Limnol. Oceanogr., 44(1), 1999, 225–229
© 1999, by the American Society of Limnology and Oceanography, Inc.

Phosphorus distribution in three crustacean zooplankton species

Abstract—The distribution of phosphorus (P) was assessed in homogeneously ^{33}P-labeled *Daphnia magna, Daphnia galeata* and *Eudiaptomus gracilis.* The specific P contents were 1.48, 1.41, and 0.50% of dry weight (DW), respectively. The results support the view of low intraspecific variability in P:DW ratios in crustacean zooplankton. The fraction of P allocated to nucleic acids, phospholipids, and other P compounds was assessed in *D. galeata* and *E. gracilis.* In both species, the major pool, 35–69% of the total P content, was associated with nucleic acids. This fraction decreased with both body size (age) of *D. galeata* and *E. gracilis* and with the reproductive rate of *D. galeata. D. magna* and *D. galeata* revealed similar patterns of P allocation between body, carapace, and eggs. The carapace contained approximately 14% of the total P content. If most P is not reabsorbed from the exoskeleton prior to molting, the molting will result in a substantial P drain both from the individual and, at times, from the entire planktonic system.

Zooplankton production has long been considered to be mainly energy (or carbon) limited, but recently much attention has been paid to the importance of food quality for the growth of crustacean zooplankton. One of the potentially important factors, which may place constraints on zooplankton growth, is phosphorus (P) (Hessen 1992; Urabe et al. 1997). Typically, daphnids have low carbon : phosphorus (C : P) ratios, while copepods have high C : P ratios in their somatic tissues (Andersen and Hessen 1991; Hessen and Lyche 1991). Furthermore, the sestonic P concentration is often below predicted and observed thresholds for P limitation of zooplankton growth (Hessen and Andersen 1992; Sommer 1992; Urabe and Watanabe 1992). This implies that in order to maintain a nearly homeostatic C : P ratio, a grazer must balance its net intake of elements relative to its bodily demands. At high C : P ratios in the food, some proportion of C (“excess C”) must be disposed off, invariably reducing the growth rate. Similarly, the relationship between the P content in zooplankton and in their food also has implications in terms of the amount of P that is recycled by zooplankton (Sterner et al. 1992).

Expected major pools of P in crustaceans are nucleic acids (deoxyribonucleic acid [DNA] and ribonucleic acid [RNA]), phospholipids, small metabolites (e.g., adenosine triphosphate [ATP], adenosine diphosphate [ADP], reduced nicotinamide adenine dinucleotide [NADH], and reduced nicotinamide adenine dinucleotide phosphate [NADPH]), and calcium-associated P (hydroxyapatite) in the exoskeleton. Only limited information exists on the occurrence and dynamics of these compounds in crustaceans. The distribution of P between these pools, as well as between reproduction and somatic growth, reflects metabolic and physiologic constraints but is also a consequence of the animals’ life histories (Elser et al. 1996). The allocation pattern will also have implications for P flow within the planktonic food web because of differences in turnover time and bioavailability between different pools of P. While the high specific P content of *Daphnia* relative to that of other zooplankton, particularly relative to that of copepods, is settled, there is no obvious reason why this is so. If a high specific P content may lead to reduced growth rates during periods with P-deficient food, there should be advantages to counterbalance this. The most obvious advantage would be that high specific P reflects a high content of RNA, promoting rapid growth rates (Hessen 1990; Elser et al. 1996; Main et al. 1997). There may also be a significant portion of P allocated to the carapace. The specific calcium content of the *Daphnia* carapace is particularly high (Hessen unpubl. data), and this may require a corresponding part of P for calcium-P bindings. Assuming that not all of this P can be retained during the molting, this carapace-associated P could represent a substantial drain of P not only for the individual *Daphnia* but potentially for the entire water body.

In this paper, the patterns of P allocation between nucleic acids, phospholipids, and other P compounds are quantified in three crustacean zooplankton species, *Daphnia magna, Daphnia galeata,* and *Eudiaptomus gracilis.* In addition, the distribution of P between somatic tissues and eggs is assessed in *D. magna* and *D. galeata.* Consequences for P fluxes in lakes are discussed.

D. magna Straus (from the culture collection of the Norwegian Institute for Water Research), *D. galeata* Sars (isolated from Lake Erken), and *E. gracilis* (Sars) (from Lake Norrviken) were fed ^{33}P-labeled algae in batch cultures in 1- to 5-liter glass or polycarbonate bottles. *D. magna* was fed *Selenastrum capricornutum, D. galeata* was fed either exponentially growing *Rhodomonas lacustris* (“*Daphnia galeata* population A”) or stationary phase *Cryptomonas* cf. *marsoni* (“*Daphnia galeata* population B”), and *E. gracilis* was fed exponentially growing *R. lacustris. Selenastrum* were grown at 18°C and 70 μE m^{-2} s^{-1} in Z8 medium (Staub 1961). *Rhodomonas* and *Cryptomonas* were grown at 15°C and 35 μE m^{-2} s^{-1} in 3 × L16 medium (Lindström 1991) modified with soil extract and B vitamins. In all experiments, ^{33}P was added to the algal cultures as carrier-free ^{33}P-orthophosphate (DuPont NEZ-080 for *Selenastrum* and Amersham BF1003 for *Rhodomonas* and *Cryptomonas*). The initial specific activity ranged between 10 and 20 μCi ^{33}P l^{-1} in each algal culture; these are tracer amounts in comparison with the ^{31}P content of the nutrient media. Algae were added to the zooplankton cultures every day or every second day. The algal concentrations in the zooplankton cultures were not determined. For *Selenastrum* and *Rhodomonas,* the food abundance was likely sufficient (above the incipient limiting level), but for *Cryptomonas,* the food concentration may have been suboptimal during the last days before harvesting the daphnids.

Animals were fed ^{33}P-labeled algae for at least 10 d in order to make them uniformly labeled. This is 2.5–5 times longer than the time required for homogeneous labeling of *Daphnia* with ^{32}P or ^{33}P (Peters and Rigler 1973; Hessen and Andersen

Table 1. Relationships between dry weight (DW, μg) and length (L, mm) for *Daphnia magna* and *D. galeata.* Regression model: $\ln DW = \ln a + b \ln L$. RMS: residual mean square.

Species	$a \times L^b$	RMS	Size range (mm)	No. individuals
D. magna	$5.08 \times L^{3.26}$	0.228	0.73–2.93	16
D. galeata	$8.59 \times L^{2.98}$	0.112	0.59–2.19	43

Table 2. Relationships between individual P content (P) of *Daphnia magna, D. galeata,* and *Eudiaptomus gracilis* and dry weight (DW). The relationship between P and DW was analyzed with linear regression of log-transformed data ($\log P = a + b \log DW$). A slope (b) of 1 indicates homeostatic P/DW.

Species	% P of DW*	(b)†	Size range (mm)	No. individuals
Daphnia magna	1.48±0.32	0.96±0.13	0.73–3.20	25
Daphnia galeata‡	1.41±0.12	1.11±0.09	0.77–2.21	28
Eudiaptomus gracilis	0.50±0.03	0.97±0.18	0.71–1.22	11

* Average ± 95% CI.
† Estimate ± 95% CI.
‡ Population A, fed exponentially growing *Rhodomonas.*

1990). The cultures were inoculated with a few adult females with eggs of *Daphnia* or *Eudiaptmus.* Their offspring, which were hatched in the laboratory and reared with ^{33}P-labeled algae as the sole food, were used in the analyses described below. Before analysis, animals were transferred to dense nonradioactive algal cultures for 30–60 min (*Daphnia*) or 60–90 min (*Eudiaptomus*) in order to empty their guts of radioactive contents. They were subsequently washed in nonradioactive medium before being anaesthetized with CO_2.

Estimates of dry weight (DW) specific P content of the animals were obtained from individual measurements of radioactivity using a liquid scintillation counter as described below. The P content of each individual was calculated by multiplying the ^{33}P content of the individual (calculated from measurements of radioactive decay) by the $^{31}P:^{33}P$ ratio in the nutrient medium. DW was estimated from regressions of ln(DW) on ln(length). Specific regressions were made for *Daphnia* spp. (Table 1), whereas for *E. gracilis,* we applied the regression from Bottrell et al. (1976). The length of the daphnids was measured from the base of the caudal spine to the top of the head (*D. galeata* did not have helmets). Body length of *E. gracilis* was measured from the anterior end of the cephalothorax to the posterior end of the furca. Individual daphnids were put in preweighed tin capsules and dried at room temperature until they had attained stable weight ($\geq$2 d). The samples were weighed on a Mettler SE30 (*D. magna*) or a Cahn 4700 (*D. galeata*) microbalance.

For an analysis of the anatomic distribution of P, anaesthetized large (>1-mm body length) *D. magna* and *D. galeata* (population A) were dissected under a dissection microscope using a small drop of nonradioactive algal medium. The carapace was cut with a sharp needle at the integument at the neck, and the major thoracic portion of the exoskeleton (termed carapace below) was removed. The carapace was geometrically estimated to constitute approximately 70% of the total exoskeleton. The eggs were gently removed from the brood pouch and transferred with a pipette to an Eppendorf vial. The carapace and the remaining part of the body (including the drop of medium) were then transferred to separate Eppendorf vials. The samples were treated with tissue solubilizer, and the activity was measured in a liquid scintillation counter as described below. The allocation of P to different fractions was calculated as the contribution of each fraction to the total activity (i.e., the sum of all three fractions) of each individual.

For biochemical separations, homogeneously labeled *D. galeata* (populations A and B) and *E. gracilis* were picked up with forceps and placed in 1.5-ml Eppendorf vials. Animals were sorted according to their size (*D. galeata*; larger or smaller than 1 mm, which roughly corresponds to the size at first reproduction) or developmental stage (*E. gracilis*; copepodite stages IV and V, adult males and adult females). The number of individuals per vial ranged between 10 and 20. Zooplankton were stored frozen and subsequently freeze-dried without being thawed. The animals were homogenized in the Eppendorf vial with a plastic piston pellet mixer and mixed with 500 μl sterile deionized water. From each vial, six subsamples of 50–55 μl were randomly assigned in duplicate to three treatments: total activity measurement, nucleic acid precipitation, and lipid extraction. Nucleic acids were precipitated with ice-cold 0.2-M perchloric acid (PCA) (Munro and Fleck 1966) for 30 min and centrifuged in a cold centrifuge for 30 min at $13{,}000 \times g$. The pellet was washed twice with 0.2-M PCA. Phospholipids were extracted with chloroform : methanol : water (1 : 2 : 0.8 by volume) (Bligh and Dyer 1959). To each subsample, 200 μl ice-cold chloroform : methanol (1 : 2, v : v) was added. The lipids were extracted for 30–60 min and subsequently centrifuged as above. The supernatant was pipetted into a new Eppendorf vial, and the extraction was repeated twice with 250 μl chloroform : methanol : water (1 : 2 : 0.8). For each sample, the supernatants were combined. The chloroform phase was separated from the methanol : water phase by addition of 200 μl 0.9% sodium chloride (NaCl) followed by thorough shaking and centrifugation (10 min, $13{,}000 \times g$). The pellets were air-dried, and the liquid phases were allowed to dry so that the volume was less than 100 μl before tissue solubilizer was added.

To *D. magna* samples, 50 μl Packard Soluene 350 tissue solubilizer was added, and the samples were dissolved over night before 1.4 ml Packard Ultima Gold scintillation fluid was added. The Eppendorf vials were shaken and put into 20-ml plastic scintillation vials, and the activity was measured in the ^{14}C window of a Packard Tri-Carb 1500 scintillation counter. For *D. galeata* and *E. gracilis* experiments, the procedure was similar, but 100 μl Biolute-S (Zinsser Analytic) was used as solubilizer, the scintillation fluid was Wallac OptiPhase HiSafe, the Eppendorf vials were put into glass vials, and the activities were measured on a LKB 1217 RackBeta scintillation counter. External standards with known ^{33}P activity were used for estimation of the counting efficiency. All results were corrected for decay (half-life, 25.3 d). Controls were included, which consisted of the treatment of nonradioactive animals using the same procedures as described above.

Table 3. Number of eggs per large (>1 mm) *Daphnia.*

Species	Population	Food alga (growth phase)	Eggs per female*	Range	No. individuals
D. magna		*Selenastrum*	4.5 ± 1.8	0–10	10
D. galeata	A	*Rhodomonas* (exponential)	5.9 ± 1.9	0–21	37
D. galeata	B	*Cryptomonas* (stationary)	0.02 ± 0.02	0–1	137

* Average ± 95% CI.

The specific P content was similar in *D. magna* and population A of *D. galeata*; 1.48 ± 0.32 and 1.41 ± 0.12% of DW (average ± 95% CI), respectively (Table 2). In *E. gracilis,* the specific P content of large copepodites and adults was 0.50 ± 0.03% of DW (Table 2). The P:DW ratio did not change across the size spectrum in *D. magna* or in *E. gracilis* (Table 2). However, there was a slight increase in DW specific P content with increasing size in *D. galeata,* as indicated by a slope larger than 1 for the regression of log(P/ind) on log(DW/ind) (Table 2).

The number of eggs per large (>1 mm) female differed both within and between *Daphnia* species (Table 3). The highest reproductive rate was found in population A of *D. galeata,* which was fed exponentially growing *Rhodomonas.* The lowest reproductive rate was found in *D. galeata* population B, which was fed stationary phase *Cryptomonas.* The number of eggs in *D. magna* was slightly lower than in population A of *D. galeata,* but the confidence intervals were overlapping.

The relative distribution of P between "body" (excluding the major carapace and eggs), carapace, and eggs was very similar in *D. magna* and population A of *D. galeata* (Fig. 1). The major part, approximately 67%, of the total P content was allocated to the somatic tissues both in *D. magna* and *D. galeata.* The carapaces contained approximately 14% of the total P content of the animals, which is a minimum estimate of P content in total exoskeleton since it includes only approximately 70% of the entire carapace. In both species, 20% of the P was allocated to the eggs. The amount of P allocated to eggs is most likely strongly dependent on clutch size, and the estimates above probably reflect the similarity in clutch size in these populations.

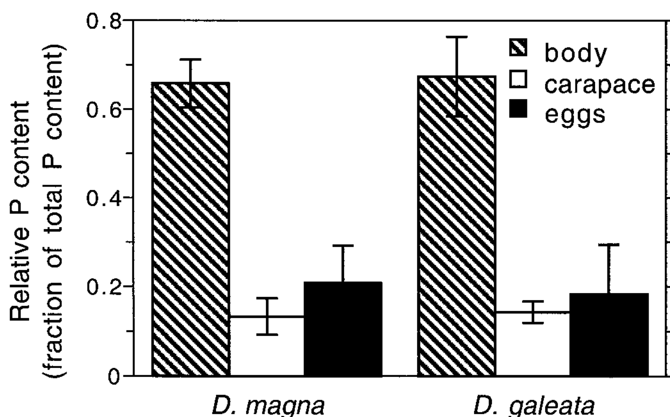

Fig. 1. Distribution of P between body (excluding carapace and eggs), carapace, and eggs in *D. magna* and *D. galeata* (population A). Error bars indicate 95% confidence interval for mean of replicate measurements of ^{33}P activity. Number of individuals, $N = 10$ for *D. magna,* and $N = 8$ for *D. galeata.*

In both populations of *D. galeata,* the fraction of P allocated to nucleic acids was slightly higher in daphnids that are <1 mm than in those that are >1 mm in length (Fig. 2). In *E. gracilis,* a larger part of the total P content of the animals was allocated to nucleic acids in copepodites than in adults. The changes in nucleic acid content with the size of the crustacean was largely mirrored by changes in the pool "other" (Fig. 2). The variation in the fraction of P allocated to phospholipids was less than the variation in nucleic acid content (Fig. 2). Significantly more P was allocated both to nucleic acids and phospholipids in population A than in population B of *D. galeata* (unpaired *t*-tests; $p < 0.0001$ both for nucleic acids and phospholipids; data pooled for small [<1-mm] and large [>1-mm] daphnids within each population) (Fig. 2).

The specific P contents of *D. galeata, D. magna,* and *E. gracilis* are close to previously published values (Andersen and Hessen 1991; Hessen and Lyche 1991). Also, the constancy in specific P content over size classes in *D. magna* and *E. gracilis* and the weak increase in P:DW in *D. galeata* support the view of a low intraspecific variability in the specific P content of crustacean zooplankton. However, this study does not test for variability in P:DW ratios induced by changes in dietary P intake. Furthermore, in contrast with our results, Carrillo et al. (1996) showed that the specific P content of the calanoid copepod *Mixodiaptomus laciniatus* decreased with increasing size. This discrepancy can be explained by the fact that their smallest size class consisted of nauplii, while in the present study, the smallest individuals were copepodites in stages IV and V. Thus, there may be changes in the P content of *Eudiaptomus,* especially during earlier stages of development, but no measurements were made during those stages. The similarity in P allocation patterns in dissected *D. magna* and *D. galeata* supports the notion that any *Daphnia* species could serve as a suitable model organism for assessing the general role of daphnids in P cycling.

Quantitatively, the most important pool of P is associated with nucleic acids, both in *Eudiaptomus* and in *Daphnia.* For *D. galeata* population B, however, the pool "other" was of comparable size. These results are well in agreement with a calculated P content in nucleic acids ranging between 84% (copepodid III) and 47% (adult) of total P content in the calanoid copepod *Euchaeta elongata* (Dagg and Littlepage 1972), assuming that 40% of the basepairs in the nucleic acids are guanine–cytosine (Smith 1964) and 0.005 μgP μg DW^{-1} (*Eudiaptomus,* this study). The percentage of P allocated to nucleic acids in *D. magna* was calculated to be 38% (McKee and Knowles 1986; assuming 0.015 μgP μgDW^{-1}, DW was calculated as the sum of protein, RNA, DNA, ADP, ATP, lipid,

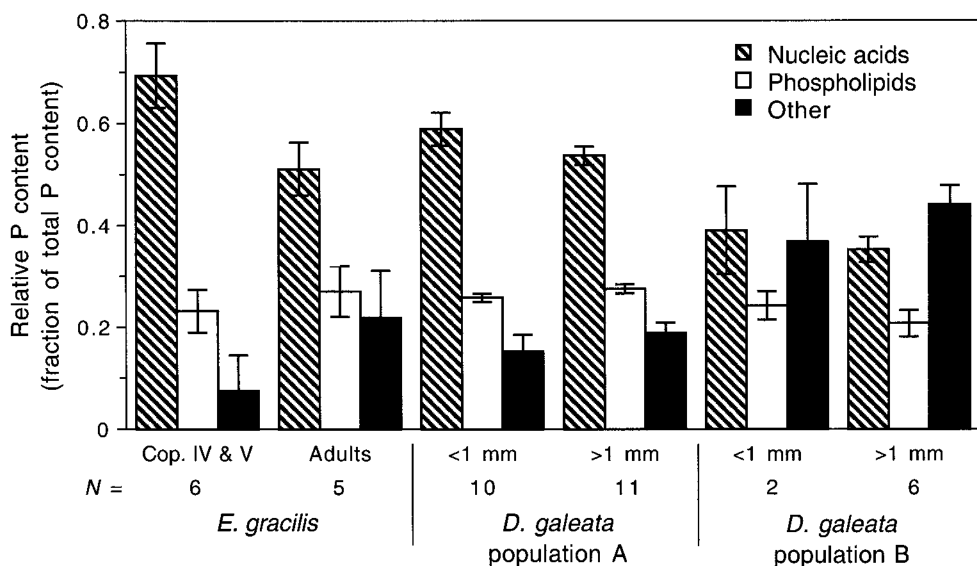

Fig. 2. Biochemical distribution of P in copepodites and adults of *E. gracilis* and small (<1-mm) and large (>1-mm) *D. galeata* (populations A and B), expressed as fraction of total P content. *E. gracilis* and population A of *D. galeata* were fed exponentially growing *Rhodomonas* while population B of *D. galeata* was fed stationary phase *Cryptomonas.* See text for separation procedures. Error bars indicate 95% confidence interval for mean value of replicate measurements of ^{33}P activity. N = number of batches; 10–20 individuals per batch.

and glycogen). This is similar to the values for population B of *D. galeata,* but considerably lower than for population A. RNA is a major part of total nucleic acids in crustaceans. It constitutes more than 95% of total nucleic acids in *D. magna* (calculated from McKee and Knowles 1986) and 55% in adults of *E. elongata* (Dagg and Littlepage 1972). The decrease in P allocated to nucleic acids from small (juvenile) to large (adult) stages both in *Daphnia* and *Eudiaptomus,* as well as the low amount of P allocated to nucleic acids in population B of *D. galeata,* can be interpreted to represent a decrease in specific RNA content in slow-growing individuals. This is supported by the generally positive relationship between RNA content and growth rate for a wide variety of invertebrate taxa (Sutcliffe 1970; Båmstedt and Skjoldal 1980) as well as decreasing RNA : DNA ratios with decreasing egg production rate in adult females of the copepod *Acartia grani* (Saiz et al. 1998). Furthermore, the RNA per DW ratio in marine copepods is negatively correlated with DW (Dagg and Littlepage 1972; Båmstedt and Skjoldal 1980). The large amount of P in the pool "other," in combination with the low levels of P in nucleic acids in population B of *D. galeata,* may indicate that overall P homeostasis in individuals with low RNA content can be maintained by retaining high concentrations of P in other compounds (e.g., as free nucleotides).

There is a striking similarity across the tested species and stages in the amount of P allocated to phospholipids. However, considering the low specific P content of *Eudiaptomus,* this means that the DW-specific phospholipid content in this species is only one-third of that in *D. galeata.* Even though Sterner (1995) suggested that differences in nucleic acid content alone could stand for the difference in specific P content between daphnids and copepods, the present study indicates that differences in phospholipid content also contribute to the difference in P : DW ratios.

The instar duration of *Daphnia* is very short, ranging between 2 and 3 d at 20°C (Lynch et al. 1986). Consequently, if as much as 14% of bodily P is lost during each molt, molting would result in a substantial drain of P from the individual during its lifetime. As an example, the cumulative losses of P at ecdysis in a *Daphnia* growing with a specific growth rate of 0.3 d^{-1} and a period of 2 d between molts would correspond to 44% of what is incorporated into new biomass during a period of 14 d (7 molts). Thus, almost one-half of the P that is incorporated into new biomass will be lost as a result of molting. Sterner et al. (1993) observed that daphnids fed P-deficient algae had problems with the molting, as the molt remained attached to the posterior margin of the new carapace. This may indicate that P is an important factor for carapace formation and molting and is consistent with the observed high P content of the carapace.

The observed 14% of total P allocated to the carapace in *D. magna* and *D. galeata* is considerably more than the approximately 3% in cast-off exuviae reported by Peters and Rigler (1973). In support of our data, Yan et al. (1989) noted the high specific P content of *Daphnia* carapaces. The contrast with the low P content in exuviae, as reported by Peters and Rigler (1973), could indicate either an efficient reabsorption of carapace-bound P prior to molting or a substantial loss to the medium. The latter hypothesis is supported by Scavia and McFarland (1982), who reported conspicuous peaks in the P release from *Daphnia* during molting. This is further supported by the observation of lower specific calcium content in cast-off exuviae relative to intact exoskeletons in *Daphnia,* accompanied by little evidence indicating bodily reclaim of calcium (Hessen

unpubl. data). Alternatively, the apparent discrepancy in P content can be explained by the presence of organic P in the epidermis, which is included in our measurements but not in measurements on cast-off exuviae because the epidermis is not molted (Skinner 1985; Stevenson 1985). It remains uncertain whether the P content of the carapace remains high after molting. If the latter case is true, molting may be an important P loss process, because carapaces will sink rapidly from the epilimnion, and, thus, the P becomes unavailable for other planktonic organisms. During periods with high abundance of daphnids, like the clearwater phase, this loss of carapace-bound P could provide a substantial drain of P from the epilimnion of lakes.

Tobias Vrede

Uppsala University
Department of Limnology
Norbyvägen 20, SE-752 36
Uppsala, Sweden

Tom Andersen
Dag O. Hessen

University of Oslo
Department of Biology
Division of Limnology
Box 1027 Blindern, N-0315
Oslo, Norway

References

Andersen, T., and D. O. Hessen. 1991. Carbon, nitrogen, and phosphorus content of freshwater zooplankton. Limnol. Oceanogr. **36:** 807–814.

Båmstedt, U., and H. R. Skjoldal. 1980. RNA concentration of zooplankton: Relationship with size and growth. Limnol. Oceanogr. **25:** 304–316.

Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37:** 911–917.

Bottrell, H. H., and others. 1976. A review of some problems in zooplankton production studies. Norw. J. Zool. **24:** 419–456.

Carrillo, P., I. Reche, and L. Cruz-Pizarro. 1996. Intraspecific stoichiometric variability and the ratio of nitrogen to phosphorus resupplied by zooplankton. Freshw. Biol. **36:** 363–374.

Dagg, M. J., and J. L. Littlepage. 1972. Relationship between growth rate and RNA, DNA, protein, and dry weight in *Artemia salina* and *Euchaeta elongata.* Mar. Biol. **17:** 162–170.

Elser, J. J., D. R. Dobberfuhl, N. A. MacKay, and J. H. Schampel. 1996. Organism size, life-history, and N : P stoichiometry: Towards a unified view of cellular and ecosystem processes. Bioscience **46:** 674–684.

Hessen, D. O. 1990. Carbon, nitrogen and phosphorus status in *Daphnia magna* at varying food conditions. J. Plankton Res. **12:** 1239–1249.

——— 1992. Nutrient element limitation of zooplankton production. Am. Nat. **140:** 799–814.

———, and A. Lyche. 1991. Inter- and intraspecific variations in zooplankton element composition. Arch. Hydrobiol. **121:** 343–353.

———, and T. Andersen. 1990. Bacteria as a source of phosphorus for zooplankton. Hydrobiologia **206:** 217–223.

———, and ———. 1992. The algae-grazer interface: Feedback mechanisms linked to elemental ratios and nutrient cycling. Arch. Hydrobiol. Beih. Ergebn. Limnol. **35:** 111–120.

Lindström, K. 1991. Nutrient requirements of the dinoflagellate *Peridinium gatunense.* J. Phycol. **27:** 207–219.

Lynch, M., L. J. Weider, and W. Lampert. 1986. Measurement of the carbon balance in *Daphnia.* Limnol. Oceanogr. **31:** 17–33.

Main, T. M., D. R. Dobberfuhl, and J. J. Elser. 1997. N : P stoichiometry and ontogeny of crustacean zooplankton: A test of the growth rate hypothesis. Limnol. Oceanogr. **42:** 1474–1478.

McKee, M. J., and C. O. Knowles. 1986. Effects of fenvalerate on biochemical parameters, survival, and reproduction of *Daphnia magna.* Ecotoxicol. Environ. Saf **12:** 70–84.

Munro, H. N., and A. Fleck. 1966. Recent developments in the measurement of nucleic acids in biological materials. Analyst **91:** 78–88.

Peters, R. H., and F. H. Rigler. 1973. Phosphorus release by *Daphnia.* Limnol. Oceanogr. **18:** 821–839.

Saiz, E., A. Calbet, A. Fara, and E. Berdalet. 1998. RNA content of copepods as a tool for determining adult growth rates in the field. Limnol. Oceanogr. **43:** 465–470.

Scavia, D., and M. J. McFarland. 1982. Phosphorus release patterns and the effects of reproductive stage and ecdysis in *Daphnia magna.* Can. J. Fish. Aquat. Sci. **39:** 1310–1314.

Skinner, D. M. 1985. Molting and regeneration, p. 43–146. *In* D. E. Bliss [ed.], The biology of Crustacea. Academic Press.

Smith, M. 1964. Deoxyribonucleic acids of Crustacea. J. Mol. Biol. **9:** 17–23.

Sommer, U. 1992. Phosphorus-limited *Daphnia*: Intraspecific facilitation instead of competition. Limnol. Oceanogr. **37:** 966–973.

Staub, R. 1961. Ernährungsphysiologisch-autökologische Untersuchungen aus an der planktischen Blaualge Oscillatoria rubescens DC. Schweiz. Z. Hydrol. **23:** 82–198.

Sterner, R. W. 1995. Elemental stoichiometry of species in ecosystems, p. 240–252. *In* C. G. Jones and J. H. Lawton [eds.], Linking species and ecosystems. Chapman and Hall.

———, D. D. Hagemeier, W. L. Smith, and R. F. Smith. 1993. Phytoplankton nutrient limitation and food quality for *Daphnia.* Limnol. Oceanogr. **38:** 857–871.

———, J. J. Elser, and D. O. Hessen. 1992. Stoichiometric relationships among producers, consumers and nutrient cycling in pelagic ecosystems. Biogeochemistry **17:** 49–67.

Stevenson, J. R. 1985. Dynamics of the integument, p. 1–42. *In* D. E. Bliss [ed.], The biology of Crustacea. Academic Press.

Sutcliffe, W. H. 1970. Relationship between growth rate and ribonucleic acid concentration in some invertebrates. J. Fish. Res. Board Can. **27:** 606–609.

Urabe, J., J. Clasen, and R. W. Sterner. 1997. Phosphorus limitation of *Daphnia* growth: Is it real? Limnol. Oceanogr. **42:** 1436–1443.

———, and Y. Watanabe. 1992. Possibility of N or P limitation for planktonic cladocerans: An experimental test. Limnol. Oceanogr. **37:** 244–251.

Yan, N. D., G. L. Mackie, and D. Boomer. 1989. Seasonal patterns in metal levels of the net plankton of three Canadian Shield lakes. Sci. Total Environ. **87/88:** 439–461.

Acknowledgments

This study was financially supported by stipends from NorFA and Malméns foundation to T.V.

Received: 23 February 1998
Accepted: 9 September 1998
Amended: 16 October 1998