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A laboratory study of phosphorus and nitrogen excretion of *Euchlanis dilatata lucksiana*

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Abstract

Phosphorus (PO_4-P) and nitrogen (NH_4-N) excretion rates of *Euchlanis dilatata lucksiana*, a rotifer, isolated from Lake Loosdrecht (The Netherlands) and cultured in the lake water at 18–19 °C, were measured in the laboratory.
In a series of experiments, the effects of experiment duration on the P and N excretion rates were examined. The rates measured in the first half-hour were about 2 times higher for P and 2–4 times for N than the rates in the subsequent three successive hours which were quite comparable.
Eight experiments were carried out in triplicate, 4 each for P and N excretion measurements, using animals of two size ranges: 60–125 μm and >125 μm. The specific excretion rates varied from 0.06 to 0.18 μg P.mg^{-1} DW.h^{-1} and 0.21 to 0.76 μg N.mg^{-1} DW.h^{-1}. Generally an inverse relationship was observed between the specific excretion rates and the mean individual weight. The excretion rates of *Euchlanis* measured by us are lower than those reported for several other rotifer species, most of which are much smaller than *Euchlanis*.
Extrapolating the excretion rates of *Euchlanis* to the other rotifer species in Lake Loosdrecht, and accounting for their density, size and temperature, rotifer excretion appears to be a significant, potential nutrient (N,P) source for phytoplankton growth in the lake. The excretion rates for the rotifers appear to be about two thirds of the total zooplankton excretion, even though the computed rotifer mean biomass is about one-third of the total zooplankton biomass.

Introduction

In a recent study on phosphorus and nitrogen excretion rates of the zooplankton community from the Loosdrecht lakes, it was demonstrated that the P excreted by zooplankton is substantial in comparison with P-demand of phytoplankton (den Oude & Gulati, 1988). This study was, however, restricted to the large-sized (>150 μm) zooplankton, mainly the crustaceans. Studies on the composition, densities and biomass of zooplankton of the Loosdrecht lakes (Gulati, in press) and calculations of P and N regeneration rates of zooplankton based on Ejsmont-Karabin (1983) show that the rotifers in summer, when they are abundant, may contribute from 45 to 95% of the P regeneration by total zooplankton. These release rates, if included in the P-flow

scheme of Lake Loosdrecht (van Liere *et al.*, in press), will largely meet the primary production requirements of phytoplankton.

The mean annual densities of rotifers in the Loosdrecht lakes are between 3 and 8 times higher than the crustacean densities. Because the mean individual weight of the rotifers (0.05 μg C ind^{-1}) in the lakes is an order of magnitude lower than that of the crustaceans (0.44 μg C ind^{-1}) (Gulati, in press), the mean specific excretion rates of rotifers will be about 2.5 times higher than the rates of crustaceans, based on the studies of Ejsmont-Karabin (1984).

Considering the aforementioned arguments, the role of P and N regeneration by rotifers in the Loosdrecht lakes needs to be investigated, and its quantitative importance in the lake ecosystem assessed. This paper deals with N and P excretion rates of *Euchlanis dilatata lucksiana* and is the first attempt to examine rotifer excretion in these lakes. The choice of *Euchlanis* sp. for measuring excretion rates, like in the feeding studies of this euchlanid (Gulati *et al.*, 1987), is based on the ease of culturing and handling this species, mainly because of its relatively large size. The animals used for the excretion studies were obtained from the same cultures as for the studies on feeding and assimilation rate (Gulati *et al.*, 1987). Besides the excretion rates experiments, the effect of experimental duration on the excretion rates was measured. The results obtained are discussed in relation to: 1) the N and P contents of the body and food of *Euchlanis*; and 2) their significance in the nutrient regeneration by the zooplankton community as a whole.

Material and methods

Isolation, culture and biomass of Euchlanis

Euchlanis was isolated from Lake Loosdrecht and identified as *E. dilatata lucksiana* (Gulati *et al.*, 1987). It was cultured in the laboratory at 18–19 °C in lake water filtered with a 33 μm mesh sieve; Therefore, the *in situ* food (< 33 μm), dominated by filamentous cyanobacteria, was the exclusive food source in the cultures. The animals were removed from the cultures using 60 μm and 125 μm sieves and categorized as small and large, respectively. On termination of each experiment, the lengths of the animals used in the replicates were measured and the mean dry weights computed from the length-weight regression formula given by Dumont *et al.* (1975). For deriving the carbon biomass, the computed dry weight was multiplied by a factor of 0.427 (Gulati *et al.*, 1987).

Excretion experiments

The excretion rates of *Euchlanis* were measured in the dark at 18–19 °C, the culture temperature. About 200 animals were transferred to a Winkler bottle containing 50 ml fresh, filtered (GFF Whatman filter) lake water. The experiments were carried out in triplicate; the controls contained only the filtered lake water.

In the 'time-course experiments' the effect of incubation time on the N and P excretion rates was studied in separate experiments using two size classes of *Euchlanis*. The excretion rates were measured after 30 min of incubation in the filtered lake water and in the subsequent 1st, 2nd and 3rd h, i.e. after each hour removing the animals and re-transferring them into the freshly filtered lake water in the Winkler bottles.

In the second set of experiments the excretion rates were measured. The animals were incubated for 30 min in the glass-fibre filtered lake water to allow them to clear their guts of the unassimilated food, and thereafter transferred to fresh filtered lake water again and excretion rates measured after 2 h of incubation. Four such experiments were carried out each for N as well as P excretion measurements separately, using two size categories. At the end of each experiment the animals were separated by filtering, killed and fixed in 4% formalin for counting and length measurements.

N and P analyses

The water from which the animals had been removed was used for N and P analyses.

Ammonium nitrogen (NH_4-N) was determined by the bis-pyrazolone method (Procházková, 1964) in which the coloured complex is extracted in chloroform and its extinction measured at 450 nm. Phosphorus (PO_4-P: molybdenum-blue reactive phosphate) was measured according to Stephens (1963), using isobutanol as the extracting solvent for the coloured complex, and the extinction values at 690 nm as measure of the P concentration. Both these methods have been used successfully in recent other excretion studies of zooplankton (e.g. Blažka *et al.*, 1982; Ejsmont-Karabin, 1984).

For calculating the nutrient recycling rates, body nitrogen and phosphorus contents of both *Euchlanis* and its food in the cultures were measured. Micro-Dumas method was used for the analysis of N using an automated C,H,N analyzer (Perkin Elmer, No. 240); for P, a known amount of the dry matter was combusted to ash, hydrolyzed and the concentration measured according to Murphy & Riley (1962).

Results

Time course experiments

The observed excretion rates of both N and P, for large as well as small *Euchlanis*, are markedly higher in the first 30 min than in the subsequent 3 h (Fig. 1; Table 1). The excretion rates in the first half-hour can be apparently higher if the egestion products contribute significantly to the excretion losses. The egestion, however, appears to contribute negligibly to the excretion because assimilation efficiencies of *Euchlanis* are rather high, invariably 100% (Gulati *et al.*, 1987). Small animals excreted N and P 5 times and 4 times faster, respectively, than the large animals. Since there was no trend in the fluctuations in the 3 successive one-hour measurements, it was implied that the excretion rates stabilized after transfer to the filtered lake water within the first 30 min.

Table 1. A comparison of the specific NH_4-N and PO_4-P excretion rates (μg N or P $\cdot mg^{-1}$ DW $\cdot h^{-1}$) of *Euchlanis dilatata lucksiana* in the first 30 min of incubation (A) with the means of the three successive one-hour incubations (B); coefficients of variation (%) are given in the parentheses.

Animal size (μg DW $\cdot ind^{-1}$)	A	B	A/B
	N excretion rates		
1.07	0.61	0.26	2.35
(12.1)	(14.2)	(9.7)	
0.51	3.31	0.77	4.30
(33.7)	(24.7)	(5.3)	
	P excretion rates		
1.01	0.17	0.078	2.05
(17.7)	(34.3)	(22.3)	
0.79	0.72	0.18	4.00
(25.7)	(20.8)	(16.4)	

Excretion rates

The mean rates of N excretion in the first three of the four experiments were quite comparable, with daily recycling via excretion of 5–6% body N (Table 2). The specific excretion rates for the smallest animals (0.514 μg DW.ind^{-1}: Exp. 4)

Table 2. Specific NH_4-N excretion rates (μg N $\cdot mg^{-1}$ DW $\cdot h^{-1}$) of *Euchlanis dilatata lucksiana* of different sizes; coefficients of variations (%) of the replicates are given in parentheses; also daily recycling of the body N is given.

Exp. No.	Size of the animal (μg DW $\cdot ind^{-1}$)	Excretion rate	Recycling (d^{-1})
1	1.09 (8.6)	0.24 (5.1)	0.06
2	1.06 (12.1)	0.25 (0.6)	0.06
3	0.62 (26.7)	0.21 (15.7)	0.05
4	0.51 (33.7)	0.76 (5.3)	0.18

were, however, about 3 times the rates of largest animals. Interestingly, these N excretion rates are almost identical to the rates measured in the time-course experiments excluding the first half-hour (Fig. 1 and Table 1).

The P recycling rates of about 14% d^{-1} for the animals weighing about 1 μg DW.ind^{-1} were on the average 2.4 times faster than of N recycling (Tables 2 and 3: Exp. 1 and 2). Such a comparison of the N and P recycling is, however, not

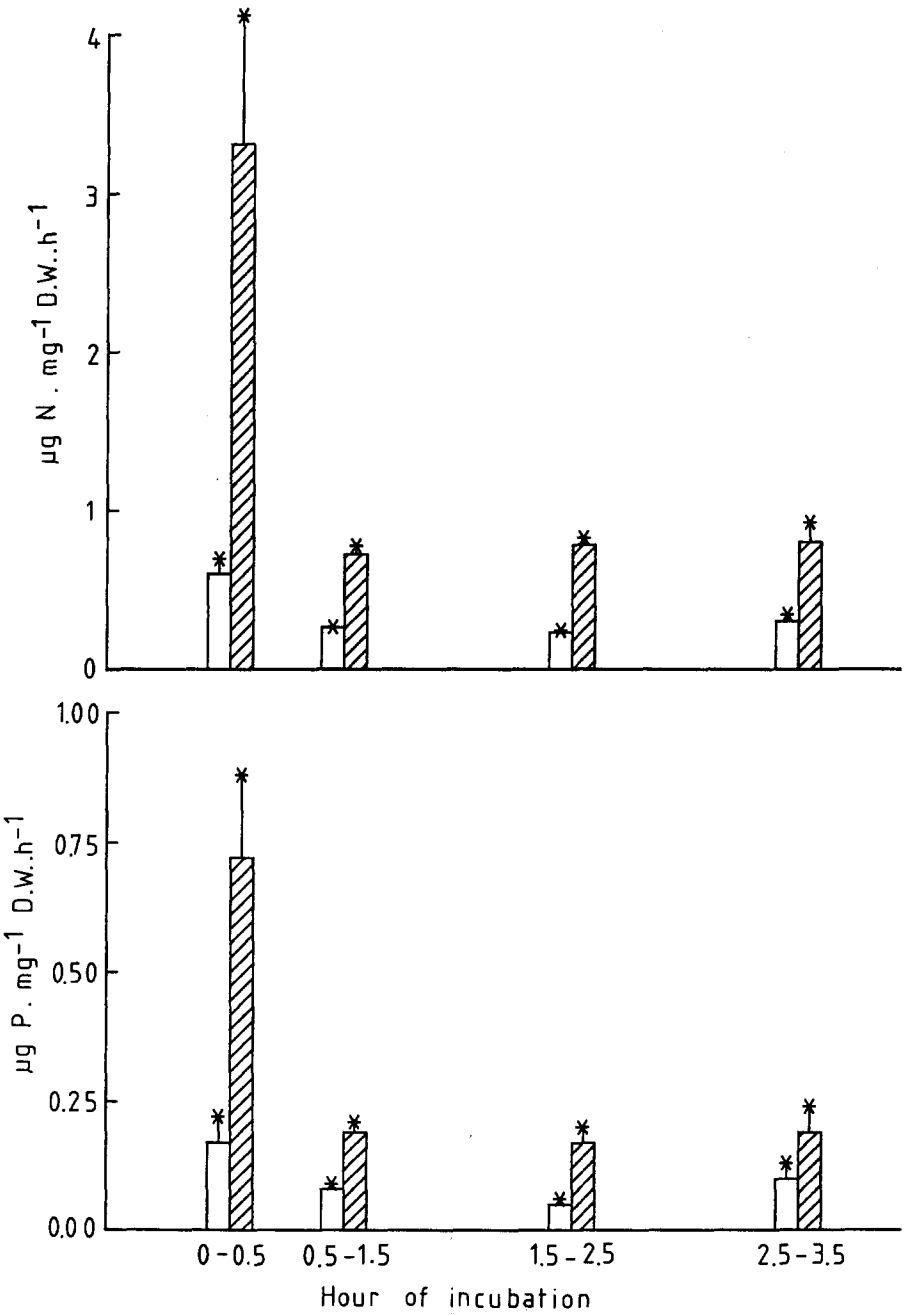

Fig. 1. Specific NH_4-N and PO_4-P excretion rates of *Euchlanis dilatata lucksiana* in the course of 3.5 h; incubation time of the first experiment was 30 min and of the subsequent three experiments 1 h each. The two bars for each experiment represent: blank bar, large animals; and hatched bars, small animals; the asterisk represents the + S.D. For more details see Table 1.

Table 3. Specific PO_4-P excretion rates ($\mu g\ P \cdot mg^{-1}\ DW \cdot h^{-1}$) of *Euchlanis dilatata lucksiana* of different sizes; coefficients of variation (%) of the replicates are given in parentheses; also daily recycling of the body P is given.

Exp. No.	Size of the animal ($\mu g\ DW \cdot ind^{-1}$)	Excretion rate	Recycling (d^{-1})
1	1.01 (17.7)	0.068 (6.8)	0.14
2	0.95 (7.3)	0.063 (24.8)	0.14
3	0.81 (25.7)	0.182 (13.8)	0.39
4	0.65 (30.4)	0.170 (17.3)	0.36

possible for the smaller animals: first, because of the greater weight differences between the animals used for N and P in the Exp. 3 and 4 (Tables 2 and 3); and secondly, also due to the high rates of P excretion for the smaller animals, both in Exp. 3 and 4 (Table 3) but of N excretion only in Exp. 4 (Table 2).

The coefficients of variation in the replicates of N and P excretion rate measurements, with one exception (Exp. 2, Table 3), were less than the variations in the size replicates of *Euchlanis*. The replicates of N excretion rates varied less than the replicates of P excretion rates in 3 out of the 4 experiments.

Discussion

This study restricts the excretion measurements to PO_4-P and NH_4-N. The measured rates of P excretion will include, besides the orthophosphates, the phosphates formed by hydrolysis of dissolved organic phosphorus compounds. These latter comprise about 20% of the total P excretion (Peters & Lean, 1973; Ejsmont-Karabin, 1984). For N, ammonia is the predominant break-down product of aquatic invertebrates including the zooplankton, which are ammoniotelic.

Factors affecting the excretion rates

Several factors may affect the rate of zooplankton excretion as well as comparability of the different studies. Important among these are: a) the water temperature; b) animal size; c) feeding history; d) concentration or extent of crowding; and e) length of the incubation period.

The temperature which has generally a significant and direct effect on rotifer excretion, with a Q_{10} of about 2.6 (Ejsmont-Karabin, 1984), was kept at 18.5 ± 0.5 °C both in the culture medium and during the incubations for excretion measurements. The excretion rates tended to increase with the decreasing weight of *Euchlanis*. The significance of the differences, however, cannot be tested because of the limited number of size classes studied. This narrow size range within a clone of a rotifer species in culture may be because of the short life span and constancy of cell number in rotifers after the embryonic development is completed (Ruttner-Kolisko, 1972). Moreover, our computed dry weights of *Euchlanis* based on the L-W regressions of Dumont (1975) are about twice the weights based on the volume-to-weight conversion (Ruttner-Kolisko, 1977), assuming dry weight to be 10% of the wet weight (see e.g. Bottrell *et al.*, 1976). Consequently, our specific excretion rates calculated using the regression-based dry weights are half as high as the rates based on the volume. Such discrepancies will affect the comparability of literature data adversely, like the errors relating to methodological and behavioural differences.

As regards feeding history and starving of the animals during the experiments, the stability of excretion rates after the first 30 min shows that starving most likely did not alter the physiology such that the rates were reduced (see also Ikeda, 1971; Mayzaud, 1976). Also, release of N and P through bacterial activity and increased minerali-

zation, which may mask the decrease in excretion rates (Le Borgne, 1979), does not appear to have played a role because we used filtered lake water and corrected our results for changes in the controls.

In our experiments we used concentrations (*ca.* 4000 ind.l^{-1}) of *Euchlanis* that are quite similar to the mean density (3770 ind.l^{-1}) of total rotifers encountered in the Loosdrecht lakes (Gulati, in press). Moreover, they are much lower than the densities (40000 ind.l^{-1}) in our *Euchlanis* stock cultures (Ejsmont-Karabin *et al.*, 1989). Therefore, crowding-related factors do not appear to be relevant in our study.

The exposure-time related changes in the specific excretion rates of *Euchlanis* differed in their magnitude for the small and large animals. Both, the N and P excretion rates are higher during the first 30 min than in the subsequent 3 h, roughly 4 times for the small animals and 2 times for the large animals (Table 1). In the first 30 min the small animals excrete both N and P 4–5 times more rapidly than the large animals. The size-dependent difference in the animal behaviour is too great to be explained on the basis of metabolism alone.

For the stabilization of the excretion rates after the first 30 min there are two possible explanations. First, the stress effects (Le Borgne, 1979) on transferring the animals from the cultures to fresh, filtered lake water without food may stimulate the animal to filter maximally in a medium devoid of food. However, this increased activity is short-lasting, tending to stabilize even before the 3 successive hourly transfers into the fresh food-less medium each time. Second, the animals apparently get quickly adapted to the short-term starvation, with no more decrease in metabolic activity than that observed in the first hour after the first 30 min in the time course experiments. In short, like in several other studies on zooplankton excretion, but especially those on the rotifers (e.g., Ejsmont-Karabin, 1983, 1984; Ejsmont-Karabin *et al.*, 1983), no symptoms of starvation, or other adverse effects, were observed, which may lead to decreased excretion in the initial few hours of incubation. Lastly, the rates in the 0.5–3.5 h of incubation were not only comparable with one another (Fig. 1), but also with those measured subsequently on the basis of 2-h incubations (Tables 2 and 3).

Ejsmont-Karabin (1984) has clearly demonstrated that the mean specific excretion rates of rotifers increase markedly with decreasing weight, e.g. P excretion rates increase from 0.19 to 35.8 μg P.mg^{-1} DW.h^{-1} corresponding to a decrease in individual weights from 0.776 to 0.013 μg DW. The range of variations is even much larger if all the extreme values are included. P excretion rates of *Euchlanis* measured by us (Table 3) are very comparable with the minimal rates (range: 0.06–0.42 μg P.mg^{-1} DW.h^{-1}; and mean: 0.19 mg P.mg^{-1} DW.h^{-1}) reported for a rotifer, namely *Asplanchna priodonta* (Ejsmont-Karabin 1984: Table 1). *Asplanchna* has a range of weights, 0.5–1.07 μg DW.ind^{-1} (Ejsmont-Karabin, 1984), that are quite similar to the weights of our *Euchlanis* (0.5–1.0 μg DW.ind^{-1}). Interestingly, these *Euchlanis* weights converted to carbon do not differ much from the mean weight (0.44 μg C.ind^{-1}) of crustacean community of the Loosdrecht lakes, in which small cladoceran species of *Bosmina* and *Chydorus* predominate (Gulati, in press). Also P excretion rates of crustaceans in these lakes of 0.17 μg P.mg^{-1} DW.h^{-1} (Den Oude & Gulati, in press: Table 1) are almost identical with the rates of *Euchlanis* measured by us (Exp. 3 and 4: Table 3).

Elemental ratios

The N : P ratios of 3.4–3.6 for the large animals and 4.2–4.5 for the small animals, based on ammonia and phosphate excretion, were constant and comparable with the ratios derived from the generalized regression formulae for the rotifers of Ejsmont-Karabin (1984), namely 3.6 for the large and 4.0 for the small animals. The higher N : P ratios for the smaller animals may be because of the relatively higher protein catabolism of these animals; moreover, the smaller animals (0.51 μg DW.ind^{-1}) in the N-experiment were much smaller than the animals (0.79 μg DW.ind^{-1}) in the P experiments (Table 1).

On comparing the elemental ratios, namely the C : N : P ratio of the *Euchlanis* food, *Euchlanis* body and the excretion products summarized below, some interesting discrepancies emerge:

Ratio	C : N : P
Food	100 : 15.4 : 0.9
Euchlanis	100 : 23.7 : 2.6
Excretion products	100 : 16.0 : 4.0

Euchlanis contains about 3 times more P and 1.5 times more N than its food. Also, per unit carbon, the excretion products contain about 50% less N, but 50% more P than N and P in the animal body. Evidently, the animal is able to maintain a lower C : N ratio in the body than its food; however, P losses as indicated by the both lower C : P and N : P ratios of the excretory products – than of the animal and its food – are difficult to explain.

Role of rotifer excretion

The *Euchlanis* excretion rates are not quite representative of the rotifer community of the Loosdrecht lakes. The rotifers in these lakes are dominated by *Keratella cochlearis* and *Anuraeopsis fissa*, with individual weights (0.01–0.02 μg DW.ind^{-1}) that are an order of magnitude lower than that of *Euchlanis*. Their excretion rates (Ejsmont-Karabin, 1984: Table 1) are, therefore, probably two orders of magnitude greater than the rates of *Euchlanis* measured under similar temperatures ($\pm$ 20 °C), using the same methods. Our computed individual mean weight of rotifers in the Loosdrecht lakes of *ca.* 0.05 μg DW.ind^{-1} (Gulati, in press) is about one-tenth the individual mean weight of crustaceans. One would, therefore, expect the rotifer specific excretion rates to be much higher and their contribution to the P and N excretion of total zooplankton in the lakes much greater than based on the crustacean : rotifer mean biomass ratio of *ca.* 2 : 1 calculated from the lake data of 1982–'87. Thus, the rotifers that contribute about 35% to the total zooplankton biomass will contribute roughly twice as much (*ca.* 65%) to the mean zooplankton excretion rates, with a range of values between 45 and 90% in summer (Gulati, in press; Van Liere *et al.*, in press).

The excretion rates of *Euchlanis* found by us, though in the range of literature values that vary widely, are low. This, like for the crustacean excretion rates (Den Oude & Gulati, 1988), may be attributed to high C : P ratios ($\pm$ 125) of sestonic lake food of *Euchlanis*, because of P-limitation of cyanobacteria that dominate the seston. Evidence of the direct effects of P-content of zooplankton food on excretory turnover rate of P has been presented in some recent studies (e.g. Lehman & Naumoski, 1985).

Finally, our study on *Euchlanis* and some comparisons of the literature data on rotifer excretion have demonstrated: 1) the specific excretion rates of rotifers – even within a species – vary enormously because of the inverse relationship between the rates and individual weights; 2) the range of excretion rates is very wide if one compares the different rotifer species, which differ in weight about 60 fold; and 3) in shallow and eutrophic lakes like Loosdrecht Lake, both the much smaller size and relatively higher densities of rotifers than of the crustaceans may contribute significantly to the importance of rotifers in nutrient regeneration, especially in summer.

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