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PHOSPHORUS AND NITROGEN EXCRETION BY LAKE ZOOPLANKTON (ROTIFERS AND CRUSTACEANS) IN RELATIONSHIP TO INDIVIDUAL BODY WEIGHTS OF THE ANIMALS, AMBIENT TEMPERATURE AND PRESENCE OR ABSENCE OF FOOD*

ABSTRACT: The relationship has been determined between the specific rate of P and N excretion by plankton rotifers and crustaceans, and ambient temperature and individual body weights. The following equations describe these relationships: for $P–PO_4$ excretion rate: Rotatoria $E_P = 0.0154\,W^{-1.27}e^{0.096T}$, Cladocera $E_P = 0.519W^{-0.230}e^{0.039T}$, Copepoda $E_P = 0.299\,W^{-0.645}e^{0.039T}$; for $N–NH_4$ excretion rate: Rotatoria $E_N = 0.0879\,W^{-1.01}e^{0.088T}$, Cladocera $E_N = 1.80\,W^{-0.191}e^{0.039T}$, Copepoda $E_N = 1.33\,W^{-0.536}e^{0.039T}$, where E_P and E_N denote the rate of P and N excretion, respectively, in $\mu g \cdot mg$ d. wt.$^{-1} \cdot h^{-1}$, W is the body weight in μg d. wt. and T is the temperature in °C. Starvation was found to cause a 1.9–fold decrease in the phosphorus excretion rate, and a 2.2–fold decrease in the nitrogen excretion rate.

KEY WORDS: Rotatoria, Cladocera, Copepoda, phosphorus, nitrogen, excretion, temperature, body weight.

Contents

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1. INTRODUCTION

The cycling of matter in ecosystems is nowadays the object of particular interest to ecologists throughout the world. It concerns in particular the nutrients considered to be factors that can limit the primary production, that is – in the case of aquatic ecosystems, primarily phosphorus and nitrogen. Hydrobiologists' attention has more and more been attracted by that part of the P and N cycle in which the flow of these elements between the pools containing them is particularly fast, and in which the producers deplete the phosphorus and nitrogen made available to them as a result of the mineralization caused by bio- and saprophages. The significant role of nutrients excretion by the zooplankton, as a mechanism of making them available to the producers, has in principle been commonly accepted (e.g., Barlow and Bishop 1965, Dugdale and Goering 1967, Hargrave and Geen 1968, Jawed 1973, Smith and Whitledge 1977, Devol 1979).

World hydrobiology has so far accumulated a considerable amount of information on the rate of P and N excretion by various taxonomic groups of aquatic animals. However, this information most often concerns marine animals (a review of studies in

Corner and Davies 1971). Particularly poor is the knowledge of the rate of excretion of phosphorus, and even more so of nitrogen, by freshwater organisms, especially rotifers. And, as pointed by Johannes (1964a), on account of their low individual body weights, animals such as rotifers can contribute to the regeneration of nutrients to the same extent as can crustaceans, even if their percentage in the total zooplankton biomass is relatively low.

The aim of the present studies was on the one hand to complete existing literature data on the relationship between the rate of P and N excretion by plankton crustaceans and their individual body weight, and on the other – to determine the relationship between this rate and individual body weights and ambient temperature for planktonic rotifers – a group which has not yet been tested for this relationship.

2. EXPERIMENTAL STUDY METHODS – BASIS, ASSUMPTIONS, TECHNIQUES

2.1. BASIS OF METHODS FOR EXPERIMENTING

In all hitherto published studies on the rate of P and N regeneration by zooplankton two basic techniques were used: (1) studying the rate of P or N excretion by natural zooplankton communities isolated from the rest of the seston (e.g., Ferrante 1976a); (2) experimental estimation of the rate of excretion by one-species animal groups isolated from a zooplanktonic community or derived from culture and subsequent transposition of the results onto natural conditions by using data on the numbers and biomass of animals found in water bodies, including those not covered by direct investigation of the rate of nutrient regeneration (e.g., Gutelmacher 1977).

A trial for separating the whole zooplankton community from the phytoplankton showed that in the eutrophic lakes of the Masurian Lakeland it was feasible only in early spring, before the phytoplankton blooms. Later on a great abundance of algae with cell and colony sizes similar to crustacean and rotifer body sizes made it impossible to effectively separate the zooplankton from the phytoplankton. For this reason, it was decided to separate various body-size zooplankton groups from the natural community by using various mesh-size nets, or picking out individuals.

As indicated by many earlier papers (Johannes 1964a, Barlow and Bishop 1965, Hargrave and Geen 1968, Jawed 1973, Mayzaud 1973, Peters and Rigler 1973, Gutelmacher 1977 and others), the rate of excretion is closely related to the body-size of the organisms in a way specific to each taxonomic animal group. A series of laboratory experiments were therefore carried out aimed at studying this relationship by selecting the widest possible

range of specific individual body weights of animals representing three taxonomic groups under study.

Tests were carried out in the absence of phytoplankton and detritus (by filtering water through Millipore membrane filters 0.45 μm) with animals that had been starved prior to the tests in order to remove food from their intestines. Due to this procedure, the animals starved during the tests, but it was possible to avoid a simultaneity of the excretion by the zooplankton and nutrient uptake by the bacteria and algae which can occur also in the dark (C o r n e r and D a v i e s 1971, R i c h e y 1979). But since food concentration, and thereby the amount of food eaten, has a strong effect on the rate of nutrient excretion by the zooplankton (P e t e r s and R i g l e r 1973, N e l s o n, S i m m o n s and K n i g h t 1979), additional tests were carried out, the aim of which was to determine the difference in the rate of P and N excretion by zooplankton starved prior to the test (i.e., with empty intestines) and zooplankton feeding till the time of the actual test (i.e., with food in the intestines at the time of exposure).

Ambient temperature is another factor strongly affecting the rate of excretion (M a r s h a l l and O r r 1961, H a r g r a v e and G e e n 1968, L a R o w 1973, P e t e r s and R i g l e r 1973, G a n f and B l a ž k a 1974, G o p h e n 1976, J a c o b s e n and C o m i t a 1976, F o u r n i e r et al. 1977). For the pelagial zone of lakes no rapid temperature changes are as a rule recorded. To approximate the relationships studied to natural conditions, the tests for the effect of temperature on the rate of excretion were carried out on animals acclimated to the experimental temperatures. For technical reasons it was impossible to carry out investigations of this type with the natural zooplankton. In the case of crustaceans, P e t e r s' and R i g l e r' s (1973) data on the effect of temperature on the rate of P excretion by *Daphnia rosea* Sars were used, and in the case of rotifers, experiments were carried out with *Brachionus calyciflorus* Pallas populations from laboratory cultures.

Studies carried out by P e t e r s and L e a n (1973) and F e r r a n t e (1976b) indicate that in the products of freshwater zooplankton excretion PO_4^- represents about 90% of the total phosphorus excreted. It was therefore only this form of phosphorus that was determined in the tests. The method used (with ammonium molybdate according to S t a n d a r d m e t h o d s 1971) allows for the determination of the soluble reactive phosphorus (SRP) which includes, in addition to orthophosphates, phosphates formed due to the acid hydrolysis of the dissolved organic phosphorus compounds (R i g l e r 1968, D o w n e s and P e a r l 1978, W h i t e and P a y n e 1980). However, the scheme of the experiment included nutrient determination in samples collected at even time intervals, the same treatment for all samples, and calculation of phosphate concentration increments as the difference between successive samples. This procedure should eliminate the error caused by an overestimation of the phosphate concentration, because the amount of phosphates

resulting from the hydrolysis of the organic phosphorus compounds contained in the water would remain the same in all the samples. Any possible increase in this phosphorus fraction as a result of dissolved organic P excretion by the zooplankton should be of little significance, because the proportion of organic P in the products of excretion is small, and only part of the organic P is hydrolysed (in the studies of P e t e r s and L e a n 1973 – phosphates formed due to hydrolysis represented only 20% of the phosphate fraction).

Aquatic invertebrates are ammoniotelic. In the literature reports can be found on the excretion of other nitrogen forms by the marine zooplankton, but in the case of freshwater zooplankton no studies of this type have been carried out. Simultaneously, it cannot be ruled out that a relatively high percentage of nitrogen compounds other than ammonia in the products of marine zooplankton excretion may have been caused by improper experimental conditions (C o r n e r and D a v i e s 1971). It may be presumed that even if freshwater invertebrates do excrete certain amounts of nitrogen forms other than ammonia, the form that is positively predominating in the excretion products is ammonia. For this reason, in the experiments described in this paper only one form of nitrogen – ammonium nitrogen – was determined.

2.2. EXPERIMENTAL MATERIAL

For the tests natural zooplankton and laboratory rotifer cultures were used. The natural zooplankton came from over a dozen eutrophic lakes and several dystrophic lakes of the Masurian Lakeland, from a small astatic water body located near the Mikołajskie Lake and a dam lake on the Vltava river and from fish-ponds near Prague.

Zooplankton samples were collected by superficial hauls with a plankton net, 30 or 60 μm in mesh size. From these samples relatively specifically pure populations of individuals were obtained by applying plankton nets of the following mesh sizes: 0.03, 0.04, 0.06, 0.08, 0.10, 0.15, 0.24, 0.50, 0.80, 1.00, 1.50 and 2.00 mm, each time in a different set up. The individuals represented the following species: *Keratella cochlearis* (Gosse), *K. quadrata* (Müller), *Polyarthra vulgaris* Carlin, *P. dolichoptera* Idelson, *Synchaeta kitina* Rousselet, *Asplanchna priodonta* Gosse, *Conchilus unicornis* Rousselet, *Bosmina longirostris* (O. F. Müller), *Daphnia cucullata* Sars, *D. longispina* O. F. Müller, *Ceriodaphnia quadrangula* (O. F. Müller), *Scapholeberis mucronata* (O. F. Müller), *Polyphemus pediculus* O. F. Müller and populations of nauplii, copepodites, adult individuals of *Mesocyclops leuckarti* (Claus) and adult individuals of *Cyclops vicinus* Uljanine and *Eudiaptomus gracilis* Sars.

Series of experiments on the effect of temperature on the rate of P and N excretion were carried out at the Institute of Zoology, the Academy of Sciences of the Byelorussian Soviet Socialist Republic in Minsk with the rotifers *Brachionus calyciflorus*. The cultures had been started from resting eggs of rotifers from fish-pond bottom sediments. The food given to them was *Chlorella* sp. cultures.

2.3. TECHNIQUES OF A STANDARD EXPERIMENT WITH MEASUREMENTS OF THE RATE OF PHOSPHORUS AND NITROGEN EXCRETION BY ANIMALS STARVED PRIOR TO EXPOSURE

The scheme of the experiment has been presented in Figure 1. The zooplankton to be used in the tests was collected in the period May-December 1978. Only those samples were chosen for tests in which it was possible to divide the animals into separate size-fractions corresponding to populations of individuals of a species, as well as to separate the animals from the phytoplankton.

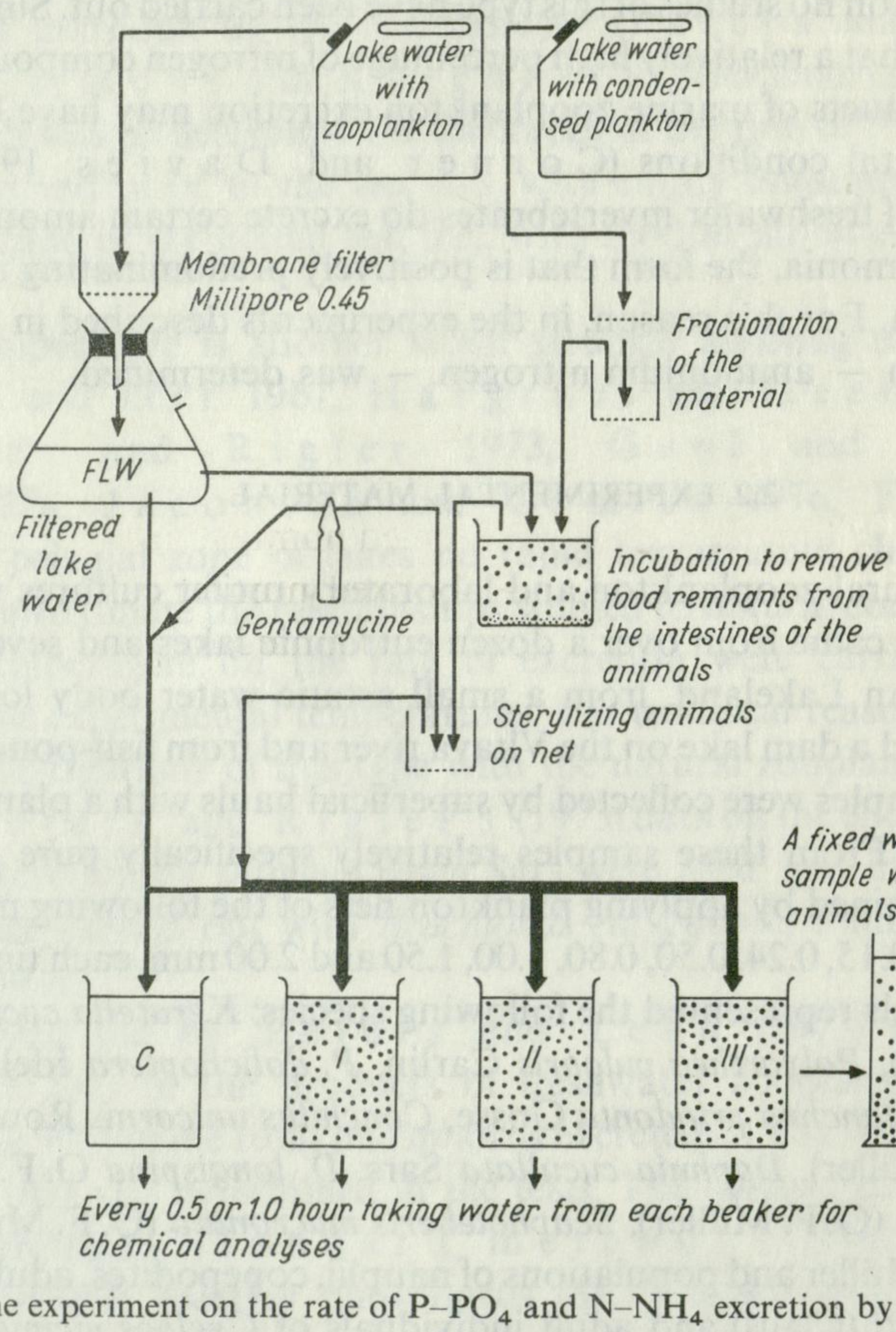

Fig. 1. Scheme of the experiment on the rate of $P–PO_4$ and $N–NH_4$ excretion by animals starved prior to exposure

The plankton-containing water taken from a lake and condensed plankton were immediately transported to the laboratory where the animals were sorted into body-size groups and separated from the phytoplankton. The lake water was filtered through 0.45 μm Millipore filters, by which procedure the seston was removed from it, therein most of the bacteria (H o b b i e and P o m e r o y 1972). The animals were placed in a beaker filled with filtered lake water (FLW) and kept for 5 to 60 minutes to remove food remnants from their intestines. In the case of crustaceans, the completion of food

removal from the intestines was determined by examining selected individuals under the microscope. This was not feasible in the case of rotifers. The assumption was therefore made that if in the intestine of *Asplanchna priodonta* food remained on an average for 14 minutes (E j s m o n t-K a r a b i n 1974), then for smaller rotifers this time should be shorter. Therefore rotifers were kept in the FLW for 5 up to 15 minutes, depending on the individual body size.

The animals were subsequently gently transferred on a net with mesh-size 40 or 60 μm and washed with gentamycin solution of the concentration of 10 mg · litre FLW^{-1}. The aim of this treatment was to inactivate the epizootic bacteria capable of accumulating phosphates (R i g l e r 1961, H a r g r a v e and G e e n 1968). Three beakers were filled with FLW containing gentamycin of the concentration of 2 mg · litre^{-1} (ensuring a complete elimination of aquatic bacteria within an hour's time – C h r ó s t 1978). After being sterilized the zooplankton was transferred to experimental beakers and incubated in 4 litres of FLW for 2 hours in the case of rotifers, and 4 hours in the case of crustaceans[1] (only for *Cyclops vicinus* was this time elongated to 8 hours), in the dark and at temperatures similar to those prevailing in the environment where the animals had been collected. Differences between the experimental and natural temperatures amounted at the most to 2°C. Every 30 minutes, in the case of rotifers, and 60 minutes, in the case of crustaceans, 350–ml water samples with animals were taken and filtered through a specified number, fixed for each experiment (5–7), of 0.45 μm Millipore filters. The use of fixed numbers of filters eliminated a possible determination error due to phosphate and ammonia adsorption on the filters (S c h i e r u p and R i e m a n n 1979).

SRP concentration in water samples was determined colorimetrically using ammonium molybdate (reagent) and stannous chloride (reducer) according to S t a n d a r d m e t h o d s (1971). Ammonium nitrogen concentration was determined by using the Orion ammonium selective electrode.

In tests with *C. vicinus* and *A. priodonta* the sensitivity of P and N determinations was increased by extracting coloured complexes by means of organic solvents: isobutanol for P (S t e p h e n s 1963) and chloroform in the bis-pyrozolone method for the determination of ammonium nitrogen (P r o c h a z k o v á 1964).

In order to study any possible changes in the chemistry of FLW, nitrogen and phosphorus concentrations were also determined in control samples without animals, at the beginning and at the end of the experiment.

Increments of N and P per unit time were calculated from differences in $P–PO_4$ and $N–NH_4$ concentrations between the successive samples. If the value of P or N increment fell during the experiment, in later calculations values obtained for the first 30 or 60 minutes of exposure were adopted. Three replications were used. The standard deviation (%) from the mean was on the average about 15% for phosphorus excretion and about 10% for nitrogen, and varied between 0 and 40% for P, and between 0 and

[1]For the criterion for choosing the exposure time and frequency of sample collecting – see Section 3.2.

36% for N. Considering the possible effects of many external factors, it was assumed that for the rate of metabolic processes this reproducibility was sufficient, so in all further analyses the mean was used.

In several cases total-P analyses were also made after burning the samples in sulphuric acid and subsequent neutralizing with sodium hydroxide (S t a n d a r d m e t h o d s 1971).

At the beginning of the experiment a 250–ml FLW sample with animals was taken and fixed in 4% formalin. This was subsequently used for a detailed determination of the numbers and biomass of the experimental animals. The same sample also was used for checking the experiment for purity, i.e., presence of phytoplankton and damaged animals. A small sample of water with live animals in it, taken at the end of the experiment, was examined immediately. This was a check for the condition of the animals and their death rate.

In later analyses the results were used from only those tests in which no phytoplankton had been found and the animals' condition had been found to be good.

2.4. TECHNIQUES OF TESTS AIMED AT ASSESSING THE EFFECT OF STARVATION AND FEEDING OF ANIMALS BEFORE AN EXPERIMENT ON THE RATE OF PHOSPHORUS AND NITROGEN EXCRETION

In July 1979, a series of experiments with taxonomically different zooplankton communities from five different lakes were carried out to determine the difference in the rate of P and N excretion between a zooplankton using its food reserves and one digesting the food contained in the intestines. The basis of the experiment was the comparison of the rates of P and N excretion by starved animals and by individuals fed prior to the actual experiment. This method has been widely used for the determination of the effect of diet and feeding intensity on the rate of nutrient excretion (J o h a n n e s 1964b, B u t l e r, C o r n e r and M a r s h a l l 1970, L a R o w 1973, I k e d a 1977, N e l s o n, S i m m o n s and K n i g h t 1979).

The scheme of the experiment has been presented in Figure 2. Water with condensed plankton was collected by horizontal hauls with a plankton net, 10 μm in mesh-size. In the laboratory the material was fractionated, by using appropriate net sets, into two fractions: (1) animals of body-size range 260 to 1500 μm, (2) food of particle-size range 20 to 100 μm. Fraction (1) was divided into two parts; one of these was kept in FLW for 30 minutes to remove food remnants from the animals' intestines. Afterwards the rate of P and N excretion was tested by the methods described earlier (Section 2.3). The other part was placed for about 2 hours in a 3–litre beaker, filled with a food suspension (fraction 2). The density of the food relative to the animals was more or less the same as the natural one. During the incubation the water containing animals and their food was carefully stirred by gentle revolutions of a magnetic agitator to prevent the food particles from falling onto the bottom of the beaker, which might decrease the availability of the food to animals. After two hours the animals were

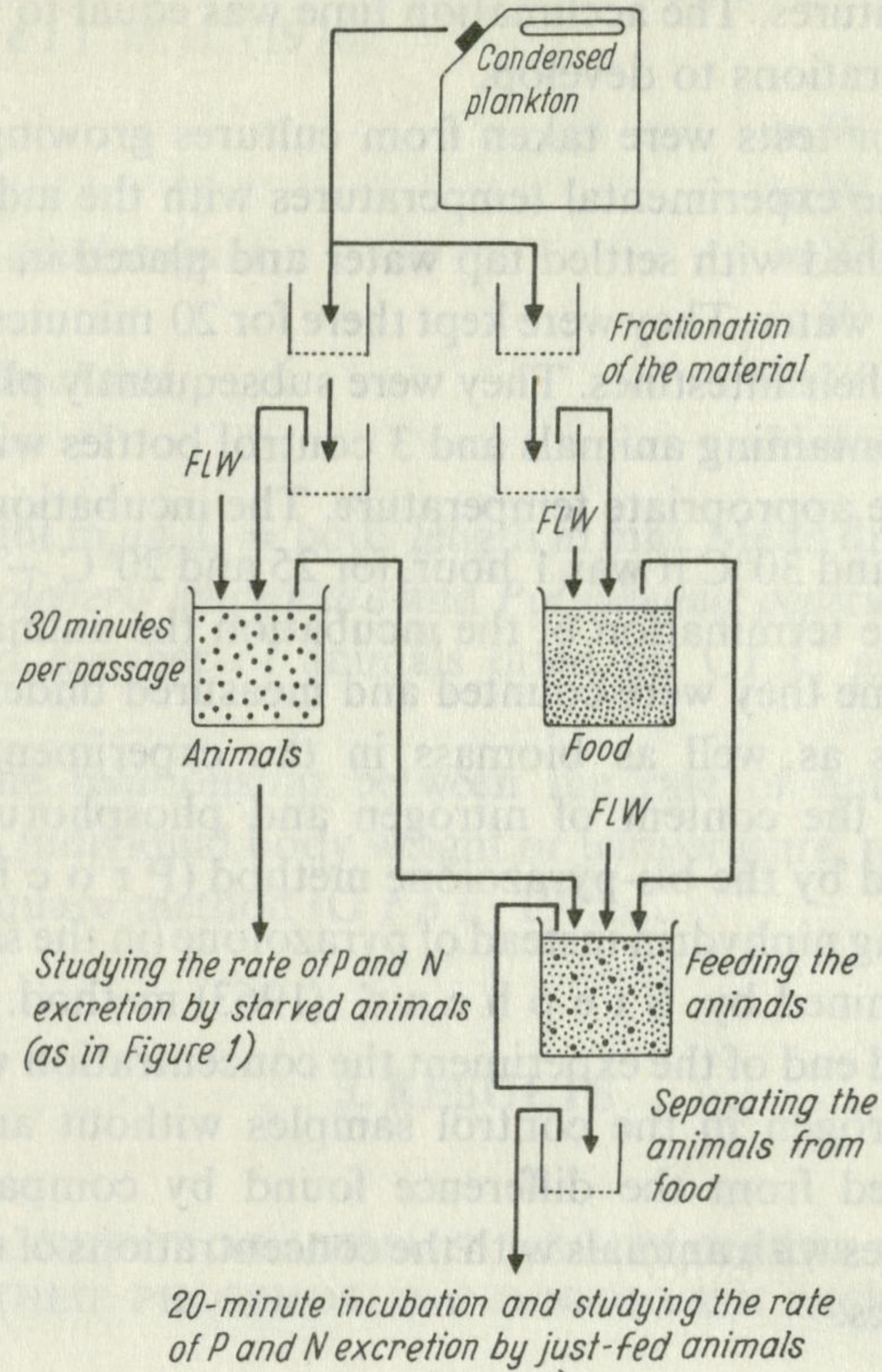

Fig. 2. Scheme of the experiment on the effect of zooplankton feeding prior to exposure on the rate of its $P–PO_4$ and $N–NH_4$ excretion

separated from the food with a net, washed with gentamycin solution and immediately placed in FLW. They were incubated in the dark for 20 minutes. The water was then filtered and tested chemically for the content of phosphorus and nitrogen. Because the density of the animals in the tests was comparatively low, and at the same time the incubation time was shorter, determination methods of a higher sensitivity were used (S t e p h e n s 1963, P r o c h a z k o v á 1964). Increments in P and N concentrations as a result of their excretion by starved and fed animals were estimated by comparing the concentration of these elements in control samples (without animals) with that in the experimental samples. All experiments were carried out in three replications.

2.5. TECHNIQUE OF THE TEST FOR THE EFFECT OF TEMPERATURE ON THE RATE OF PHOSPHORUS AND NITROGEN EXCRETION BY *BRACHIONUS CALYCIFLORUS*

In the tests material from *Brachionus calyciflorus* cultures was used. Six experimental temperatures were applied: 10, 15, 20, 25, 30 and 35°C. The animals were acclimated

to each of these temperatures. The acclimation time was equal to the time needed for several *Brachionus* generations to develop.

The animals used for tests were taken from cultures growing under good food conditions at each of the experimental temperatures with the aid of a plankton net, 70 μm in mesh-size, washed with settled tap water and placed in the thermostat in a beaker filled with settled water. They were kept there for 20 minutes to cause a removal of food remnants from their intestines. They were subsequently placed in three 50–ml bottles. Three bottles containing animals and 3 control bottles without animals were placed in the dark at the appropriate temperature. The incubation time depended on the temperature: for 35 and 30°C it was 1 hour, for 25 and 20°C – 2 hours, for 15 and 10°C – 4 hours. On the termination of the incubation the animals were filtered off, fixed, and after some time they were counted and measured under the microscope to estimate their numbers as well as biomass in the experiment. The filtrate was immediately tested for the content of nitrogen and phosphorus. The ammonium nitrogen was determined by the bis-pyrazolone method (P r o c h a z k o v á 1964) slightly modified by using ninhydrin instead of pyrazolone (in the same concentration). Phosphorus was determined by S t e p h e n s' (1963) method.

At the beginning and end of the experiment the concentration was also determined of phosphorus and nitrogen in the control samples without animals. The rate of excretion was calculated from the difference found by comparing the P and N concentrations in samples with animals with the concentrations of these elements in the terminal control samples.

2.6. A WAY OF ELABORATING THE RESULTS OF EXPERIMENTS, AND METHODS FOR DETERMINING THE BODY WEIGHTS OF EXPERIMENTAL ANIMALS

The difference found during the experiments between the concentrations of nutrients in the successive samples, or between their concentrations in the experimental and control samples were considered to be the result of excretion. In further sections of this paper the value will be given of specific excretion rate expressed as follows: μg P or N $\cdot$ mg d. wt.$^{-1}$ $\cdot$ hour^{-1}.

The body-weights of the animals and their biomass were estimated on the basis of quantitative samples taken at the beginning of the exposure. The samples were examined in their entirety under the microscope and all individuals were counted, and the body-breadth and length of 20 individuals of each rotifer species, and the body-length of 30 individuals of each crustacean species and growth stage were measured with the aid of a graduated scale. The wet weight of rotifers was calculated on the basis of the relationship "body length and breadth, and body weight" defined by the equations obtained by comparing the animals to solid figures corresponding to their shapes (acc. to R u t t n e r-K o l i s k o 1977). Dry weight was estimated on the basis of wet weight, adopting, after B o t t r e l l et al. (1976), a proportion of dry weight in wet weight equal to 3.9% for *Asplanchna* and 10% for the remaining rotifers.

The dry weight of the crustaceans was calculated by using the equations given in the paper by Bottrell et al. (1976):

Bosmina	$\ln W = 2.71 + 2.53 \ln L$
Daphnia	$\ln W = 1.44 + 2.77 \ln L$
Ceriodaphnia quadrangula	$\ln W = 2.56 + 3.34 \ln L$
Diaptomidae	$\ln W = 1.24 + 2.26 \ln L$
Mesocyclops leuckarti	$\ln W = 1.27 + 2.26 \ln L$
Cyclops vicinus	$\ln W = 1.45 + 2.12 \ln L$

where W = dry weight in μg, L = body length in mm. Mean dry weights of individuals of the species *Scapholeberis mucronata* and *Polyphemus pediculus* were determined by weighing a specified number of animals dried on GF/C glass filters at 60°C for 72 hours.

To determine the relationship between the rate of nutrient excretion by the zooplankton and its individual body weight or temperature, regression analyses were made by the least square method (Greń 1975).

3. RESULTS

3.1. EFFECT OF ANIMALS' DENSITY ON THE RATE OF THEIR PHOSPHORUS AND NITROGEN EXCRETION

To obtain P and N concentration increments that could be determined by the chemical analysis employed in the experiments it was necessary to use animal densities much above the natural density in their environment.

In the relevant literature a lack has most often been reported of an effect of animal density on the rate of P or N excretion and the respiratory rate (Jawed 1973, Mayzaud 1973, Szyper et al. 1976, Fournier et al. 1977, Gutelmacher 1977, Smith and Whitledge 1977), regardless of whether or not the animals had been starved prior to the tests. At the same time, however, Satomi and Pomeroy (1965) recorded an increased rate of metabolic processes following overcrowding, and Hargrave and Geen (1968) found a decrease under similar conditions. It seems, therefore, that plankton animals may react in a different way to an increase in density, and the direction and intensity of the reaction may depend on many factors. According to Zeiss (1963), animals that do not occur in high densities in their natural environment show a weaker relationship between the rate of their metabolic processes and density than that found for animals occurring in greater densities in their natural environment.

The experiments concerned with this problem were carried out with *Cyclops vicinus* and *Asplanchna priodonta* in November 1978 (Fig. 3). As indicated by these investigations, *C. vicinus* individuals derived from a dam lake (I) and a small pond (II) reacted by lowering the rate of P and N excretion and respiratory rate by about 30% following a

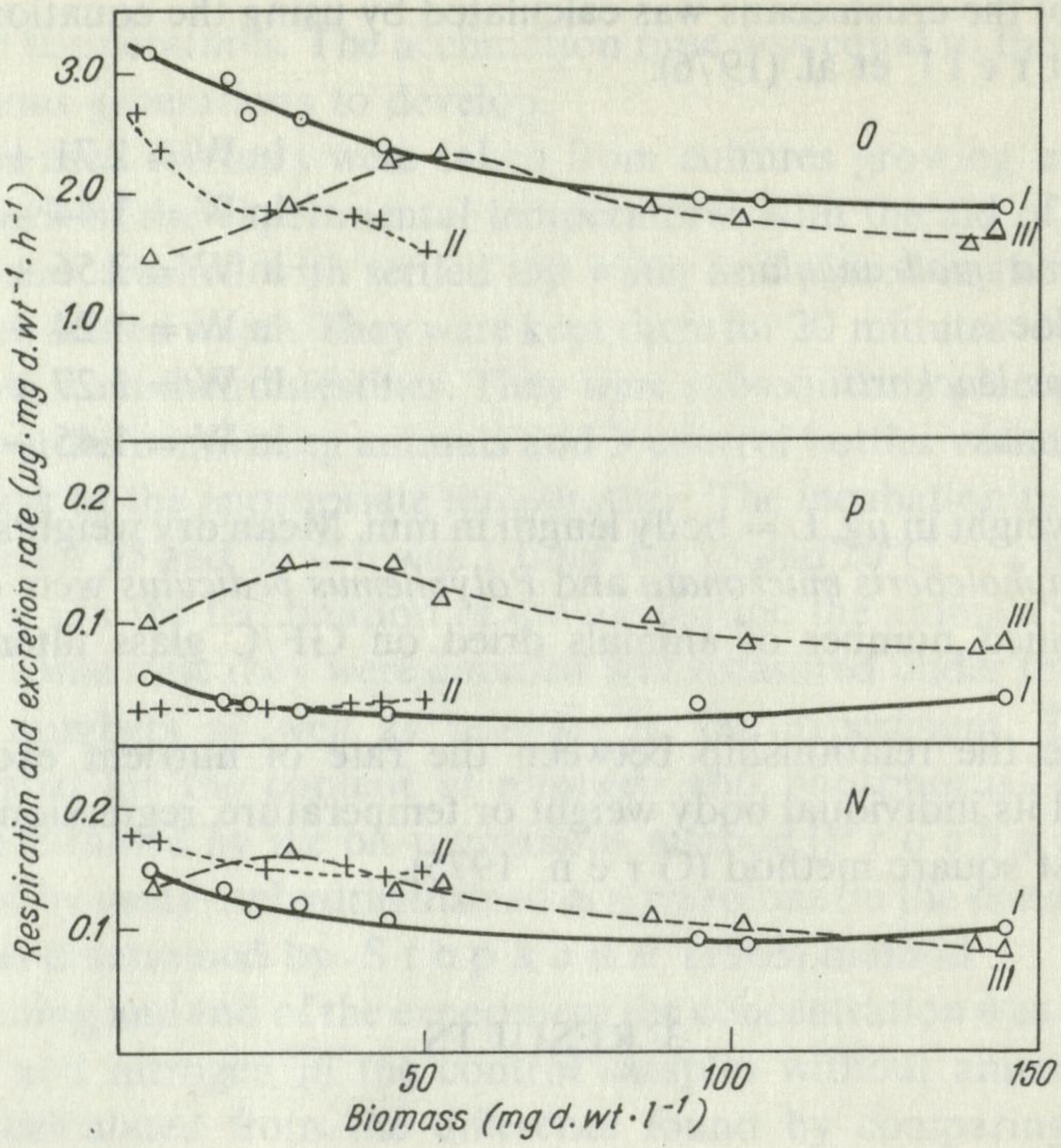

Fig. 3. Effect of animal density in experiment (I, II – *Cyclops vicinus*; III – *Asplanchna priodonta*) on the rate of respiration (O) and of phosphorus (P) and nitrogen (N) excretion

growth in density by 5 to about 50 mg dry weight of animals per litre. In the case of *A. priodonta* (Fig. 3, III) over a density range of about 5 to 30 mg of dry weight $\cdot 1^{-1}$ there was an increase in excretion rate by about 50% of the initial value for P, and about 25% for N. With a further growth in density the rate of excretion dropped to values below the initial level.

With the varying animal reaction to density, as indicated by both the literature and the results presented above, it would not have been purposeful to use high densities in the experimental populations and then extrapolate the results onto natural conditions, an approach used by H a r g r a v e and G e e n (1968). For this reason, in all later analyses the results were used of only those experiments in which the density of animals did not exceed a level similar to the highest level recorded for the natural environment, of the range of 5 mg in the case of crustaceans, and 1 mg of dry weight $\cdot 1^{-1}$ in the case of rotifers.

3.2. EFFECT OF EXPOSURE TIME ON THE RATE OF EXCRETION

In the standard experiment (described in Section 2.3.) the exposure time was established on the basis of the results from three experiments carried out in September 1977 in which the effect was estimated of experimental conditions on the

animal death rate (i.e., increased percentage of dead animals in the initial numbers recorded during an hour). In those experiments densities were used approaching the maximum level. The animals were sedimented for 1 up to 5 hours in funnels, each fitted with a rubber tube and clamp. On the termination of the exposure the sediment accumulated on the bottom of the funnel was carefully siphoned off and then examined, in its entirety, under the microscope, and the dead animals were counted. The remainder of the sample was fixed and the live individuals were counted (in the case of rotifers it was assumed that all those individuals that shrank during the fixation of the sample were living). Six experimental variants were used: I – starved rotifers (incubated in FLW), in the presence of gentamycin in the same concentration as in the standard experiment; II – starved rotifers without gentamycin; III – rotifers in the presence of natural food and gentamycin; IV – rotifers in the presence of food but without gentamycin; V – starved crustaceans in the presence of gentamycin; VI – starved crustaceans without gentamycin. An approximate theoretical death rate was calculated on the basis of data on the life span of rotifers (G a l k o v s k a j a 1965) and crustaceans (M a n u i l o v a 1964).

Both the rotifers and crustaceans used in the experiments showed a mortality rate much higher than the theoretical mortality (Fig. 4). This was probably due to the effect of the conditions of material collecting and sorting, since the highest death rate was recorded for large individuals of the genus *Polyarthra* (Fig. 5), which are particularly sensitive to mechanical injuries (E j s m o n t-K a r a b i n 1979).

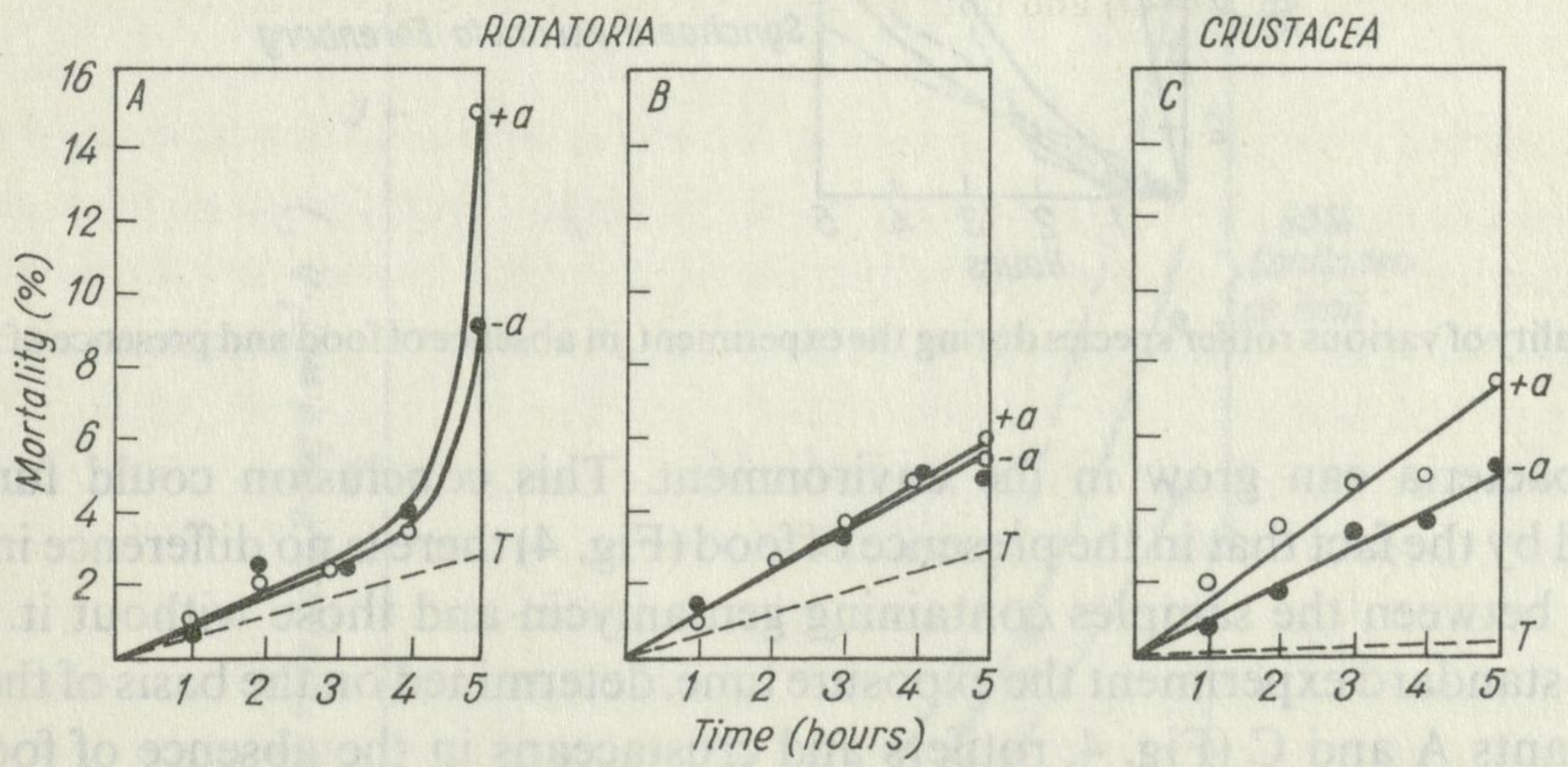

Fig. 4. Mortality of animals in a 5 hours' experiment (percentage of dead individuals relative to initial numbers) in the presence of food in the environment (B) and during starvation (A and C); –a = in absence of antibiotic; +a = in presence of antibiotic; T = theoretical mortality

In samples containing food the increase in the percentage of dead rotifers was rather uniform, but in the absence of food after a 4-hour exposure a rapid growth of mortality was seen (Fig. 4), the death rate being lower in the absence of gentamacin. This can be explained as resulting from the exhaustion of food reserves (bacteria + particulate organic matter + food stored in the bodies) during 3–4 hours, and consequently an increased mortality. In the absence of the antibiotic there may be higher food supplies,

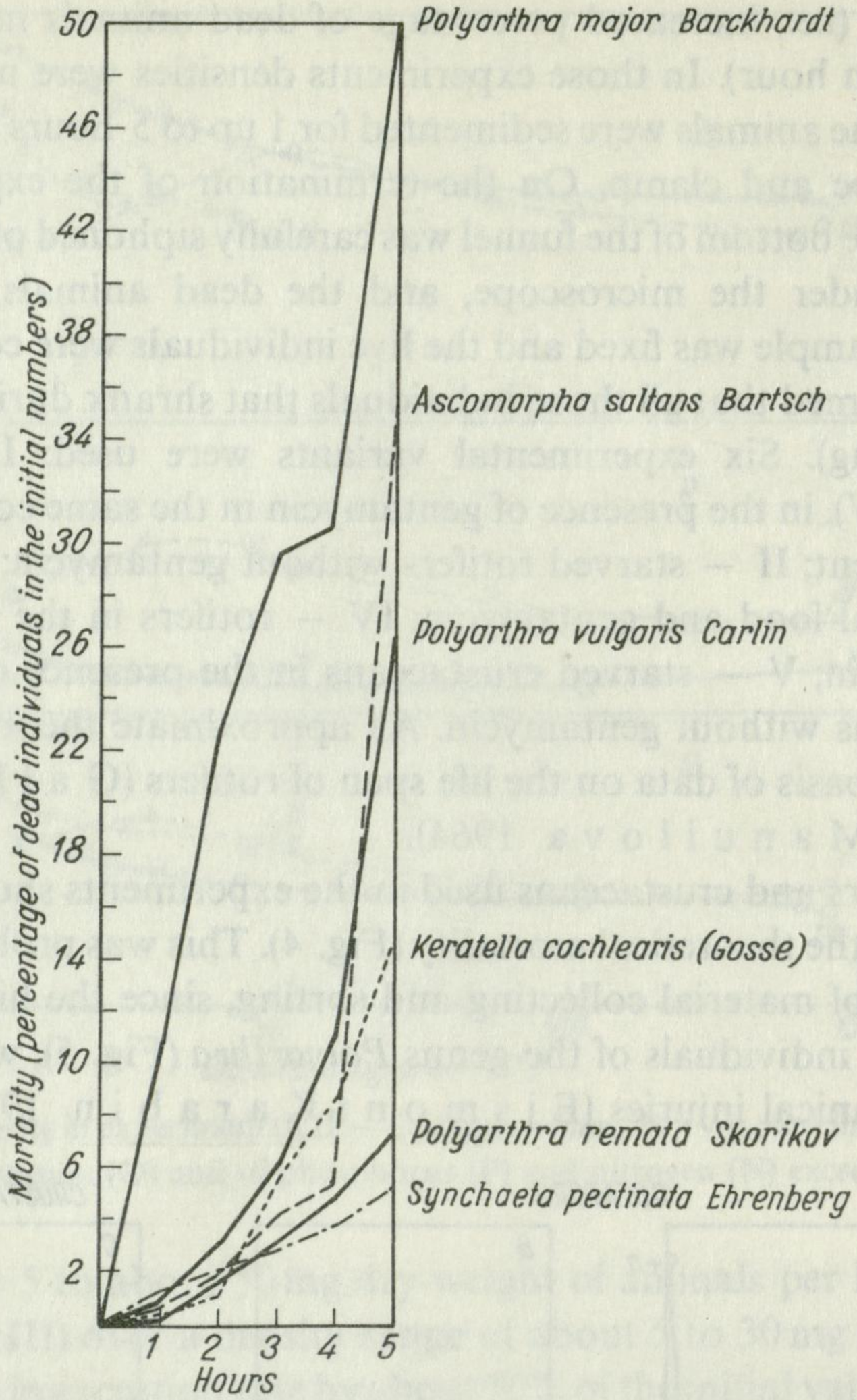

Fig. 5. Mortality of various rotifer species during the experiment, in absence of food and presence of antibiotic

because bacteria can grow in the environment. This conclusion could further be supported by the fact that in the presence of food (Fig. 4) there is no difference in animal mortality between the samples containing gentamycin and those without it.

In the standard experiment the exposure time, determined on the basis of the results from variants A and C (Fig. 4, rotifers and crustaceans in the absence of food), was 4 hours. However, the food reserves of small rotifers could be depleted within a shorter time. For this reason, the adopted exposure time was 2 hours for rotifers and 4 hours for crustaceans, and for bigger crustaceans the time was elongated up to 8 hours. In this time the death rate should not be significantly higher than 10% of the initial abundance, this being the level at which the mineral P removed from the dead animal bodies should not represent a significant fraction of P derived from excretion (C o o p e r 1935).

Starving of animals during an experiment may cause an increased death rate, and should have a considerable effect on the rate of nutrient excretion if the food supplies stored in the body are depleted before the termination of the exposure. It was therefore necessary to check for changes in the excretion rate during an experiment. Water

samples for analyses were taken every 30 minutes for rotifers and every 60 minutes for crustaceans to record changes in the rate of excretion during exposure. In the case of rotifers a 30–minute time seems too long, but it cannot be shortened for technical reasons (necessity to obtain measurable P and N concentration increments, much time needed for chemical treatment of successive samples). It was assumed that an assessment of changes in the rate of excretion throughout the incubation would make it possible to record a possible effect of a prolonged exposure time on the animals' metabolism.

3.3. EFFECT OF TEMPERATURE ON THE RATE OF PHOSPHORUS AND NITROGEN EXCRETION BY *BRACHIONUS CALYCIFLORUS*

In *Brachionus calyciflorus* a rise of temperature from 10 to 35°C caused a considerable growth of the rate of $P–PO_4$ excretion from 0.24 to 2.40 $\mu g \cdot mg\,d.\,wt.^{-1} \cdot hour^{-1}$ (for mean values from several replications) and of $N–NH_4$ excretion from 0.44 to 2.85 $\mu g \cdot mg\,d.\,wt.^{-1} \cdot hour^{-1}$. The nature of the changes in the excretion rate with a rising temperature, in the case of P and N, is similar and indicates an exponential relationship (Figs. 6, 7). For the relationship between the rate of

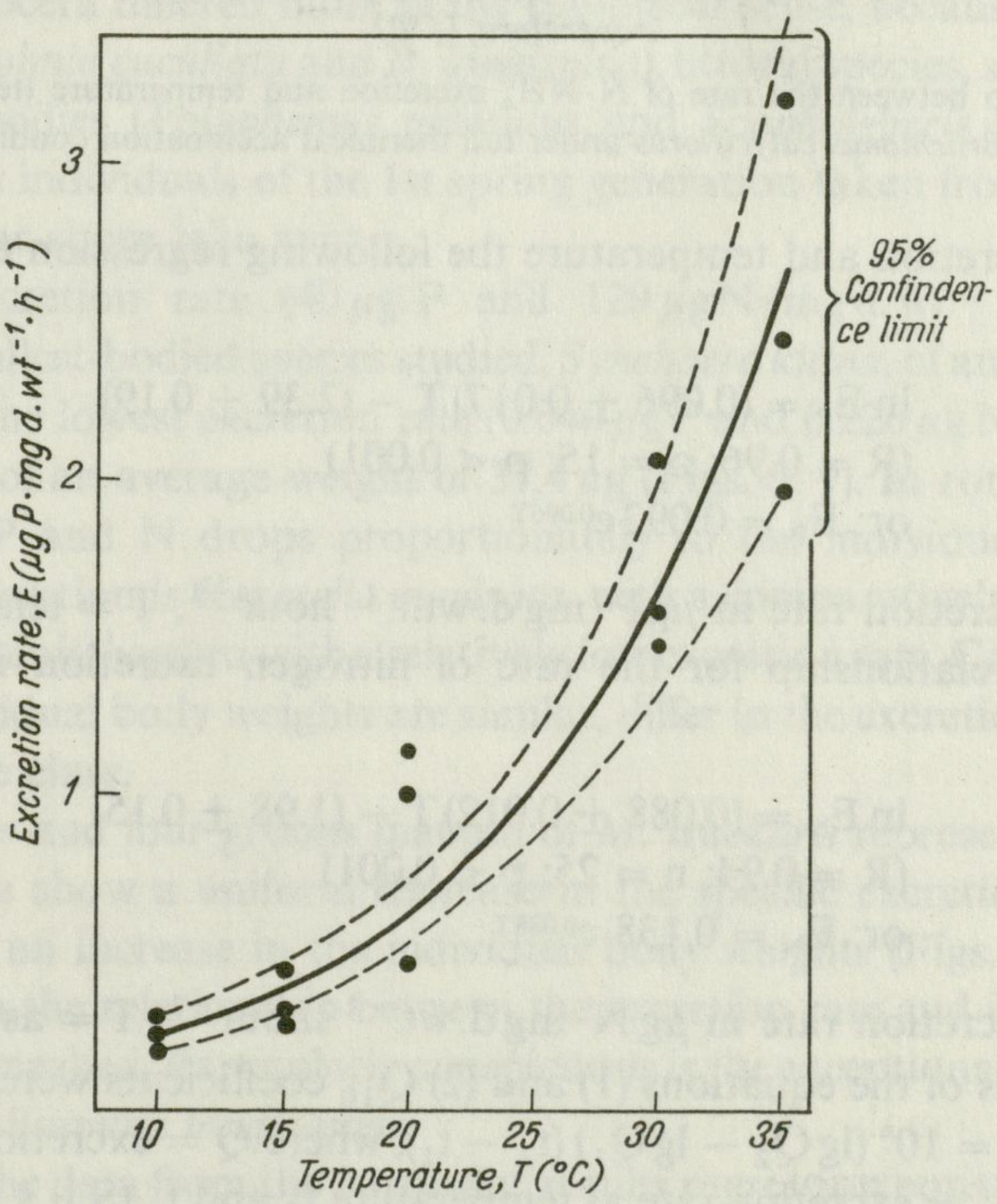

Fig. 6. Relationship between the rate of $P–PO_4$ excretion and temperature (tests were carried out on *Brachionus calyciflorus* under full thermical acclimation conditions)

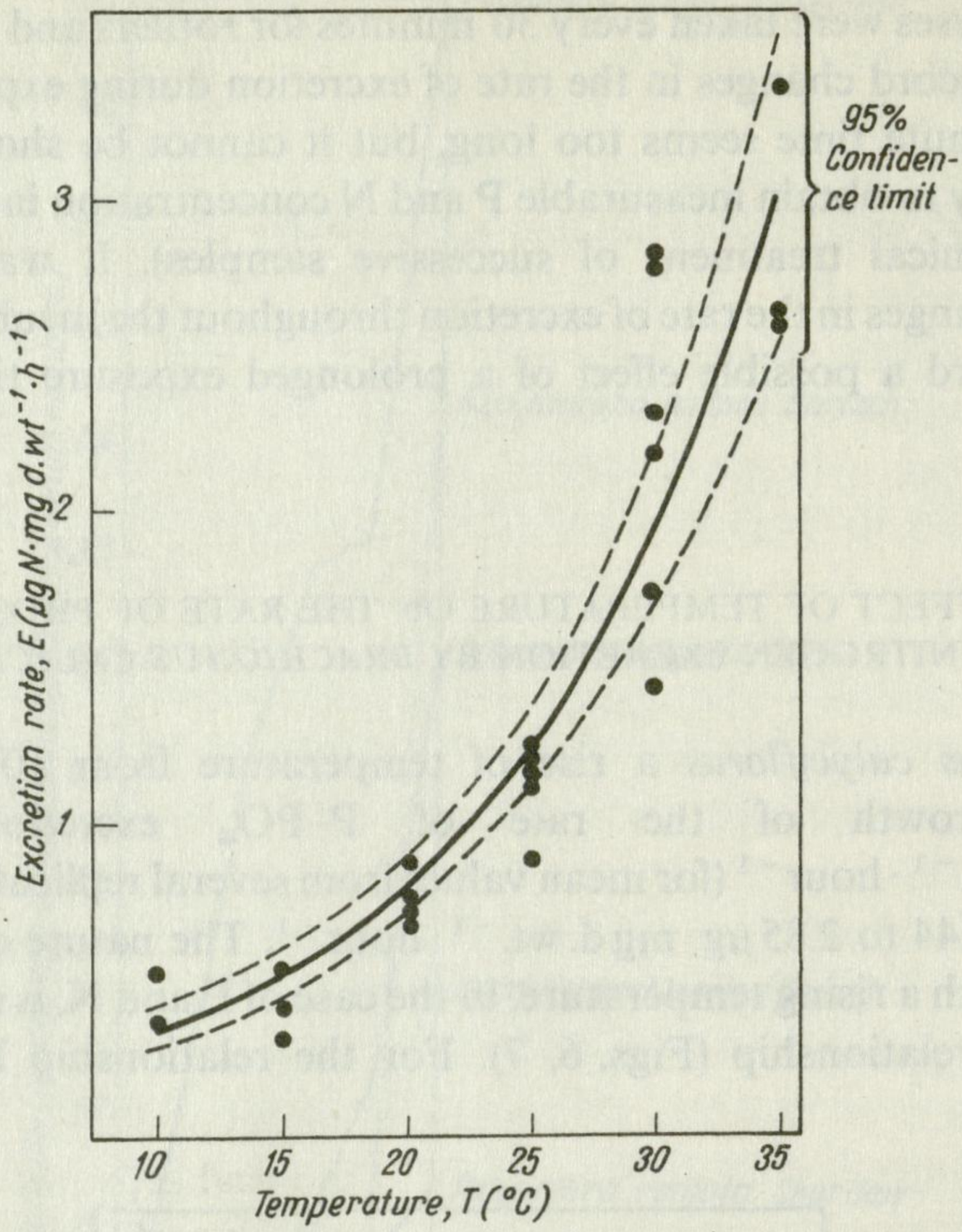

Fig. 7. Relationship between the rate of $N\text{–}NH_4$ excretion and temperature (tests were carried out on *Brachionus calyciflorus* under full thermical acclimation conditions)

phosphorus excretion and temperature the following regression equation was obtained:

$$\ln E_P = (0.096 \pm 0.017)\,T - (2.39 \pm 0.19)$$
$$(R = 0.96;\ n = 15;\ p < 0.001)$$
$$\text{or: } E_P = 0.092\, e^{0.096T} \qquad (1)$$

where: E_P = excretion rate in $\mu g\,P \cdot mg\,d.\,wt.^{-1} \cdot hour^{-1}$, T = temperature in °C.

The same relationship for the rate of nitrogen excretion is described by the equation:

$$\ln E_N = (0.088 \pm 0.012)\,T - (1.98 \pm 0.15)$$
$$(R = 0.94;\ n = 25;\ p < 0.001)$$
$$\text{or: } E_N = 0.138\, e^{0.088T} \qquad (2)$$

where: E_N = excretion rate in $\mu g\,N \cdot mg\,d.\,wt.^{-1} \cdot hour^{-1}$; T = as above.

On the basis of the equations (1) and (2) Q_{10} coefficients were calculated from the formula $\lg Q_{10} = 10^{\circ}\,(\lg Q_2 - \lg Q_1)\,(t_2 - t_1)$, where Q = excretion rate, t = temperature, Q_1 and Q_2 = excretion rate at temperature t_2 and t_1 (S u š č e n j a 1972). The value of this coefficient over the temperature range 10–35°C was 2.6 – for phosphorus excretion rate and 2.4 – for nitrogen excretion rate.

3.4. EFFECT OF INDIVIDUAL BODY WEIGHT ON THE RATE OF PHOSPHORUS AND NITROGEN EXCRETION, AND THE N:P RATIO IN EXCRETION PRODUCTS

A total of 78 tests were carried out, the aim of which was to study the rate of $P–PO_4$ excretion by plankton rotifers and crustaceans in relationship to their individual body weights, and 66 tests for the rate of $N–NH_4$ excretion. The results of these investigations have been presented in the form of mean, for each species, values of individual body weights and excretion rate, and ranges of the values obtained (Table I). The excretion rate values have been given for the temperature of 20°C. Data from tests carried at temperatures other than 20°C have been adjusted to this temperature by using equations (1) and (2) for rotifers, and for crustaceans the relationship $E = a\,e^{0.039T}$, where a = a coefficient representing the function of the body weight (acc. to P e t e r s and R i g l e r 1973).

A growth of individual body weight was accompanied by a clear decrease in the specific excretion rate of both phosphorus and nitrogen (Table I, Figs. 8, 9). This is particularly conspicuous in the rotifers and copepods. In the Cladocera this relationship is not so clear, probably because of the relatively narrow range of individual body weights in this group, amounting to 0.6–6 μg, while the same range for the Rotatoria amounted to 0.01–1 μg, and for the Copepoda – 0.3–93 μg d. wt. Apart from this, the Cladocera differed more in the ecological sense, because they included pelagial species (*Daphnia cucullata* and *D. longispina*), littoral species, species typical of dystrophic water bodies (*Polyphemus pediculus* and *Scapholeberis mucronata*) and *Bosmina longirostris* individuals of the 1st spring generation taken from a population gathering in the near-shore lake zone.

The highest excretion rate (40 μg P and 129 μg N · mg d. wt.$^{-1}$ · hour^{-1}) was recorded for the smallest-bodied species studied, *Synchaeta kitina*, of an average weight of 0.0110 μg d. wt. The lowest excretion rate (0.040 μg P and 0.220 μg N) was recorded for *Cyclops vicinus* of an average weight of 37.4 μg (Figs. 8, 9). In rotifers the rate of excretion of both P and N drops proportionately to the individual body weight increase; here an exception is *Keratella quadrata*, with a comparatively high excretion rate, and *Polyarthra dolichoptera* with a relatively low excretion rate. Consequently, the species, whose individual body weights are similar, differ in the excretion rate by more than one magnitude class.

The three species and four groups (nauplii of *M. leuckarti* represented a separate group) of Copepoda show a uniform decrease in the specific excretion rate of both nutrients, following an increase in the individual body weights (Figs. 8, 9).

In the Cladocera the relationship between the excretion rate and individual body weight is much less-marked. Particularly conspicuous is the exceptionally low rate of P and N excretion in *Bosmina longirostris*.

On the basis of the data from the particular results regression equations have been calculated for the relationship between the rate of P and N excretion and individual body weight of the animals of three taxonomic groups separately, and of the

Table I. Mean excretion rate and its range (in μg $P-PO_4$ and $N-NH_4$ mg dry wt.$^{-1}$ · hour^{-1}) and mean individual weights and their range (in μg dry weight) for experimental animals of different species

Species	Phosphorus			Nitrogen		
	no. of exper.	mean indiv. body wt. and its range	mean excretion rate and its range	no. of exper.	mean indiv. body wt. and its range	mean excretion rate and its range
Rotatoria						
Synchaeta kitina	1	0.0110	40.9	1	0.0110	129
Keratella cochlearis	6	0.0127 (0.0111–0.0138)	35.8 (15.6–45.0)	7	0.0133 (0.0111–0.0162)	43.1 (10.7–129)
Conochilus unicornis	1	0.0400	7.48	1	0.0400	16.1
Polyarthra vulgaris	2	0.0417 (0.0204–0.0631)	7.26 (7.21–7.31)	2	0.0417 (0.0204–0.0631)	13.6 (5.62–21.6)
Keratella quadrata	1	0.0531	14.0	1	0.0531	40.9
Polyarthra dolichoptera	6	0.0720 (0.0560–0.0900)	0.626 (0.280–0.911)	4	0.0670 (0.0560–0.0809)	1.77 (1.17–2.86)
Synchaeta pectinata	3	0.240 (0.0752–0.368)	0.707 (0.176–1.70)	2	0.322 (0.277–0.368)	1.62 (0.800–2.44)
Asplanchna pridonta	3	0.776 (0.499–1.07)	0.194 (0.0600–0.421)	2	1.83 (0.760–1.07)	0.231 (0.113–0.350)
Cladocera						
Bosmina longirostris	3	0.733 (0.640–0.840)	0.263 (0.140–0.472)	3	0.733 (0.640–0.840)	0.841 (0.382–1.16)
Scapholeberis mucronata	5	1.08 (0.650–1.89)	0.516 (0.187–0.775)	5	1.08 (0.650–1.89)	1.59 (0.580–2.27)

Ceriodaphia quadrangula	14	1.12 (0.509–2.32)	0.583 (0.0770–1.38)	10	1.21 (0.570–2.32)	2.23 (0.924–3.01)
Daphnia cucullata	6	3.60 (1.16–6.20)	0.498 (0.243–0.661)	3	5.12 (3.85–6.20)	0.997 (0.771–1.12)
Polyphemus pediculus	1	4.40	0.295	1	4.40	0.328
Daphnia longispina	5	4.45 (1.12–6.52)	0.394 (0.139–0.715)	4	4.53 (1.12–6.52)	2.61 (1.13–5.19)
Copepoda						
Mesocyclos leuckarti nauplii	3	0.313 (0.180–0.470)	0.845 (0.520–1.12)	3	0.313 (0.180–0.470)	3.13 (1.16–5.30)
Mesocyclos leuckarti copepodites + adults	11	1.57 (1.00–2.13)	0.337 (0.100–0.895)	10	1.52 (1.00–2.09)	1.38 (0.220–5.01)
Eudiaptoms gracilis	2	3.30 (3.10–3.50)	0.196 (0.106–0.285)	2	3.30 (3.10–3.50)	0.827 (0.635–1.02)
Cyclops vicinus	5	37.4 (20.5–93.0)	0.0400 (0.0269–0.0478)	5	37.4 (20.5–93.0)	0.220 (0.158–0.275)

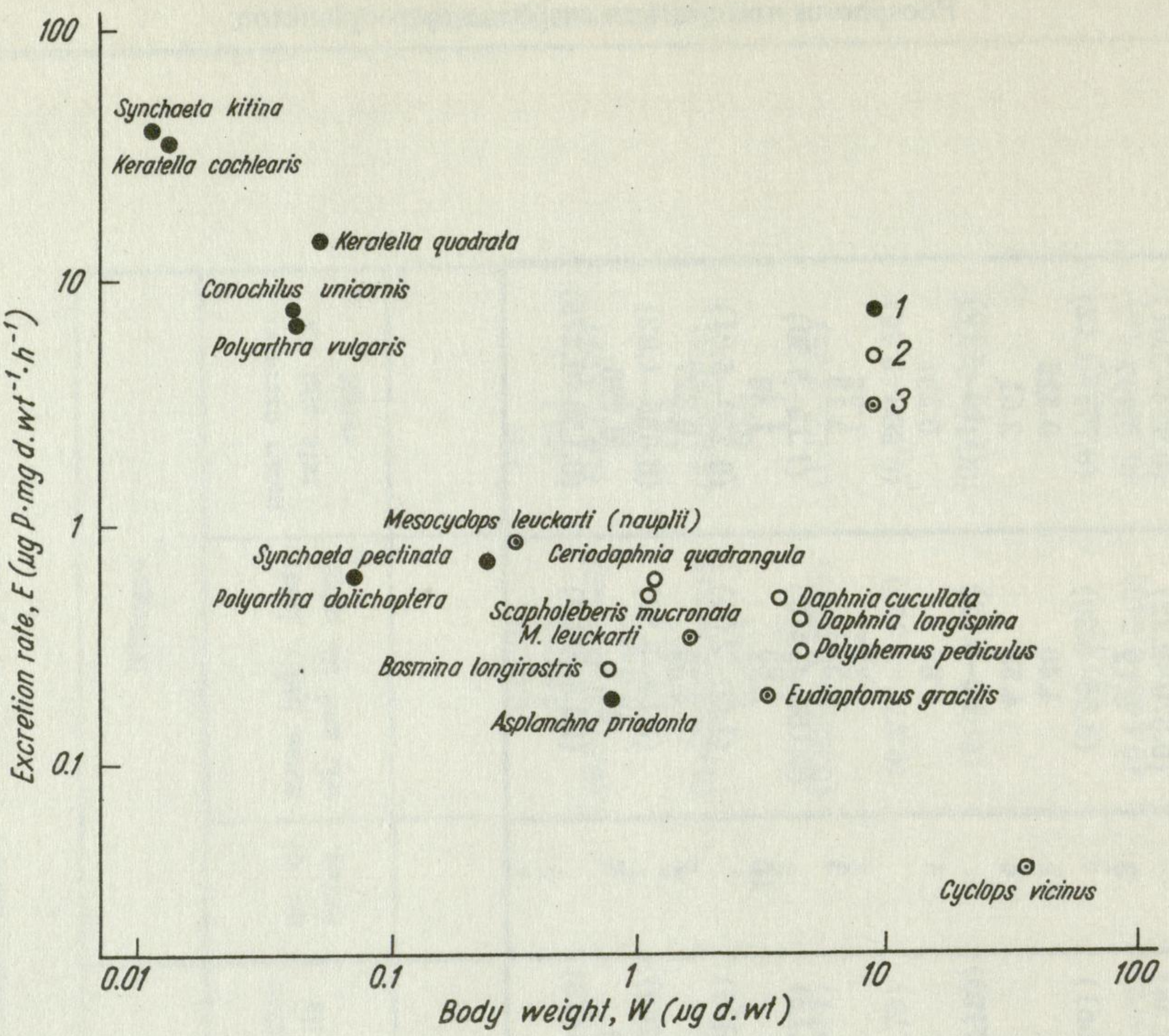

Fig. 8. Relationship between the rate of $P–PO_4$ excretion and individual body weights of the particular species of rotifers (1), cladocerans (2) and copepods (3), on the basis of the mean values of both parameters for each species

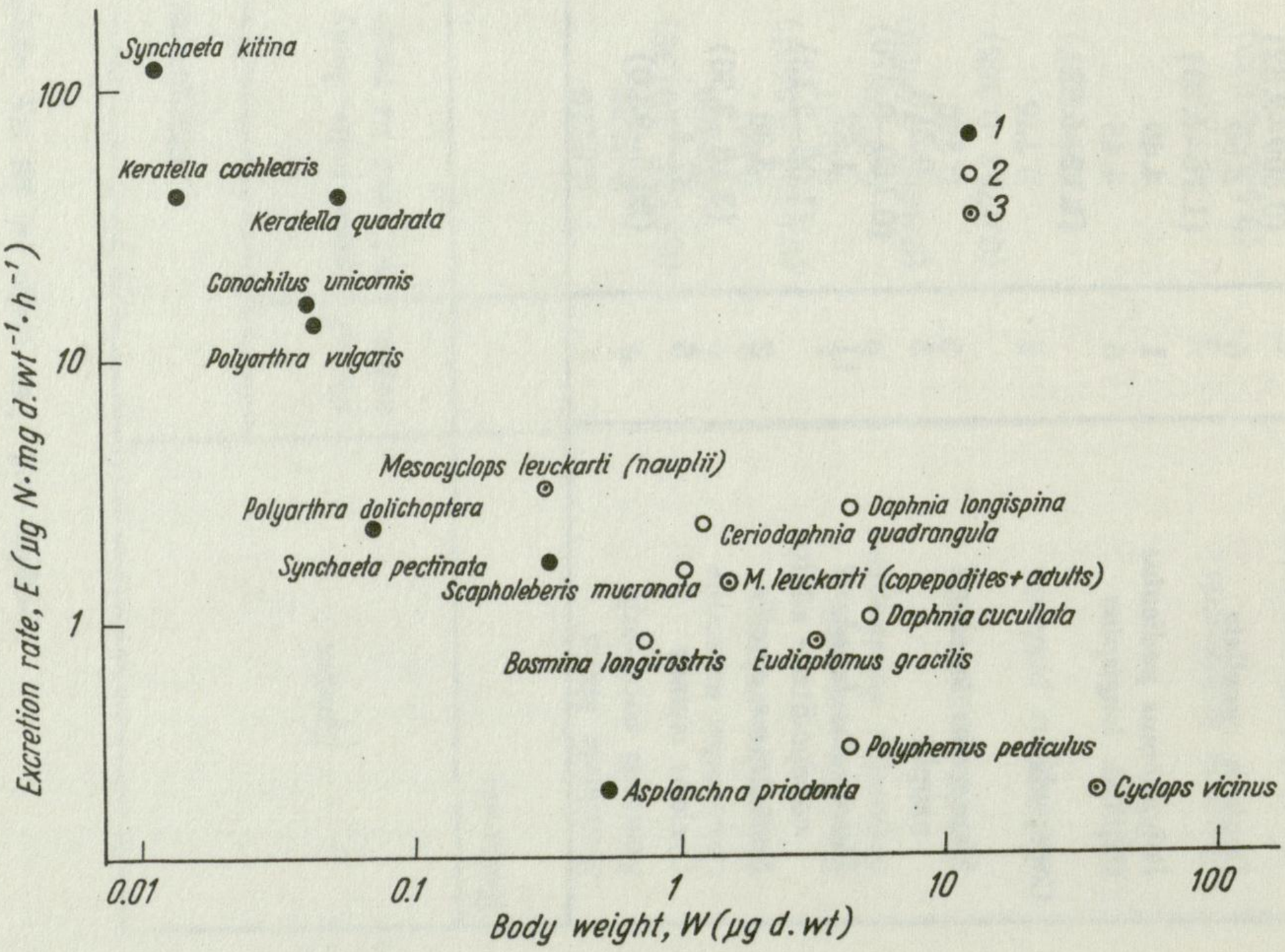

Fig. 9. Relationship between the rate of $N–NH_4$ excretion and individual body weights of the particular species of rotifers (1), cladocerans (2) and copepods (3), on the basis of the mean values of both parameters for each species

zooplankton as a whole. The regressions have been represented graphically in Figures 10 and 11.

The following regression equations describe the relationship between the rate of P–PO_4 excretion and individual body weight of animals:

for Rotatoria:

$$\ln E_P = -(1.43 \pm 0.30) \ln W - (3.12 \pm 0.45)$$
$$(R = 0.91;\ n = 23;\ p < 0.001)$$
$$\text{or: } E_P = 0.0443\, W^{-1.43} \qquad (3)$$

for Cladocera:

$$\ln E_P = -(0.230 \pm 0.254) \ln W - (0.781 \pm 0.112)$$
$$(R = 0.31;\ n = 34;\ p = 0.1)$$
$$\text{or: } E_P = 0.458\, W^{-0.230} \qquad (4)$$

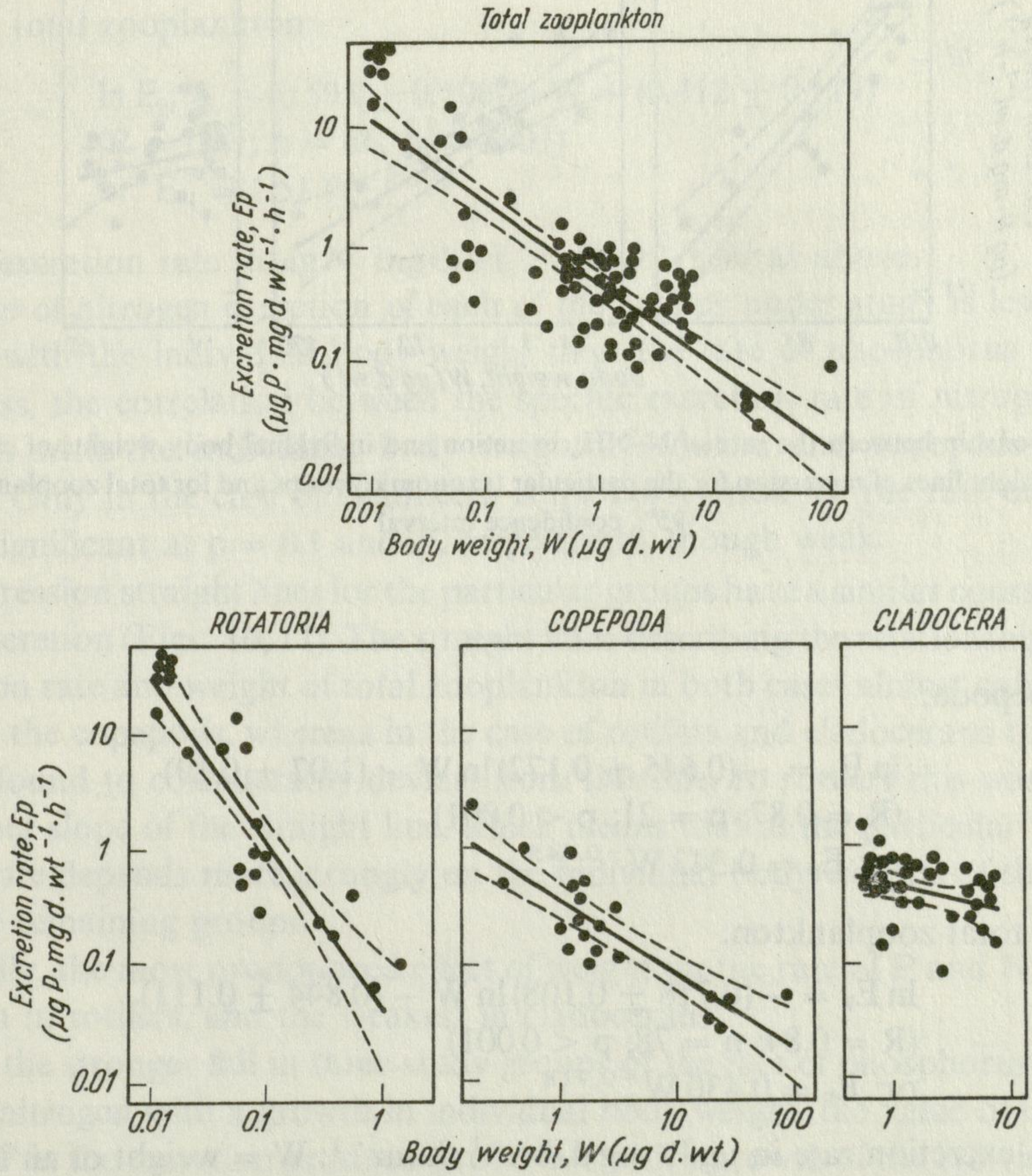

Fig. 10. Relationship between the rate of P–PO_4 excretion and individual body weights of experimental animals, and straight lines of regression for the particular taxonomic groups and for the zooplankton as a whole with a 95% confidence interval

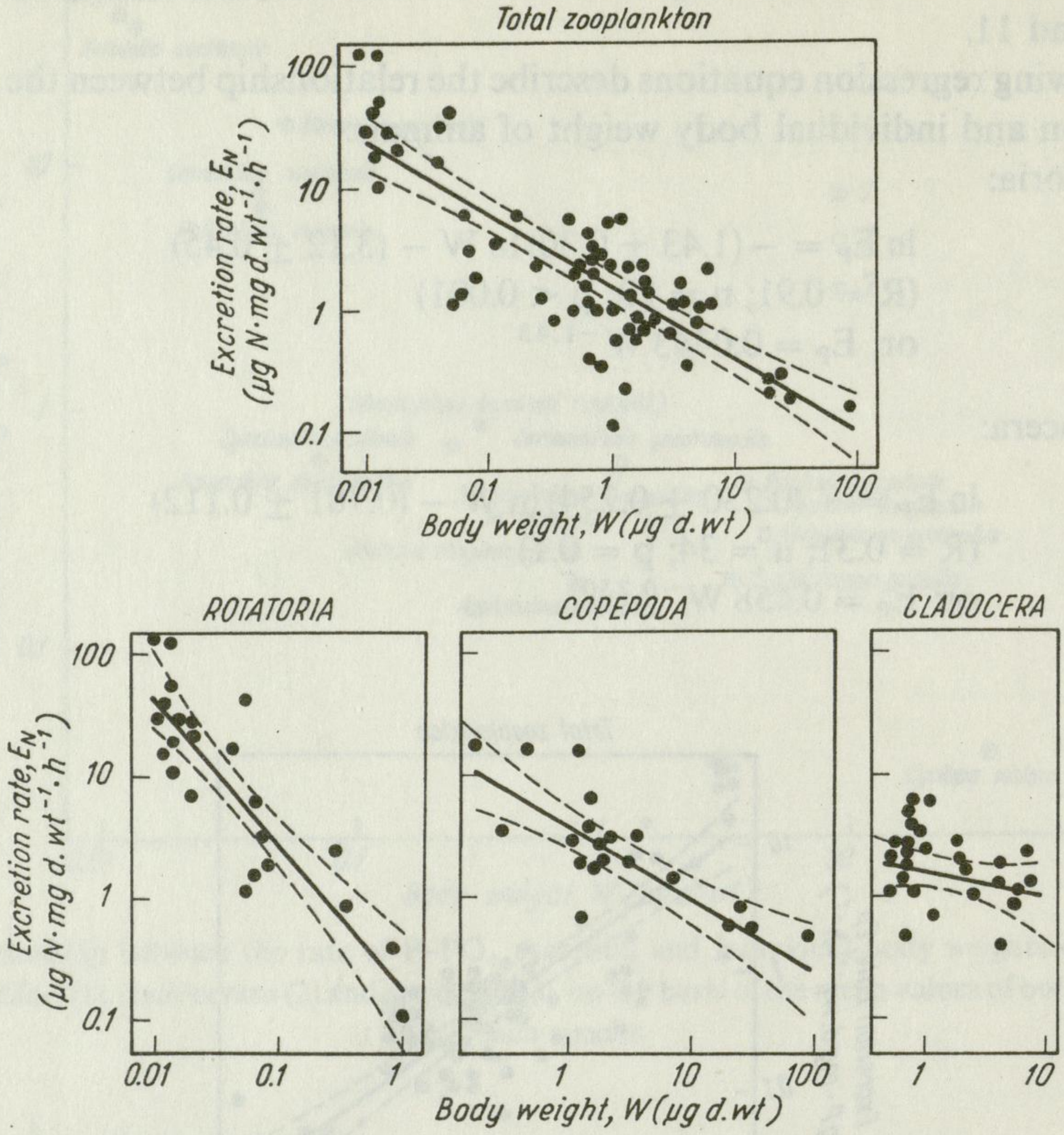

Fig. 11. Relationship between the rate of $N–NH_4$ excretion and individual body weights of experimental animals – straight lines of regression for the particular taxonomic groups and for total zooplankton with a 95% confidence interval

for Copepoda:

$$\ln E_P = -(0.645 \pm 0.172) \ln W - (1.07 \pm 0.19)$$
$$(R = 0.87;\ n = 21;\ p < 0.001)$$
$$\text{or: } E_P = 0.343\, W^{-0.645} \qquad (5)$$

and for total zooplankton:

$$\ln E_P = -(0.718 \pm 0.108) \ln W - (0.844 \pm 0.111)$$
$$(R = 0.84;\ n = 78;\ p < 0.001)$$
$$\text{or: } E_P = 0.430\, W^{-0.718} \qquad (6)$$

where E_P = excretion rate in $\mu g\, P \cdot mg\, d.\, wt.^{-1} \cdot hour^{-1}$; W = weight of an individual in μg of dry weight.

The relationship between the rate of $N–NH_4$ excretion and individual body weight can be described by the following regression equations:

for Rotatoria:

$$\ln E_N = -(1.21 \pm 0.31) \ln W - (1.70 \pm 0.50)$$
$$(R = 0.89;\ n = 20;\ p < 0.001)$$
$$\text{or: } E_N = 0.183\, W^{-1.21} \quad (7)$$

for Cladocera:

$$\ln E_N = -(0.191 \pm 0.311) \ln W + (0.464 \pm 0.148)$$
$$(R = 0.25;\ n = 26;\ p = 0.3)$$
$$\text{or: } E_N = 1.59\, W^{-0.191} \quad (8)$$

for Copepoda:

$$\ln E_N = -(0.536 \pm 0.208) \ln W + (0.278 \pm 0.035)$$
$$(R = 0.79;\ n = 20;\ p < 0.001)$$
$$\text{or: } E_N = 1.32\, W^{-0.536} \quad (9)$$

and for total zooplankton:

$$\ln E_N = -(0.593 \pm 0.108) \ln W + (0.412 \pm 0.119)$$
$$(R = 0.81;\ n = 66;\ p < 0.001)$$
$$\text{or: } E_N = 1.51\, W^{-0.593} \quad (10)$$

where: E_N excretion rate in $\mu g\,N \cdot mg\,d.\,wt.^{-1} \cdot hour^{-1}$; W as above.

The rate of nitrogen excretion of each of the groups under study is less strongly correlated with the individual body weight than the rate of phosphorus excretion. Nevertheless, the correlation between the specific excretion rate of nitrogen and of phosphorus with the individual body weight of rotifers and copepods is highly significant. Only in the case of Cladocera is the correlation for the rate of P and N excretion significant at $p = 0.1$ and .3, respectively, though weak.

The regression straight lines for the particular groups have a similar course for both P and N excretion (Figs. 10, 11). The straight lines describing the relationship between the excretion rate and weight of total zooplankton in both cases almost coincide with the line for the copepods, whereas in the case of rotifers and cladocerans the straight lines were found to considerably deviate from this line. In rotifers this was due to a much steeper slope of the straight line, which means that in the particular group the excretion rate depends more strongly on the individual body weights of the animals than in the remaining groups.

Generally, the most pronounced effect of weight on the rate of P and N excretion can be seen in rotifers, and the weakest in cladocerans.

Due to the stronger fall in three study groups of the rate of phosphorus excretion relative to nitrogen with a growth in individual body weight, the value of the weight ratio of N:P in the excretion products grows fast with a growth of the individual body weight of the animals (Fig. 12). The growth is most intense in rotifers, being least intensive in cladocerans.

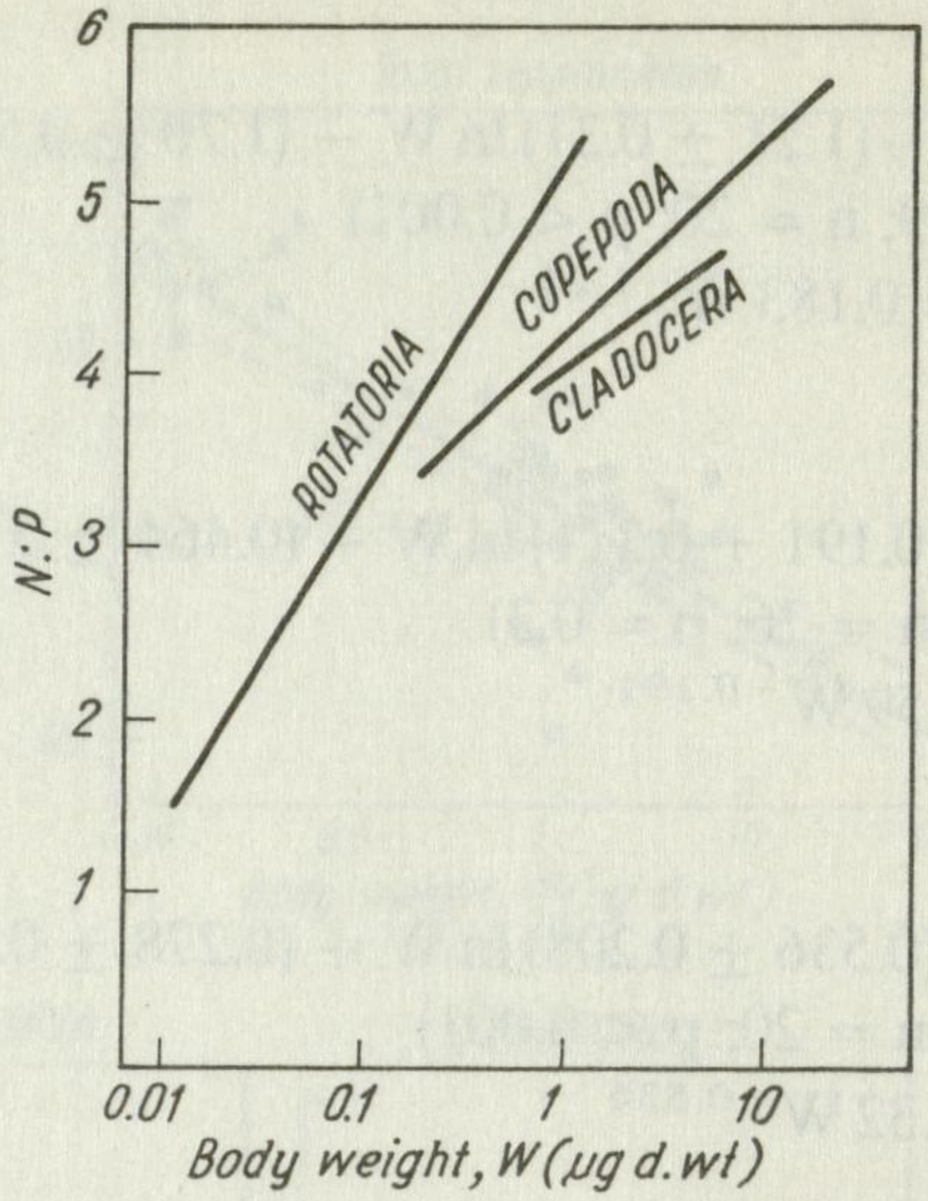

Fig. 12. Variations of the N:P ratio in the excretion products of 3 taxonomic groups of the zooplankton, following changes in individual body weight of the animals (on the basis of regression equations, 13–18, for the relationship between the rate of P and N excretion and individual body weight)

3.5. EFFECT OF FOOD ON THE RATE OF PHOSPHORUS AND NITROGEN EXCRETION

Six experiments have been carried out with a natural zooplankton, the specific composition of which was not homogeneous, and in which there was a strong

Table II. Comparison of the rate of phosphate–P and ammonium–N excretion by zooplankton: non-starved (E_F) and starved prior to exposure (E_S) (in brackets values are given of standard deviation for n = 3)

Taxonomic composition	Excretion rate in µg · mg d. wt.$^{-1}$ · hour^{-1}				$E_F \cdot E_S^{-1}$	
	phosphorus		nitrogen			
	E_F	E_S	E_F	E_S	P	N
Mixed zooplankton with *Mesocyclops leuckarti* dominating	0.162 (0.006) 0.260 (0.062) 0.332 (0.033) 0.075 (0.007)	0.091 (0.007) 0.100 (0.003) 0.156 (0.012) 0.047 (0.004)	0.735 (0.271) 0.621 (0.066) 0.478 (0.046) 0.373 (0.078)	0.270 (0.014) 0.267 (0.027) 0.234 (0.048) 0.156 (0.037)	1.8 2.6 2.1 1.6	2.7 2.3 2.0 2.4
Mixed zooplankton with *Synchaeta pectinata* dominating	0.200 (0.010) –	0.140 (0.005) –	0.981 (0.072) 0.246 (0.007)	0.566 (0.093) 0.103 (0.008)	1.4 –	1.7 2.4
Average					1.9	2.2

dominance of *Mesocyclops leuckarti* in 4 experiments, and of *Synchaeta pectinata* in 2 experiments.

Starvation of experimental animals caused an about 2–fold decrease in the rate of nutrient excretion (Table II). The mean value of the ratio of the rate of P excretion by animals not starved before the measurement (E_F) to the rate of P excretion by starved animals (E_S) was 1.9; the value of this ratio for nitrogen was 2.2, its variations being small in spite of the fact that the composition of both the zooplankton and its food was different in each experiment.

4. DISCUSSION

4.1. EFFECT OF TEMPERATURE ON THE PROCESS OF PHOSPHORUS AND NITROGEN EXCRETION

The speed of most chemical reactions is doubled or trebled with each temperature growth by 10°C, thus the temperature coefficient of these reactions Q_{10} = reaction speed $(T + 10°C) \cdot [\text{reaction speed } (T)\,°C]^{-1}$ should remain within the limits from 2 to 3 (H o c h a c h k a and S o m e r o 1978). The metabolism consists of many biochemical reactions the Q_{10} of which varies. A thermal reaction of such indices of metabolic changes as, e.g., excretion constitutes a certain average quantity of all the metabolic pathways. Moreover, when encountering adverse temperatures, animals (also poikilotherms) can use a strategy involving compensatory changes in the cell chemistry in a direction ensuring the maintenance of the metabolic rates at a safe level (H o c h a c h k a and S o m e r o 1978). It may thus be expected that the reaction of the excretion rate to temperature changes will vary considerably.

A survey of the literature concerned with the effect of temperature on the excretion rate (Table III) indicates that in spite of the fact that the test animals differed taxonomically, the Q_{10} coefficient was in most cases approaching 2, for both the rate of phosphorus excretion (e.g., B u t l e r, C o r n e r and M a r s h a l l 1970, G a n f and B l a ž k a 1974) and of nitrogen excretion (e.g., B u t l e r, C o r n e r and M a r s h a l l 1970, G a n f and B l a ž k a 1974, G o p h e n 1976). The range of its variation from taxon to taxon was 1.00–2.59 – for phosphorus, 0.87–2.74 – for nitrogen (Table III). The values of Q_{10} obtained in the present series for *Brachionus calyciflorus* (for P = 2.6; for N = 2.4) are thus within the upper limits of the values given in the literature for other animal species.

In most studies a logarithmic or exponential relationship was found between the excretion rate and temperature (Table III, P e t e r s and R i g l e r 1973). Also the values obtained in the present investigation were best approximated by the exponential function (Figs. 6, 7).

In the lake pelagial zone (except the thin surface layer) temperature changes occur gradually, owing to which aquatic animals are given the time necessary for setting in motion the appropriate adaptive mechanisms. In laboratories experiments are carried

Table III. Effect of temperature on the rate of phosphate–P and ammonium–N excretion by zooplankton – a comparison of literature data

P, N	Taxonomic group	Range of study temperatures in °C	Q_{10} values	Comments	Author
$P–PO_4$	*Cyclops* *Daphnia* *Diaptomus*	10–20 10–20 10–20	1.75; 1.64; 1.63 1.77; 1.84; 1.77 1.81; 1.45; 1.54	for 3 levels of food concentration	LaRow (1973)
	Daphnia	10–21	1.50		Peters and Rigler (1973)
	Thermocyclops hyalinus	20–35	2.00	$\log_{10} P = 0.0304\ t - 0.045$, P = excretion rate in $\mu g\ P \cdot mg\ d.wt.^{-1} \cdot hour^{-1}$	Ganf and Blažka (1974)
	Calanus finmarchicus	6–18	2.00 1.97 2.00	for females for males for 5th stage copepodite	Butler, Corner and Marshall (1970)
	C. finmarchicus	5–15	1.00–2.29	for 6 hours' experiments	Marshall and Orr (1961)
	Acartia tonsa	10–20	2.59		Hargrave and Geen (1968)
	mixed marine zooplankton	10–20	1.81		Fournier et al. (1977)
	Cyclops *Daphnia* *Diaptomus*	10–20	1.81; 1.70; 1.59 1.70; 2.00; 1.98 1.76; 1.73; 1.49	for 3 levels of food concentration	LaRow (1973)
	Daphnia pulex	15–25	2.74	$\log_{10} E = (0.043)\ T + 0.153$, E = excretion in $\mu g\ N \cdot mg\ d.wt.^{-1} \cdot day^{-1}$	Jacobsen and Comita (1976)

$N-NH_4$	*Ceriodaphnia reticulata*	15–27	2.08		Gophen (1976)
	Thermocyclops hyalinus	21–35	2.00	$\log_{10} N = 0.0302\ t + 0.4822$, N = excretion in $\mu g\ N \cdot mg\ d.\ wt.^{-1}\ hour^{-1}$	Ganf and Blažka (1974)
	Calanus finmarchicus	6–18	2.08 2.12 2.21	for females for males for 5th stage copepodite	Butler, Corner and Marshall (1970)
	mixed marine zooplankton	10–20	1.67		Fournier et al. (1977)
	Temora stylifera	13–21	1.83	$E = 2.799 \cdot 1.062^T$	
	Calanus helgolandicus	13–23	1.37	$E = 3.819 \cdot 1.032^T$	
	Acartia clausi	13–23	0.87	$E = 5.683 \cdot 0.986^T$	
	Centropages typicus	13–22	1.34	$E = 4.511 \cdot 1.03^T$ E = excretion in $\mu g\text{–at} \cdot 10^{-3} \cdot \mu g\text{–at}\ N^{-1} \cdot hour^{-1}$	Nival et al. (1974)

out without acclimating animals to experimental temperatures in order to study the so-called "sharp metabolic responses", that is, the metabolic reaction of the body, as a rule rapid, to strongly changed thermical conditions. In the present investigations the animals were acclimated to the experimental temperatures to avoid this mistake. The relationships found for *Brachionus* were generalized to include other rotifer species (because of the lack of appropriate data). Most of the rotifers found in the pelagial zone of the lakes in the temperate zone, including *B. calyciflorus*, are considered to be eurithermical (K u t i k o v a 1970). It is therefore probable that the relationships between the rate of P and N excretion and temperature in other rotifer species are similar to those found for *B. calyciflorus*.

Experimental conditions similar to those described in the present paper (i.e., thermal acclimation and control of other environmental factors) were maintained in the investigations carried out by P e t e r s and R i g l e r (1973) with *Daphnia rosea*. For this reason, they were used for cladocerans and also in the case of copepods if no appropriate data were available. The equation obtained by the above authors is as follows: $E = a e^{0.039T}$, where a = coefficient as a weight function, T = temperature in °C, E = specific excretion rate. In view of the lack of data it was also used for the rate of nitrogen excretion. It must be noted that the Q_{10} values described in this paper and in the literature for phosphorus and nitrogen excretion did not in fact differ much (Table III, Figs. 6, 7).

4.2. EFFECT OF INDIVIDUAL BODY WEIGHT ON THE RATE OF PHOSPHORUS AND NITROGEN EXCRETION

One of the basic features of the excretion rate observed by many investigators was its relationship to the body weight of the individuals studied; a growth in individual body weight was followed by a decrease in the specific rate of P and N excretion (J o h a n n e s 1964a, B a r l o w and B i s h o p 1965, H a r g r a v e and G e e n 1968, M u r a v s k a j a 1968, 1973, 1978, P e t e r s and R i g l e r 1973, P e t e r s 1975, G u t e l m a c h e r 1977).

In many studies this relationship was described by the power function ($E = a W^b$), taken from earlier generalizations concerning the effect of body weight on the respiratory rate. It has therefore been assumed that since this function has been accepted for the respiratory rate, then it should also be correct for another aspect of the metabolism – nitrogen and phosphorus excretion. Studies of the ratio of the amount of oxygen taken to the nitrogen and phosphorus excreted have shown that its value should be constant for a particular group of organisms under specific trophical conditions (S a t o m i and P o m e r o y 1965). The authors of the papers concerned with this problem (S a t o m i and P o m e r o y 1965, M u r a v s k a j a 1973, G a n f and B l a ž k a 1974, G u t e l m a c h e r 1977, I k e d a 1977) suggested that the rate of excretion also can always be calculated from known respiratory rate values. This approach has been used in modelling the phosphorus cycling in water bodies (e.g., S c a v i a 1979).

The power nature of the relationship between the metabolic rate and body weight has been formulated on the basis of Rubner's "surface law". This theory has been checked against an immense number of experimental data relating to animal respiratory rate, and it has been demonstrated that the average value of the power regression coefficient (b) is 0.75 (if the respiratory rate is expressed per unit biomass, it will take the value of −0.25) (W i n b e r g 1976), regardless of whether it concerns homoiotherms or poikilotherms, uni- or multicellular, provided that the animals studied represented a group with a sufficiently wide range of body weights.

A review of the literature dealing with the problem of body weight effect on the specific excretion rate shows that the values of the coefficient of power found for phosphorus excretion vary between −0.16 (G u t e l m a c h e r 1977) and −0.92 (B a r l o w and B i s h o p 1965), for nitrogen excretion between −0.20 (calculated on the basis of the paper by N i v a l et al. 1974) and −0.81 (calculated from data in S m i t h' s 1978 paper). Moreover, even in studies carried out by one author different values of coefficient b were obtained for different groups of organisms; e.g., for two groups of marine organisms J o h a n n e s (1964a) obtained b values of −0.33 and −0.67 (according to P e t e r s and R i g l e r 1973), whereas for epilimnion cladocerans B a r l o w and B i s h o p (1965) obtained a value of b = −0.69, but b = −0.92 for hypolimnion copepods.

The range of the b coefficient obtained in the present study considerably exceeds the values quoted above. For phosphorus excretion the value of b ranged from −0.23 for cladocerans to −1.43 for rotifers (equations 3, 4 and 5), whereas for the excretion of nitrogen from −0.191 for cladocerans to −1.21 for rotifers (equations 7, 8 and 9). The differences seem to be caused primarily by the diet of the organisms studied, although the effect of some other, mainly internal, factors may also be expected.

Here a significant role should be ascribed to the bacterial diet. According to P o m e r o y (1975), there is up to 5% P and 15% N in the dry bacterial weight. If bacteria are eaten by the detritus-bacteria-eating zooplankton containing 1–2% P in the dry body weight and up to 10% N, then after meeting its energetic and building requirements the surplus of mineral P and N, resulting from the quantitative ratios of these elements between the bacterio- and zooplankton, must be excreted. Thus the rate of P and N excretion by the zooplankton depends on the difference between the chemical composition of the food and that of its consumers. The lowest relative excretion rate may be expected in "pure" herbivores, the food of which probably contains slightly less P and N in dry weight than its consumers, and in predators, the prey of which have a chemical body composition most similar to their own body, but a higher one in detritus-herbivores, and the highest in detritus-bacteria-eaters.

A comparison of the specific excretion rate of different species (Figs. 8, 9) confirms this supposition. In the case of rotifers, for instance, the above conclusions can explain the earlier occurrence of differences in P and N excretion rate between the detritus-eating species *Keratella quadrata* and the herbivorous *Polyarthra dolichoptera*. Moreover, in rotifers an increase in individual body weights proceeds in parallel with the changes of the trophic status of the species: detritus-feeders → detritus-plant-

feeders → herbivores → predators. A growth in individual body weight is thus accompanied by a change in diet in such a way that it results in an ever-decreasing excretion rate, irrespective of the effect of the change in individual body weights. This may account for the strikingly low value of the coefficient b (equal to −1.43), that is, a very strong, relative to other groups dependence of the excretion rate on the individual body weights.

Similar phenomena can be observed among crustaceans, e.g., the predaceous *Polyphemus pediculus* has a much lower excretion rate, with a similar body weight, than the detritus-plant-eating *Daphnia*. The rate of P and N excretion by cladocerans, in whose diet bacteria represent a considerable proportion, is characteristically higher (except *Bosmina longirostris* for which a lower than expected consumption rate was found in studies carried out by Starkweather and Bogdan 1980) than in predatory cyclopids and herbivorous Diaptomidae.

In the literature there is a relatively large amount of information on the rate of phosphorus excretion by animals of a specified body weight (Fig. 13 A), for both freshwater (Rigler 1961, Barlow and Bishop 1965, Peters and Rigler 1973, Ganf and Blažka 1974, Peters 1975, Gutelmacher 1977) and marine zooplankton (Johannes 1964a, 1965, Satomi and Pomeroy 1965, Hargrave and Geen 1968, Butler, Corner and Marshall 1970). In spite of the considerably wide dispersion of the

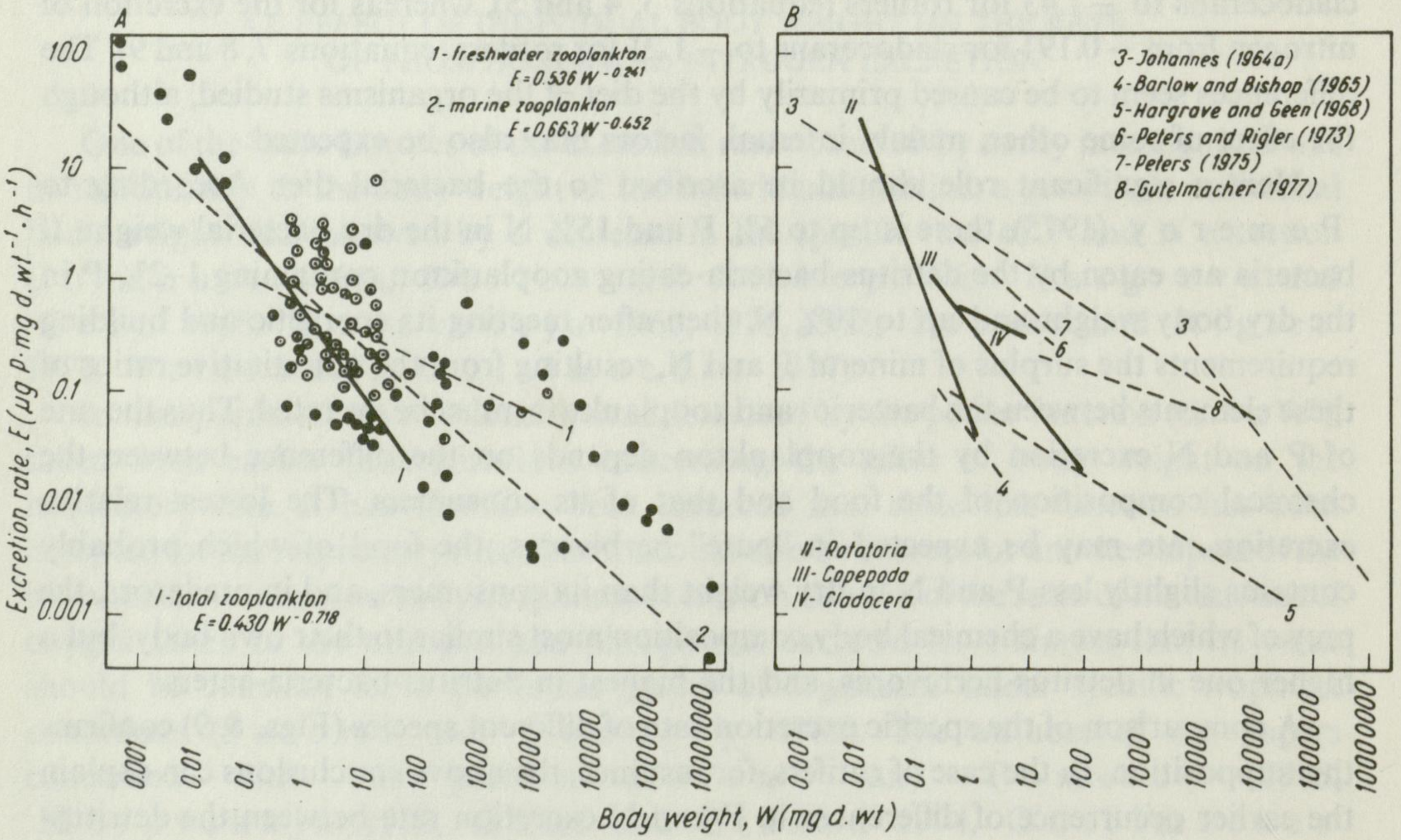

Fig. 13. Effect of individual body weights of animals on the rate of $P–PO_4$ excretion – comparison of the obtained regressions with literature data; A – summarized data from the literature, B – summary of regressions; 1–8, regressions from the literature; I–IV, regressions on the basis of the author's own studies

excretion rate values for the same body weight, as was pointed earlier by P e t e r s and R i g l e r (1973), a clear fall can be seen of the specific rate of P excretion following an increase in body weight (Fig. 13 A). The regression equations calculated from these data for freshwater ($E = 0.536\,W^{-0.243}$) and marine zooplankton ($E = 0.663\,W^{-0.452}$) differ, but it is difficult to establish to what extent this is due to the salinity of the habitat they live in. The relationship found in the present study for total zooplankton applies to the range of values obtained by other authors, although the b exponent has a value incomparably lower (-0.72) than those quoted above. This very low value is the result of a very high rate of P excretion by the group of the smallest organisms (rotifers) of the size-class of several score $\mu g\,P \cdot mg\,d.\,wt.^{-1}$ (Table I, Figs. 8–11). However, values of the same class were also obtained by F e r r a n t e (1976a) for a size fraction of the zooplankton including primarily rotifers.

The relationship between the specific excretion rate and body weight, found by different authors for different taxa vary considerably in respect of both the excretion level and its dependence on the individual body weights. The regressions obtained in the present investigations do not in principle differ from those obtained by other authors (Fig. 13 B).

A comparison of the rates of phosphorus excretion by different species of the genus *Daphnia* (Fig. 14) shows that apart from the very low values obtained by B a r l o w and B i s h o p (1965) for *Daphnia* sp. from the epilimnion of Lake Cayuga, all the data including the regression obtained in the present study ($E = 0.688\,W^{-0.426}$), are very similar. This has been taken to be a verification of the results obtained in the present study for cladocerans, and in spite of the low correlation, it was decided to use the relationship found between the excretion rate and body weight in cladocerans in the further part of the investigation.

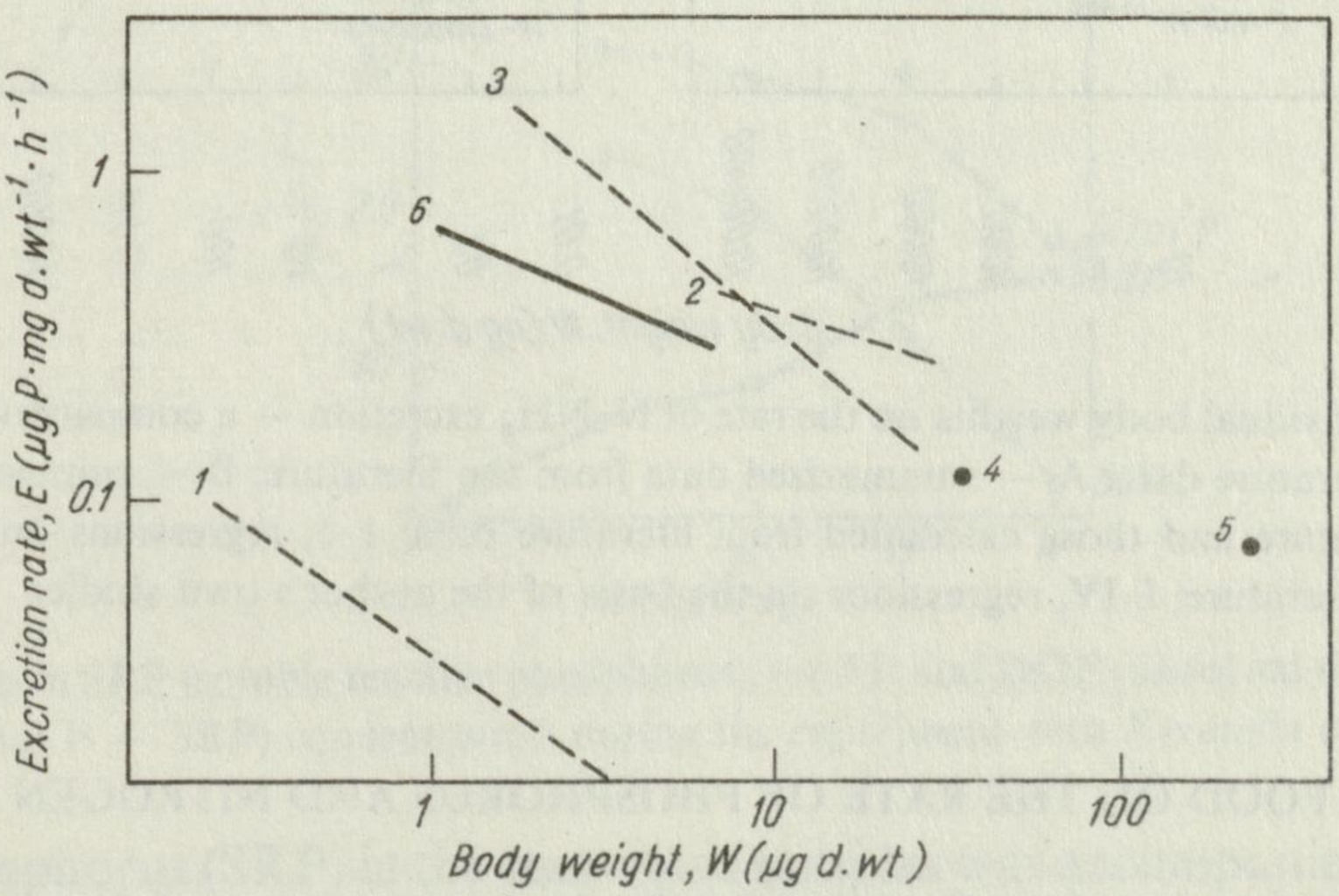

Fig. 14. Effect of individual body weights on the rate of $P–PO_4$ excretion by *Daphnia* – a comparison of the regression obtained in the present study (6) with literature data (1 – *Daphnia* sp. B a r l o w and B i s h o p 1965; 2 – *D. rosea* Sars, P e t e r s and R i g l e r 1973; 3 – *D. galeata* Sars, P e t e r s 1975; 4 – *D. pulex* (De Geer) G u t e l m a c h e r 1977; 5 – *D. magna* Straus, R i g l e r 1961)

A comparison of the literature data on the relationship between the rate of N excretion and individual body weights (Fig. 15) (C o r n e r and N e w e l l 1967, B u t l e r, C o r n e r and M a r s h a l l 1970, J a w e d 1973, M a y z a u d 1973, G a n f and B l a ž k a 1974, N i v a l et al. 1974, G o p h e n 1976, S m i t h and W h i t l e d g e 1977, M u r a v s k a j a 1978, S m i t h 1978, N e l s o n, S i m m o n s and K n i g h t 1979) leads to the same conclusions as in the case of phosphorus. Although less information has been found for nitrogen than for phosphorus, e.g., N excretion by freshwater zooplankton was so far almost left out during investigations, it can be estimated even in this case that the b coefficient of the relationship found during the present study for total zooplankton is much lower (-0.593) than most of the values obtained before (-0.2–-0.4); an exception here is the data reported by S m i t h (1978), on the basis of which a regression analysis has been made, and a value of the coefficient b of -0.805 has been obtained. The level of N excretion by total zooplankton, as well as by its three taxonomic groups does not differ much from the levels found by other investigators (Fig. 15).

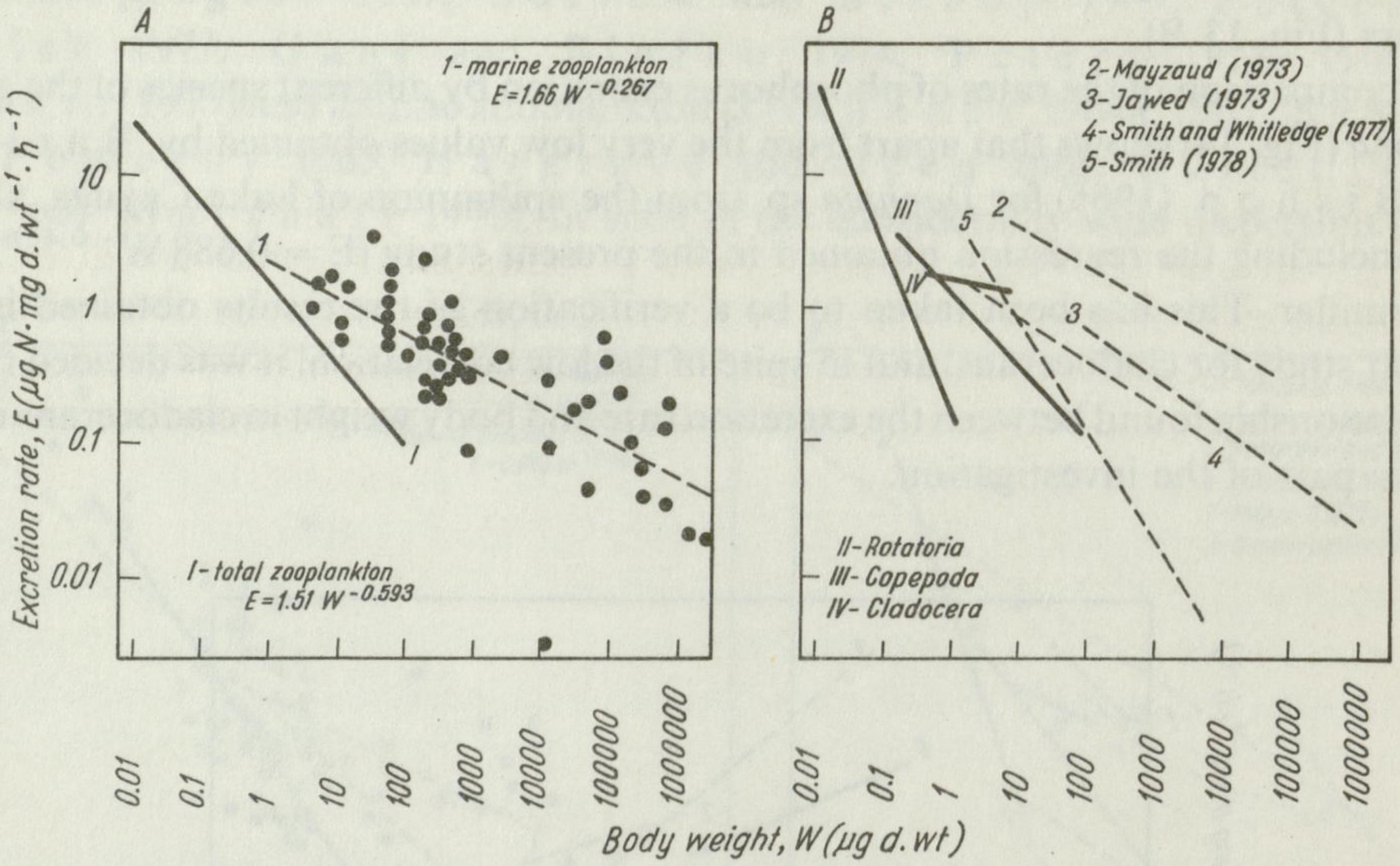

Fig. 15. Effect of individual body weights on the rate of N–NH_4 excretion – a comparison of the obtained regressions with literature data; A – summarized data from the literature; B – summary of regressions quoted in the literature and those calculated from literature data; 1–5, regressions on the basis of the literature; I–IV, regressions on the basis of the author's own studies

4.3. EFFECT OF FOOD ON THE RATE OF PHOSPHORUS AND NITROGEN EXCRETION

The rate of excretion is influenced by both the quantity of food eaten (M a r s h a l l and O r r 1961, J o h a n n e s 1964b, H a r g r a v e and G e e n 1968, B u t l e r, C o r n e r and M a r s h a l l 1970, L a R o w 1973, P e t e r s and R i g l e r 1973, T a k a h a s h i and I k e d a 1975 and others),

its chemical composition (Hargrave and Geen 1968, Martin 1968, Ferrante 1976a, Szyper et al. 1976, Nelson, Simmons and Knight 1979), as well as the assimilability of the nutrients contained in the food (Nelson, Simmons and Knight 1979).

During the present investigation experiments on the effect of body weight on the rate of nutrient excretion were carried out with animals starved before the experiments and then incubated in lake water filtered through filters 0.45 μm in pore size. It is assumed that such water contains almost exclusively dissolved organic and mineral compounds. Hargrave and Geen (1968) report that animals placed in filtered water could feed on plaquette-like aggregates which formed in the foaming water in the vacuum flask during the filtering. Moreover, they found a considerable difference in the excretion rate depending on the presence or absence of aggregates in the water. This indicates that the animals really were able to effectively utilize this food source. The method used for the preparation of water for the experiments described in this paper was similar to that given by the above authors, and during the observation of live samples taken at the end of incubation "floccules" were found, the origin of which is hard to explain. The problem here is whether the experimental animals were really starved.

An analysis of the rate of phosphorus excretion by small rotifers of the genus *Keratella* has shown that during their incubation these animals excreted 2–3 times more phosphorus than they contained in their bodies. Since no increase in mortality was recorded at that time, it was assumed that the animals had access to some food. Additional analyses of changes in the concentration of total P during tests with *K. cochlearis* (Fig. 16) have shown that a growth of the concentration of soluble

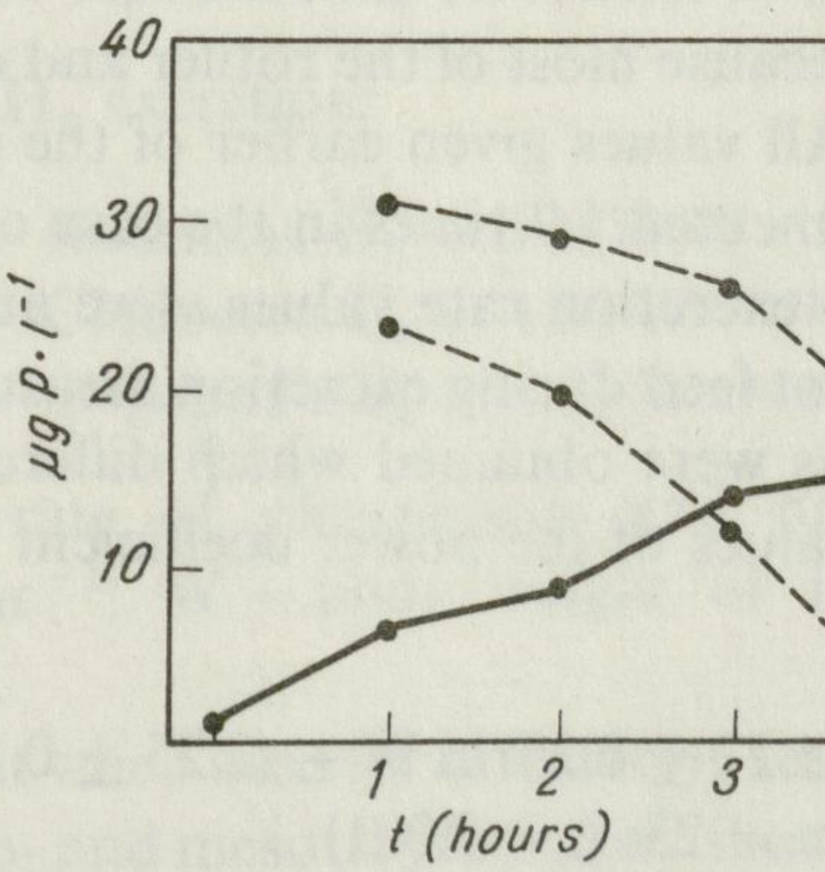

Fig. 16. Changes in SRP (soluble reactive phosphorus), total P and DOP (dissolved organic phosphorus = total P − SRP) concentration during the experiment with *Keratella cochlearis*

reactive phosphorus (SRP) in the successive samples was accompanied by a decrease in the concentration of organic phosphorus compounds (Fig. 16). The fall of organic P concentration was always greater than the growth in SRP concentration, which indicates that a part of the P taken from the environment was incorporated for good into the animals' bodies or remained on the filters in the form of particles larger than the

pores. Small-bodied sedimentators, such as *K. cochlearis*, are a case where it is hard to determine the lower size limits for the food particles available to them (J ö r g e n s e n 1966). It was therefore assumed that rotifers capable of feeding on detritus (*Keratella* and *Conochilus* – P o u r r i o t 1977) may have been feeding during the measurement of excretion. Species of the genera *Polyarthra, Synchaeta* and *Asplanchna* do not utilize detritus (P o u r r i o t 1977, B o g d a n, G i l b e r t and S t a r k w e a t h e r 1980) it should therefore be presumed that these animals really starved during the tests.

Experiments on the effect of feeding on the rate of P and N excretion (Table II) have shown that starving animals (without food in the environment and their intestines during the experiment), that is to say, all except the detritus-eating rotifers, should have a P excretion rate lower about 1.9 times, and an N excretion rate lower about 2.2 times that the respective rates for animals that fed, up to the start of the experiment, on mixed food of a concentration similar to the natural one in eutrophic lakes (i.e., animals which could digest the contents of their intestines). This agrees with the results of P e t e r s and R i g l e r (1973) who found a 2.47–fold difference in the excretion rate of *Daphnia* starved and fed with an optimum quantity of food. Similarly, H a r g r a v e and G e e n (1968) report a difference in excretion rate of 1.38 up to 2.37. But T a k a h a s h i and I k e d a (1975) found as great an increase as 5 up to 9–fold in the excretion rate for *Euphausia* and *Metridia* following a growth in phytoplankton density, while N e l s o n, S i m m o n s and K n i g h t (1979) a 3.7 to 5.9–fold growth of the excretion rate in shrimps feeding on food of different kinds (always in excess).

To approximate estimates of the rates of P and N excretion by lake zooplankton to natural conditions the fact must be taken into account that under these conditions the zooplankton seldom starves, because most of the rotifer and crustacean species find a wide spectrum of food there. All values given earlier of the excretion rate found for starved animals should be augmented 1.9 times in the case of P, and 2.2 times in the case of N. In the rotifer group excretion rate values were augmented only for those species which most likely did not feed during excretion measurements. As a result, for this group regression equations were obtained which differed from those presented earlier (3) and (7) also in the values of the power coefficient b:

for $P–PO_4$ excretion rate:

$$\ln E_P = -(1.27 \pm 0.25) \ln W - (2.25 \pm 0.14)$$
$$(R = 0.91;\ n = 23;\ p < 0.001)$$
$$\text{or: } E_P = 0.105\, W^{-1.27} \qquad (11)$$

for $N–NH_4$ excretion rate:

$$\ln E_N = -(1.01 \pm 0.28) \ln W - (0.661 \pm 0.198)$$
$$(R = 0.87;\ n = 20;\ p < 0.001)$$
$$\text{or: } E_N = 0.511\, W^{-1.01} \qquad (12)$$

where E_P and E_N = excretion rate in μg P or N · mg d. wt.$^{-1}$ · hour^{-1}, W = weight of 1 individual in μg d. wt.

5. CONCLUSIONS

On the basis of the results from the three types of experiments already presented equations have been constructed to describe the effect of the body weight of plankton animals and temperature on the rate of phosphate phosphorus and ammonium nitrogen excretion.

In rotifers the relationship between the excretion rate and temperature was determined on the basis of the results from tests with *Brachionus calyciflorus* described by equations (1) and (2). The effect of body weight for this animal group is described by equations (11) and (12) which take into account the effect of food on the rate of excretion. The relationship between the excretion rate and temperature in crustaceans has been described on the basis of data taken from P e t e r s' s and R i g l e r' s (1973) model. The effect of body weight on the rate of P and N excretion in cladocerans is described by equations (4) and (8), in copepods by equations (5) and (9). It was assumed that in the presence of food in concentrations typical of eutrophic lakes the rate of excretion in this animal group should be 1.9 times (for P) and 2.2 times (for N) as high as that in starved animals. For this reason, the a coefficient (in $E = aW^b$) has been multiplied by these values. Using the partial equations obtained earlier and the above assumptions a "model" has been obtained of the effect of factors such as temperature and body weight on the excretion rate:

for the rate of $P–PO_4$ excretion:

$$\text{Rotatoria } E_P = 0.0154\, W^{-1.27} e^{0.096T} \quad (13)$$
$$\text{Cladocera } E_P = 0.519\, W^{-0.230} e^{0.039T} \quad (14)$$
$$\text{Copepoda } E_P = 0.299\, W^{-0.645} e^{0.039T} \quad (15)$$

for the rate of $N–NH_4$ excretion:

$$\text{Rotatoria } E_N = 0.0879\, W^{-1.01} e^{0.088T} \quad (16)$$
$$\text{Cladocera } E_N = 1.80\, W^{-0.191} e^{0.039T} \quad (17)$$
$$\text{Copepoda } E_N = 1.33\, W^{-0.536} e^{0.039T} \quad (18)$$

where: E_P and E_N = rate of phosphorus and nitrogen excretion, respectively, in $\mu g \cdot mg\,d.wt.^{-1} \cdot hour^{-1}$; W = body weight of 1 individual in μg dry weight; T = temperature in °C.

It must be noted that when used for calculating the rate of nutrient excretion by the zooplankton from oligo- and mesotrophic lakes, these equations may give results that are a little too high, because the concentration of available food in such lakes is lower.

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6. SUMMARY

Three series of laboratory experiments have been carried out. The first of them, concerned with the relationship between the rate of phosphorus and nitrogen excretion and animal body weight, has been carried out by using a procedure (Fig. 1)prepared during earlier methodological experiments in which the effect was assessed of animal overcrowding (Fig. 3) and exposure time (Figs. 4, 5) on the rate of excretion and zooplankton condition. The second experimental series (Fig. 2) was devoted to the evaluation of the effect of feeding and starving of animals on the level of excretion. The third experimental series concerned the relationship between the rate of P and N excretion and temperature in *Brachionus calyciflorus* acclimated to experimental temperatures.

The rate of excretion is strongly dependent on the temperature, this relationship being of exponential nature (Figs. 6, 7). Over the temperature range 10–35°C the Q_{10} for the rate of phosphorus excretion was 2.6, for nitrogen – 2.4. A power relationship has also been demonstrated between the rate of excretion of both the elements studied and the individual body weight of animals representing three taxonomic groups: Rotatoria, Cladocera and Copepoda (Table I, Figs. 8–11).

A greater decrease – following an increase in animal body weight – in the rate of the excretion of phosphorus than of nitrogen causes an increase in the N:P ratio in excretion products following a growth of body weight (Fig. 12). Starvation of animals prior to measurement caused a decrease in the excretion rate: 1.9–fold in the case of P and 2.2–fold in the case of N (Table II).

The results were compared with literature data on the relationship between the excretion rate and temperature (Table III) and individual body weight of the animals studied (Figs. 13–15).

Additional analyses of the course of the experiment with the small-bodied detritus-feeding rotifer *Keratella cochlearis* have demonstrated that the species could feed during the exposure time with organic suspension (Fig. 16), which fact was taken into account in the construction of the overall relationship of the excretion of P and N by rotifers and individual body weights and temperature.

Using the results from all the experiments and some literature data, it was possible to build equations describing the effect of body weight and temperature on the rate of $P–PO_4$ excretion:

for Rotatoria $E_P = 0.0154\, W^{-1.27} e^{0.096T}$

Cladocera $E_P = 0.519\, W^{-0.230} e^{0.039T}$

Copepoda $E_P = 0.299\, W^{-0.645} e^{0.039T}$

and $N–NH_4$:

for Rotatoria $E_N = 0.0879\, W^{-1.01} e^{0.088T}$

Cladocera $E_N = 1.80\, W^{-0.191} e^{0.039T}$

Copepoda $E_N = 1.33\, W^{-0.536} e^{0.039T}$

where: E_P and E_N = rate of P and N excretion, respectively, in $\mu g \cdot mg\ d.\ wt.^{-1} \cdot hour^{-1}$; W = body weight of one individual in μg dry weight; T = temperature in °C.

7. POLISH SUMMARY

Wykonano 3 serie eksperymentów laboratoryjnych. Pierwsza z nich, poświęcona zbadaniu zależności tempa ekskrecji fosforu i azotu od ciężaru ciała zwierząt, została wykonana przy zastosowaniu procedury (rys. 1) przygotowanej uprzednio przez eksperymenty metodyczne, w których oceniono wpływ nadmiernego zagęszczenia zwierząt (rys. 3) i czasu ekspozycji (rys. 4, 5) na tempo ekskrecji i kondycję zooplanktonu. W drugiej serii eksperymentów (rys. 2) oceniono wpływ odżywiania się zwierząt i głodowania na poziom ekskrecji. Seria trzecia była poświęcona zbadaniu zależności tempa ekskrecji P i N od temperatury u *Brachionus calyciflorus*, aklimowanego do temperatur eksperymentalnych.

Tempo ekskrecji jest silnie uzależnione od temperatury, przy czym jest to zależność wykładnicza (rys. 6, 7). Q_{10} w zakresie temperatur 10–35°C dla tempa ekskrecji fosforu wynosiło 2,6, azotu – 2,4. Wykazano również potęgowy charakter zależności między tempem ekskrecji obu badanych pierwiastków i ciężarem

osobniczym zwierząt z trzech grup taksonomicznych: *Rotatoria, Cladocera* i *Copepoda* (tab. I, rys. 8–11). Silniejszy spadek – przy wzroście ciężaru osobniczego zwierząt – tempa ekskrecji fosforu niż azotu powoduje, iż stosunek N:P w produktach ekskrecji rośnie ze wzrostem ciężaru ciała (rys. 12). Głodzenie zwierząt przed pomiarem powodowało spadek tempa ekskrecji: 1,9-krotny w przypadku P i 2,2-krotny w przypadku N (tab. II).

Otrzymane wyniki porównano z podawanymi w literaturze danymi dotyczącymi zależności tempa ekskrecji od temperatury (tab. III) i od ciężaru osobniczego badanych zwierząt (rys. 13–15).

Dodatkowe analizy przebiegu eksperymentu z drobnym detrytusożernym wrotkiem *Keratella cochlearis* wykazały, że gatunek ten mógł się odżywiać w trakcie ekspozycji zawiesiną organiczną (rys. 16), co uwzględniono przy konstruowaniu ogólnej zależności tempa ekskrecji P i N przez wrotki od ciężarów osobniczych i temperatury.

Wyniki wszystkich eksperymentów, przy częściowym wykorzystaniu danych literaturowych, pozwoliły na skonstruowanie równań opisujących wpływ ciężaru ciała i temperatury na tempo ekskrecji $P–PO_4$:

dla *Rotatoria* $E_P = 0{,}0154\, W^{-1{,}27}\, e^{0{,}096T}$

Cladocera $E_P = 0{,}519\, W^{-0{,}230}\, e^{0{,}039T}$

Copepoda $E_P = 0{,}299\, W^{-0{,}645}\, e^{0{,}039T}$

oraz $N–NH_4$:

dla *Rotatoria*: $E_N = 0{,}0879\, W^{-1{,}01}\, e^{0{,}088T}$

Cladocera $E_N = 1{,}80\, W^{-0{,}191}\, e^{0{,}039T}$

Copepoda $E_N = 1{,}33\, W^{-0{,}536}\, e^{0{,}039T}$

gdzie: E_P i E_N = tempo ekskrecji P i N, odpowiednio, w $\mu g \cdot mg\,s.\,m.^{-1} \cdot h^{-1}$; W = ciężar ciała 1 osobnika w μg suchej masy; T = temperatura w °C.

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