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Anna STAŃCZYKOWSKA

SOME METHODICAL PROBLEMS IN ZOOMICROBENTHOS STUDIES*

The effect of following factors upon the estimate of the number of microbenthos organisms has been analyzed: 1) sampling methods (size of catching surface of the apparatus, depth of slime layer taken, number of samples per station) and 2) segregation methods (preservation of samples, storage of unpreserved samples, rinsing on sieve, floatation).

Studies on microbenthos are being carried out from some 30 years, but it is only during recent years that a distinct intensification of work can be noted. Methods of sampling and preparation of quantitative samples are most frequently the same as those for macrobenthos, what not in all cases seems to be justified. The smaller size of zoomicrobenthos organisms requires the application of more precise methods.

In our studies among microbenthos animals there were classified *Turbellaria*, *Rotatoria Gastrotricha*, *Nematoda*, *Oligochaeta* (juvenile forms), *Cladocera*, *Copepoda*, *Ostracoda*, *Tardigrada*, and *Chironomidae* (juvenile forms), thus organisms, which body size is included within limits from 0.5 – 3.5 mm.

The work aimed at the analysis of a question, which method should be applied in the elaboration of microbenthos in order to obtain most reliable results during possibly short time. Material, on which the analysis was based, has been taken from 3 Mazurian lakes (Mikołajskie, Śniardwy, and Tałtowisko)

*From the Institute of Ecology Polish Academy of Sciences, Warszawa.

and from 3 lakes in Bulgaria (Biełosław, Błatnica, and Szabła) during 1964 and 1965. Altogether there were taken 30 samples with the Ekman-Birge's sampler, 15 ones – with tube sampler with catching surface of 43 cm^2 and 545 – with a sampler with 10 cm^2 area (altogether 590 samples).

In the following discussion I shall consider subsequently two phases of microbenthos analysis: sampling and the segregation of obtained material.

SAMPLING

a) Size of the catching surface of the apparatus

For the collection of quantitative samples in the field, various bottom samplers are in use (Welch 1948, Žadin 1956, Hrbaček et al. 1962) both with large catching surface (e.g. sampler of Ekman-Birge's type with an area of 225 cm^2) and with small one (with an area of several square centimetres) (Cvetkov 1959). Some research workers (among others Cejeb 1937) take microbenthos samples with calibrated tubes or pipettes as subsamples of large samples, or in shallow portions of a reservoir directly from the bottom. The application of the latter method is in its assumption restricted to shallow portions of reservoirs.

In order to determine the most favourable size of apparatus from the standpoint of result reliability, there were taken comparative series of samples (from the same depths, on the same stations and at the same time) with bottom samplers with various catching surface. In July 1964 in Mikołajskie Lake there were taken 3 series of samples (for the comparison of the number of organisms obtained with the aid of pneumatic apparatus with plexiglass tube with a catching surface 43 and 10 cm^2 large), while during the October and November 1964 in three Bulgarian lakes one series was taken (for the comparison of zoomicrobenthos number obtained by Ekman-Birge's sampler with the area of 225 cm^2 and in tube apparatus of Cvetkov's type with the area of 10 cm^2).

Following to the conversion of results obtained with various bottom samplers into a comparable area unit (1 m^2) there were found no fundamental nor regular differences between them (Tab. I and II). Numbers of organisms were similar with slight deviations in both directions without an increase or discrease proportional to changes in apparatus size. In the above analysis there was taken into consideration the number of: *Nematoda*, *Oligochaeta*, *Cladocera*, *Copepoda*, *Ostracoda*, and *Chironomidae*. No differences in the number of any from above mentioned groups have been found in relation to the size of catching surface in apparatus in use.

The comparison of number of zoomicrobenthos (per 1 m^2 of bottom surface) taken with bottom samplers with various area (Mikołajskie Lake, 1964)

Tab. I

Series	Bottom sampler with an area of			
	43 cm^2		10 cm^2	
	number of samples	number of individuals	number of samples	number of individuals
I	5	7, 440	15	6, 030
II	5	9, 067	15	10, 210
III	5	4, 882	15	6, 100

The comparison of number of zoomicrobenthos (per 1 m^2 of bottom surface) taken with bottom samplers with various area

Tab. II

Lakes	Bottom sampler with an area of			
	225 cm^2		10 cm^2	
	number of samples	number of individuals	number of samples	number of individuals
Biełosław	10	40, 228	30	45, 101
Błatnica	10	15, 424	30	14, 780
Szabła	10	16, 176	30	19, 873

b) The depth of taken layer of slime

The height of apparatus, or the depth of slime layer, from which the sample is obtained is also of prime significance in microbenthos sampling. Data from literature indicate that living zoomicrobenthos organisms are to be found down to 15–20 cm of slime volume (Moore 1939, Cole 1955), but mainly are grouped within its surface layers, generally within upper 1 cm layer. In order to get more accurate data, to examine to what depth live microbenthos animals and to determine the depth of slime layer taken for studies, there have been taken 8 series (10 samples each) from lakes: Mikołajskie, Śniardwy, and Tałtowisko. Samples have been divided into following layers, beginning with the surface: 0–5, 5–20, 20–40, 40–80, 80–120, and 120–160 mm. Samples were taken by pneumatic sampler with plexiglass tube with 10 cm^2 catching surface, adjusted to the division of sample into horizontal layers (Kajak, Kacprzak, and Polkowski 1965).

It was found that the microbenthos occurs down into the slime to the depth of 160 mm. The surface layer down to 20–40 mm had the highest density of animals. In fact 90% of living organisms occurred within this layer (Fig. 1).

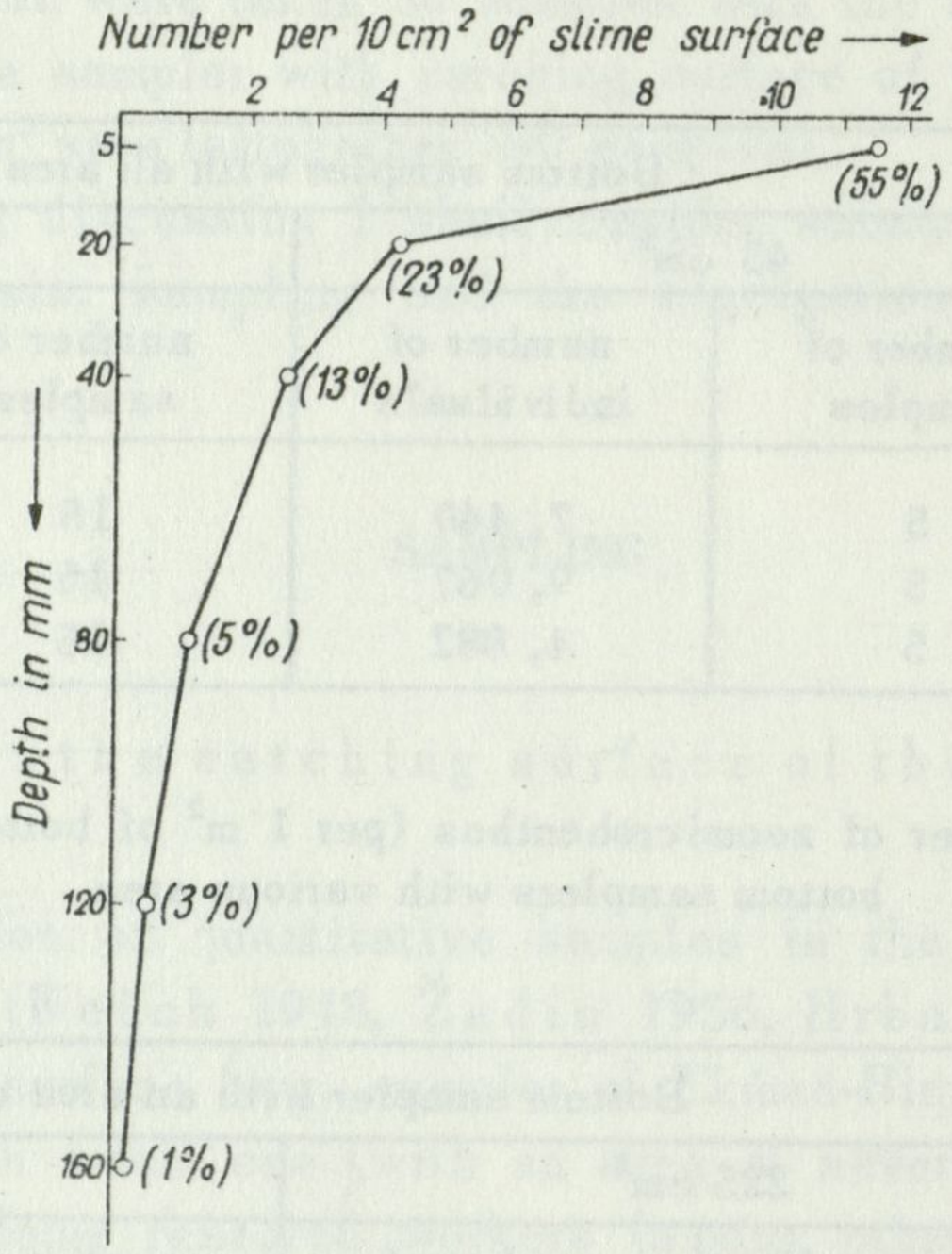

Fig. 1. Vertical distribution of zoomicrobenthos within individual layers of sediment
Per cents presented in parentheses have been calculated in relation to the total number of organisms found within all layers

c) Number of samples taken within one station

In order to determine the variation in number within one station there were taken 11 series (10 samples each) in Mazurian and Bulgarian lakes differing both in respect to their environment conditions and numbers of zoomicrobenthos. In all cases samples were taken with bottom sampler with 10 cm² catching surface and following animal groups have been selected: *Nematoda*, *Oligochaeta*, *Cladocera*, *Copepoda*, *Ostracoda*, and *Chironomidae*.

There was found a great variation in numbers within one station (Tab. III). Calculated error of arithmetic mean is generally similar to the error, with which macrobenthos materials are burdened (Kajak 1963). Thus it seems possible to use analogical criteria for the estimate of the number of samples characterizing definite environment, as in works on macrobenthos.

THE SEGREGATION OF MICROBENTHOS

a) Comparison of preserved and unpreserved samples

The knowledge of time, during which the living material can be kept in an unchanged state, is of great importance, particularly in the elaboration of

Variation in zoomicrobenthos numbers within one station
(within each station a series of 10 samples has been taken)

Tab. III

Lakes	Station	Mean number of individuals per sample	Range of fluctuations	Arithmetic mean error*
Mikołajskie	I	24.6	10 – 37	9.6
	II	8.6	2 – 25	5.7
	III	45.3	18 – 93	58.5
	IV	26.3	11 – 38	7.0
	V	11.2	6 – 20	2.1
	VI	67.9	36 – 131	7.1
Bielosław	I	12.7	10 – 17	0.4
	II	22.4	10 – 35	4.2
Błatnica	I	28.8	8 – 59	47.7
	II	65.3	31 – 105	67.7

*Calculated according to formula: $m = \frac{1}{n(n-1)} \left[\sum X^2 - n\bar{X}^2\right]$,

where: n – number of samples, $\bar{X}$ – arithmetic mean, X – number in individual samples.

animal groups, which cannot be preserved or require complex fixation processes. In the standard fixation of samples in 4% formaldehyd or 70% alcohol, the presence of certain taxonomic groups of microbenthos animals (*Protozoa, Turbellaria, Rotatoria, Gastrotricha*) practically cannot be distinguished in the mass of detritus remnants. The segregation of, at least, a portion of samples or "subsamples" in living state is thus necessary. This can be done with aid of e.g. Žadin's (1956) method.

The comparison of a number of remaining groups (*Nematoda, Oligochaeta, Cladocera, Copepoda, Ostracoda, Chironomidae,* and *Tardigrada*) in living and preserved material did not indicated any regular differences; for instance, in a series of 10 samples taken from Mikołajskie Lake in July 1965, the mean number of individuals in an unpreserved sample amounted to 14.2, while in preserved one – to 18.3. In a corresponding series from lake Śniardwy calculated means have related values and amounted to 32.8 and 32.2; only in lake Tałtowisko there was found somewhat higher number in unpreserved samples – 71.6 than in fixed ones – 52.1.

b) Storage of unpreserved samples

Collected samples of slime with unpreserved microbenthos, when stored at room temperature in closed test tubes, are subjected to rather rapid alterations

(among others Žadin 1956, Lackey 1961). In order to determine the type of occurring alterations and their rate, there were carried out 4 series of experiments with the material collected from Mikołajskie and Szabła lakes. Sample series were taken in environments characteristic with a small spatial differentiation in microbenthos (average error of arithmetic mean did not exceed 5%). Collected samples were placed in cylinders and stored in laboratory: a) at room temperature of ca 18°C (from Mikołajskie Lake) and at 22°C (from lake Szabła), and b) at a temperature of ca 8°C in vacuum flasks with cold water (two series of samples). From each series containing 20 samples 4 have been examined following to 4, 12, 24, 48 and 60 hours. Mean numbers of living and dead individuals have been determined and results compared with average initial values.

It was found that the number of microbenthos in unpreserved samples, stored in vacuum flasks with cold water from the profundal (with temperature about 8°C) undergoes first, visible alterations after some 48 hours of exposure (Fig. 2a), when an increase in mortality is noted. In samples stored at room

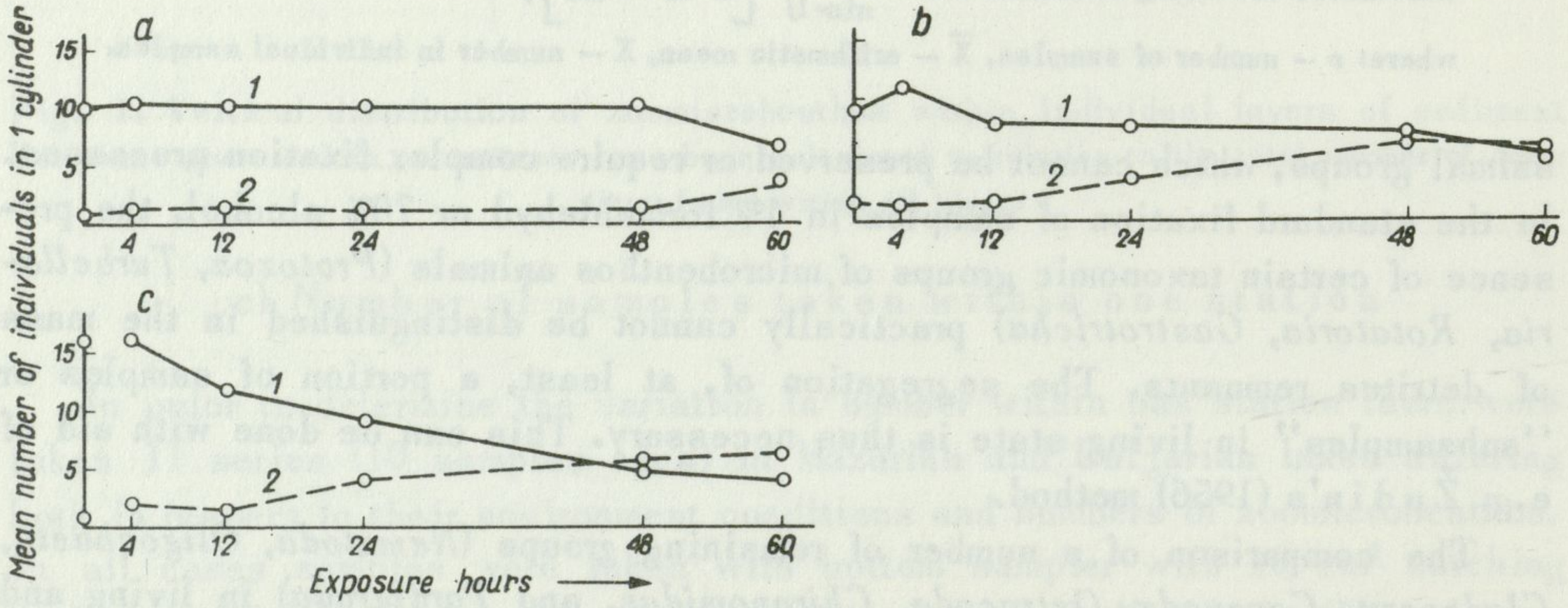

Fig. 2. The effect of exposure duration in unpreserved samples upon the number of zoomicrobenthos organisms

a – temperature ca 8°C (samples from the Mikołajskie Lake), b – temperature ca 18°C (samples from the Mikołajskie Lake), c – temperature ca 22°C (samples from the lake Szabła); 1 – living individuals, 2 – dead individuals.

temperature first alterations occurred already after 12 hours (Fig. 2b and c). More rapid course of changes has been noted in the material taken from lake Szabła (Fig. 2c), than that from Mikołajskie Lake (Fig. 2b). This is probably connected with the higher temperature in Bulgaria.

c) Rinsing of samples

The rinsing of microbenthos samples is generally applied. It has been carried out by Cvetkov (personal communication) and Cejeb (1958) directly

in the field. In connection with high time consumption of rinsing, in our studies we have moved this phase of material preparation to the laboratory, what resulted at the same time in increased accuracy. The sieve (in a shape of cylinder) with a bottom made of Perlon gauze, with a surface of ca 8 cm^2 and mesh diameter 45 μ was used for rinsing. The slime was poured to the sieve in portions and did not covered the net with a layer thicker than 0.5 cm. The sediment retained on sieve has been rinsed to jars, preserved again and examined under cytoplast with 50 × magnification.

When comparing the material from rinsed samples and those segregated without rinsing, there were found distinct differences in the number of zoomicrobenthos. In 60 samples taken in three series from lakes: Mikołajskie, Śniardwy and Tałtowisko during July 1965, in all cases the number has been higher in samples following to rinsing (Tab. IV). There was found also in them rather numerous proportion of certain groups (first of all *Tardigrada*), which have not been practically noted in fixed, but not rinsed material.

The comparison of the mean number of zoomicrobenthos (per sample) in rinsed and unrinsed samples

Tab. IV

Lakes	Rinsed samples*	Unrinsed samples*
Mikołajskie	76	60
Śniardwy	58	46
Tałtowisko	144	114

*series with 10 samples each

d) Floatation of samples

The method of floatation with sugar solution (concentration 1.12 g/ml) used with such success to the macrobenthos (Kajak, unpublished data) fails to give satisfactory results in the case of microbenthos. In the slime remaining after floatation there are to be found on an average some 60% of zoomicrobenthos organisms. In a series of 20 samples taken from Mikołajskie Lake, only in 4 cases less than 50% of organisms remained in slime. Perhaps the use of sugar solution in another concentration, or other solutions would enable the application of the method also to zoomicrobenthos.

CONCLUSIONS

1) In the connection with a high similarity of the number of zoomicrobenthos organisms obtained with bottom samplers with varying surface (225, 43, and

10 cm²) it seems more purposeful to do sampling with a small apparatus (with catching surface of 10 cm²), than with a greater one. This reduces the time consumed during the preparation of individual samples and at the same time it enables to take the greater number of samples which would yield an information about the degree of differentiation within one station.

2) The depth of sampling may be limited to the surface, 2–4 cm layer of bottom sediments, because in most cases within it occurs some 90% of organisms.

3) The variation in zoomicrobenthos number within one station is similar to that in macrobenthos, what enables the application of the same criteria for sample number estimate as in work with macrobenthos.

4) In the elaboration of samples in laboratory in order to obtain reliable results it is recommended to rinse the material on a sieve made of Perlon gauze before the segregation.

5) Since the number of zoomicrobenthos in samples, even that stored at a low temperature (ca 8°C) is subjected to alterations following to rather short time (48 hours), it is impossible to elaborate the whole material without preservation. In a face of fact that not all groups of animals can be noted in preserved material, it seems reasonable to segregate a portion of samples from a series, or so-called „subsamples" in a fresh state.

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REFERENCES

1. Cejeb, J.J. 1937 – K metodike količestvennogo učeta mikrofauny pelegena v svjazi s jej primenenem na solenych ozerach Krima – Zool. Žurn. 16: 499–510.
2. Cejeb, J.J. 1953 – Sostav i količestvennoje razvite fauny mikrobentosa nizovjev Dniepra i vodojomov Krima – Zool. Žurn. 37:3–10.
3. Cole, A.G. 1955 – An ecological study of the microbenthic fauna of two Minnesota lakes – Amer. Midl. Nat. 53: 213–231.
4. Cvetkov, L. 1959 – O mikrobentose bołgarskich pribrežnych ozer i značeni mikrobentosa dla ryboproduktivnosti vodojomov – Tr. VI sov. po probl. biol. vnutr. vod, 223–230.
5. Gurvič, V.V. 1961 – Formirovanije fauny mikrobentosa i pridonnogo planktona – Avtoref. dissert. Otdel. Biol. Nauk AN USSR, Kiev, 1–16.
6. Hrbaček, J. 1962 – Hydrobiologicke metody – Praha, 130 pp.
7. Kajak, Z. 1963 – Analysis of quantitative benthic methods – Ekol. Pol. A, 11: 1–52.
8. Kajak, Z., Kacprzak, K., Polkowski, R. 1965 – Chwytacz rurowy do pobierania prób dna – Ekol. Pol. B, 11: 159–165.

9. Lackey, J.B. 1961 – Bottom sampling and environmental niches – Limnol. Oceanog. 6: 271–279.
10. Moore, G.M. 1939 – A limnological investigation of the microscopic benthic fauna of Douglas Lake, Michigan – Ecol. Monog. 9: 37–582.
11. Sacharova, M.I. 1965 – Nekatoryje rezultaty isledovanija mikrobentosa Učinskogo vodochranilišča – Bull. Mosk. Obšč. Ispit. Prir. 5: 119–122.
12. Welch, P.S. 1948 – Limnological methods – New York-Toronto-London, 381 pp.
13. Žadin, V.I. 1956 – Žizn presnych vod (t. IV) – Moskva, Leningrad, 467 pp.

NIEKTÓRE ZAGADNIENIA METODYKI BADANIA ZOOMIKROBENTOSU

Streszczenie

Analizowano problem, jakie metody należy stosować przy pobieraniu i opracowywaniu prób mikrobentosu, aby w możliwie krótkim czasie osiągnąć najbardziej wiarygodne wyniki. W tym celu pobrano na terenie jezior mazurskich i w Bułgarii (w latach 1964 i 1965) kilkanaście serii prób (łącznie 590 prób) różnego typu chwytaczami oraz przeprowadzono kilka eksperymentów laboratoryjnych.

Porównywano chwytacze dna o różnej powierzchni chwytnej (225, 43 i 10 cm^2) i stwierdzono, że najkorzystniejsze jest posługiwanie się chwytaczem dna o powierzchni chwytnej 10 cm^2 (tab. I i II). Badania rozmieszczenia pionowego zoomikrobentosu wskazały na obecność żywych organizmów do 16 cm w głąb mułu, jednak około 90% zwierząt zgrupowanych jest w powierzchniowej (2–4 cm) warstwie (fig. 1). Pozwala to na ograniczenie wysokości próby praktycznie do tej górnej warstwy. Stwierdzono, że zróżnicowanie liczebności zoomikrobentosu w obrębie jednego stanowiska jest podobne jak makrobentosu, co umożliwia stosowanie takich samych kryteriów oceny liczby prób jak w pracach nad makrobentosem.

Przy analizie metod sortowania uzyskanego materiału wykazano, że lepsze wyniki uzyskuje się przy przebieraniu materiału uprzednio płukanego (tab. IV). Stwierdzono nieprzydatność stosowania metody flotacji roztworem cukru o stężeniu 1,12 g/ml, ponieważ około 60% organizmów zoomikrobentosu pozostaje w mule pozostałym po flotacji. Nie jest możliwe opracowywanie całego materiału bez konserwowania, ponieważ, jak stwierdzono w eksperymentach laboratoryjnych, liczebność zoomikrobentosu w próbach niekonserwowanych, nawet trzymanych w niskiej temperaturze, zmienia się w stosunkowo krótkim czasie (fig. 2). Wydaje się celowe przebieranie części prób w serii lub tzw. podpróbek bez konserwowania, ponieważ nie wszystkie grupy zwierząt są możliwe do zaobserwowania w materiale konserwowanym.

AUTHOR'S ADDRESS:
Dr. Anna Stańczykowska
Institute of Ecology
Warszawa, ul. Nowy Świat 72,
Poland