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Excretion Rates and Excreted components of the Rotifer *Brachionus plicatilis* O.F. MÜLLER in Culture

Hachiro HIRATA*1 and Warren D. NAGATA*1,2

Abstract

The excretion rates and excreted components of the rotifer, *Brachionus plicatilis*, cultured on the Chlorophycean, *Chlorella saccharophila*, were measured under constant conditions of: 20℃, 20 ppt salinity, and 1 klux light intensity. Analyses for: NH_4-N, urea-N, total persulfate nitrogen, and PO_4-P were carried out on the resulting experimental water. Rotifer density, fecundity, and algal density were examined as dependent variables. The uptake kinetics of dissolved nutrients by *C. saccharophila* were also determined as a control.

Under the above conditions, *B. plicatilis* excretes the majority of its dissolved nitrogen as ammonium and urea, with urea comprising almost an equal amount of the total nitrogen as NH_4-N. The rates of excretion by *B. plicatilis* were found to be high in comparison to other zooplankton excretion rates, and dependent on all the tested variables. Thus, *B. plicatilis* most likely plays a very important role in the cycling of nutrients in both natural and artificial ecosystems.

Introduction

The Rotifera are a very unique group of animals, which not only exhibit both universal and cosmopolitan distribution, but are often found to bloom and dominate the planktonic communities in a variety of aquatic ecosystems (PENNACK, 1953; ITO, 1963; PILARSKA, 1972; and MULLIGAN *et al.*, 1980). Never the less, the role of rotifers in the regeneration of nutrients through excretion of dissolved nitrogen and phosphorus within these systems has been little investigated. HARGRAVE and GEEN (1968) measured phosphorus excretion by *Asplanchna* sp., a brackish water monogonont rotifer; while DROOP and SCOTT (1978) utilized an indirect assay technique to estimate nitrogen excretion of *Brachionus plicatilis*. However, problems were encountered in both studies due to experimental design.

Recent studies have utilized monospecific zooplankton cultures fed on one phytoplankton or bacterial species in order to measure species specific excretion rates of dissolved nitrogen and phosphorus (MOFFETT and FISHER, 1978; LEHMAN, 1980; and GARDNER and SCAVIA, 1981). Cultured zooplankton, fed on a defined food source, eliminate many of the constraints imposed by

*1 Laboratory of Aquacultural Physiology, Faculty of Fisheries, Kagoshima University, Shimoarata 4, Kagoshima, 890, Japan.

*2 Present address: Laboratory of Planktology, Faculty of Fisheries, Hokkaido University, Minato-machi 1, Hakodate, 041, Japan.

studies on natural zooplankton populations, but at the same time provide a model system comparable to natural ecosystems.

The monogonont rotifer, *Brachionus plicatilis* O.F. MÜLLER, cultured on the Chlorophycean, *Chlorella saccharophila* KRUGER, provides an ideal sytem for the study of nutrient cycling between a micro-zooplanktonic herbivore and its algal prey. This animal can be easily grown to naturally high densities; and since the newly hatched individual is virtually identical to the adult morphologically, it possesses no distinct larval stages. The system is further simplified by the persistence of the amictic reproductive cycle (diploid parthenogenesis), thus eliminating the presence of the heteromorphic male.

In recent years *B.plicatilis* has become an indespensable food organism in the larval culture of many crustacean and marine fish species (HARADA, 1970; FUJITA, 1973); and is usually cultured on a species of marine *Chlorella* (HIRATA, 1980). However the dynamics of nutrient cycling within this much used culture system are poorly understood. The objects of this study were to quantitatively and qualitatively observe dissolved nitrogen and inorganic phosphorus excretion by *B. plicatilis*, and to determine the kinetics of nutrient cycling within the *B. plicatilis/C. saccharophila* culture system. Rotifer density, fecundity, and algal density were also examined as dependent variables influencing excretion rates.

Materials and Methods

Chlorella saccharophila was cultured in 4 l. acrylic cylinders as serially renewed batch cultures in sunlight at 25℃, using Yashima I medium (HIRATA, 1964), modified to contain no urea and replacing Ca-superphosphate with potassium phosphate mono and dibasic. *Brachionus plicatilis* was cultured at 20 ppt. salinity, 20±0.2℃, with a 16 : 8 hr. photoperiod, in 1 liter pyrex beakers. The rotifers were fed daily on *C. saccharophila* to yield an initial density of 6 to 9 x 10^6 cells per ml within the rotifer cultures. Rotifers from the same clone (Kagoshima University, Cultivation Physiology Laboratory) were precultured for over one year under similar conditions.

Fifteen hours prior to experimentation, *C. saccharophila* cells were centrifuged, washed, and resuspended in Medium ASP (PROVASOLI *et al.*, 1957), modified to contain no tris buffer and diluted to 20 ppt. The resuspended phytoplankton were then acclimated at 20℃ until the following day. Following this period, *B. plicatilis* were resuspended into the algal culture to yield a rotifer density similar to that of the original culture.. This was accomplished by filtering the rotifer culture through a short plastic cylinder, closed at one end with 30 μm. plankton netting. The rotifers were then rinsed several times with ASP medium before being removed to the algal culture. The rotifer/algal culture was then incubated at 20℃ and 1 klux. light intensity for a 2 hr. acclimation period. Before and after this period samples were taken for rotifer and algal density determinations, in 5 replicates. Algal cell densities were enumerated by direct counts using a hemocytometer, while rotifer densities were observed under a binocular dissecting microscope. The dissolved oxygen concentration of the combined culture was then adjusted to

saturation by slowly bubbling O_2 gas through it. Next a 145 ml stoppered glass reagent bottle was slowly filled with the aggitated rotifer/algal culture, then stoppered to exclude air bubbles. A duplicate bottle was then filled with the same rotifer/algal culture from which the rotifers had been removed. The first bottle served as the experimental vessel for excretion measurements, while the second served as a control for the uptake of nutrients by the phytoplankton. A third bottle was filled with the same medium, from which both the rotifers and algal cells had been removed. This served as the control for changes in nutrient levels due to bacterial activity and bottle adhesion. The bottles were then incubated for a three hour experimental period, under the same conditions as the previous acclimation period.

Upon termination of the experimental period samples were taken for algal counts and rotifer dry weight determinations. The latter were made by filtering volumes of the rotifer culture onto tared pieces of 30 μm plankton netting and dried at 60℃ for 24 hr. The rotifers and algae were then removed from the culture medium by filtration and centrifugation, respectively; and the resulting supernatant analyzed, within one hour of termination of the experimental period, for: ammonium by the method of SOLÓRZANO (1969), urea by the method of MCCARTHY (1970), soluble reactive phosphate by the method as described by STRICKLAND and PARSONS (1972), and total dissolved persulfate nitrogen (TPN) by the method of SOLÓRZANO and SHARP (1980). All analyses were scaled to 10 ml of sample and carried out in four replicates.

Total body nitrogen of the rotifers was determined with a Shimadzu Model 1A CHN analyzer, against a standard of acetanilide. Rotifer volumes were determined by volume displacement, while algal volumes were calculated by $v=r^3$ (4/3). These volumes were used to correct the controls for volumes occupied by the animals or algal cells which were absent in the controls.

Results and Discussion

Nutrient uptake by *C. saccharophila*

Nutrient uptake by phytoplankton is generally considered to follow Michaelis-Menten kinetics as expressed by the equation:

$$(1) \qquad S=Vm\ (s/v)-Ks$$

where S is the substrate concentration, v the uptake velocity, Vm the maximum uptake velocity, and ks the half saturation substrate concentration (EPPLY *et al.*, 1969).

When the data from this study were analyzed, it was found that *C. saccharophila* can utilize ammonium, urea, and orthophophate which were remineralized by *B. plicatilis*. Upon fitting the data to the above equation, Vm and Ks values of: 5.70×10^{-10} μg-at. hr^{-1}. $cell^{-1}$ and 97.27 μg-at.; 6.50×10^{-10} μg-at. hr^{-1}. $cell^{-1}$ and 53.57 μg-at.; and 1.0×10^{-10} μg-at. hr^{-1}. $cell^{-1}$ and 16.00 μg-at.; for NH_4-N, urea-N, and PO_4-P, respectively. Vm values for urea and ammonium were very similar, but Ks for urea was about half that of ammonium. This indicates a similar uptake mechanism for both these nutrients, since the urea molecule consists of two ammonia

radicals joined to a carbon atom.

Never the less, *C. saccharophila* exhibited considerable deviations from Michaelis-Menten kinetics for all three nutrients. Rapid uptake (>Vm) was observed for ammonium, urea, and orthophosphate, while slow or inhibited uptake was observed for ammonium and orthophosphate. Rapid uptake of dissolved nitrogen and phosphorus can possibly be attributed to a condition of partial nutrient depletion of the algal cells, which was probably induced by their resuspension in the virtually nitrogen and phosphorus free ASP medium. Rapid uptake of nutrients following nutrient depletion has been demonstrated by McCARTHY and GOLDMAN (1979) for ammonium uptake by *Thalassiosira pseudonana*; LEHMAN (1980) for orthophosphate uptake by *Chlamydomonas reinhardii*; and HORRIGAN and McCARTHY (1981) for urea uptake by *T. pseudonana*. Slow uptake, on the other hand, cannot be explained as a result of nutrient depletion; but possibly can be attributed to competitive inhibition of the same pathway, or the existence of more than one transport mechanism for the same nutrient (BROWN *et al.*, 1978).

A correction to zooplankton excretion rates is essential when phytoplankton are provided as food organisms, due to their rapid absorption of the excreted nutrients (GANF and BLAZKA, 1974; TAKAHASHI and IKEDA, 1975; and LEHMAN, 1980). TAKAHASHI and IKEDA (1975) attempted to remedy this problem by calculating correction factors, assuming that phytoplankton nutrient uptake always followed Michaelis-Menten kinetics; but they noted deviations at low substrate concentrations. Considerable deviations from Michaelis-Menten kinetics were also observed in this study. LEHMAN (1980), on the other hand, saturated the nutrient uptake pathways with the addition of known amounts of nutrients to the algal cultures, thus resulting in constant uptake rates. However, variations in Vm and, consequently, Ks were observed among experiments, possibly due to differences in the nutritional status of the algal cells. During this study the 2 hr acclimation period of the mixed rotifer/algal culture facilitated saturation of the uptake mechanisms of *C. saccharophila*, utilizing only rotifer excreted nutrients. In some trials, never the less, saturation of nutrient uptake could not be attained, thus resulting in rapid nutrient uptake due to a persistent state of nutrient depletion of the algal cells.

From the above data it could be concluded that correction factors for the uptake of zooplankton excretory products by phytoplankton cannot be estimated by Michaelis-Menten kinetics, but must be measured individually for each experimental trial. The fact that *C. saccharophila* is able to directly utilize both urea and NH_4-N excreted by the rotifers leads to the conclusion that nitrogen is conserved within this culture system. BORAAS (1980) also noted the conservation of nitrogen within a *Brachionus calyciflorus/Chlorella pyrenoidosa* culture system.

Excreted components and excretion rates of *B. plicatilis*

Rotifer excretion rates were corrected for uptake of nutrients by *C. saccharophila*, and loss or gain due to bacterial or physical processes. The former were also adjusted to account for grazing losses. The mean net excretion rates calculated were: $1.41 \pm 0.87 \times 10^{-4}$ μg-at. NH_4-N hr^{-1}. $individual^{-1}$ for ammonium; $1.17 \pm 1.31 \times 10^{-4}$ μg-at. urea-N hr^{-1}. ind^{-1}. for urea; $3.51 \pm 3.11 \times 10^{-4}$ μg-at. NO_3-N hr^{-1}. ind^{-1}. for total persulfate nitrogen; and $0.27 \pm 0.29 \times 10^{-4}$ μg-at.

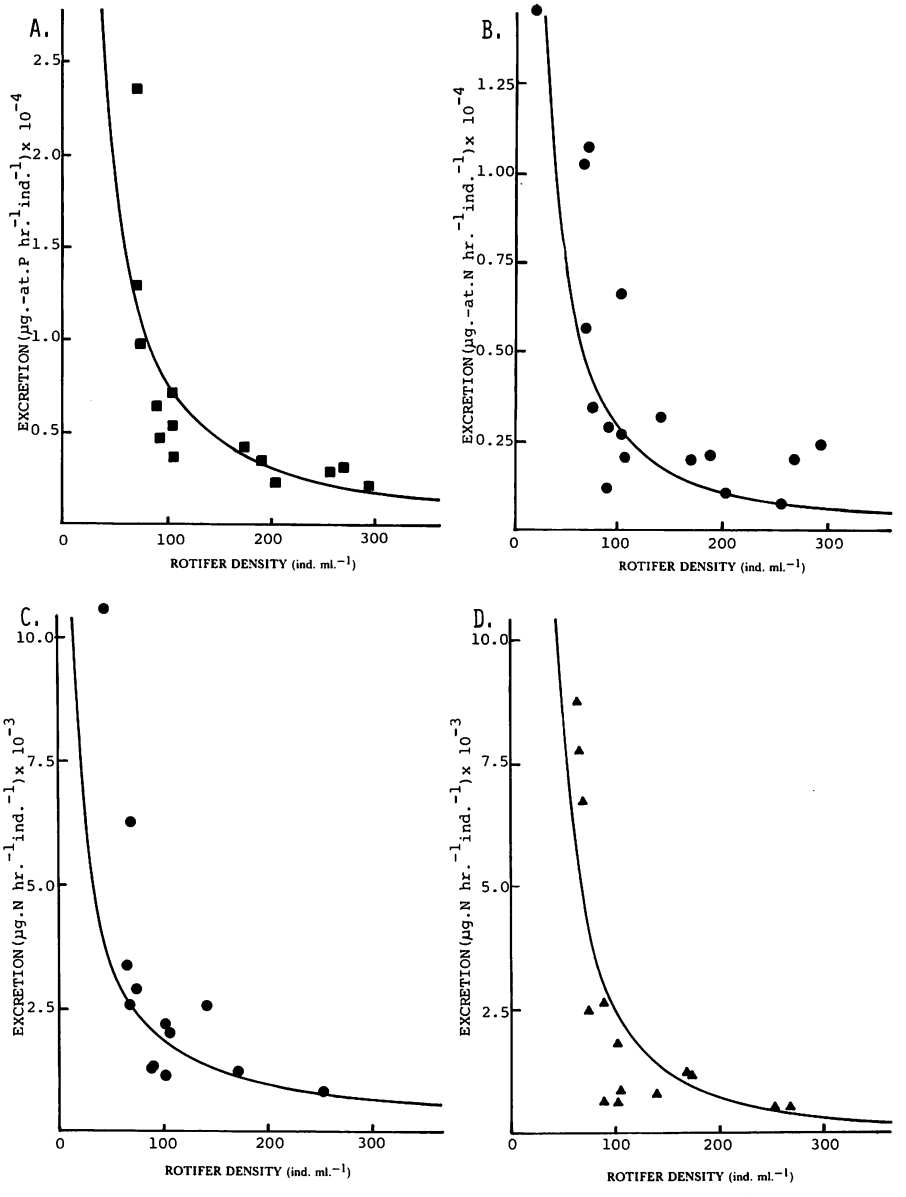

Figure 1. Dependence of excretion rates on density in *Brachionus plicatilis*, A. PO_4-P, B. TPN, C. NH_4-N, and D. Urea-N excretion. All data fitted to $y=a\cdot x^b$.

PO_4-P hr^{-1}. ind^{-1}. for orthophosphate. Never the less, considerable variation in excretion rates among trials is indicated by the large standard errors of the means. The percentage of the total nitrogen occupied by ammonium, urea, and ammonium plus urea averaged: 44.85 ± 12.92%, 34.76 ± 15.74%, and 76.60 ± 14.62%, respectively.

The large percentage of the total persulfate nitrogen occupied by ammonium is not surprising,

since aquatic invertebrates are thought to be primarily ammonotelic (PANDIAN, 1975). However the large proportion excreted as urea by *B. plicatilis* has not been previously reported for any specific zooplanktonic animal. CORNER and NEWELL (1967) reported about 11% of the total nitrogen excretion of *Calanus helgolandicus* as urea; while JAWED (1969) reported only 1.19% of the total nitrogen excreted by *Euphausia pacifica* as urea. EPPLEY *et al.* (1973), on the other hand, observed roughly half of the nitrogenous excretion of small mixed zooplankton as urea. The fact that *B. plicatilis* excretes urea in such high proportions leads to many ecological and evolutionary implications, thus warrants further investigation. Other organic forms of nitrogen such as: amino acids, purines, uric acid, trimethyl amine, and taurine may constitute the remaining portion of the total nitrogen not accounted for by urea or ammonium. All of these compounds have been known to be excreted by a variety of aquatic invertebrates (PANDIAN, 1975).

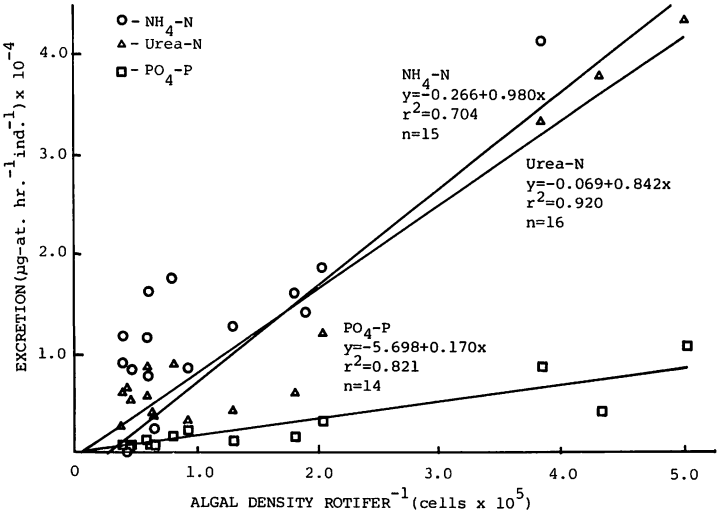

Figure 2. Dependence of excretion rates on rotifer specific algal densities in *B. plicatilis*.

The mean excretion rates of *B. plicatilis* are high in comparison to previous zooplankton excretion studies. This is due to the high metabolic rate of these rotifers (DOOHAN, 1973), which stems from the fact that small herbivores have faster metabolic rates per unit biomass (LEHMAN, 1980). The wide range in excretion rates reported here were due to the previously mentioned dependent variables of rotifer density, fecundity, and algal density, and will be discussed forthwith.

Effect of rotifer density on excretion rates

Densities of *B. plicatilis* utilized in this study ranged from 66 to 293 ind. ml^{-1}. (Table 1). These densities represent natural culture densities, since the animals were not artificially concentrated prior to introduction into the experimental vessels. HIRATA and MORI (1967) have cultured *B.*

plicatilis up to densities of 2000 ind. ml^{-1}., while ITO (1960) observed natural densities of over 3000 ind. ml^{-1}. of this rotifer from eel culture ponds.

The excretion data were fitted to the power regression $y = a \cdot x^b$ to yield the non-linear regressions for: NH_4-N ($r^2 = 0.692$), urea-N ($r^2 = 0.694$), TPN ($r^2 = 0.610$), and PO_4-P ($r^2 = 0.723$). As can be seen in Figure 1, all excretion rates decline rapidly to a density of about 100 ind. ml^{-1}., then level off, but still decline gradually with increasing density. This rapid decline in excretion rates, and the non-linear nature of the excretion curves suggests the influence of other dependent variables. Although the derived regressions can only be considered as rough approximations of the density effect on excretion rates, a density of 100 ind. ml^{-1} does represent the point of flection.

A considerable density effect was observed by HARGRAVE and GEEN (1968) in phosphorus excretion by *Acartia tonsa*. They noted a great decrease in excretion rates of this copepod as density approached five times their natural density. Similar crowding effects were also observed in NH_4-N excretion of *Artemia salina* (MOFFETT and FISHER, 1978) and *Pseudocalanus* sp. (CHRISTIANSEN, 1978). The rotifer densities used in this study were natural culture densities, therefore these animals were not under crowded conditions. However, the density dependence of excretion rates in *B. plicatilis* may be described as a response to their own population density which manifests at a low threshold level, and is due to either increased competition for food or metabolite accumulation (BADER *et al.*, 1976). This may represent a mechanism by which *B. plicatilis* may prevent toxic metabolite accumulations during periods of increased population density. The production of urea by this rotifer species may serve a similar purpose.

Another possibility is that high ingestion/excretion rates at densities below 100 ind. ml^{-1}. may represent the phenomenon of superfluous feeding as described by BEKLEMISHEV (1962), with usual rates being exhibited above this density.

Effect of algal density on excretion rates

Densities of *C. saccharophila* for each excretion trial are listed in Table 1. When these values were converted into rotifer specific algal densities (i.e. algal density per rotifer), significant linear regressions were obtained for NH_4-N, urea-N, and PO_4-P (Figure 2) $P<0.0005$ in all cases. Therefore the effect of algal concentration on excretion rates is seen to be dependent on rotifer density. As algal densities per rotifer increase, so do excretion rates. Similar trends were observed by TAKAHASHI and IKEDA (1975) in excretion experiments with *Metridia pacifica* and *Euphausia pacifica*. During all trials in this study food levels were sufficient to sustain high algal concentrations throughout the experimental period, thus at no time was food level limiting to the rotifers.

Effect of fecundity on excretion rates

In this study fecundity was defined as the average number of eggs per individual. The fecundity values for each excretion trial are also listed in Table 1. When these values were multiplied by the reciprical of rotifer density and plotted against excretion rates, significant linear regressions were obtained for NH_4-N, urea-N, and PO_4-P, $P<0.0005$ in all cases. Thus, it

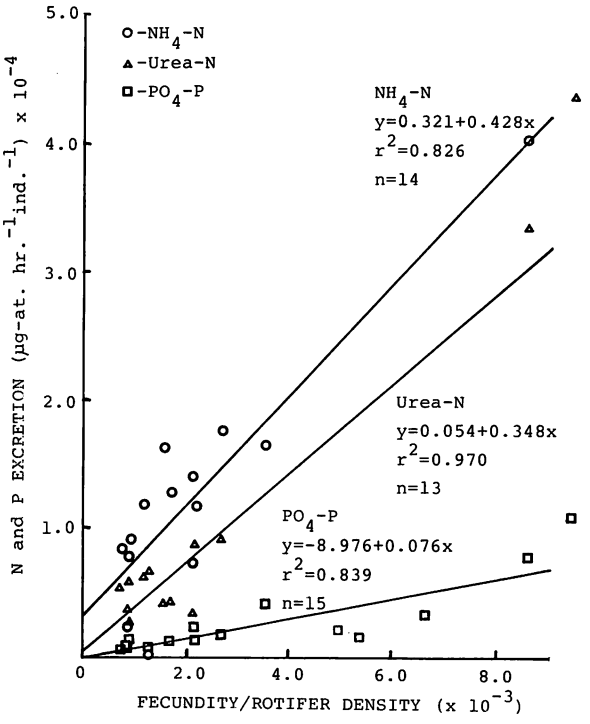

Figure 3. Dependence of excretion rates on fecundity in *B. plicatilis*.

appears that the relations between fecundity and excretion rates are also density dependent, with excretion rates increasing with increased fecundity. However at higher rotifer densities, food would become limiting resulting in reduced fecundity. Since a large amount of body nitrogen and phosphorus is transferred to eggs during production in zooplankton (CORNER and DAVIES, 1971) it is quite reasonable to assume that ingestion rates and, consequently, excretion rates increase proportionally to increased fecundity. This is further supported by the finding of DOOHAN (1973) that *B. plicatilis* is more efficient at converting assimilated food into reproduced eggs than into body material. She also measured higher respiratory rates, thus higher activity, in ovigorous females as opposed to non-ovigorous ones.

Comparative excretion rates

Beginning with the work of HARRIS (1959), several species of both limnic zooplankton (PETERS and RIGLER, 1973; GANF and BLAZKA, 1974; LEHMAN, 1980; and GARDNER and SCAVIA, 1981) and marine zooplankton (JOHANNES, 1964; CORNER *et al.*, 1965; CONOVER and NEWELL, 1967; HARGRAVE and GEEN, 1968; CONOVER and CORNER, 1968; JAWED, 1969; MAYZAUD, 1973; IKEDA, 1974, 1977; TAKAHASHI and IKEDA, 1975; and LE BORGNE, 1979) have been studied within the context of nutrient cycling and excretion physiology. However the derived excretion rates vary with the many different experimental conditions imposed on a wide array of mainly crustacean

Table 1. Filtration rates, fecundity, and nitrogen excretion rates in *Brachionus plicatilis*

Trial	Density (ind. ml.$^{-1}$)	Fecundity (eggs ind.$^{-1}$)	*Chlorella* sp. Density (cells ml.$^{-1}$) X 10^{6}	Acclimation Filtration Rate (ml. min.$^{-1}$ ind.$^{-1}$) X 10^{-5}	Experimental Filtration Rate (ml. min.$^{-1}$ ind.$^{-1}$) X 10^{-5}	E TPN (μg-at. hr.$^{-1}$ ind.$^{-1}$) X 10^{-4}	E NH_4^++Urea-N (μg-at. hr.$^{-1}$ ind.$^{-1}$) X 10^{-4}
1.	66	0.84	33.20	—	—	10.36	6.56
2.	70	0.60	27.00	—	—	10.82	7.36
3.	68	0.24	29.48	—	—	5.67	5.50
4.	89	0.44	45.26	—	—	1.16	0.94
5.	91	0.49	16.49	—	1.40	2.89	2.22
6.	106	0.18	13.75	—	1.94	2.07	1.72
7.	103	0.22	9.53	—	4.14	—	1.20
8.	104	0.28	8.44	—	3.06	2.69	2.67
9.	141	0.22	9.01	—	1.26	3.18	2.04
10.	171	0.20	6.72	—	2.04	—	1.80
11.	203	0.26	8.63	0.58	0.78	1.08	0.67
12.	255	0.22	16.63	—	0.21	0.70	0.60
13.	293	0.22	13.36	1.83	0.98	2.40	1.38
14.	172	0.15	10.34	1.95	1.07	2.02	1.37
15.	268	0.25	10.58	2.19	1.80	2.01	1.18
16.	189	0.41	11.12	2.96	1.79	2.13	2.05
17.	74	0.49	15.11	3.44	2.34	3.46	3.09
$\bar{X}$ (S.E.)		0.34(0.18)			1.75(1.03)	3.51(3.11)	2.49(2.04)

Table 2. Review of some zooplankton excretion rates

Species	Dry wt. (μg.)	Temp. (°C.)	Food Supplied	NH^4–N (μg. N mg. dry wt.$^{-1}$ day^{-1})	Urea–N (μg. N mg. dry wt.$^{-1}$ day^{-1})	Total N (μg. N mg. dry wt.$^{-1}$ day^{-1})	PO_4–P (μg. P mg. dry wt.$^{-1}$ day^{-1})	Reference
Acartia clausi	5.00	13.0	USW	43.10	—	—	—	HARRIS (1959)
Calanus finmarchicus A	24.60	10.0	FSW	21.60	—	—	—	CORNER *et al.*(1965)
Calanus finmarchicus N, CI, CII	5.50	10.0	FSW	38.10	—	—	—	CORNER *et al.*(1965)
C. helgolandicus	148.00	10.0	*Skeletonema costatum*	3.33	0.50	4.48	—	CORNER and NEWELL (1967)
C. hyperboreus	1.51×10^3	5.0	USW	0.71	—	—	—	CONOVER and CORNER (1968)
Acartia sp. N	—	15.5	USW	—	—	—	1.37	HARGRAVE and GEEN (1968)
Euphausia pacifica	1.71×10^4	10.0	FSW (0.45 μm)	2.02	0.02	1.68	—	JAWED (1969)
Psuedocalanus sp.	14.00	2.0	USW	55.00	—	—	—	CHRISTIANSEN (1968)
C. helgolandicus	—	10.0	*Biddulphia sinensis*	8.18	—	9.05	—	CORNER *et al.*(1972)
Mixed zooplankton (Station 1)	103.00	20.0	USW	0.44	0.57	—	0.15	EPPLEY *et al.*(1973)
Acartia clausi	13.20	13.0	FSW (0.22 μm)	52.04	—	62.11	—	MAYZAUD (1973)
Sagitta setosa	85.92	13.0	FSW (0.22 μm)	50.68	—	70.81	—	MAYZAUD (1973)
Calanus sp.	180.00	19.5	FSW (0.45 μm)	4.76	—	—	—	JAWED (1973)
Thermocyclops hyalinus (FW)	0.93	27.3	FFW (0.70 μm)	49.44	—	—	12.42	GANF and BLAZKA (1974)

Table 2. (Continued).

Species	Dry wt. (μg.)	Temp. (°C)	Food Supplied	NH_4–N (μg. N mg. dry wt.$^{-1}$ day^{-1})	Urea–N (μg. N mg. dry wt.$^{-1}$ day^{-1})	Total N (μg. N mg. dry wt.$^{-1}$ day^{-1})	PO_4–P (μg. P mg. dry wt.$^{-1}$ day^{-1})	Reference
Metridia pacifica	102.00	8.0	*Nitzscia sp.* *S. costatum*	1.91	—	—	1.22	TAKAHASHI and IKEDA (1975)
C. finmarchicus	260.00	5.0	FSW (0.22 μm)	0.90	—	—	—	MAYZAUD (1976)
Paracalanus parvus	6.00	10.0	FSW (0.70 μm)	4.93	—	—	1.60	IKEDA (1977)
Artemia salina A	770.00	25.0	*Platymonas sp.*	10.11	—	—	—	MOFFETT and FISHER (1978)
Artemia salina N	2.00	13.0	FSW (1.0 μm)	4.27	—	—	—	MOFFETT and FISHER (1978)
Gaussia princeps	700.00	5.5	FSW (0.4 μm)	0.39	—	—	—	QUETIN *et al.*(1980)
Daphnia pulex (FW)	20.00	20.0	*Chlamydomonas sp.* N. D.*	0.67	—	—	0.04	LEHMAN (1980)
Daphnia pulex (FW)	20.00	20.0	*Chlamydomonas sp.* N.S.	1.54	—	—	0.06	LEHMAN (1980)
Artemia sp. (axenic)	—	25.0	Nutrient medium	5.18	—	—	—	HERNANDORENA and KAUSHIK (1980)
Daphnia magna (FW)	30.00	20.0	UFW and Soy flour	0.88	—	—	—	GARDNER and SCAVIA (1981)
Brachionus plicatilis	0.42	20.0	*Chlorella saccharophila*	112.03	93.27	280.88	48.15	This study

Key: USW–Unfiltered seawater FSW–Filtered seawater (pore diameter) FFW–Filtered freshwater (pore diameter)
UFW–Unfiltered freshwater A–Adult N–Nauplii CI–Copepodite I CII–Copepodite II FW–Freshwater species
*N. D.–Nutrient deficient cells N. S.–Nutrient sufficient cells

species. The problems and constraints encountered during these expriments have been reviewed by: CORNER and DAVIES (1971), REEVE (1977), IKEDA (1977), and LEBORGNE(1979).

Table 2 presents a short review of previously derived zooplankton excretion rates. The most obvious trend from this table is the increase in excretion rates with decreasing body size (JOHANNES, 1964; CORNER *et al.*, 1965). Within this table, *B. plicatilis* possesses the smallest body size and also the highest daily excretion rates. Thus, it has been estimated that this rotifer is capable of turning over an amount of nitrogen equivalent to its body weight (body equivalent excretion time; JOHANNES, 1964) in about 10.6 hr., through nitrogenous waste excretion. The importance of rotifers and other micro-zooplankton in the recycling of nutrients was proposed by JOHANNES (1964), GANF and BLAZKA (1974), and LEHMAN (1980). The data from this study substantially supports their point of view.

In conclusion, *B. plicatilis* has been found to possess high excretion rates of ammonium, urea and orthophosphate, which are directly available for utilization by phytoplankton; and these excretion rates are at least are dependent on its density, fecundity, and food density. Thus, *B. plicatilis* may play a very important role in the cycling of nutrients in both natural and artificial ecosystems.

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