KOMITET EKOLOGICZNY - POLSKA AKADEMIA NAUK

EKOLOGIA POLSKA - SERIA A

Tom XVI Warszawa 1968 Nr 13

CHAIR OF HYDROBIOLOGY, UNIVERSITY OF WARSAW, WARSZAWA

Curator: Prof. Dr.K. Petrusewicz

Zbigniew Maciej GLIWICZ

THE USE OF ANAESTHETIZING SUBSTANCE IN STUDIES ON THE FOOD HABITS OF ZOOPLANKTON COMMUNITIES

A description is given of the method used for field experiments which made it possible to examine the food composition and feeding rate of a whole natural zooplankton community in situ. The principle of this method is comparison of the abundance of nannoplankton, bacteriaplankton and detritus in lake water with active zooplankton and in lake water with anaesthetized zooplankton, after exposing this water for a definite period in transparent organic glass apparatus. Initial results are given to illustrate this method.

Studies on the food habits of zooplankton are usually carried out in two directions. The first of these is formed by analyses of the gut contents of animals caught directly from the natural habitat (e.g. Komarova 1966 – *Cladocera*, Esterly 1916 – *Calanoida*, Fryer 1957a – *Cyclopoida*, Erman 1962a – *Rotatoria*), and the second is formed by laboratory investigation of the filtering rate and feeding rate of single species of *Crustacea* (review of literature – Rigler in press) and *Rotatoria* (e.g. Erman 1956, 1962b), and also of the mechanism of food intake by plankton animals (e.g. Storch 1924 – *Cladocera*, Storch 1929 – *Calanoida*, Fryer 1957b – *Cyclopoida*, Pourriot

1965 – *Rotatoria*). In numerous publications concerned with the gut contents of different species of zooplankton information can be found on the various forms which are included in the food composition of a given species, but this provides little information as to their actual participation in the food. On the other hand the great majority of experimental studies give the value of the feeding rate or filtering rate obtained under purely laboratory conditions, most often in experiments with completely artificial food – monocultures of algae or bacteria and in general with animals obtained from cultures. On the other hand the few experimental studies in which natural food is used take into consideration only certain of its elements, being limited to algae (e.g. Corner 1961, Nauwerck 1963) or bacteria (e.g. Beljackaja-Potaenko 1964). In addition the results of experimental studies as a rule refer to single species of zooplankton, and usually to some definite stages of development, so that it is extremely difficult or even impossible to refer these results to a real situation in a body of water.

The aim of the study is to present a method which makes it possible to investigate the actual food composition, and also the feeding rate of a whole natural zooplankton community, or more precisely – a community of plankton primary consumers, filtrators and sedimentators.

DESCRIPTION OF METHOD

1. The principle of the method is comparison of the abundance of particles of potential food of zooplankton (cells of small algae[1], bacteria and detritus particles) in lake water with active grazing zooplankton and lake water without active grazing zooplankton, after exposing this water in situ for a definite period in transparent 3-litre apparatus made of organic glass. The lake water with active zooplankton acts as a control. Water with anaesthetized zooplankton plays the part of an experimental variant by means of automatically added anaesthetizing substance, which is neutral in relation to algae and bacteria.

At the same time the experiment was set up control samples were taken by means of the same apparatus in order to ascertain the initial abundance of particles of potential food.

Conclusions were drawn as to the amount and composition of the food eaten by zooplankton from the differences between the abundance of small

[1]On the basis of observations made of the mechanism of food consumption, and also analyses of gut contents (for references – see above) it was found that the large forms of net phytoplankton are inaccessible as food to non-predatory species of zooplankton.

algae, bacteria and detritus particles in the control and experimental variants after a given period of exposure.

Exposure took place in situ, not only in the sense of the lake itself, but also of depth, as the special construction of the apparatus made this possible without bringing up water to the surface. This protected the material exposed from sudden changes in habitat conditions (pressure, temperature, oxidisation etc.) and also prevented deformation of the composition of zooplankton communities, e.g. as the result of introducing of air bubles under the shells of *Cladocera*.

The use of anaesthetizing substance made it possible to eliminate complicated preparatory operations consisting in separating the zooplankton from all the other components of the seston[2]. The method which is technically the simplest and most often used for separating zooplankton is to filter water through nylon net of suitable mesh size, such as was done in food experiments, inter alia by Beljackaja-Potaenko (1964), Corner (1961), Geen and Hargrave (1966). There is, however, a serious danger inherent in this method – filtration eliminates animals but at the same time deprives the filtered water of net phytoplankton and net tripton, which elements, although not the direct food of the dominating species of zooplankton, yet from the food supply and medium for bac eria which may play a dominating rôle in the food of the former. Filtration is also most often connected with the necessity for bringing the material up to the surface of the water, which seriously divorces the experimental conditions from the natural ones.

2. The anaesthetizing substance – physostigminum salicylicum (syn. eserinum) was used at a concentration $5 \cdot 10^{-5}$g/ml (0.005% solution). Its effect was to paralyse the crustaceans and rotifers in the experimental variant as the result of blockade of the synapses of the peripheral neuromuscular system (depolarisation as the result of halting holin esterasis, which decomposes acetyloholin). The strong effect of this drug on the synaptic transmission in the nervous system of other species of crustaceans was established by Prosser (1940) and Wiersma and Schallek (1948).

In order to find the concentration of physostigminum required to act effectively on all species, microscopic observations were made of zooplankton

---

[2]Investigation of food composition, feeding rate and filtering rate is usually made on the principle of comparing samples with and without zooplankton after a given period of exposure (review of literature – Rigler in press). Differences in the abundance of food particles are usually treated as the number of particles consumed. The method of through-flowing "food habitat" is also used, and then the differences between abundance at inflow and abundance at outflow are treated as the number of particles eaten (e.g. Erman 1958). Isotope methods, also frequently used (review of literature – Sorokin 1966) are suitable only for experiments with uniform food, e.g. only with algae, or only with bacteria, etc.

The effect of different concentrations of physostigminum salicylicum on the zooplankton species dominating in the pelagial of the lakes examined

The table shows the time (in minutes), after which the following are observed: first manifestations of paralysis (1), complete paralysis (2) and partial return of activity (3)

Tab. I

Species	Concentration of physostigminum salicylicum (in g/ml)												
	$1 \cdot 10^{-4}$			$5 \cdot 10^{-5}$			$1 \cdot 10^{-5}$			$5 \cdot 10^{-6}$			0
	1	2	3	1	2	3	1	2	3	1	2	3	1
Diaphanosoma brachyurum	1	3	–	1	3	–	3	10	–	3	15	80	–
Daphnia cucullata	1	3	–	1	5	–	3	15	–	3	20	80	–
Bosmina coregoni	1	4	–	1	5	–	3	10	–	3	20	60	–
Chydorus sphaericus	1	4	–	1	5	–	3	10	–	3	20	60	–
Eudiaptomus graciloides (adults)	1	10	–	1	10	–	1	25	–	5	–	40	–
Calanoida (copepodits and nauplii)	1	15	–	1	20	–	3	25	–	5	–	40	–
Mesocyclops leuckarti (adults)	1	15	–	1	15	–	3	25	–	5	–	40	–
Cyclopoida (copepodits and nauplii)	1	15	–	1	20	–	3	25	–	5	–	40	–
Keratella cochlearis	5	15	–	10	15	–	10	–	–	20	–	–	–
Kellicottia longispina	5	15	–	10	20	–	10	–	–	20	–	–	–
Asplanchna priodonta	10	25	–	10	25	–	15	–	–	–	–	–	–
Conochilus unicornis	5	15	–	10	20	–	15	–	–	–	–	–	–

With *Crustacea*, complete paralysis of natatorial and antennal legs observed in second stage, with *Rotatoria* – disturbance in the function of the coronal ciliation and paralysis of the muscles of the mastax (in both cases the animals sink to the bottom of the jar in which exposure is carried out).

organisms from the eutrophic Mikołajskie Lake when exposed in different concentrations of this substance. Parallel examination was made of the behaviour of animals in lake water without physostigminum and lake water with the addition of physostigminum and distilled water (the physostigminum solutions added to experimental samples were made in distilled water).

The effect of this preparation at a concentration of $5 \cdot 10^{-5}$ g/ml was most rapid in the case of *Cladocera* (complete paralysis of the legs took place after 3–5 minutes), slower in the case of *Copepoda* (complete paralysis of the legs after 10–20 minutes) and slowest in the case of *Rotatoria* (disturbance in the work of the coronal ciliation and paralysis of muscles of the mastax (Tab. I). The animals sunk to the bottom of the jar as the result of the action of physostigminum. Greater concentrations ($1 \cdot 10^{-4}$ g/ml) were not much more effective, but lesser concentrations ($1 \cdot 10^{-5}$ and $5 \cdot 10^{-6}$ g/ml) were distinctly slower in their action. A concentration of $5 \cdot 10^{-6}$ g/ml was not observed to exert any distinct effect on rotifers, and crustaceans were only transitorily paralysed: from 40–80 minutes later active movement of the legs returned (Tab. I). The effect of the following preparations was also ascertained in the same way: pantocainum, piperazinum, santonimum, curara and chloral hydrate. Chloral hydrate is the most effective, but it was not established with complete certainty whether its effect is neutral in relation to algae and bacteria.

The effect of physostigminum salicylicum at a concentration of $1 \cdot 10^{-3}$ on nannophytoplankton and bacteriaplankton was examined by the oxygen method of light and dark bottles. Water from Mikołajskie Lake filtered through nylon net of approx. 50 microns net mesh size was exposed for 10 hours for this purpose. Filtered water was used in order to eliminate the effect of animals (grazing). The simultaneous filtering off of net phytoplankton and net tripton was not of any real importance in this case as the effect of anaesthetizing substance on the large phytoplankton forms is less interesting here. Exposure was made under standard conditions (light intensity 2500 lux, temperature 16°C). Five repeats each of water taken from two different depths: 0.5 and 5.0 m were exposed. On the basis of the value of primary production in the case of samples with and without physostigminum it may be concluded that this substance is neutral in relation to nannophytoplankton even at far greater concentrations than that used in the experiment (Tab. II). The absence of differences with a concentration of 0_2 in dark bottles with and without physostigminum justifies the conclusion that it is also neutral in relation to bacteria. If it had an anaesthetizing effect on bacteria, concentration 0_2 would be greater in the samples with physostigminum (as the result of reduced intensivity of respiration of the microorganisms), and if a stimulating effect – the reverse. We find however that these differences are not significant (Tab. II).

Effect of physostigminum salicylicum on algae and bacteria
Oxygen concentration (in mg/l) in lake water taken from two depths, filtered free of net seston, exposed 10 hours in standard conditions in bottles of 100 ml capacity; Mikołajskie Lake, June 8th 1966, mean values from 5 repeats
A — light bottles, B — dark bottles, C — gross primary production

Tab. II

Depth (in m)		Water with physostigminum		Water without physostigminum	
		Mean	Range	Mean	Range
0.5	A	10.65	10.41–10.77	10.64	10.52–10.79
	B	10.46	10.24–10.55	10.49	10.34–10.72
	C	0.19		0.15	
5.0	A	10.87	10.75–10.97	10.95	10.91–11.04
	B	10.74	10.68–10.83	10.79	10.62–10.90
	C	0.13		0.16	

3. The construction and operation of the apparatus in which exposure was carried out is shown in Figure 1. Apparatus of 3 l capacity, made of organic glass, operate on the principle of a Bernatowicz type plankton sampler (Bernatowicz 1960). A concentrated solution of anaesthetizing substance, or in the case of the control, filtered lake water, is placed in the inner containers, which are also made of organic glass (capacity 15 ml). The open apparatuses are immersed in turn to the same depth and closed by means of messengers. The upper and lower closing valves which were connected with each other, automatically opened the containers with concentrated physostigminum solution ($1 \cdot 10^{-2}$ g/ml) or filtered lake water, which, due to the violent eddies caused by the movement of the valves, mixed with the whole volume of lake water contained inside the apparatus. Mixing of the water when the apparatuses closed was checked by means of coloured detector ink placed in the inner containers.

Gentle mixing of the water during the exposure period was ensured by turning the apparatus upside down every 20 minutes by means of nylon lines fastened to the lower edge of the collars.

This apparatus was also used in the second modification of the experiment, in which unfiltered water was used as a control variant and water deprived of zooplankton (and net phytoplankton) by filtering as the experimental variant. In connection with this the apparatus have collars of organic glass at both ends, the lower of which serves to secure the cones of nylon net filtering the water entering as the apparatus is immersed (experimental), while the upper prevents accidental entrance of the zooplankters to the interior when the apparatus is being closed in the case of the experimental variant also. As a result the apparatus is firmly closed at the moment it is arrested at the depth at which the experiment was carried out. Analysis of

abundance of the animals in the apparatuses showed that this provides sufficient security. There was complete absence of the net forms of zooplankton in the apparatus with filtered water.

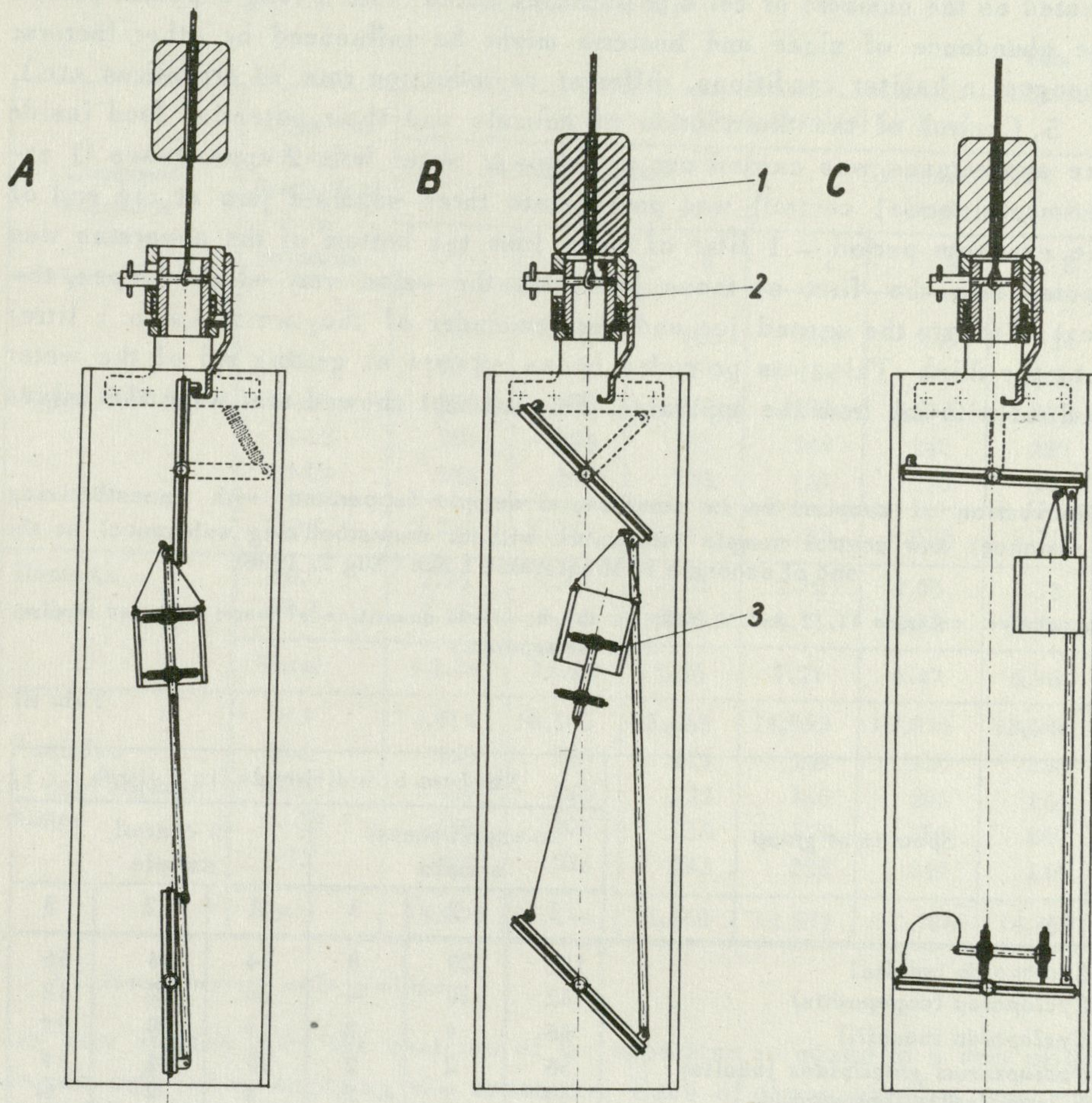

Fig. 1. Diagram of construction and operation of apparatuses used for investigations of the feeding habits of zooplankton (modification of Bernatowicz's plankton sampler, Bernatowicz 1960)

A — apparatus in course of immersion, B — apparatus showing valves in course of closing and container opening, C — apparatus after being closed and contents of container mixed 1 — messenger, 2 — release mechanism, 3 — container for concentrated solution of anaesthetizing substance

4. The exposure period was 4 hours. This was a sufficiently long period for the differences in abundance of the various elements of the food in the experimental and control variants to be adequately distinguishable at the time exposure ended, and at the same time sufficiently short for them to be treated as the numbers of cells or particles eaten (with a long exposure period the abundance of algae and bacteria might be influenced by other factors: changes in habitat conditions, different reproduction rate of organisms etc.).

5. Control of the distribution of animals and their potential food inside the apparatuses was carried out as follows: water from 2 apparatuses (1 experimental and 1 control) was poured into three separate jars at the end of the exposure period – 1 litre of water from the bottom of the apparatus was poured into the first of these jars with the valve very slightly open, the next litre into the second jar and the remainder of the water (also 1 litre) into the third. This was preceded by an attempt at getting rid of the water stained with ink from the apparatus. This attempt showed that when the valves

Distribution of zooplankton in experimental sample (apparatus with anaesthetizing substance) and control sample (apparatus without anaesthetizing substance) at the end of exposure in Mikołajskie Lake (Aug 2, 1966)

Successive columns (1, 2 and 3) refer to the one-litre quantities of water released in turn from the apparatus

Tab. III

Species or group	Numbers of individuals (in 1 litre)					
	in experimental sample			in control sample		
	1	2	3	1	2	3
Cyclopoida (adults)	138	20	8	34	54	46
Cyclopoida (copepodits)	32	10	–	26	12	18
Cyclopoida (nauplii)	46	4	2	14	28	22
Eudiaptomus graciloides (adults)	36	2	2	20	24	14
E. graciloides (copepodits)	10	2	2	2	2	6
E. graciloides (nauplii)	16	2	–	2	6	4
Daphnia cucullata	88	8	2	22	12	24
Diaphanosoma brachyurum	30	4	2	12	20	10
Bosmina coregoni and *B. longirostris*	18	6	–	8	10	12
Chydorus sphaericus	96	30	4	58	66	42
Keratella cochlearis	52	22	10	28	44	40
Other rotifers	16	8	8	14	14	18
Total	578	118	40	240	292	256

Distribution of nannophytoplankton, bacteriaplankton and particles of dead organic matter in experimental sample (apparatus with anaesthetizing substance) and control sample (apparatus without anaesthetizing substance) at the end of exposure in Mikołajskie Lake (Aug 2, 1966)

Successive columns (1, 2 and 3) refer to the one-litre quantities of water released in turn from the apparatus

Tab. IV

Compared elements	Diameter of cells (particles) in microns	Number of cells (particles) in 1 ml					
		in experimental sample			in control sample		
		1	2	3	1	2	3
Algae cells	<3	659	765	605	463	445	534
	3–6	641	570	659	356	401	365
	6–9	303	347	365	231	294	222
	9–12	285	294	325	249	240	258
	>12	338	267	303	231	276	347
	Total	2,226	2,243	2,257	1,530	1,656	1,726
Bacteria cells*	<0.5	2.95	1.48	3.10	2.10	2.05	1.73
	>0.5	9.21	9.74	8.95	5.11	4.42	5.23
	Total	12.16	11.22	12.05	7.21	6.47	6.96
Particles of dead organic matter	<3	14,614	16,162	15,255	14,062	12,816	13,546
	3–6	445	356	374	249	320	338
	6–9	320	365	222	160	205	169
	9–12	196	285	214	178	196	267
	>12	329	276	303	222	249	169
	Total	15,904	17,444	16,368	14,871	13,786	14,489

* Number of bacteria cells in millions

are very slightly open the contents of the apparatus is mixed to a very slight degree only when emptying the apparatus. Each of these samples was conserved and elaborated separately by the method described below. The results of this analysis (Tab. III and IV) show that the reversal of the apparatus every 20 minutes in the case of the control variant was a sufficiently reliable way of ensuring even distribution of the whole material, and in the case of the experimental variant – of ensuring even distribution of algae and bacteria (the animals sank down as a result of the action of the anaesthetic substance).

Distribution of larger animals (*Copepoda*, *Cladocera*, *Asplanchna* sp.) was also controlled by the method of direct observations made when the

apparatus was drawn up at the end of the exposure period. In the control variant the animals were not found to congregate in the lower layers of water isolated in the apparatus, despite the fact that the final reversal of the apparatus took place about 20 minutes before the end of the exposure period.

6. The samples were preserved and elaborated in the following way: water exposed in the apparatus, and also that taken in at the time of setting up the experiment (initial control) was filtered through plankton net (net mesh size about 50 μ). Net plankton was preserved in about 3% formalin, and sub-samples were taken from the filtered water for nannoplankton, detritus and bacteria.

For this purpose 0.5 l of filtered water was poured into 500 ml cylinders and preserved with Utermöhl's fluid (Utermöhl 1925) with the addition of formalin. After the whole of the seston had been densified to 5 ml by two-degree sedimentation, the nannophytoplankton and dead organic particles in 5 classes of size: < 3 μ, 3–6 μ, 6–9μ, 9–12μ and > 12 μ, were counted in these samples.

The remaining 2.5 l of filtered water was thoroughly mixed for 3 minutes. Three 1-millilitre sub-samples were then taken from the water and preserved in 3% formalin. After tenfold dilution they were strained through Coli 5 membrane filters. The material on the filters was fixed for 24 hours on filter paper saturated with 40% formalin, then stained for 24 hours on filter paper saturated with 5% erythrosin solution. After decolouring the filters and rendering them transparent with immersion oil, the bacteria on them in the two following classes of size: < 0.5 μ and > 0.5 μ were counted. This is a modification of the method described by Rodina (1965) used in the Chair of Microbiology of Warsaw University.

7. Elaboration of material. Differences in the abundance of a given class of size of algae or bacteria after the exposure period in experimental and control samples were treated as the number of cells of this class eaten during the course of the experiment.

The percentage of consumed cells of the given class of size was calculated as the ratio of this difference to numbers in experimental samples × 100.

In the case of detritus the difference between the abundance of particles in a given class of size in experimental and control samples could not be treated as the number of particles consumed for the following reason. The difference was the result not only of consumption of dead organic material but also defecation of undigested food, which in *Copepoda* only takes the form of enveloped small lumps, whereas in other animals it reaches the water in the form of small particles. Bearing in mind the mechanical character of food preferences it was assumed that the same percentage of detritus particles was consumed as that of the algae cells in the respective classes

of size. Taking the numbers of particles in the given class of size in the initial control sample[3] as 100% calculation was made of the number of detritus particles of this class eaten.

Next summing up the volume of the cells (particles) eaten from the different classes of size of algae, bacteria and detritus (the number of eaten cells or particles multiplied by mean volume[4]), the volume of food eaten during a period of 4 hours is obtained (4-hour feeding rate of zooplankton from 1 l). Knowing the volume of the phytoplankton, bacteria and detritus consumed, calculation was made of the percentage of these elements in the food of the zooplankton community isolated in the apparatus.

INITIAL RESULTS

The initial results set out in Tables V, VI and VII to illustrate the method were obtained from the experiments made in two lakes of the Mazurian Lake District: the eutrophic Mikołajskie Lake and the mesotrophic lake Tałtowisko. Exposure was made at a depth of 4 to 6 m, depending on where the current maximum abundance of dominating species was found.

A comparison is given in Table V of the value of the daily feeding rate of natural zooplankton communities in the two lakes (in mg of food per 1 g of biomass of the animals) with the value of the feeding rate of single species (in these same units), calculated on the basis of laboratory data.

Comparison is given in Table VI of the filtering rate of natural zooplankton communities, for which it was decided to accept rate of consumption of the most intensively consumed class of size, with the theoretical filtering rate of these communities calculated on the basis of data in literature for different species (Beljackaja 1964 – for *Crustacea* and Nauwerck 1959 – for *Rotatoria*). The data given by these authors, who obtained them in experiments with natural food, were taken for calculations of theoretical filtering rate. All other publications known to the author give far lower values for filtering rate (even 15 times lower) for the species in which we are interested.

[3] The final numbers in the experimental variant were not taken as 100%, as the numbers of detritus particles at the end of the exposure period were also the result of the action of other factors, e.g. cutting off the inflow of sedimentating tripton or the process of settlement of detritus particles on the walls of the apparatus, which altered the abundance of different classes of size in favour of the larger, as the result of the coagulation of smaller particles into larger ones.

[4] The mean volume of cells or particles from the given class of size was accepted on the basis of microscopic measurements of materials from lakes Mikołajskie, Tałtowisko and Piłakno, made in July and August 1966 (taken jointly measurement was made of 60 cells and particles in each class of size).

Comparison of daily feeding rate a zooplakton community (in percentages of its biomass) obtained as the result of an experiment with anaesthetizing substance and of the daily feeding rate of 2 species of zooplankton (in percentages of their biomass) obtained as the result of laboratory experiments

A – *Daphnia longispina*, laboratory observations by Monakov and Sorokin (1960), B – *Brachionus calyciflorus*, laboratory observations by Erman (1962)

Tab. V

Lake and date of experiment (1966)		Biomass of zooplankton from 1 litre (in mg)	4-hour feeding rate of zooplankton from 1 litre (in mg)	Daily feeding rate of a zooplankton community (in %% of its biomass)	Daily feeding rate of 2 species of zooplankton (in %% of their biomass)	
					A	B
Mikołajskie	9 VII	2.273	0.669	176	66–197	23–104
	2 VIII	5.063	1.014	118		
Tałtowisko	11 VII	3.893	0.313	48		
	4 VIII	3.038	0.823	162		

Comparison of filtering rate of zooplankton communities (from 1 litre volume) understood as consumption rate of algae in the most intensively eaten class of size (A) and theoretical filtering rate of the same communities (B) calculated on the basis of data for the various species obtained in laboratory experiments by Beljackaja-Potaenko (1964) for *Crustacea* and Nauwerck (1959) for *Rotatoria*

Tab. VI

Lake and date of experiment (1966)		Algae in most intensively eaten class of size (in microns)	%% of consumption during 4 hours of experiment	A (ml/hour)	B (ml/hour)
Mikołajskie	9 VII	0–3	39.6	99.0	43.7
	2 VIII	3–6	40.3	100.8	114.0
Tałtowisko	11 VII	9–12	23.9	59.7	79.2
	4 VIII	3–6	46.7	116.8	53.7

It would therefore appear that in the majority of cases the data obtained in a laboratory are artificially reduced in relation to the real situation in a lake[5],

[5] When calculating the theoretical filtering rate neither *Copepoda* nauplii (lack of data in literature), copepodits nor adult individuals of *Cyclopoida* were taken into account. It would seem, however, that this causes only very slight reduction

Percentages of algae, bacteria and detritus in mass of food eaten during 4 hours of the experiment by natural zooplankton communities

Tab. VII

Lake and date of experiment (1966)		Algae	Bacteria	Detritus
Mikołajskie	9 VII	14.9	67.9	17.2
	2 VIII	6.0	85.6	8.4
Tałtowisko	11 VII	24.8	56.0	19.2
	4 VIII	13.3	72.4	14.3

the more so as the filtering rate obtained in the author's experiments is also most probably too low as a result of the assumption that the most intensively consumed class of size is equally intensively consumed by all the species.

Table VII gives the percentages of algae, bacteria and detritus in the mass of food consumed by the zooplankton communities of the two lakes. These results confirm the generally held opinion on the important role of bacteria in the food of plankton animals, an opinion documented inter alia by Neuwerck's classic investigations (1963).

APPLICATION OF THE METHOD

The results given above, despite their considerable degree of agreement with the results of laboratory investigations, should on account of the small number of repeats be treated as a guide only. Application of this method to obtain absolute figures for values such as feeding rate, or filtering rate of zooplankton community must be supplemented by methodical studies.

In the first place in experiments carried out in situ it is necessary to take into account the great differences in distribution of different components of potential food, and also in the distribution of zooplankton, which most certainly affects the size of the dispersion of repeats. This makes it essential to carry out a larger number of repeats than in experiments carried out under laboratory control conditions, in which the water with the whole seston could be mixed at the time of setting up the experiment.

Attention must be drawn here to the phenomenon of reduction in abundance of nannoplankton (and to a lesser degree of bacteriaplankton) during exposure,

---

of the theoretical filtering rate, since the feeding rate of nauplii is probably very small, and the majority of *Cyclopoida* are predators.

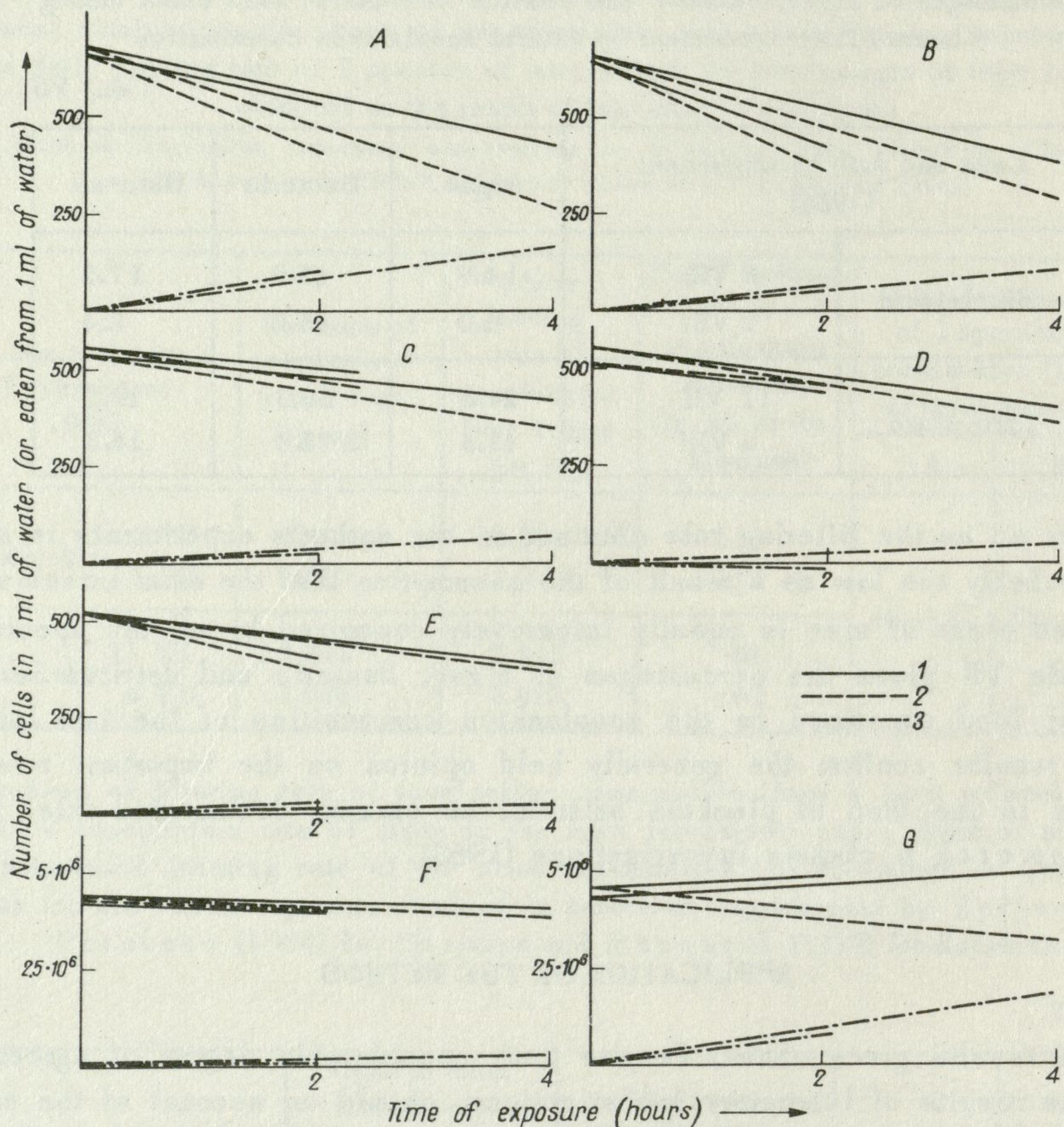

Fig. 2. Variations in abundance of algae and bacteria in the experimental and control variants during 2 — and 4—hour exposure periods

A — algae of < 3 μ diameter, B — 3–6 μ, C — 6–9 μ, D — 9–12 μ, E — > 12 μ, F — bacteria of < 0.5 μ diameter, G — bacteria of > 0.5 μ diameter

1 — abundance in experimental variant, 2 — abundance in control variant, 3 — number of cells eaten from 1 ml

as is established by comparing initial control samples with final control samples and experimental samples. The decrease in abundance is of a linear character, as shown by two experiments, carried out one directly after the other in Mikołajskie Lake: 2-hour and 4-hour (Fig. 2). Geen and Hargrave (1966) observed a similar phenomenon when exposing filtered and unfiltered lake water in 2 polyethylene pipes. They found that the abundance of phytoplankton decreased in both pipes, the decrease being far greater in the tube

with unfiltered water, probably as the result of consumption by zooplankton. The abundance of nannoplankton varied similarily in the experiments made by the author using the second modification, referred to above, of the method also consisting in parallel exposure of filtered and unfiltered water.

If the experimental conditions fully correspond to the natural ones, most probably an increase in the abundance of algae and bacteria in the experimental variant and the maintenance of a constant level of abundance in the control variant would be observed. The decrease in numbers is thus certainly caused by some unfavourable conditions which may be created as the result of isolating the exposed water from the whole mass of water. This may perhaps be the effect of the absence of constant currents, settlement of cells on the walls of the apparatus or else the slight shading of the water inside the apparatus by the few non-transparent parts of the construction, or again the presence of small areas of metalparts.

It may, however, be considered that the factors causing decrease in the numbers of algae and bacteria act equally intensively in both the control and experimental variants, and thus do not significantly affect the final result of the experiment.

Finally some difficulty is encountered here in referring the calculated values of the feeding rate to a definite time, on account of the different rate of anaesthetisation of different species of zooplankton. The opinion is justified, however, that with 4-hour exposure there is no great error incurred by referring this value to the whole of the exposure time, since the time of anaesthetisation of all the species does not exceed 25 minutes, which constitutes only about 10% of the exposure time.

It would seem that despite these weak points in the method the general principle of the experiment is useful for application not only in investigations of the feeding habits of zooplankton, but also in determination of the time of generation of bacteria, or in investigations of the reproduction rate of algae, in short, in searching for solutions to problems into which the effect of grazing by zooplankton has to be taken into consideration.

I am extremely grateful to Dr. Wojciech Kostowski, of the Department of Experimental Pharmacology of the Warsaw Medical School, both for his assistance in the search for a suitable anaesthetizing substance, and his invaluable advice on its application.

I should also like to take this opportunity of thanking Piotr Łebkowski, M. Sc. for his help in constructing the apparatus used for the investigations.

REFERENCES

1. Bernatowicz, S. 1960 – The methods of investigations of vascular plants in lakes – Roczn. Nauk roln. 77 B: 61–78. (Polish, English summary).
2. Beljackaja-Potaenko, J. S. 1964 – Količestvennye dannye po bakterjalnomu pitaniju zooplanktona – Biolog. osnovy rybnogo chozjajstva na vnutrennych vodoemach pribaltiki, Trudy naučnoj konf., Minsk, 277–282.
3. Corner, E. D. S. 1961 – On the nutrition and metabolism of zooplankton. I. Preliminary observations on the feeding of the marine copepod *Calanus helgolandicus* (Claus) – J. Mar. biol. Ass. U. K. 41: 5–16.
4. Erman, L. A. 1956 – O količestvennoj storone pitanija kolovrotok – Zool. Ź. 35: 965–971.
5. Erman, L. A. 1958 – Novye laboratornye ustanovki dla kultirovanija kolovrotok i izučenija ich pitanija – Naučn. dokl. vysš. šk., Biol. nauki, 4: 11–15.
6. Erman, L. A. 1962a – Ob ispolzovanii trofičeskich resursov vodoemov planktonnymi kolovrotkami – Bjull. mosk. Obšč. Isp. Prir., Otd. biol. 67: 32–47.
7. Erman, L. A. 1962b – Pitanie i rozmnoženie planktonnych kolovratok *Brachionus calyciflorus* Pall. v massovych kulturach – Dokl. Akad. Nauk SSSR, 144: 926–929.
8. Esterly, C.O. 1916 – The feeding habits and food of pelagic copepods and the question of nutrition by organic substances in solution in the water – Univ. Calif. Publ. Zool. 9: 253–340.
9. Fryer, G. 1957a – The food of some freshwater cyclopoid copepods and its ecological significance – J. Anim. Ecol. 26: 263–286.
10. Fryer, G. 1957b – The feeding mechanism of some freshwater cyclopoid copepods – Proc. Zool. Soc. Lond. 129: 1–25.
11. Geen, G. H., Hargrave, B. T. 1966 – Primary and secondary production in Bras d'Or Lake, Nova Scotia, Canada – Verh. int. Vereinig. Limnol. 16: 333–340.
12. Komarova, I. V. 1966 – Izučenie pitanija *Ceriodaphnia quadrangula*, *C. pulchella*, *Moina rectirostris*, *M. brachiata*, *M. macrocopa* i *Diaphanosoma brachyurum* – Trudy vsesojuzn. nauč. – issled. Inst. prudov. rybn. Choz. 14: 213–225.
13. Monakov, A. V., Sorokin, J. I. 1960 – Eksperymentalnoe izučenie pitanija dafni s pomoščju C^{14} – Dokl. Akad. Nauk SSSR, 135: 1516–1518.
14. Nauwerck, A. 1959 – Zur Bestimmung der Filtierrate limnischer Planktontiere – Arch. Hydrobiol. Suppl. 25: 83–101.
15. Nauwerck, A. 1963 – Die Beziechungen zwischen Zooplankton und Phytoplankton im See Erken – Symb. bot. upsaliens. 17: 1–163.
16. Pourriot, R. 1965 – Recherches sur l'ecologie des rotiféres – Vie et Milieu, Suppl. 21; 1–224.
17. Prosser, C. L. 1940 – Action potentials in the nervous system of the crayfish. Effects of drugs and salts upon synaptic transmission – J. Cellular Comp. Physiol. 16: 25–38.
18. Rigler, F. H. (in press) – Quantitative methods for studying the feeding of planktonic *Crustacea* (Grazing and Secondary Production in Freshwater Communities, Ed. W. T. Edmondson) – London, New York.
19. Rodina. A. G. 1965 – Metody vodnoj mikrobiologii – Moskva, Leningrad, 363 pp.
20. Sorokin, J. I. 1966 – Carbon 14 Method in the Study of the Nutrition of Aquatic Animals – Int. Rev. Hydrobiol. 51: 209–224.
21. Storch, O. 1924 – Morphologie und Physiologie des Fangapparat der Cladoceren und Euphyllopoden – Ergeb. Fortschr. Zool. 6: 125–230.

22. Storch, O. 1929 – Analyse der Fangapparat niederer Krebs auf Grund von Mikro--Zeitlupenfnahmen – Biologia Generalis, 5: 40–58.

23. Utermöhl, H. 1925 – Limnologische Phytoplanktonstudien – Arch. Hydrobiol. Suppl. 5: 1–527.

24. Wiersma, C. A. G., Schallek, W. 1948 – Influence drugs on response of a crustacean synapse to preganglionic stimulation – J. Neurophysiol. 11: 491–496.

ZASTOSOWANIE SUBSTANCJI INAKTYWUJĄCEJ W BADANIACH NAD ODŻYWIANIEM SIĘ ZESPOŁÓW ZOOPLANKTONU

Streszczenie

Zaproponowano rozwiązanie metodyczne, które umożliwia badania realnego składu pokarmu oraz racji pokarmowej całego, naturalnego zespołu zooplanktonu, a ściślej – zespołu planktonowych konsumentów I rzędu, filtratorów i sedymentatorów.

Zasadą proponowanej metody jest porównanie liczebności cząstek potencjalnego pokarmu zooplanktonu (komórek drobnych glonów, bakterii i cząstek detrytusu): 1) w wodzie jeziornej z aktywnym, żerującym zooplanktonem i 2) w wodzie jeziornej z zooplanktonem inaktywowanym, po określonym czasie ekspozycji. Ekspozycję przeprowadza się in situ (w sensie jeziora i głębokości) w przezroczystych, 3-litrowych aparatach ze szkła organicznego (fig. 1). Woda jeziorna z aktywnym, pobierającym pokarm zooplanktonem odgrywa rolę kontroli. Rolę wariantu eksperymentalnego spełnia woda z zooplanktonem inaktywowanym, nie pobierającym pokarmu. Różnica pomiędzy liczebnością cząstek pokarmu w wariancie kontrolnym i eksperymentalnym mówi o tym, ile cząstek z różnych klas wielkości pobrał w okresie ekspozycji cały zooplankton izolowany w aparacie.

Zooplankton w wariancie eksperymentalnym inaktywuje się (przy użyciu substancji inaktywującej – physostigminum salicylicum) na początku eksperymentu, in situ, w momencie zamykania aparatu na określonej głębokości. Substancja inaktywująca nie oddziałuje na glony i bakterie, co stwierdzono przez porównanie intensywności fotosyntezy i oddychania nannoplanktonu (metodą tlenową jasnych i ciemnych butelek) w wodzie jeziornej z physostigminum i bez physostigminum (tab. II). Stężenie $5 \cdot 10^{-5}$ g/ml tej substancji powoduje całkowity paraliż odnóży skorupiaków i zakłócenia w pracy aparatów wrotnych wrotków (tab. I).

Wstępne wyniki uzyskane tą metodą (tempo filtracji zespołu zooplanktonowego, racja pokarmowa zespołu, udział żywych glonów, bakterii i detrytusu w pokarmie zespołu) pokrywają się w ogólnych zarysach z teoretycznymi wartościami odpowiednich parametrów wyliczonych na podstawie danych literatury dla pojedynczych gatunków zooplanktonu (tab. V, VI i VII).

AUTHOR'S ADDRESS:
Dr Zbigniew Maciej Gliwicz
Katedra Hydrobiologii
Uniwersytetu Warszawskiego,
Warszawa, Nowy Świat 67,
Poland.