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APPLICATION OF THE FLOTATION TECHNIQUE TO ASSESSMENT OF ABSOLUTE NUMBERS OF BENTHOS

The authors demonstrate that flotation in a sugar solution of unrinsed and unpreserved samples ensures, when certain conditions are maintained, that almost 100% of the individuals in all stages of development are collected from extra-littoral ooze habitats. This method also permits of obtaining live material for experiment. Other solutions used for flotation – NaCl, $MgSO_4$, waterglass, cause a high degree of mortality among the organisms, and make it impossible to ascertain the full numbers.

The fact is generally known that benthos methods, in particular sorting benthos material, are extremely laborious and this is the reason why a constant search is made for new methods and solutions to this problem. One of the means employed is the flotation method, which has been known for a fairly long time in its application to investigations of both soil (Caveness and Jensen 1955, Teal 1960) and benthos (review of literature – Žadin 1956, Anderson 1959, Schwoerbel 1966) although not as yet in general use. There are very few studies containing accurate quantitative data or investigations of the reliability of the method. For instance, considerable deviations are exhibited by the data given by different authors on the time that the organisms float on the surface of the solution. This is probably due to lack of exactitude in presenting results; the differences in floating time of different

individuals, even those belonging to the same species, may be very considerable. The paper by Anderson (1959) recommending flotation in a sugar solution and containing an evaluation of the reliability of this method (unfortunately based on a small amount of material) constituted an important step forward. One of the aims of the present study was to evaluate this method on the basis of a large amount of material from different habitats. More attention was paid to a different aspect of benthos methods. It is a known fact that investigation of only certain stages of development, omission or incomplete quantitative treatment of the youngest stages measuring only a few millimetres, form a serious deficiency in studies on benthos. Some degree of progress in this field has recently been noticeable, namely the use of fine sieves of $0.2 \times 0.2 - 0.25 \times 0.25$ mm mesh (Jonasson 1955, Mundie 1957, Kajak 1958, 1963[1]). Judging by the dimensions of the youngest stages of several species of macrobenthos, even the use of such fine sieves as those described does not permit of grasping their total abundance. The use of very fine sieves creates serious problems in sorting large amounts of material. By using fine sieves the amount of material would be even further increased, and the sediment is hardly rinsed out at all since the openings in the sieve are blocked. It is of course possible to get round this by mechanical shaking etc. but this is not a method for general use. In addition when sorting material rinsed through very dense sieves a great difficulty arises with quantitative selection of organisms, even when optical instruments are used.

The basic aim of this study was to show whether the flotation method applied to samples not rinsed through a sieve makes it possible to obtain the absolute numbers of all stages of development of macrobenthos. The possibility of quantitative assessment of all, including the youngest, stages of development of macrobenthos would appear to be the more important on account of the fact that contrary to zooplankton, in the case of many of the dominating forms of benthos it is impossible to make an exact estimate of the number of eggs; thus when it is desired to analyse variations in numbers over the development cycle it is necessary to take the youngest stages of development, hatched from the eggs, as a point of reference. In addition attention has been paid in this study to the question of survival of the organism after flotation in connection with the experimental work done (Kajak and Dusoge 1967, Kajak, Dusoge and Stańczykowska 1968).

The objectives of this study were therefore as follows:

– to check the reliability of the flotation method, using a large amount of material and applying different solutions, particularly sugar solutions, and to analyse many of the aspects of this method (concentration of solution, ratio of height of the layers of solution and ooze etc.);

[1] Recently the use of the elutriation method (Lauff et al. 1966) was also suggested, but it has not as yet been sufficiently checked. The same applies to the method of driving down the microfauna by cooling the sample from the top (Uhlig 1964).

– to examine the possibility of applying this method to material not rinsed through a sieve, in order to obtain a quantitative picture of all the benthos organisms regardless of their size;

– to analyse the possibility of obtaining whole live natural benthos communities, in good condition, for experimental purposes.

Flotation was carried out in glass aquaria 10 cm high, the base measuring 15 × 10 cm. Part of the sample was placed in the aquarium, ensuring that the layer of ooze was not more than 1 cm thick, then the sugar solution was poured in. The initial concentration of the solution was empirically determined on the basis of repeated measurements of the differences of solution density before and after combining it with the sample. In methodical analyses the concentration of the solution was checked after flotation. The contents of the aquarium was stirred and all the organisms floating to the surface of the solution removed. Material was usually floated simultaneously in several aquaria placed near each other, which made it possible to float the whole sample at one time. As it was necessary to wait a little while after stirring the contents of the aquarium until the suspension had settled, sorting carried out in several aquaria simultaneously proved a great time-saver. The use of several small aquaria instead of one large one seems to make it easier to descry the organisms. The sample was stirred several times.

In all the experiments either part or the whole of a sample was examined after flotation under a stereoscopic microscope to check whether any organisms had remained in it. After flotation the sample was rinsed on a very fine sieve (0.05 × 0.05 mm), which makes the organisms easier to see under the microscope (Stańczykowska 1966). The operation recommended by Anderson (1959) of repeatedly placing the sample in water (in order to allow the organisms to regain their original specific gravity) was not used on account of the amount of time required for such manipulation.

The work was carried out on material obtained from the sublittoral and profundal zones of the lakes. In the habitats we examined there were no molluscs or caddis fly larvae with their houses, to sort which the flotation method is of course unsuitable.

I. NUMBERS OF BENTHOS USING SIEVES OF DIFFERENT FINENESS AND FLOTATION OF UNRINSED MATERIAL IN A SUGAR SOLUTION

1. Comparison of material rinsed and not rinsed through sieves

It is obvious that only part of the benthos is caught by means of coarse sieves, and many authors have drawn attention to this (Jonasson 1955, 1958, Mundie 1957, Kajak 1958). It proved that even the use of a relatively fine sieve (mesh 0.4 × 0.4 mm) gives numbers of *Chironomidae* larvae several

Comparison of numbers of *Chironomidae* in unrinsed material floated in sugar solution (A) and material rinsed through sieves with mesh of different fineness: 0.2 × 0.2 mm (B) and 0.4 × 0.4 mm (C) sorted in the traditional way

Tab. I

Lake	Series number	Depth (in metres)	Numbers (per 1 m²) A	Ratio of numbers	
				A/B	A/C
Mikołajskie	I	4	4,650	2.2	–
	II	4	6,500	1.4	–
Śniardwy	III	5	8,000	0.9	–
	IV	5	4,100	1.1	–
	V	6	8,100	2.0	3.9
	VI	7	4,300	1.4	4.8
Mikołajskie	VII	16	1,100	–	2.2
Śniardwy	VIII	6	3,900	–	1.9
	IX	6	32,400	–	20.1
	X	4	6,450	–	3.5

Total numbers of *Chironomidae* and percentage of individuals in the class up to 3 mm in length, in material unrinsed and floated in a sugar solution, and rinsed through sieves of different fineness of mesh

Tab. II

Lake	Total numbers (individuals/m²) in unrinsed material	Percentage of ⩽ 3 mm class of length		
		Unrinsed samples	Samples rinsed through 0.2 × 0.2 mm mesh sieve	Samples rinsed through 0.4 × 0.4 mm mesh sieve
Mikołajskie	320	54	37	22
Tałtowisko	6,500	80	65	50
Śniardwy	485	63	50	17

times smaller, and when there is domination of small-dimension species (series IX), twenty times smaller than those obtained by floating unrinsed material (Tab. I). Also very dense sieves (0.2 × 0.2 mm) usually give numbers 1.5–2.0 times smaller. The participation of forms with small dimensions in materials elaborated by different techniques differs very considerably (Tab. II). The numbers of other groups of organisms: *Ephemeroptera, Heleidae, Trichoptera* and *Nematoda* exhibited analogical regularity to that of the numbers of *Chironomidae,* when rinsed and unrinsed materials were compared. *Oligochaeta,* however, exhibited greater variability; lower numbers were sometimes observed in unrinsed material than in material rinsed through a sieve. This is probably

Rapidity of sinking of *Tubificidae* in 1.125 g/ml concentration sugar solution

Material preserved in 4% formalin solution, floated 10 days after preserving; 20 individuals in each series

Tab. III

Flotation time (in minutes)	Percentage of individuals sinking to the bottom
1	0
7	10
10	30
13	60
16	65
20	100

due to the frequently observed great unevenness of the spatial distribution of this group of invertebrates. The frequently lower numbers of *Oligochaeta* in floated unrinsed samples was not due to the poorer floating properties of these organisms, since even preserved individuals floated for a sufficiently long time in sugar solution (Tab. III), longer than would appear from Anderson's data (1959). Possibly the concentration of the solution which he used (1.11 g/ml) was too weak.

2. Comparison of the effectiveness of floating live and preserved materials

The effectiveness of flotation of live material not rinsed through a sieve is generally several times greater than preserved material. It is sometimes very considerably greater, e.g. for *Chironomidae* from several to twenty times greater; some groups, such as, e.g. *Nematoda*, despite their fairly great numbers in live material, were not found at all in preserved material (Tab. IV).

In experiments with live and preserved representatives of *Tubificidae* placed in the ooze shortly before flotation their capacity for floating to the surface was 100% in both cases. Keeping dead individuals in ooze for one day caused a certain degree of decomposition, but did not reduce the degree to which they floated to the surface during flotation. Neither were differences found in the floating capacity or length of floating of whole or fragmented, and live and preserved individuals, in experiments without ooze.

All these facts would appear to indicate that the differences found in the numbers of benthos organisms when floating live and preserved material obtained from natural conditions, are not due to a change in the floating capacity of

Effect of preserving samples in 4% formalin solution on estimate of numbers of organisms in material not rinsed through a sieve, sorted by the sugar solution flotation method

N – numbers of organisms (per 1 m²) in unpreserved material; R – ratio of numbers of organisms in unpreserved material to numbers in preserved material; symbol „–" indicates absence of given group in one or both compared variants; I–V – series numbers

Tab. IV

Taxonomic group	Lake and depth									
	Mikołajskie (4 m)				Śniardwy (6 m)				Mikołajskie (16 m)	
	I		II		III		IV		V	
	N	R	N	R	N	R	N	R	N	R
Chironomidae (total numbers)	4,790	1.2	9,530	2.0	21,400	2.0	5,440	1.2	605	1.6
Chironomidae (< 3 mm)	2,700	1.2	8,020	2.4	16,580	3.1	1,790	–	25	–
Oligochaeta	2,790	2.6	470	1.2	860	26.4	4,535	7.0	2,605	2.5
Hydracarina	47	9.3	–	–	116	–	46	–	–	–
Trichoptera	560	2.4	58	1.0	60	0.9	–	–	–	–
Ephemeroptera	325	3.5	230	0.7	30	–	–	–	–	–
Nematoda	140	3.0	810	–	116	–	23	–	–	–

the animals following preservation, but to the fact that these organisms under natural conditions live in cylindrical cases or have particles of ooze adhering to them which increase their weight. The live individuals leave their cases under the irritating action of the sugar solution[2] and float up to the surface of the solution, and as a result almost 100% of them are caught. In the case of some other forms (dragonflies, mayflies etc.), which are capable of actively clinging to the bottom, it is possible that Anderson's recommendation (1959) that material should be refloated after preservation is justified.

The above data indicate that the flotation of unpreserved material is useful if it is desired to obtain a complete absolute number of organisms. Since it is not always possible to sort the organisms when taking large series of samples, it is important to ascertain how long material can be kept in the live state without serious changes in the numbers of organisms. It was found that when material was kept at room temperature the changes in numbers taking place during the first and even the second day are relatively small (Tab. V). As from the third day changes are generally very considerable, although it does sometimes happen that even on the fifth day numbers remain only very slightly changed (Kajak, Dusoge and Stańczykowska 1968). It is important that for two days no differences were found in the numbers of organisms kept

[2] If the sugar solution alone is not sufficient a small amount of weak acid solution can be added for this purpose.

Variations in numbers of organisms in unrinsed material kept for 2 days at room temperature

Numbers indicate mean numbers of individuals per sample; series of 10 samples each with area of 45 cm² (total 50 samples); unpreserved material was floated in a sugar solution with 1.12 g/ml concentration

Tab. V

Taxonomic group	Number of hours since sampling				
	4	7	10	25	44
Chironomidae	22.0	23.6	24.6	23.3	25.6
Oligochaeta	17.7	20.8	19.7	19.6	19.8

in jars between samples with undisturbed structure[3] and samples in which the ooze had been disturbed. The material can be kept for 3 days at a temperature of +4°C without any changes in numbers taking place.

As stated above, the effectiveness of flotation of live material is very high. Examination of material after flotation showed that not more than 0.5% of the macrobenthic organisms remain in the sediment. On the other hand the groups considered as microbenthos: *Copepoda*, *Nematoda* and others, only partially float to the surface. The flotation method cannot therefore form a quantitative method for these organisms and samples must be examined under a stereoscopic microscope (Stańczykowska 1966).

The conclusion that almost 100% of the macrobenthos is obtained by flotation of live material applies to ooze habitats. In the case of material from clayey habitats to which the flotation method is applied, only a certain percentage of the organisms floats up, and this method cannot therefore be applied to such material. With materials from this type of bottom only 19.4—42.9% of the organisms were obtained. Also in cases where there is a large amount of plant detritus in the sediments flotation is difficult, as the detritus floats up to the surface of the solution and makes it more difficult to descry the benthos.

3. Effectiveness of flotation with different ratios of height of floated sediment layer and flotation solution and with different concentrations of the solution

The final problem to which attention was directed when analysing effectiveness of flotation was the ratio of height of the two layers – the floated sediment with organisms and the flotation solution. This ratio cannot be too

[3] Samples of undisturbed structure are obtained by using the experimental cylinder technique (Kajak, Kacprzak and Polkowski 1965, Kajak 1966).

Dependence of effectiveness of flotation on the ratio of height of ooze layer and height of flotation solution layer
In all 12 experiments were made, with total number of approximately 1,300 individuals; sugar solution with 1.12 g/ml concentration was used

Tab. VI

Ratio of height of ooze layer to height of flotation solution layer	Percentage of individuals floating to the surface	
	mean	range
1 : 2.5	78.8	68.2– 85.2
1 : 5.0	95.2	90.4– 97.7
1 : 10.0	97.9	95.8–100.0

Effectiveness of flotation (% of individuals floating up) with different concentrations of the solution and different ratio of height of ooze layer and flotation solution layer
A total of 20 experiments were made, with total number of approx 1,000 individuals; flotation in sugar solution

Tab. VII

Ratio of height of ooze layer to height of flotation solution layer	Concentration of flotation solution (in g/ml)						
	1.10	1.12	1.14	1.16	1.10	1.12	1.16
	Live material				Preserved material		
1 : 2.5	80.4	78.8	94.7	–	36.0	–	–
1 : 5.0	90.6	95.2	–	95.3	68.4	83.7	57.7

low (Tab. VI). Presumably when the layer of solution is too thin and in consequence the suspension very dense when the sample is stirred, the sedimenting suspension simply ,,blankets" part of the organisms, preventing them from floating to the surface.

The effect of the height of the flotation solution layer differs with different concentrations of the solution (Tab. VII). When floating live materials the greater concentration of the solution has a favourable effect on the "floatability" of the organisms, evening up the unfavourable effect of the thin solution layer. The matter becomes more complicated when preserved material is used. It is probable that the questions of favourable and unfavourable effect of the stronger solution overlap here: on the one hand better "floatability" of the organisms and on the other their more rapid soaking with the solution.

II. EFFECT OF FLOTATION ON THE SURVIVAL OF THE BENTHOS

1. Survival after flotation in solutions of different substances

The question of the effect of flotation on the survival of benthos was examined in order to check whether this method can be used for obtaining different benthic organisms quickly for experimental purposes. This is important when, inter alia, experimenting with a natural community of organisms under conditions similar to natural ones (Kajak 1966, Kajak and Dusoge 1967). The flotation technique permits of more delicate sorting of the benthos organisms than with other methods, the material is sorted far more quickly and, very important, all size groups of the macrobenthos are obtained.

When comparing the influence of different substances on the survival of *Tubificidae* it was found that the action of sugar differs significantly from the majority of the other most frequently applied substances. After floating *Tubificidae* for 10 minutes in a sugar solution with concentration of 1.13 g/ml the survival after 5 days was 100%, whereas flotation in solutions of other substances: NaCl, $MgSO_4$, waterglass (recommended by Hell 1960) causes 100% mortality within the first hour. Live *Tubificidae* underwent fragmentation in the waterglass solution.

Chironomidae exhibited far greater resistance. After flotation in a NaCl solution survival after 3 days was: for small forms (up to 6 mm) – 31%, for large forms (mainly those of the genus *Chironomus*) – 8.8%. When floated in a 1.125 g/ml sugar solution the survival of *Chironomus anthracinus* after 4 days was 100% (whether floated for 10 or 20 minutes).

2. The importance of flotation time, concentration of solution and condition of the organisms to their survival after flotation

The complete harmlessness of the sugar solution to *Tubificidae* demonstrated above, applied to individuals in good condition. Samples were taken from an aquarium with well-oxygenated water, filled with ooze obtained from a lake. The effect of the sugar solution on *Tubificidae* in bad condition, kept for several days without ooze in tap water with a slow through flow, was completely different. In this case survival after one day was only 40% (with flotation time 10 minutes). After being kept for 20 minutes in the sugar solution survival was only 20% after one day (as early as 10–60 minutes after flotation from 40–80% of the individuals died).

The use of different concentrations of the sugar solution showed that with 10-minute flotation the range of concentration used did not affect survival

Survival of *Tubificidae* (%% in relation to initial numbers) after 5 days from moment of flotation in sugar solution, depending on concentration of solution and flotation time

Initial numbers – each 20 individuals 20–25 mm long; individuals in good condition (from long-term culture in aquarium with ooze)

Tab. VIII

Concentration of solution (in g/ml)	Survival after flotation:	
	10-minute	20-minute
1.10	100	90
1.13	100	60
1.16	100	20

Effect of flotation time on survival of *Tubificidae*

Concentration of sugar solution – 1.12 g/ml; initial numbers – 20 individuals each

Tab. IX

Flotation time (in minutes)	Percentage of live *Tubificidae* after one hour from end of flotation
5	100
8	100
12	100
20	70
40	0

Survival of *Tubificidae* of different size (in %% in relation to initial numbers) after 5 days from moment of flotation, depending on flotation time

Concentration of sugar solution – 1.125 g/ml; individuals in good condition

Tab. X

Classes of size (in mm)	Survival after flotation:	
	10-minute	20-minute
2–8	95	35
8–16	100	70
> 16	95	80

Effect of flotation time on survival of *Tubificidae* of different size, in bad condition (A – kept for 3 days, B – for 5 days in vessels without ooze, in tap water)
Concentration of sugar solution – 1.125 g/ml; initial numbers – 20 individuals each

Tab. XI

Size of individuals (in mm)	Condition	10-minute flotation		20-minute flotation	
		Survival (in %%) after			
		24 hours	5 days	24 hours	5 days
2–6	A	100	–	60	–
	B	60	60	25	25
6–12	A	90	–	30	–
	B	75	75	30	30
>12	A	70	–	10	–
	B	44	39	5	0

Effect of flotation time on survival of *Tubificidae* in bad condition (kept for 5 days in vessels without ooze, in tap water)
Concentration of sugar solution – 1.16 g/ml; initial numbers – 20 individuals each

Tab. XII

Flotation time (in minutes)	Survival (in %%) after		
	2 days	3 days	5 days
10	45	30	15
20	10	5	0

after 5 days, but with 20 minute flotation the unfavourable effect of greater concentrations of the solution was very distinct (Tab. VIII). With 40 minute flotation time 100% mortality occurred after one hour (Tab. IX). When kept for longer in the flotation solution the older individuals exhibited greater resistance than the younger (Tab. X). This finding applies to *Tubificidae* in good condition. Material in bad condition in general exhibited a lower survival rate and reverse dependence on size of the individuals: the younger survived better than the older individuals (Tab. XI). In addition the mortality of individuals in bad condition, floated in a solution with higher concentration, increased with time (Tab. XII), whereas the mortality of individuals in good condition was negligible throughout the whole of the period analysed (Tab. VIII) (in both cases – with 10-minute flotation).

III. RECOMMENDED METHODS

The procedure elaborated on the basis of the present study for materials taken for the purpose of estimating numbers was as follows:

The upper layer, several centimetres thick, of the sample was floated without previously sifting it through a sieve, in order to obtain all the developmental stages of the organisms. The remainder of the sample was sifted through a sieve (mesh 0.4 × 0.4 mm) and sorted in the traditional way, by picking out the fauna after pouring the sample into a flat dish, or also by the flotation method.

Flotation was carried out in small aquaria (described above), using 1 cm layer of ooze and about 8 cm layer of solution above the ooze. A sugar solution with initial concentration of 1.13–1.14 g/ml was used, so that the concentration after mixing with the sample was not less than 1.125 g/ml. The way in which the organisms were removed was described above. Of course in cases of occurrence of organisms with high specific gravity such as molluscs, caddis larvae in their houses, or *Tubificidae* cocoons, the material must be sorted in the traditional way after flotation and sifting through a sieve.

The sugar solution can be used again for flotation after straining it through a fine net. A centrifuge supplied with fine net can be used for this purpose. In the case of flotation of live materials the sugar solution was kept in a refrigerator, as it otherwise quickly fermented.

CONCLUSIONS

1) Flotation in a sugar solution of 1.12 g/ml concentration of live material not rinsed through a sieve ensures that almost 100% of the organisms in extralittoral ooze habitats are obtained. This method does not, however, give good results in habitats with firm consistency of sediments (clay etc.) or with a large amount of plant detritus, and also of course for organisms with high specific gravity (molluscs, caddis larvae in their houses etc.). Greater concentration of solutions can be applied if this does not cause a decrease in transparency of the solution by maintaining too large a number of particles in the suspension.

2) Effectiveness of flotation of preserved material is far smaller and more variable than in the case of live material (not preserved).

3) Keeping material in room temperature for one day, and at a temperature of 4°C for 3 days, does not result in differences in the numbers of organisms obtained by flotation of live, unrinsed materials, even if the structure of the samples is disturbed.

4) It is necessary to ensure that the layer of flotation solution is sufficiently high – 7–10 times higher than the layer of sediment floated. Too thin a layer greatly reduces the effectiveness of flotation.

5) When individuals of *Tubificidae* and *Chironomidae* in good condition are floated there is no significant effect on their survival, if we use appropriate concentration of the solution and the flotation time is not too long. This makes it possible to obtain natural communities of benthos for experimental purposes. Other substances, such as NaCl, $MgSO_4$ or waterglass, caused very high mortality, particularly in the case of *Oligochaeta*.

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ZASTOSOWANIE TECHNIKI FLOTACYJNEJ DO OCENY BEZWZGLĘDNEJ LICZEBNOŚCI BENTOSU

Streszczenie

Postawiono sobie za cel wypracowanie metody oceny bezwzględnej liczebności organizmów bentosowych, która to ocena jest konieczna przy szczegółowych badaniach nad dynamiką liczebności, jej przyczynami i mechanizmami. Jak wiadomo, sita o powszechnie stosowanych gęstościach nie pozwalają na ilościową ocenę najmłodszych stadiów rozwojowych organizmów bentosowych. Odnosi się to w pewnym stopniu także do sit bardzo gęstych, których stosowanie zmusza przy tym do opracowywania bardzo dużych ilości materiału i nastręcza duże trudności techniczne przy jego sortowaniu. Ocena ilościowa stadiów młodocianych jest szczególnie ważna w wypadku form, u których nie jest możliwa ścisła ocena liczby jaj (np. *Chironomidae*). Również w badaniach eksperymentalnych nad przyczynami i mechanizmami decydującymi o liczebności bentosu ważna jest możliwość uzyskania pełnych, naturalnych zespołów fauny dennej.

Opierając się na zaproponowanej przez Anderson (1959) metodzie flotacji roztworem cukru sprawdzono ją na dużym materiale, w różnych sytuacjach, i zaproponowano pewne jej modyfikacje. Stwierdzono, że:

1) Flotacja roztworem cukru o stężeniu 1,12 g/ml prób nie konserwowanych i nie płukanych przez sito pozwala uzyskać praktycznie 100% organizmów z mulistych środowisk pozalitoralnych (tab. I–II). Metoda ta nie daje natomiast dobrych rezultatów w wypadku prób pochodzących ze środowisk o bardzo zwięzłej konsystencji osadów (glina, mada) luh z dużą ilością szczątków roślinności. Rzecz jasna, nie można stosować metody flotacji do organizmów o dużym ciężarze właściwym (mięczaki, chruściki w domkach itp.). Można stosować roztwory o wyższym stężeniu, jeśli nie powoduje to spadku klarowności roztworu (utrzymywanie zbyt dużej liczby cząstek w zawiesinie).

2) Efektywność flotacji materiałów konserwowanych jest znacznie mniejsza i bardziej zmienna niż materiałów niekonserwowanych (tab. IV).

3) Przetrzymywanie materiałów w temperaturze pokojowej przez jeden dzień, a w temperaturze 4°C przez trzy dni nie powoduje różnic w uzyskiwanych liczebnościach organizmów przy flotacji żywych materiałów niepłukanych, nawet jeśli struktura prób jest zaburzona (tab. V).

4) Należy przestrzegać tego, by warstwa roztworu flotacyjnego była odpowiednio wysoka (7–10 cm); zbyt cienka warstwa znacznie zmniejsza efektywność flotacji (tab. VI–VII).

5) Flotacja w roztworze cukru *Tubificidae* i *Chironomidae* (osobniki o dobrej kondycji) nie zmniejsza zupełnie ich przeżywalności, jeśli zastosujemy odpowiednie stężenie roztworu i niezbyt długi czas flotacji (tab. VIII–XII). Pozwala to na uzyskanie naturalnych zespołów bentosu do celów eksperymentalnych. Inne substancje, jak NaCl, $MgSO_4$ czy szkło wodne powodują bardzo wysoką śmiertelność organizmów, zwłaszcza *Oligochaeta*.

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