Content and turnover rates of phosphorus in *Daphnia pulex:* Effect of food quality

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Abstract

The content of phosphorus in individual *Daphnia pulex* and the rates at which the element turns over are functions of the P contents of algal cells fed to the zooplankton. *Chlamydomonas* and *Ankistrodesmus* were grown in semi-continuous cultures containing 2 μM and 10 μM PO_4 to produce differences in cellular P of the algae. Animals reared on the cultures high in P contained 60 percent more P than did animals of equal size that were fed low P cells. *Daphnia* with high body contents of P turned over that P faster than did the animals fed low P algae. Measured turnover rates imply that large differences exists between rates of gross P assimilation and P excretion for animals fed high and low P sources.

Introduction

Rates of release of P from crustacean zooplankton have been the subject of many inquiries in recent years (see Harrison, 1980; Korstad, 1980; Lehman, 1980a for reviews). Rates are often equivalent to the loss each hour of at least several percent of the P-content of the animals (Lehman, 1980b). Much of the recycling owes to egestion and metabolism of recently ingested food and rapid declines in P-release occur when animals are separated from their diet (Scavia & Gardner, 1982). Measured rates of P-release from well fed individuals may thus overestimate rates of which zooplankton catabolize or turn over their structural components. Release rates measured from starved plankton may likewise lead to erroneous conclusions because unfed animals may alter their physiology to accommodate their deprived state. Rates of nutrient turnover in the body mass of herbivorous zooplankton are important ecologically. They probably determine the speed and extent to which animals can change tissue stoichiometries in response to changes in quality and abundance of food. That, in turn, influences rates and proportions of nutrients available to higher trophic levels and thereby affects nutrient flux throughout the community.

Radiotracers have been used successfully to measure metabolism of P in *Daphnia* (Peters & Rigler, 1973). The approach is simple and direct in concept and we adopted it for our work. We studied the effects of algal food type and algal P content on the content and turnover of P in *Daphnia pulex.* The investigation suggests that herbivorous zooplankton like *Daphnia* adjust very quickly to the nutrient quality of their food. Our animals renewed their body masses of P within a few hours under appropriate conditions.

Methods

Cultures of *Daphnia pulex* were maintained in screw-top glass vessels containing 4 l of filtered epilimnetic water from Third Sister Lake, Michigan. *Chlamydomonas reinhardtii* and *Ankistrodesmus falcatus* were grown in semi-continuous cultures in MWC medium (Lehman, 1976) with the PO_4 con-

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centration altered among treatments. For each species separate cultures were designated 'Low-P' (2 μM PO_4) and 'High-P' (10 μM PO_4). Each day one-half of the volume of each culture was removed and fresh sterile medium was added. Cells were harvested by centrifugation and were added to the *Daphnia* cultures. Optical densities of the *Daphnia* cultures (500 nm, 5 cm path) were monitored to assure that food did not become depleted. Calibration curves related cell abundances of each algal species to light absorbance. Abundances did not fall below 4×10^4 cells ml^{-1} in any treatment. All cultures were maintained at 20 °C on a 16:8 light:dark cycle with illumination at c. 100 μEin m^{-2} s^{-1} from cool-white fluorescent bulbs.

Relations between animal body size and P-content were determined for each treatment. Individual *Daphnia* were removed from their culture vessels and measured to the nearest 0.04 mm from head to base of tail spine. They were placed in 50 ml distilled, deionized water and digested with potassium persulfate for 60 min (Menzel & Corwin, 1965). The digests were neutralized and measured for SRP (Strickland & Parsons, 1972).

Rates of P-turnover were determined by measuring the increase in specific activity of animals fed a radioactive diet. Algae were labelled with ^{33}P by incubating the food cultures with c. 50 μCi carrier-free $H_3{}^{33}PO_4$ (New England Nuclear) for 24 h. Labelled algae were harvested by centrifugation and were fed to *Daphnia* that had been preconditioned with identical but unlabelled food for 7 days. Individuals were then removed at intervals over the next 5 days, rinsed in non-radioactive medium, and measured for body length. They were transferred to 0.5 ml Protosol (New England Nuclear) in scintillation vials, digested at 55 °C overnight, and 10 ml OCS (Amersham) was added. At the end of the experiment all samples were counted as a batch on a Beckman LS-210 liquid scintillation counter with the detection window optimized manually for ^{33}P.

Results

The nutrient content of algal food had a marked effect on P-content of *Daphnia pulex* (Fig. 1). For both *Chlamydomonas* and *Ankistrodesmus*, animals fed from high-P treatments contained more P than did animals fed low-P algae (analysis of covariance). P-content of the animals varied approximately with the cube of body length in all treatments except low-P *Chlamydomonas* where the power of the relation was less than 3 ($P<0.05$). The main effect of food quality, however, did not involve the slope of P-content *vs* length; rather it concerned the elemental content of *Daphnia* body tissue. Adjusted to equivalent body length, high-P food resulted in higher P content in *Daphnia* ($P<0.0001$) by a factor of 1.65 for *Chlamydomonas* and 1.59 for *Ankistrodesmus*. The main effect is unquestionably the P content of the algae because there are no differences between the effects of food species (*Chlamydomonas vs Ankistrodesmus*) at either high or low levels ($P>0.5$). In our semi-continuous cultures at harvest the low-P *Chlamydomonas* contained 3.4×10^{-9} μmol P $cell^{-1}$ and high-P *Chlamydomonas* contained 7.8×10^{-9} μmol P $cell^{-1}$; low-P and high-P *Ankistrodesmus* contained 3.3×10^{-9} and 8.9×10^{-9} μmol P $cell^{-1}$, respectively.

Time courses of isotope equilibrium are shown in Fig. 2. Specific activities for individual *Daphnia* were calculated from cpm $animal^{-1}$ (measured directly) and the body mass of P (nmol P $animal^{-1}$) estimated from the regressions plotted in Fig. 1. Analyses were confined to animals greater than 1 mm length, because smaller animals were poorly represented in Fig. 1. Moreover, the smaller sizes include animals newborn in the labelled food and we were interested only in rates of isotope exchange in animals that originally contained large unlabelled pools of P. Curves were fit to the data by non-linear regression using the model:

$$A = A_{max}(1-e^{-kt}) \quad (1)$$

where A is specific activity, A_{max} is the asymptotic maximum, t is time and k is a rate constant ($time^{-1}$). Activities are reported as cpm because all samples were counted with identical efficiency (c. 90%) and the results are comparable.

Turnover times for P in the body tissue of *Daphnia pulex* are listed in Table 1. Animals fed algae low in P-content turned over their body-P in 2 days or less. When fed algae high in P, however, the animals exchanged their body-P in a matter of several hours.

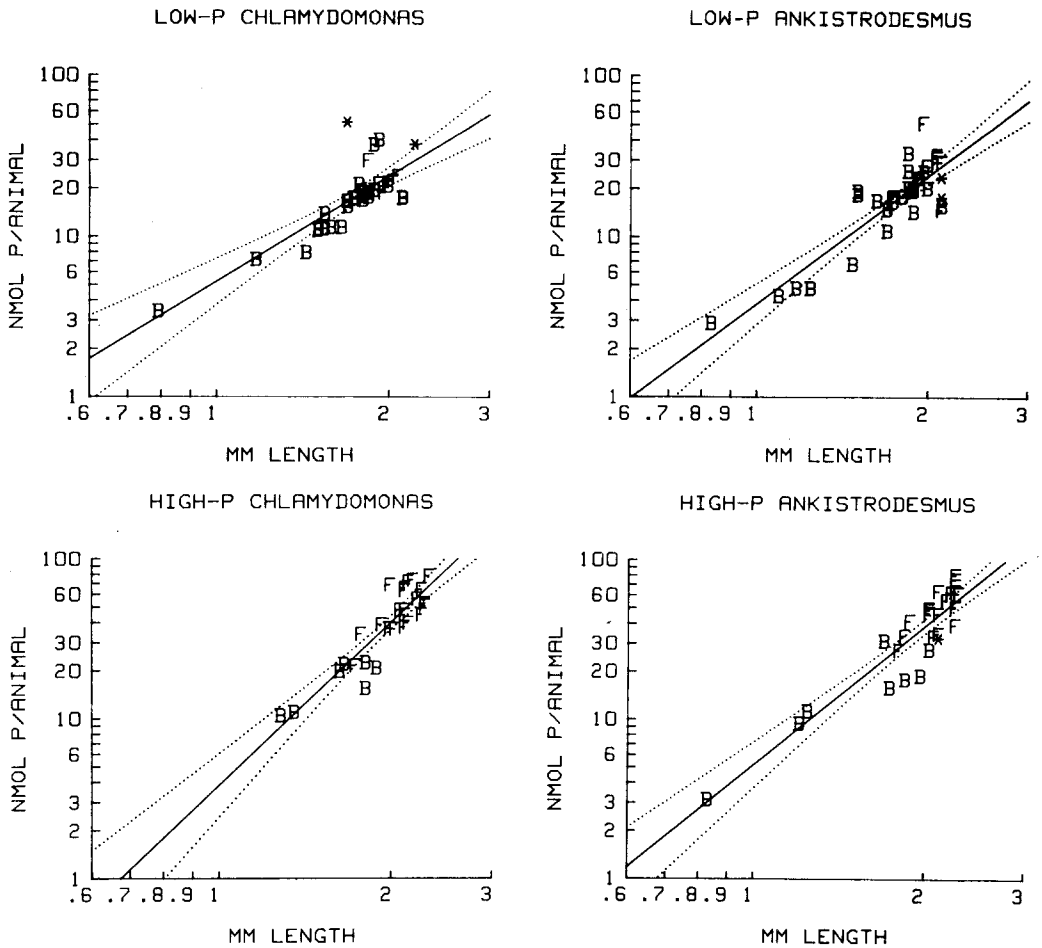

Fig. 1. Log-log relationships between body size and P content of individual *Daphnia pulex* fed high-P and low-P cultures of *Chlamydomonas* (left) and *Ankistrodesmus* (right). Symbols indicate whether the individuals were without eggs (B), egg-bearing (F), or ephippial (*). Linear regressions are fit to each data set, and 95% confidence intervals of the regressions are shown.

Table 1. Turnover times of P in *Daphnia pulex* computed as 1/k by fitting equation (1) to the data in Fig. 2.

	Turnover time (h)
Low-P *Chlamydomonas*	47.6
Low-P *Ankistrodesmus*	36.0
High-P *Chlamydomonas*	6.7
High-P *Ankistrodesmus*	4.7

Discussion

The rates at which *Daphnia* turn over the P bound in their body tissue evidently depend greatly on the amount of P available to them in their diet. Animals husband the nutrient most efficiently when it is less available in their food. Overall tissue contents of P are lower among *Daphnia* fed a diet poor in P. The animals thus contain less P and they retain it more efficiently when it is less abundant.

The results seem reasonable from both ecological and physiological points of view. The implications they hold for models of nutrient dynamics in aquatic ecosystems have not been considered previously, however. Even in models which include variable nutrient contents for the algae, zooplankton are treated as though they possess constant, fixed stoichiometry. Our results show not only that this is unlikely, but that the animals turn over their constitutive P so fast in some cases that they may re-

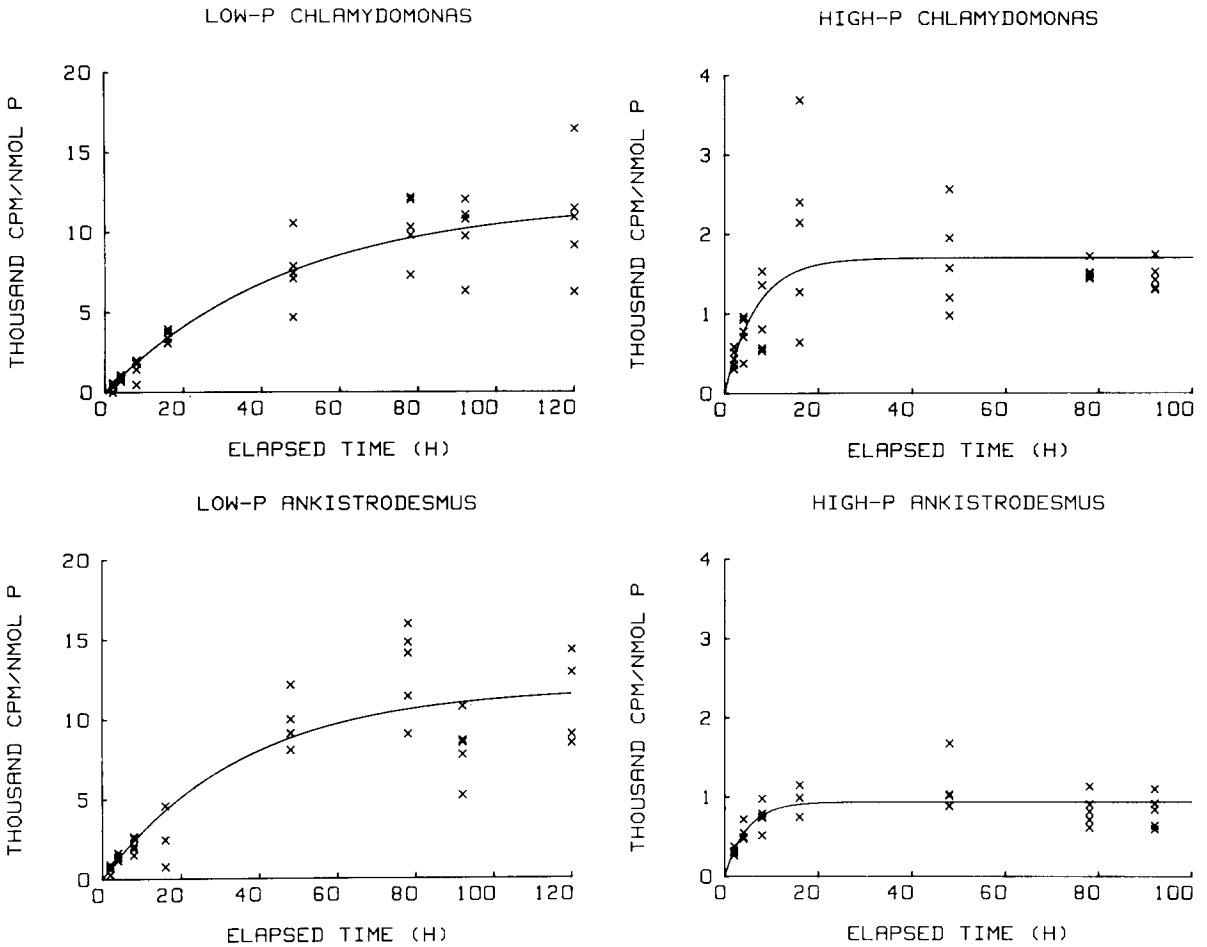

Fig. 2. Radiophosphorus content of the total mass of P in individual *Daphnia pulex* after the addition of labelled food. Curves were fit by non-linear regression using equation (1). Turnover times inferred from the plots are listed in Table 1.

spond rapidly to changes in food quality. *Daphnia* body tissue does not mimic the composition of ingested food, but it is constrained and influenced by it.

We did not purge or dissect gut contents from the *Daphnia* prior to our analyses, but differences among algal cells contained in those guts do not affect our interpretations. Based on Geller's (1975) data for *Scenedesmus, Daphnia pulex* 2 mm in length can pack c. 2×10^6 μm^3 of that green alga into their guts. We estimated the maximum differences between animals that could be ascribed to differences in the P-content of food held in the gut from the sizes and cellular P contents of our algae. These differences were less than 0.1 nmol P animal^{-1} whereas measured differences in P content between 2 mm *Daphnia* fed low-P and high-P algae were greater than 15 nmol animal^{-1}. The gut contents contributed to less than one percent of the measured differences.

The turnover times in Table 1 are qualitatively consistent with the findings of Lehman (1980b) that *Daphnia* fed with P-deficient *Chlamydomonas* released P equivalent to 1.24% h^{-1} of their body mass of P whereas animals fed nutrient-sufficient cells released 1.95% h^{-1}. Our turnover times are much faster than these excretion rates imply, however, and they even exceed rates that might be expected from smaller metazoa or protozoa based on body equivalent excretion rates (Taylor & Lean, 1981).

The reason for this discrepancy is that the isotop-

ic equilibria we measured integrate more of the animals' physiology than do excretion rates alone. Conover & Francis (1973) described a 4 compartment model that is appropriate to our experimental conditions. In addition to dissolved P (P_D) and P in algae (P_Q), the total body P of *Daphnia* (P_Z) is presumed to consist of 2 compartments, P_1 and P_2. P_1 is a metabolically active exchangeable pool and P_2 is a larger, structural component. This description of *Daphnia* conforms to empirical work by Peters & Rigler (1973), and it parallels the patterns of C metabolism in these animals (Lampert & Gabriel, 1984). The equations which describe changes in specific activities of all these pools can be derived by the approach of Lampert & Gabriel (1984). For pool P_1:

$$dP_1/dt = (\rho_{Q1} - \rho_{1D} - \rho_{12})P_1 + \rho^2 P_2 \quad (2)$$

$$dP_1^*/dt = (\rho_{Q1}a_Q - \rho_{1D}a_1 - \rho_{12}a_1)P_1 + \rho_{21}a_2P_2 \quad (3)$$

where ρ_{Q1} is the rate of gross assimilation of P by *Daphnia,* ρ_{1D} is excretion rate, and ρ_{12} and ρ_{21} are the exchange rates between pools 1 and 2, (% total *Daphnia* body P h^{-1}), P_1^* is radiophosphorus content (dpm) and a_Q, a_1, a_2 are the specific activities of alga and animal P pools (dpm nmol-P^{-1}). Similarly,

$$dP_2/dt = \rho_{12}P_1 - \rho_{21}P_2 \quad (4)$$

$$dP_2^*/dt = \rho_{12}a_1P_1 - \rho_{21}a_2P_2 \quad (5)$$

The specific activity of the algae in our experiments can change during an incubation because the radioactive cells are present together with animals that are initially unlabelled. Excreted P can be incorporated into the algae:

$$dQ/dt = \rho_{DQ}Q - \rho_{Q1}P_1 \quad (6)$$

$$dQ^*/dt = \rho_{DQ}a_DQ - \rho_{Q1}a_QP_1 \quad (7)$$

where ρ_{DQ} is the rate of uptake of P (% algal cell P h^{-1}); and a_D is the specific activity of P incorporated by the algae (dpm nmol-P^{-1}). Under experiment conditions concentrations of PO_4 were sufficiently available that we feel justified claiming that ρ_{DQ} is a constant, independent of phosphate levels. The differential equations describing the changes in specific activity of individual pools are:

$$\frac{da_Q}{dt} = \frac{d(Q^*/Q)}{dt} = \frac{1}{Q}\frac{dQ^*}{dt} - \frac{a_Q}{Q}\frac{dQ}{dt} = \rho_{SQ}(a_D - a_Q) \quad (8)$$

$$da_1/dt = \frac{\rho_{Q1}}{S_1}(a_Q - a_1) + \frac{\rho_{21}}{S_1}(a_2 - a_1) \quad (9)$$

$$da_2/dt = \frac{\rho_{12}}{S_2}(a_1 - a_2) \quad (10)$$

where S_1 and S_2 are the fractions of total *Daphnia* P in pools 1 and 2 respectively. Because the experiment situation is such that algae obtain nutrients released by the *Daphnia* we will assume $a_D = a_1$. To solve this system we use the additional information that the fractions S_1 and S_2 remain constant, so

$$a_Z = a_1S_1 + a_2S_2 \quad (11)$$

This constraint forces a dependency among coefficients ρ_{Q1}, ρ_{1D}, ρ_{21}, ρ_{12}, and S_2 as described by Lampert & Gabriel (1984; their equation 4).

The equations (8) to (10) describe a linear homogeneous system with constant coefficients, which has an interdependent family of solutions. For the specific activity of whole *Daphnia* (a_Z), which we measured, the general solution is:

$$a_Z(t) = A_{max}(1 - e^{\lambda t}) \quad (12)$$

where λ is a (negative) eigenvalue obtained by solving the quadratic equation:

$$\lambda^2 + b\lambda + c = 0 \quad (13)$$

with

$$b = \rho_{DQ} + \frac{\rho_{Q1}}{S_1} + \frac{\rho_{21}}{S_1} + \frac{\rho_{12}}{S_2}$$

$$c = \frac{\rho_{DQ}\rho_{21}}{S_1} + \frac{\rho_{DQ}\rho_{12}}{S_2} + \frac{\rho_{Q1}\rho_{12}}{S_1S_2}.$$

Equation (12) is effectively identical to equation (1), fit to our data. We find that the rate of approach to isotope equilibrium depends on rates of gross assimilation, internal transfers among pools, and

on rates of uptake by the algae, which in turn are dependent on nutrient regeneration. For characteristic values of the parameters (e.g., Table 1: Lampert & Gabriel, 1984), λ is most sensitive to values of ρ_{12}, followed by ρ_{QI} and ρ_{DQ}; the other independent coefficients are of lesser significance.

Other authors have made simplifying assumptions about experimental conditions in order to decipher the behavior of radiotracers introduced in an alga-herbivore system (e.g., Conover & Francis, 1973; Lampert & Gabriel, 1984), with much success. Nonetheless, it is especially important to consider the overall behavior of equations (8) to (10) when interpreting the changes in turnover rate we report in Table 1. Lampert (1977), for instance, reports rates of gross assimilation of C at 60% d^{-1} (=0.025 h^{-1}) for animals c. 2 mm in length. Gross assimilation reported by Peters (1975), for P, was equivalent to 0.025 h^{-1}. We measured rate constants for high-P cells of 0.15 and 0.21 h^{-1}, which exceed gross assimilation. The reason for this is that when *Daphnia* are fed food high in P content, the transfer rate between internal pools (ρ_{12}) becomes elevated. In the most extreme limit of this phenomenon, *Daphnia* might be regarded as a single pool of P, so that a_Z is substituted for a_1 in equations (8) to (10) and $a_1 = a_2$. Then

$$a_Z(t) = \frac{\rho_{QI} a_Q(t_o)}{\rho_{QI} + \rho_{DQ}} [1 - e^{-(\rho_{QI} + \rho_{DQ})t}] \qquad (14)$$

and isotope dynamics depend both on the rates of gross assimilation of P by *Daphnia* and on the rates at which excreted P is incorporated by the algae.

Based on the preceeding line of reasoning we conclude that the body tissues of *Daphnia* can equilibrate to new food regimes faster than one would conclude from excretion measurements or gross assimilation. The differences we measured between *Daphnia* as a function of food quality consequently reflect differences in assimilation of P from the algal food and internal exchange dynamics. Peters & Rigler (1973) reported that *Daphnia* species fed *Rhodotorula* contained constant amounts of P per unit mass regardless of temperature, food availability or body size. The phosphorus content of the food was variable in their experiments; the reported standard deviation of P $cell^{-1}$ was almost 50% of the mean value. Combining their discoveries with our own, we conclude that the amount of P per unit mass in their diet is a major determinant of P-content and turnover rates in the tissue of these herbivores.

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