Kinetics of nitrogen and phosphorus release in varying food supplies by *Daphnia magna**

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Abstract

Rates of nitrogen and phosphorus release from individual *Daphnia magna* were determined by measuring ammonia and soluble reactive phosphorus in successive 10-min incubations in small (0.05 ml) vessels after the animals were removed from their food. Release rates of both nutrients were generally highest initially and decreased with time after removal. The ratio of nitrogen to phosphorus released increased with time after animals were removed from an artificial detritus/bacterial food; ratios were lower and changed with time less for animals fed algae. These data suggest errors may be introduced by assumptions of constant stoichiometry for nutrient release in varying environments.

Introduction

Phytoplankton abundance in lakes and oceans is controlled by balances between growth and removal processes. Since epilimnetic biomass concentrations often are maintained during periods of summer stratification, nutrients must be supplied to the photic zone at rates sufficient for growth to balance combined losses by sinking, grazing, and other removal processes. Zooplankton often play a major role in providing available nitrogen and phosphorus to the photic zone (e.g., Ketchum 1962; Barlow & Bishop 1965; Ganf & Blazka 1974; Lehman 1980a, b; Richey 1979; Scavia 1979). In conceptualizations of nutrient regeneration by zooplankton, nitrogen and phosphorus have often been assumed to be released in constant stoichiometry (e.g., Chen & Orlob 1975; Canale *et al.* 1976; Walsh 1976, 1977; DiToro *et al.* 1977; Scavia 1980). The assumption of constant stoichiometry is reasonable for experiments with relatively constant food sources (e.g., cultured phytoplankton), but may not be valid in natural systems, where food sources vary. Phytoplankton are a major nutritional source for zooplankton, but often comprise only 10–50% of the particulate organic carbon in the ocean (Parsons 1963) and lakes (e.g., Saunders 1972; Stadelmann & Munawar 1974). Therefore, since detritus often forms the major component of the seston, it is reasonable to assume that detritus is a major component of the diet for filter-feeding zooplankton. Although assimilation efficiency is lower for detritus than for algae, detritus can be assimilated (Schindler 1968; Saunders 1972) and may be necessary to meet the energy requirements of zooplankton, especially between periods of rapid algal production (Saunders 1969).

Because of the important role zooplankton play in providing nutrients for algal production and because detritus may be a major component of their diet, we measured rates of ammonia and phosphorus release from *Daphnia magna* fed algae and artificial detritus.

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Methods

Release rates

Nitrogen release rates were determined for individual animals as described by method B of Gardner & Scavia (1981). For this method, high performance liquid chromatographic components interfaced an incubation flow cell (0.05 ml) with the injection loop of an ammonium (NH_4) analyzer to measure ammonium after separation from primary amines by cation exchange (Gardner 1978). Phosphorus release rates were measured in the same way, except that a Technicon AutoAnalyzer was interfaced to receive samples from the injection loop. Soluble reactive phosphorus (SRP) was detected with the ascorbic acid-molybdate method (Murphy & Riley 1962), with automated Technicon methodology. Rate kinetics were obtained for nitrogen and phosphorus by measuring nutrients accumulated in successive 10-min incubations. Nitrogen and phosphorus release rates were determined in parallel systems for two separate animals taken from the same culture.

As described in Gardner & Scavia (1981), an animal was gently drawn from culture into the incubation flow cell by suction from a large syringe attached to the outlet end of the flow cell. After reassembling and attaching the flow cell, flow (1.1 ml min^{-1}) of control water was directed through the cell for 1.5 min to purge the cell of water and food that was transfered with the animal. Thus nutrient release rates were measured for animals in the absence of food (Gardner & Scavia 1981). Control of sample valves and flow rates for both animals was automated by using a solvent programmer (Beckman, Model 420) and pneumatically operated sample valves.

Standards and blanks were run by loading the sample loop with either control water or nutrient standard prepared in control water prior to injection. Control water contained 20 mg $CaCl_2 \cdot 2H_2O$, 10 mg $MgSO_4$, and 20 mg $NaHCO_3$ per liter of distilled deionized water (Lehman 1980a).

The frit in the outlet end of the incubation flow cell (Gardner & Scavia 1981) held back only particles greater than *ca.* 10 μm in diameter (determined by analysis with a Model TA II Coulter Counter). Thus, nutrients associated with small particles (<10 μm) released by the animals could account for some of the measured NH_4 and SRP. Particle effects may be more important to phosphorus analysis than to nitrogen analysis because phosphorus is readily sorbed to particles, but may be released by exposure to acid during SRP analysis. Sorption of phosphorus by the flow cell frit, checked by observing removal of standard PO_4 solutions from the incubation flow cell with and without the frit, was not a serious problem.

Experimental design

To determine effects of diet on release rates, two experiments were performed with animals exposed to different food regimes. In the first experiment, animals were cultured at 20 °C in a 9 l vessel under constant flourescent lighting. Soy flour was fed in excess at 1–2 day intervals. The soy flour (*ca.* 4–20 μm diameter) with microbes in the culture tank served as artificial detritus. The chemical compositions of ground whole grain soy flour, natural detritus, and cultured phytoplankton (Table 1) indicate that soy flour is a reasonable detritus analog. Protein, carbohydrate, lipid, and nitrogen content of phytoplankton, detritus, and soy flour are all similar. Although phosphorus levels in all three materials sometimes overlap, phytoplankton often contain more phosphorus than detritus or soy flour (Table 1). This may be because algal cells have the capacity to store phosphates in great excess of their immediate needs.

A second experiment was designed to measure nutrient release from animals experiencing changes in food type. Animals were placed initially in 9 l of 0.45 μm filtered Lake Michigan water at 20 °C under constant fluorescent lighting for 24 h. Thereafter, 0.3 g of artificial detritus (soy flour) was mixed into the culture at the end of each day. Nutrient release was measured for 2–4 new animals each day for 11 days after the 24-h starvation period. This design was intended to examine the relative effects of detritus and microbes as food sources. Because filtration should reduce the density of microbes in lake water, we presumed that the soy flour–lake water mixture would initially have a low microbial content, but microorganism density would increase with time in the culture. An algal bloom (2.6–3.9 μm round, non-motile green Chlorococcales, probably *Chlorella,* S. J. Tarapchak, personal communication) was observed on the 6th

Table 1. Chemical composition of cultured phytoplankton, detritus, and soy flour (% dry weight ± standard error). Number in parentheses is sample size.

Protein	Total carbohydrate	Lipid	Nitrogen	Phosphorus	Source
Phytoplankton					
39 ± 3.9[a]	23 ± 3.3	8 ± 1.5	6.2 ± 0.6	1.8 ± 0.3	Parsons *et al.* (1961)[i]
43 ± 4.7(13)	26 ± 2.5(13)	14 ± 2(17)	4.7 ± 0.5(17)	1.3 ± 0.2(14)	Strickland (1960)[c]
			1.0 - 12.5	0.1 - 10.0	Holm-Hanson (1972)[d]
Detritus					
			2.3 ± 0.2(47)		Tenore (1981)[e]
46, 51[a]			7.4, 8.1	0.9, 1.3	Holm-Hanson (1972)[b,f]
24[a]			3.8		Holm-Hanson (1972)[b,g]
20–48	15–30		3.0–8.0	0.5–0.8	Holm-Hanson (1972)[b,h]
Soy flour					
37	30	20	6.5	0.6	Watt & Merrill (1963)

[a] Nitrogen × 6.25.
[b] Assumes dry weight is 50% carbon.
[c] Nutrient-deficient cultures omitted.
[d] Laboratory cultures only.
[e] Oven-dried, ground seaweeds.
[f] Particulate organic matter (POM) from 300 m, 400 m; Lake Tahoe.
[g] POM from 175–600 m; off Southern California.
[h] POM from 300–2 000 m; off Baja, California, assume protein and carbohydrate are 52% and 44% carbon.
[i] N = 11 for all contents.

day and was dominant during and after the 7th day. Thus, food conditions changed from one approximating detritus and bacteria to one dominated by phytoplankton and bacteria. Since we did not measure the relative concentrations of bacteria, detritus, and algae during the 11 days, the relationship between food type and nutrient release rates is not quantitative. However, the dramatic changes in measured release rate patterns (see below) with the appearance of the algal bloom suggests that food quality may strongly affect the release rates and composition of nutrient release products.

Nutrient release rates based on wet weight measurements were converted to a dry weight basis by a constant factor (dry weight:wet weight ± SE = 0.100 ± 0.005) determined by weighing 47 animals from an algal-fed culture both wet and dry. Wet weights were determined by gently drying each animal with tissue (*ca.* 3 min) and weighing on a Sartorius 6-place balance (accurary = *ca.* 1 μg). Animals were dried for 48 h at 60 °C in preweighed aluminum planchetts before dry-weight measurements were made.

All experiments were carried out at 20 °C. Temperature was maintained by submerging the incubation flow cell and inlet tubes in an insulated water bath maintained at 20 °C by adding ice periodically.

Results and discussion

Release kinetics

Simultaneous SRP and NH_4 release rates, determined in our first experiment for animals taken from an artificial detritus culture maintained over a 2-week period, were highest immediately after removal from food [4.8 ± 0.5 nmol-P mg^{-1} h^{-1} (N = 10), 80.5 ± 12.4 nmol-N mg^{-1} h^{-1} (N = 12)] and decreased rapidly to more stable rates of 1.2 (SE = 0.3, N = 10) and 41.3 (SE = 5.1, N = 12) nmol mg^{-1} h^{-1} for SRP and NH_4, respectively, after approximately 1.5 h (Fig. 1). These initial rates approximate those of well-fed animals. Because the animals were removed from their food prior to release measurements, these rates do not include confounding effects of algal nutrient uptake [discussed by Taka-

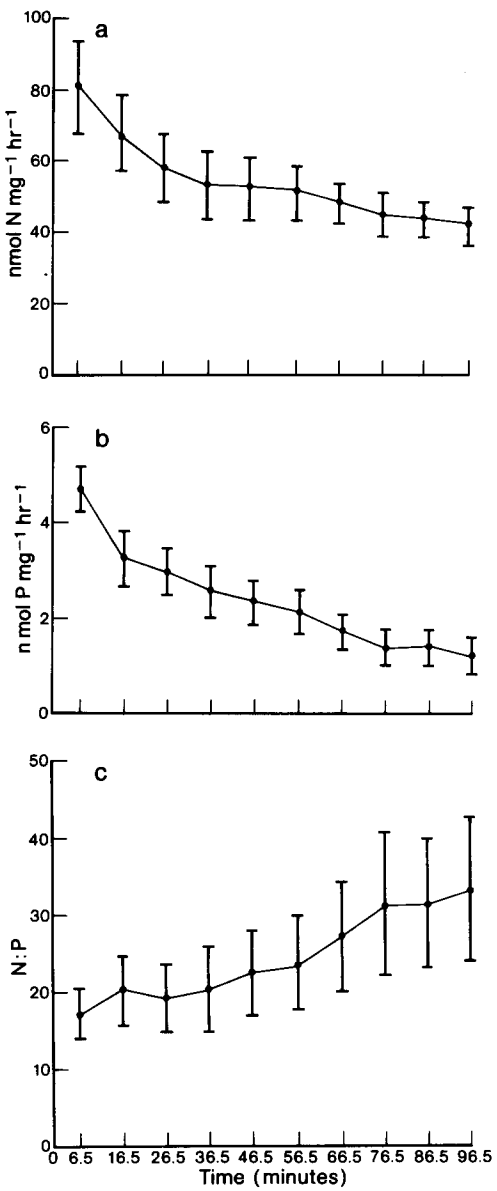

Fig. 1. Mean ± 1 SE rates of release of NH_4 (a) and SRP (b), and the molar ratio of nitrogen to phosphorus (c) vs. time after *Daphnia magna* were removed from their food.

hishi & Ikeda (1978), Lehman (1980a), and Gardner & Scavia (1981)]. The patterns of release (i.e., decreasing with time) are similar to those observed earlier for NH_4 by *D. magna* (Gardner & Scavia 1981) and for radiolabeled PO_4 by daphnids under a somewhat different experimental design (Peters & Rigler 1973).

The ratio of NH_4 to SRP release for the first experiment (Fig. 1) was calculated as the ratio of means from 12 N-release and 10 P-release determinations to compare the changes of NH_4 and SRP release with time after the animals were removed from food. Molar nitrogen to phosphorus ratios during the first three 10-min periods averaged 18.7 (SE = 1.1, N = 3) and rose gradually to 32.1 (SE = 0.7, N = 3) during the last three periods (70–100 min after the animals were removed from food). The initial ratios should approximate the ratio of NH_4 to SRP released while animals are actively feeding. The animals in this experiment produced ratios somewhat higher than those found for marine and freshwater animals feeding on phytoplankton (7.3, 18.4, Harris 1959; 6.8, Martin 1968; 8.2, Ganf & Blazka 1974; 7.2–11.1, Lehman 1980a; 11.4, Lehman 1980b), indicating that phosphorus release, relative to nitrogen, is lower with our artificial detritus food.

The increasing ratio of nitrogen to phosphorus with time after the animals were removed from food indicates SRP release rates decreased faster than did NH_4 release rates. This suggests that phosphorus release is more dependent on food supply than is NH_4 release; phosphorus release responded more rapidly to the removal of food.

Food effects

To examine the effects of food type on NH_4 and SRP release, we measured release rates from animals taken from a culture where the food environment changed during a 2-week interval from one of artificial detritus to one dominated by algae.

Release rates after 24 h without food (day 0) were low (0.15 ± 0.03 nmol-P mg^{-1} h^{-1}, N = 4; 21.7 ± 5.9 nmol-N mg^{-1} h^{-1}, N = 4) and did not decrease with time after initiation of measurements. Initial release rates (0–10 min) generally increased over the next 11 days (Fig. 2). Although variability was quite large (r^2 = 0.48 for SRP, 0.20 for NH_4), a simple linear regression with time resulted in significant (α = 0.05) positive slopes for initial release rates of both SRP (0.43 ± 0.08 nmol mg^{-1} h^{-1} day^{-1}) and NH_4 (2.58 ± 0.81 nmol mg^{-1} h^{-1} day^{-1}). Analysis of residuals about the regression lines clearly demonstrated increased variance for SRP and uniform variance for NH_4 release over the 11 day period.

The increased day-to-day variability, after the

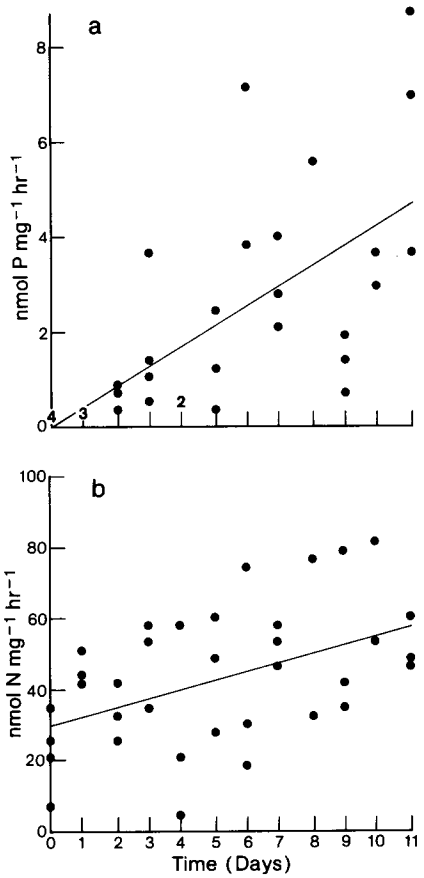

Fig. 2. Rates of release of SRP (a) and NH_4 (b) during the first 10 min after each animal was removed from food vs. time after new culture was started. Each point represents an individual animal; numbers indicate overlapping rates. The lines through the data were fitted by linear least-squares regression against time (SRP: $r^2 = 0.48$, $p < 0.05$; NH_4: $r^2 = 0.20$, $p < 0.05$).

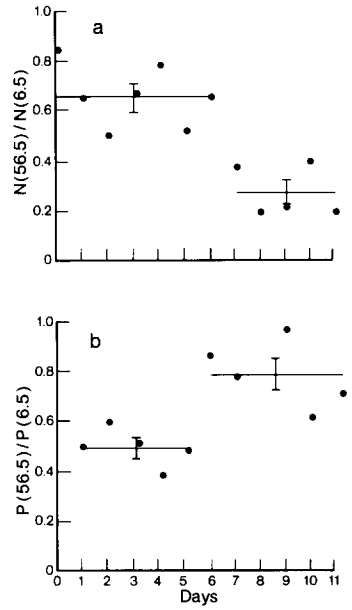

Fig. 3. Daily mean ratios of rate determined 1 h after the animal was removed from food, N (56.5), to rate determined during the first 10 min, N (6.5) for NH_4 (a) and SRP (b) vs. time after new culture was started. Dots represent daily means (N = 2–4) and the horizontal lines represent means of grouped days. (See Table 2.) Error bars represent ± 1 SE around the grouped means.

Table 2. Comparison of mean ratios of rates 1 h after removal from food to rates immediately after removal for periods before and after the onset of algal bloom (day 6–7). N = number of samples.

Days	Mean	Variance	N	P*
SRP				
1–5	0.50	0.006	5	
6–11	0.80	0.019	5	<0.01
NH_4				
0–6	0.66	0.017	7	
7–11	0.28	0.011	5	<0.01

* T-test for comparison of means.
Both differences are significant at given P values.

algal bloom (~ day 6), suggests the changing diet may have affected release rates. While the rates showed gradual increases as the culture aged, other properties showed dramatic changes when the algal bloom became dominant. For example, the ratio of daily mean (N = 2–4) release rates 1 h after removal from food to rates immediately after removal changed after the algal bloom for both SRP and NH_4 (Fig. 3, Table 2). Prior to the algal bloom, SRP release rates dropped an average of 50% during 1 h. After the bloom, initial (0–10 min) rates continued to increase (Fig. 2), and the mean rates after 1 h dropped only to 80% of the initial rates. Increased ratios (Table 2) after the bloom illustrate a slower reduction in release rates; that is, the release rates remained high longer after the animals were removed from food. During the first 6 days, NH_4 release dropped to an average of 66% of the initial rates during the first 1 h after the animals were removed from food. After the algal bloom, initial rates continued to increase (Fig. 2), but unlike SRP, the ratio of 1-h rates to initial (0–10 min)

rates decreased (Fig. 3, Table 2), indicating a rapid decrease in NH_4 release during the first hour after removal from food.

Changes in the ratios of nitrogen to phosphorus release with time after animals were removed from their food (Fig. 4) further illustrate the differences in nutrient release during the transition to algal dominance in the culture tank. Prior to the algal bloom, mean daily initial nitrogen to phosphorus ratios averaged 34.9 ± 6.1 ($N = 4$). After the bloom, initial nitrogen to phosphorus ratios fell to 17.7 ± 4.8 ($N = 6$). These latter values are closer to values cited above for animals fed only algae (Harris 1959; Martin 1968; Ganf & Blazka 1974; Lehman 1980a, b). Nitrogen to phosphorus ratios decreased with time during the first 30 min after removal from food throughout the 11 days (Fig. 4). After the onset of the algal bloom (days 6–7), the ratios remained low throughout the entire hour of determination; however, prior to the algal bloom, decreased release of SRP relative to NH_4 after the first 30 min caused a dramatic shift to higher nitrogen to phosphorus ratios. Lower and relatively constant nitrogen to phosphorus ratios for animals after the bloom were caused by elevated and relatively constant SRP release rates and constant NH_4 release rates after an initial 30 min decrease. This suggests that the proportions of nitrogen and phosphorus released are different for the algal food than for the artificial detritus and that, given the algal food, nitrogen and phosphorus release rates apparently responded similarly with time after removal from food.

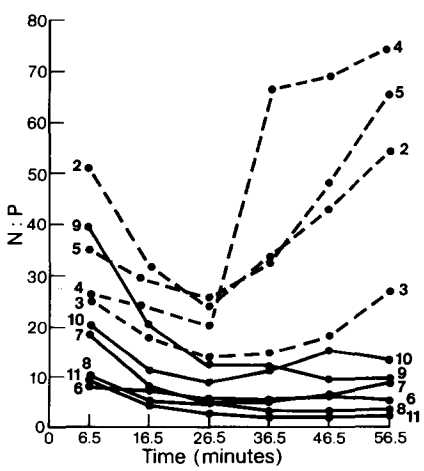

Fig. 4. Daily mean molar ratios of NH_4 to SRP release vs. time after the animals were removed from their food. Numbers refer to days after the new culture was started, solid lines for after the onset of the algal bloom. (See text for details.)

Conclusions

Changes in the ratio of released NH_4 and SRP during the period after animals were removed from food illustrate differences in nitrogen and phosphorus regeneration mechanisms. Results from the experiment on animals experiencing a changing food supply demonstrated a gradual increase in release rates as the cultue became colonized by bacteria and algae (Fig. 2). Patterns of nutrient release changed after the algal bloom, but NH_4 and SRP release patterns were modified in different ways. Prior to the algal bloom, SRP release was low and decreased more rapidly than NH_4 release after the animals were removed from food. These results (broken lines in Fig. 4) are similar to those for the artificial detritus food in the first experiment (Fig. 1) and indicate a condition of relatively low phosphorus supply. After the bloom, both initial NH_4 and SRP release rates were higher. The SRP release rates also remained high for a longer period after the animals were removed from food, suggesting that phosphorus was supplied to the animals in greater quantities after the bloom. The high initial NH_4 rates for animals observed after the bloom decreased more rapidly during incubation than did those for animals examined before the bloom. While the presence of algae in the diet appeared to increase protein catabolism, as evidenced by higher initial NH_4 release rates, more rapid decrease in those rates after removal from food suggests that the algal diet provided a relatively labile protein pool that was rapidly catabolized. Lower initial rates and relatively slow decrease in rates prior to the bloom suggest that protien in the artificial detritus was catabolized, but at a slower rate.

Results from these experiments illustrate differences in the kinetics of nitrogen and phosphorus release with time after removal from food and changes in the proportion of nitrogen and phosphorus released when the food source changed from an artificial detritus/bacterial base to an algal/bacterial base. These results suggest that simple stoichiometric relationships between nitrogen and phosphorus release may introduce errors in models

of nutrient cycling. Further work is needed to accurately define regeneration models in terms of nutrient content and quantity of available food. This may be especially critical for lakes that undergo dramatic oscillations between detrital and algal dominance of potential food supply.

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