic and inorganic constituents of the sample. This, perhaps the most tedious part of the whole procedure, has generally been carried out in two steps: sieving, followed by hand sorting. Many workers have used the "floatation method" (i.e. animals are floated off from the detritus using various aqueous solutions of specific gravity greater than that of the animals but less than that of the detritus) to minimize the time spent on this operation. Some, such as Kajak, Dusoge and Prejs (1968) have attempted to eliminate the sieving step by using the floatation technique directly on the sample, but generally the technique is carried out on sieved samples.

A variety of chemicals and concentrations have been used including sucrose, S.G. 1.11 by Anderson, 1959; S.G. 1.18 by Carveness and Jensen, 1955; various S.G. by Kajak et al., 1968,

magnesium sulphate, S.G. 1.11 by Ladell, 1936; S.G. 1.28 by Slack, 1965, and calcium chloride, a strong solution by Beak, 1938.

The present investigation compares floatation times for live and preserved animals of the various stages of representative taxa of aquatic invertebrates, in various solutions. It examines the effect these chemicals have on the mortality of the chironomid larvae, Chironomus tentans in order to establish whether the technique is useful for sorting larvae required for rearing purposes. The rehydration procedure used by Anderson (op. cit) for double checking efficiency of this sorting technique is also investigated.

#### Methods

Detritus may adhere to the animals, interfere with their floatation properties, and cloud up the water making observations difficult. Accordingly, these experiments were carried out using animals only and no detritus. Six solutions were made up as follows: D-mannitol, S.G. 1.05, sodium chloride, S.G. 1.18, Magnesium sulphate, S.G. 1.20, Sucrose S.G. 1.10 and 1.13, and calcium chloride, S.G. 1.24.

The various solutions were then introduced into sets of 6, 16 fl. oz, wide mouthed jars to a depth of 7 cm, (Kajak et al., op. cit. showed that if an insufficient "height" of fluid is used a blanketing effect occurs because of crowding

of the animals) a drop of liquid detergent being added to each jar to minimize surface tension effects. Solutions were renewed after each experiment.

About three months before the experiments commenced a field collection was made from the Assiniboine R., Manitoba and the animals collected, together with second, third and fourth instar chironomids from a laboratory culture were divided into two groups; half being preserved in 70% alcohol and half in 10% formalin. The day before the experiment a further collection was made from these two locations to provide live animals.

A series of experiments were then set up as shown in Table 1. Two basic observations being made a) the time taken for the first animals in the container to sink and b) floatation time as defined by Anderson (op. cit.) - the time required for 50% of the organisms to sink, to a maximum of thirty minutes, which was regarded as ample time to sort a sample. From this table it can be seen: that floatation time is not directly dependant on specific gravity, is perhaps most efficient with live animals, and varies with preservative and different taxa.

Results using caddisflies (Trichoptera) with stone cases, Gastropods and Sphaerids (Sphaerium sp.) are not included in this report because none of the solutions were of sufficiently high specific gravity to float these animals. This is in accordance with the findings of most other workers referred to

Table 1. Flootation time (minutes) for live, alcohol preserved and formalin preserved animals in the various solutions a) is time required for one animal to sink b) time for 50% of animals to sink.

		D- mannitol	S.G. 1.05	sucrose	S.G. 1.10	sucrose	S.G. 1.13	sodium chloride	S.G. 1.18	magnesium sulphate	S.G. 1.20	calcium chloride	S.G. 1.24	number of animals used per jar
		a)	b)	a)	b)	a)	b)	a)	b)	a)	b)	a)	b)	
<u>LIVE ANIMALS</u>														
2nd instar	<u>Chironomus tentans</u>	10 <sup>1</sup>	15	>30	-	>30	-	10	10	>30	-	15	>30	16
3rd instar	<u>C. tentans</u>	>30	-	>30	-	>30	-	15	15	>30	-	>30	-	24
4th instar	<u>C. tentans</u>	>30	-	>30	-	>30	-	15	15	>30	-	>30	-	24
Ephemeroptera	<u>Baetis sp</u>	10	30	>30	-	>30	-	30	>30	>30	-	>30	-	9
	<u>Heptagenia sp.</u>	25 <sup>1</sup>	>30	>30	-	>30	-	>30	-	>30	-	>30	-	6
Plecoptera	<u>Acroneuria sp.</u>	3	3	>30	-	>30	-	>30	-	>30	-	>30	-	8
Hemiptera	Corixids	no live material collected												
Trichoptera	Caseless	>30	-	>30	-	>30	-	>30	-	>30	-	>30	-	8
	Cased	no live material collected												
	Mean	19.7	24	30	30	30	30	22.9	22.9	30	30	27.9	30	
<u>PRESERVED-10% FORMALIN</u>														
2nd instar	<u>C. tentans</u>	20	>30	>30	-	20	>30	5	10	20	>30	5	10	30
3rd instar	<u>C. tentans</u>	>30	-	>30	-	>30	-	10	15	>30	-	10	15	25
4th instar	<u>C. tentans</u>	>30	-	>30	-	>30	-	15	20	>30	-	15	20	14
Ephemeroptera	<u>Baetis sp.</u>	<1	<1	30	>30	>30	-	15	>30	>30	-	25	>30	10
	<u>Heptagenia sp.</u>	<1	<1	>30	-	>30	-	20	>30	>30	-	>30	-	10

Plecoptera	<u>Acroneuria</u> sp.	<1	<1	15	>30	>30	-	>30	-	>30	-	>30	-	15
Hemiptera	Corixids	<1	<1	>30	-	>30	-	>30	-	>30	-	>30	-	10
Trichoptera	Caseless	>30	-	>30	-	>30	-	>30	-	>30	-	>30	-	3
	Cased <sup>2</sup>	>30	-	>30	-	>30	-	>30	-	>30	-	>30	-	3
	Mean	16	17.1	28.3	30	28.9	30	20.6	25	28.9	30	22.8	25	

PRESERVED 70% ALCOHOL

2nd instar	<u>C. tentans</u>	3	5	5	10	5	15	1	3	3	15	3	5	40
3rd instar	<u>C. tentans</u>	3	10	20	>30	10	>30	5	10	15	>30	10	15	25
4th instar	<u>C. tentans</u>	5	10	15	>30	15	>30	5	10	20	>30	10	>30	13
Ephemeroptera	<u>Baetis</u> sp.	15	20	10	>30	25	>30	10	15	25	>30	5	>30	9
	<u>Heptagenia</u> sp.	15	25	10	>30	20	>30	15	20	15	>30	5	>30	9
Plecoptera	<u>Acroneuria</u> sp.	20	>30	>30	-	>30	-	30	>30	>30	-	>30	-	15
Hemiptera	Corixids	>30	-	>30	-	>30	-	>30	-	>30	-	>30	-	10
Trichoptera	Caseless	>30	-	>30	-	>30	-	>30	-	>30	-	>30	-	3
	Cased <sup>2</sup>	25	30	>30	-	>30	-	>30	-	>30	-	>30	-	3
	Mean	15.6	21.1	20	27.8	21.7	28.3	17.2	19.78	22	28.3	17	25.6	

<sup>1</sup> These specimens were able to swim to the bottom or sides of the jars and hold thus the times given in the first column are not accurate.

<sup>2</sup> These results are only from caddis larvae with organic cases, experiments with larvae with stone cases were totally unsuccessfully, as were experiments with large sphaeriids (Sphaerium sp.) and gastropods.

in this paper.

A second series of experiments (Table 2) was set up as before using the 4th instar chironomids from the 10% formalin and 70% alcohol experiments described above. These animals were rehydrated in preservative for one hour, tested, then transferred to water for one hour, then retested. From the results it can be seen that this technique will give an extra few minutes of floatation and that either rehydration in water is less efficient than in preservative or successive rehydrations are less efficient.

A third series of experiments was carried out to investigate the effect of length of immersion time in the various solution on the mortality of C. tentans. Sixty-four larvae were immersed in each of the 6 solutions plus an additional 64 in water to act as a control. Sixteen specimens were removed from each of the seven jars at 3, 5, 10 and 15 minutes after immersion, transferred to aerated water and mortality after 24 and 48 hours recorded. The results (Table 3) show a decided increase in mortality with increase of immersion time in most of the solutions.

#### DISCUSSION

Anderson (1959) suggested that although initial floatation is dependent on the specific gravity of the fluid used, the floatation time is more closely related to the osmotic pressure

Table 2. Floatation times (in minutes) for preserved 4th instar C. tentans with two rehydration procedures a) being time to sinking of 1st animal, b) time to sinking of 50% of animals.

S.G.	D-mannitol		sucrose		sucrose		sodium chloride		magnesium sulphate		calcium chloride	
	1.05		1.10		1.13		1.18		1.20		1.24	
	a)	b)	a)	b)	a)	b)	a)	b)	a)	b)	a)	b)
70% preserved original floatation (Table 1)	5	10	15	>30	15	>30	5	10	20	>30	20	>30
after rehydration, alcohol	3	10	15	25	3	25	3	3	10	15	10	15
after rehydration, water	1	15	10	25	10	25	3	10	15	25	3	3
10% formalin preserved original floatation (Table 1)	>30	-	>30	-	>30	-	15	20	>30	-	15	20
after rehydration, 10% formalin	8	25	15	25	20	25	7	20	25	>30	7	20
after rehydration, water	3	25	20	25	5	15	5	10	10	25	5	10

Table 3. Percentage survival of *C. tentans* after immersion in the various floatation solutions.

		Control <sup>1</sup> (aerated water)	D- mannitol <sup>1</sup>	sucrose <sup>1</sup> 1.10	sucrose <sup>1</sup> 1.13	sodium chloride	magnesium <sup>1</sup> sulphate	calcium chloride
% survival immersion	3 mins	24 hrs 100	100	100	100	100	100	75
	48 hrs	100	100	100	93.75	93.75	93.75	50
% survival immersion	5 mins	24 hrs 100	100	100	100	93.75	93.75	19
	48 hrs	100	100	100	87.5	87.5	87.5	6.25
% survival immersion	10 mins	24 hrs 100	100	100	100	37.5	93.75	0
		48 hrs 100	93.75	93.75	87.5	25	81.25	0
% survival immersion	15 mins	24 hrs 100	100	100	100	0	81.25	0
	48 hrs	93.75	87.5	93.75	81.25	0	75	0

<sup>1</sup> specimens from these solutions reared to imagines

of that fluid. He also suggests that since sucrose has a high molecular weight it should have a floatation time longer than that for most inorganic salt. The expected success of the various solutions should thus be in the order: sucrose 1.10, sucrose 1.13, D-mannitol, magnesium sulphate, calcium chloride, sodium chloride. In all of the experiments it was immediately obvious that the D-mannitol was of insufficiently high specific gravity to cause the initial floatation to occur. Calcium and sodium chloride performed rather poorly as was expected. However, there was little difference between the remaining three-solutions, sucrose 1:10 perhaps being a little too dilute to function efficiently.

The results (Table 1) indicate that live material "floats" longer than preserved and that material preserved in 10% formalin "floats" longer than that preserved in 70% alcohol. This, too, is perhaps not unexpected since according to Howmiller (1971) weight loss of preserved specimens is larger in 70% alcohol than in 10% formalin. It would also seem reasonable from Howmiller's results to expect material preserved in 100% formalin to respond in the same manner as live material. Since he found that material preserved in 100% formalin did not undergo any significant weight loss.

It should also be noted that the individual groups responded differently to particular solutions and that this appears to be correlated to the cuticle permeability and/or surface area to body volume ratio of the given animal. The

mayfly Heptagenia which show a much higher degree of dorso-ventral compression apparently floating for a longer time than the fish-shaped Baetis, also animals with thick, relatively impermeable exoskeletons such as the Acroneuria sp. and the corixids appear to float longer than the thin "skinned" chironomids.

From the second series of experiments (Table 2) it is obvious that the rehydration technique is of limited value, since the time available for a second picking over of a sample would be restricted. A further experiment in which rehydration with water is carried out before rehydration with the preservative, would solve the question of whether or not successive rehydrations are less efficient.

Most of the families of aquatic insects are so inadequately described in the immature stages that imagines are required for positive species identification. The floatation technique has been used to provide large number of larvae, in a short time, for rearing to adults and it would therefore be useful to know which fluids are least toxic to aquatic insects and what length of time the larvae can be safely immersed in any given solution. Table 3 lists the results of some acute toxicity tests carried out on C. tentans with the six floatation fluids. From these results it can be seen that sodium and calcium chlorides are totally unsuitable for this purpose and the use of magnesium sulphate would be somewhat suspect. Of the remaining three solutions sucrose 1.10 would apparently

be the most suitable since D-mannitol is not a very efficient floatation solution while sucrose 1.13 appears to be slightly toxic. Since imagines were reared from all four of these solutions it seems reasonable to assume that there were no serious sublethal effects, however, the possibility of live, but deformed, imagines being produced must still be considered.

### Conclusions

The experimental results clearly show that live material responds better to the floatation technique than does preserved material providing a dense enough solution is used, and that material preserved in 10% formalin responds better than that preserved in 10% alcohol. They also indicate that the rehydration technique is useful to a limited degree, but does not return the animals to their original "floatability" even after prolonged immersion in water. Survival of animals after floatation varies from solution to solution and with immersion time but several solutions are apparently (at least within the immersion times used) reasonably safe. The inability of these solutions to float insects with stone cases and molluscs means that for general quantitative samples the method is inadequate. However, in situations where these animals are either not present or not important and in qualitative samples, there would appear to be a place for this technique.

Sucrose seems to be the best all round floatation medium, the specific gravity being chosen according to individual requirements.

#### Acknowledgements

I am much indebted to Miss Janet Bach, summer assistant, who assisted with the floatation and toxicity tests and to Drs. O.A. Saether and R.D. Hamilton for reading and providing useful criticisms of this manuscript.

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