ECOPHYSIOLOGY

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Stoichiometry in an ecological context: testing for links between *Daphnia* P-content, growth rate and habitat preference

Abstract We used laboratory experiments with ten *Daphnia* taxa to test for links between *Daphnia* P-content, growth rate and habitat preference. The taxa represent a wide range of body sizes and most show distinct preferences for one of three habitats: shallow lakes, deep, stratified lakes or fishless ponds. Previous studies show that taxa from shallow lakes and fishless ponds experience high predation risk and rich food resources, whereas taxa from deep lakes experience low predation risk, strong food limitation and potentially P-deficient resources. Thus, we predicted higher P-content and higher maximal growth rates in taxa from ponds and shallow lakes and lower P-content, lower maximal growth but reduced sensitivity to P-limitation in taxa preferring stratified lakes. In each of 25 experiments, a clonal *Daphnia* cohort was cultured for 4 days on a P-sufficient (molar C:P ratio 70) or a P-deficient (C:P 1,000) diet of a green alga at a high concentration (1 mg C l^{-1}). The P-content of adult *Daphnia* fed the P-sufficient diet ranged from 1.52 to 1.22% mass. Small-bodied taxa from shallow lakes had higher P-content than larger-bodied taxa from deep lakes or fishless ponds. However, we found a nonsignificant negative correlation between P-content and growth on the P-sufficient diet, rather than the positive relationship predicted by the growth rate hypothesis. The P-deficient diet resulted in declines in both growth rate and P-content compared with the P-sufficient controls and the extent of the declines differed between taxa. Taxa from ponds showed a marginally greater decline in growth with the P-deficient diet compared with taxa from shallow or deep lakes. However, contrary to stoichiometric theory, no relationship was found between a species' P-content and growth depression on the P-deficient diet. Although we found evidence for habitat adaptations, our results show that factors other than *Daphnia* P-content are important in determining differences between *Daphnia* species in both maximal growth rate and sensitivity to P-limited growth.

Introduction

Stoichiometric theory makes predictions about the relationship between a consumer's element contents and two key traits: maximal growth rate and sensitivity to element-limited growth (Sterner and Elser 2002). The first prediction, called the *growth rate hypothesis* links an organism's P-content to maximal growth through RNA and protein synthesis (Elser et al. 1996; Acharya et al. 2004). The growth rate hypothesis predicts that organisms with high body P-content will have high RNA content and high maximal growth rates when feeding on P-sufficient diets. Second, stoichiometric models predict a tradeoff between maximal growth rate and sensitivity to P-limited growth (Sterner and Hessen 1994). Organisms with high P-requirements and hence high maximal growth rates, should fare poorly when resources are P-deficient.

The relative advantage of high maximal growth rate versus low sensitivity to P-limited growth varies markedly between freshwater lakes along gradients of lake size, depth and food web structure (Tessier and Woodruff 2002a, b). *Daphnia* species specialize by lake type and members of the genus are often the dominant zooplankton grazers in habitats ranging from vernal ponds to deep, stratified lakes. Thus, daphniids provide a good system for testing for links between body P-content, growth rate and habitat preference. Tessier and Woodruff (2002a, b) found that *Daphnia* species from fishless ponds and shallow, unstratified lakes show higher maximal growth rates than species typically from deep lakes, whereas *Daphnia* species from deep lakes are less sensitive to food limitation. Consistent with these findings, *Daphnia* in ponds (Dudycha 2001, 2004) and shallow lakes (Tessier et

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al. 1992) experience high predation risk and high birth and death rates, conditions that should favor species with high maximal growth rates. In contrast *Daphnia* in deep lakes largely avoid fish predation by migrating into the deep water refuge during day, resulting in higher population densities, more intense grazing and stronger food limitation (DeMott et al. 2004a). DeMott and Tessier (2002) found that seston from shallow lakes was a P-sufficient resource during summer (C:P ratio<300), while seston from deep, stratified lakes was P-deficient (C:P ratio>300). Thus, stoichiometric theory predicts that shallow lakes and fishless ponds should favor taxa that have higher P-content and, therefore, higher maximal growth rates, whereas deep, stratified lakes should favor taxa that have lower P-content and, therefore, lower sensitivity to P-limited growth. Since Tessier and Woodruff (2002a, b) did not measure *Daphnia* P-content, it is unclear whether their habitat-specific results support or contradict predictions of stoichiometric theory.

Applications of stoichiometric theory have often involved comparisons between diverse taxa that differ markedly in their P-content. For example, Elser et al. (2003) found support for the growth rate hypothesis in comparisons among bacteria, zooplankton and insects. The strongest support for application of the growth rate hypothesis to zooplankton comes from a study that compared adults and juveniles of three *Daphnia* species, *Scapholebris*, an exceptionally high P littoral daphniid, and *Bosmina*, a low P, nondaphniid cladoceran (Main et al. 1997). As predicted by the growth rate hypothesis, a positive, linear relationship was found between a grazer's P-content and its growth rate when fed a high concentration of a P-sufficient green alga.

A few laboratory and field studies also support the prediction that high P cladocerans are more sensitive to P-limited growth than lower P taxa. For example, in laboratory experiments, Schulz and Sterner (1999) found that *Bosmina*, a low P cladoceran was much less sensitive to P-limited growth than was a higher P species, *D. obtusa*. This contrast between *Daphnia* and *Bosmina* was further supported by a 9-year study in three eutrophic Dutch lakes (DeMott and Gulati 1999) and subsequent experimental tests (DeMott et al. 2001).

Only one previous study directly tested for links between *Daphnia* P-content, growth rate and habitat preference. Comparisons between *D. pulex* from Arctic ponds and closely related *D. pulicaria* from Wisconsin lakes supported both key predictions of stoichiometric theory (Elser et al. 2000). Clones from the Arctic ponds exhibited higher P-content, higher maximal growth rate with P-sufficient diets and greater sensitivity to P-limitation. The authors argued that the short growing season in the Arctic selects for high growth rates and that a relatively small difference in P-content between the two taxa, about 10%, resulted in the observed modest difference in maximal growth rate and marked difference in sensitivity to P-limitation. Because earlier laboratory and field studies did not detect variation in the P-content of zooplankton species in response to variation in dietary P (reviewed by Sterner and Hessen 1994), Elser et al. (2000) measured the P-content of field-collected animals and did not consider that differences in diet may have caused the observed differences in P-content between the two *Daphnia* species. Recently, both laboratory (DeMott et al. 1998; DeMott 2003; Plath and Boersma 2001) and field studies (DeMott et al. 2001, 2004a, b) have documented declines in P-content (as percent of mass) in daphniids feeding on P-deficient diets. Thus, comparisons of P-content between consumer species need to control for dietary P-content.

In agreement with Elser et al. (2000), we expected that evolutionary tradeoffs in zooplankton P-content should be evident in comparisons between closely related taxa that have adapted to habitats with strongly differing selective regimes. In this study, we tested the effects of P-sufficient and P-deficient laboratory diets on the growth and P-content of ten *Daphnia* taxa including eight species and two interspecific hybrids. We used many of the same taxa studied by Tessier and Woodruff (2002a, b) and our clones came from lakes studied by these authors. Moreover, our taxa include representatives of all three *Daphnia* subgenera. Our experiments included three taxa representing each of three habitats: fishless ponds, shallow and deep lakes. Thus, we tested for links between P-content, maximal growth rate, sensitivity to P-limitation and habitat preference. Our goal was to answer to two interrelated questions: (1) Is stoichiometric theory useful in predicting the distribution of *Daphnia* species across habitats that consistently differ in mortality risk, growth rate selection and resource stoichiometry? and (2) Does stoichiometric theory help explain the evolutionary diversification of this important grazer? Our data allow evaluation of the predicted positive relationship between P-content and maximal growth rate on P-sufficient diets and the predicted negative relationship between P-content and sensitivity to P-limitation. We were especially interested in comparisons between three members of the *D. pulex* complex because of previous research on this group and recent documentation of very strong, habitat-specific differences in mortality risk (Dudchya 2001, 2004).

Materials and methods

Experimental organisms and culture conditions

This study tested the effects of P-deficient diets on the growth and P-content of *Daphnia* in short-term (4 days) laboratory experiments. Our experiments used monoclonal cultures of ten *Daphnia* taxa, including eight species and two interspecific hybrids. With one exception, all taxa were originally collected from ponds and lakes near Kellogg Biological Station in southwestern Michigan and maintained in culture by A.J. Tessier, Michigan State University. The identity of the hybrids and their parental species was confirmed using diagnostic allozyme markers (A.J. Tessier, personal communications). A clone of one nonnative species, *D. magna*, was obtained from Carolina Biological Supply Company.

Daphnia were cultured in a P-free artificial medium (Tollrian 1993) and were fed the green alga *Ankistrodesmus falcatus*. The algae were grown under P-sufficient (molar N:P=17:1) and P-deficient conditions (N:P=360:1) in batch cultures. P-sufficient algae had a molar C:P ratio of about 70, while P-deficient algae had a C:P ratio of about 1,000 (see DeMott 2003 for further details on algal culture and the measurement of element composition and biomass). Since the threshold for P limitation is a resource C:P ratio of about 200–300 (Sterner and Hessen 1994), our resources can be characterized as *P-rich* and *extremely P-deficient*. *Daphnia* stock cultures and experiments were kept at 20°C in an environmental chamber at low light.

Growth assays

Each experiment included three replicate beakers of a particular clone fed P-sufficient algae and three beakers fed P-deficient algae. Each experiment used a cohort of *Daphnia* born within a 24-h interval to mothers fed a high concentration of P-sufficient *Ankistrodesmus*. At the beginning of each experiment, we collected animals for estimating initial mass and randomly distributed the remaining animals among the six experimental beakers. To reduce differences in biomass, greater numbers of smaller species were added to the beakers. Depending on body size, 4–20 individuals were placed in each 250- or 500-ml beaker. Both treatments contained the same high food concentration (1 mg C l^{-1}) and the medium and food were replaced each day.

Daphnia show moderate declines in P-content between the last juvenile instar and the first adult instar (DeMott 2003). Therefore, we designed our laboratory experiments so that most animals reached the first adult instar by the end of the experiment. Thus, the animals were fed P-sufficient *Ankistrodesmus* for 3 days before the start of each experiment and then were allowed to feed on P-sufficient or P-deficient algae for 4 more days. This experimental design provided laboratory data on P-content that could be compared with data for adults of the same species collected from lakes and field enclosures (DeMott et al. 2004b). By the last day of the experiments, age 7 days, most animals were in the first adult, egg-bearing instar.

One possible criticism of our experimental design is a potential bias toward finding higher growth rates with larger-bodied taxa. The experiments ran from ages 3 to 7 days and spanned from juvenile to adult stages. Other studies show that growth tends to slow in the adult stage (DeMott 2003) and that larger species have longer juvenile development (Lynch 1980). Thus, the growth experiments from age 3 to 7 days would have included a longer period of fast juvenile growth in the larger taxa than in the smaller taxa, potentially creating a bias against high growth rates in small, quickly maturing species. However, experiments in which the growth of five daphniids was measured between ages 1–4 and 4–7 days suggest that this potential bias is small (DeMott 1998).

At the end of each 4-day experiment, the animals were collected, counted, measured and dried on glass slides in a desiccator. Survivorship was high in all experiments. All animals from a single beaker were weighed together on an ultra balance (Sartorius M3E) to the nearest microgram. The specific growth rate (day^{-1}) was calculated from the difference of the natural logarithms of the final and initial mass per individual, divided by time (4 days).

After weighing, the *Daphnia* from a beaker were placed in a volumetric flask, ashed at 550°C and then analyzed for P using the molybdate-absorbate method described by DeMott et al. (1998). Most samples, those with a range in mass from about 50–250 μg, were run in 10-ml flasks, while larger samples (about 200–500 μg) were run in 50-ml flasks.

Data analysis

We tested the effect of dietary phosphorus on two key variables: growth in mass (day^{-1}) and P-content (expressed as percent of mass). Our statistical testing was based on values of these two variables and the difference between the P-sufficient and P-deficient treatments. Thus, the growth differential is the difference in growth rate between the P-sufficient and P-deficient treatments (see DeMott et al. 2001). Similarly, the P-content differential is the difference in P-content, as percent of mass, between the two treatments.

We used the homeostasis coefficient eta (H) to quantify changes in *Daphnia* P-content relative to changes in resource P-content (Sterner and Elser 2002). H is calculated by regressing *Daphnia* P versus algal P, both expressed as percent of mass, and both on logarithmic scales. H is the inverse of the slope of the linear regression. H approaches infinity for perfect homeostasis and approaches 1.0 when a consumer's element content varies in direct proportion to the element content of its food.

We conducted 25 experiments over about 12 months with 3- to 7-day-old animals. Although we attempted to run each experiment under the same conditions, some experimental variation was undoubtedly due to uncontrolled variation over time. Thus, we took a conservative approach in statistical testing that emphasized differences that were repeatable over time. We used one-way ANOVA to test for differences between species, where the overall mean for each treatment in each experiment was considered a replicate. For testing predictions of stoichiometric theory and for testing differences between habitats, the overall mean for each taxon was considered a replicate, giving a sample size of nine or ten taxa.

Results

Comparisons across taxa and between habitats

Our experiments included three taxa representative of each of three habitats: fishless ponds and shallow and deep lakes (Table 1). One species, *D. dentifera*, commonly occurs in both shallow and deep lakes and was therefore excluded from comparisons between habitats. Size at maturity was smallest for taxa from shallow lakes, intermediate for deep lakes and largest for taxa from fishless ponds (Table 1).

Across all ten taxa, the P-content of first instar adult females on the P-sufficient diet ranged from 1.52% in *D. parvula* to 1.22% in *D. pulicaria* and the *D. pulicaria × pulex* hybrid (one-way ANOVA, species differences, $F_{7,15}=8.2$, $P<0.001$). Small-bodied *Daphnia* from shallow lakes (*D. parvula*, *D. ambigua* and *D. dubia*) exhibited higher P-content when fed P-sufficient algae than larger-bodied taxa from deep lakes or fishless ponds (Fig. 1a; one-way ANOVA, $F_{2,6}=9.16$, $P=0.015$). However, taxa from deep lakes and fishless ponds showed similar, overlapping P-content. Since taxa from deep lakes and fishless ponds experience contrasting selection regimes, their similarity in P-content is contrary to predictions of stoichiometric theory. Taxa within species complexes showed very similar values for P-content with the P-sufficient diet (*D. pulex* 1.24%, *D. pulicaria* 1.22% and their hybrid 1.22%; *D. dentifera* 1.34%; *D. mendotae* 1.29% and their hybrid 1.31%).

Growth rate on the P-sufficient diet ranged from 0.41 day^{-1} for the *D. pulex × pulicaria* hybrid to 0.21 day^{-1} for *D. dubia* and differed between taxa (Fig. 1b; one-way ANOVA, $F_{7,15}=3.18$, $P=0.029$). Growth with the P-sufficient diet was highest for the pond taxa (mean 0.34 day^{-1}), intermediate for the deep-lake taxa (0.28 day^{-1}) and lowest for shallow-lake species (0.25 day^{-1}) but these differences are not statistically significant (Fig. 1b; one-way ANOVA, $F_{2,6}=2.91$, $P=0.13$).
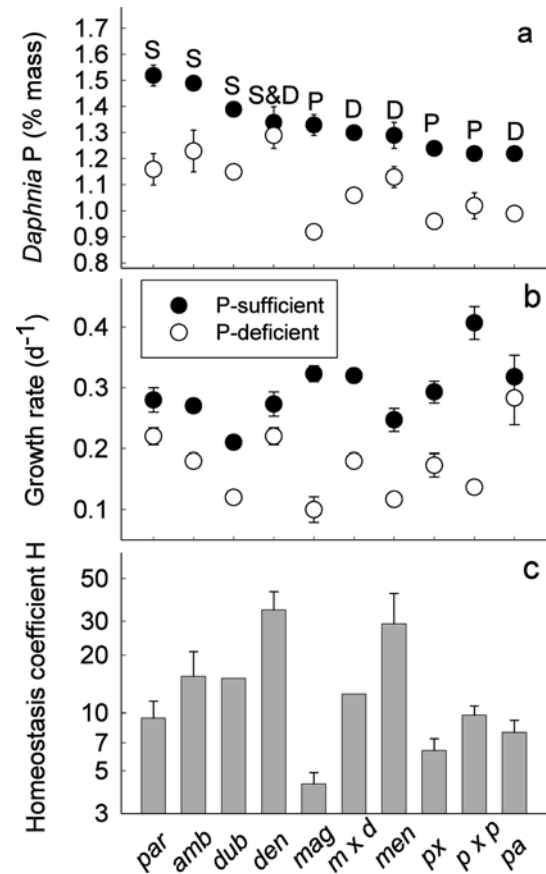
Fig. 1 Effect of dietary P on **a** *Daphnia* P-content, **b** growth rate and **c** homeostasis coefficient (note log scale) for ten *Daphnia* taxa. Taxa are ranked according to their P-content on the P-sufficient diet. Habitat preferences for each taxon are shallow lakes (*S*), deep lakes (*D*) and fishless ponds (*P*). Data are means ± SE for one to four replicate experiments with 3- to 7-day-old animals of each taxon. Abbreviations of taxonomic names are given in Table 1.

Sensitivity to P limitation, measured by the difference in growth between the P-sufficient and P-deficient diets, differed between taxa (one-way ANOVA, $F_{7,15}=20.5$, $P<0.001$). Taxa from ponds showed a marginally greater decline in growth with the P-deficient diet (mean growth

Table 1 List of study taxa grouped by habitat preference. *Size at maturity* is the mean length (excluding tail spine) of at least 20 egg-bearing females from the P-sufficient treatment. Habitat preferences are primarily from Tessier and Woodruff (2002a, b). Abbreviations are shown in *parentheses*. Experiments with each taxon were repeated one to four times over a period of about 12 months

	Size at maturity (mm)	No. of experiments
Shallow lakes		
D. ambigua (amb)	0.90	2
D. dubia (dub)	1.21	1
D. parvula (par)	0.87	2
Shallow and deep lakes		
D. dentifera (den)	1.48	3
Deep lakes		
D. mendotae (men)	1.61	3
D. mendotae × dentifera (m × d)	1.61	1
D. pulicaria (pa)	2.00	4
Fishless ponds		
D. magna (mag)	2.87	3
D. pulex (px)	2.25	3
D. pulex × pulicaria (p × p)	2.01	3

differential=0.21 day^{-1}) compared with taxa from shallow (0.08 day^{-1}) or deep (0.10 day^{-1}) lakes (one-way ANOVA, $F_{2,6}$=4.88, P=0.055). The *D. pulex* × *pulicaria* hybrid, a pond taxon, showed the strongest decline in growth with the P-deficient diet (growth differential, mean ± SE, 0.27±0.02 day^{-1}, n=3 experiments; Fig. 1b). In contrast, its deep-lake parent species, *D. pulicaria*, showed the smallest decline in growth on the P-deficient diet (growth differential, 0.04±0.02, n=4).

Taxa differed in degree of P homeostasis, measured by the homeostasis coefficient H (Fig. 1c, one-way ANOVA on log transformed data, $F_{7,15}$=8.2, P<0.001). *D. dentifera* showed the tightest homeostasis (P-content differential, 0.05±0.01%, n=3 experiments) whereas *D. magna* showed the weakest homeostasis (P-content differential, 0.41 ±0.06%, n=3 experiments; Fig. 1a, c). Homeostasis did not differ with habitat preference (one-way ANOVA, $F_{2,6}$=1.43, P=0.31).

Testing predictions of stoichiometric theory

The growth rate hypothesis predicts a positive linear relationship between *Daphnia* P-content and growth rate with the P-sufficient diet. Contrary to this prediction, we found a negative but nonsignificant relationship between P-content and growth with the P-sufficient diet (Fig. 2a; r^2=0.26, P=0.13).

A second major prediction of stoichiometric theory is that higher P taxa should be more sensitive to P-limited growth than lower P taxa. Contrary to this prediction, the growth differential was unrelated to P-content with P-sufficient algae (Fig. 2b; r^2=0.16, P=0.25). We did, however, find that *Daphnia* P-content was inversely related to body size at maturity (Fig. 2c; r^2=0.51, P=0.02). The nonnative species, *D. magna*, was an outlier in this relationship. *D. magna* is also the only member of the subgenus *Ctenodaphnia* in our data set. If the data for *D. magna* are excluded, the fit is markedly improved (r^2=0.92, P<0.001). Growth rate was not significantly related to body size at maturity (data not shown; r^2=0.21, P=0.19).

Results from the *D. pulex*/*pulicaria* complex strongly conflict with the predicted relationships between *Daphnia* P-content and both maximal growth rate and sensitivity to P-limitation. These three large-bodied taxa had the lowest P-content with the P-sufficient diet among the ten taxa, yet all three showed high growth and the hybrid showed the highest maximal growth rate of all ten taxa (Fig. 1a, b). This pattern of low P-content, high growth rate is the opposite to that predicted by the growth rate hypothesis. The two species and their interspecific hybrid showed very similar responses in P-content to the two diets (Fig. 3; two-way ANOVA on P-content; Diet, $F_{1,14}$=119, P<0.001; Taxon, $F_{2,14}$=0.19, P=0.83). However, the three taxa showed strong differences in their growth responses to the two food types, as indicated by a significant Diet × Taxon interaction term (two-way ANOVA on growth rates, Diet, $F_{1,14}$=6.88, P<0.02; Taxon, $F_{2,14}$=2.78, P<0.10; and
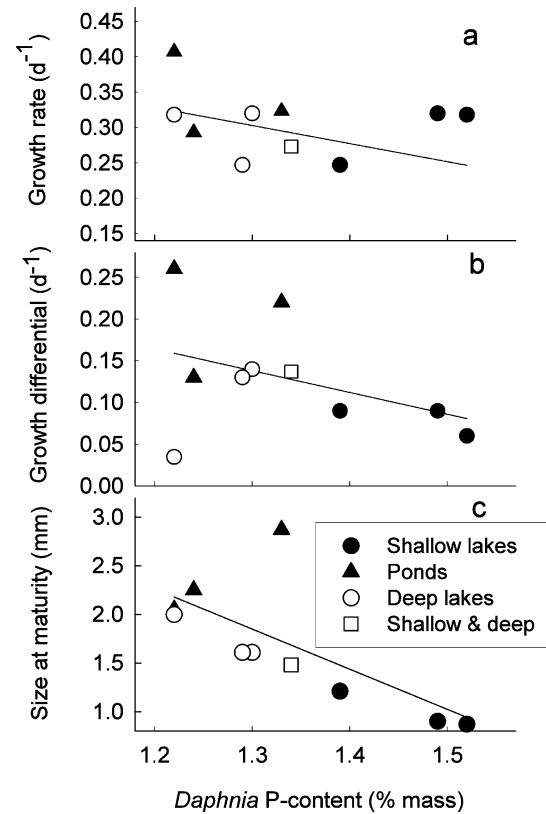

Fig. 2 Relationship between *Daphnia* P-content on the P-sufficient diet and **a** growth rate on P-sufficient diet, **b** growth differential, and **c** body size at maturity on P-sufficient diet. The growth differential is the difference in growth between the P-sufficient and P-deficient treatments. Each data point is the overall mean for a taxon preferring shallow lakes, deep lakes or fishless ponds.

Diet × Taxon interaction, $F_{2,14}$=3.9, P=0.044). The hybrid was most sensitive to P-limitation and *D. pulicaria* was least sensitive to P-limitation of the ten taxa studied, although P-content data for the two taxa were virtually identical. Thus, results from the *D. pulex* group are contrary to both the growth rate hypothesis and the notion that P-content with a high P-diet is a good predictor of sensitivity to P-limitation. Moreover, taxa from habitats with contrasting growth selection regimes exhibited essentially identical P-content. Comparisons between the hybrid and the two parental species are consistent with a tradeoff between maximal growth rate and sensitivity to P-limitation, albeit a tradeoff independent of *Daphnia* P-content.

Discussion

Our laboratory comparisons included ten *Daphnia* taxa representing wide ranges in body size and differences in habitat preference. The ten taxa exhibited variation in both growth rate and P-content in laboratory experiments with P-deficient and P-sufficient algae. Moreover, the variation between taxa was consistent when experiments were repeated over time. The range in P-content between taxa
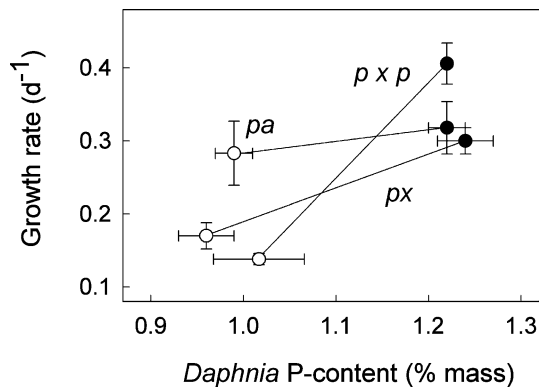
Fig. 3 Reaction norms showing changes in P-content and growth rate for three members of the *Daphnia pulex* group feeding on P-sufficient and P-deficient *Ankistrodesmus*. Taxa include *D. pulex* (*px*), *D. pulicaria* (*pa*) and their interspecific hybrid (*p × p*). The P-sufficient treatment is shown by *solid circles* in the *upper right* (*filled circle*); whereas *open circles* to the lower left represent the P-deficient treatment (*open circle*). Data are means ± SE for three or four experiments with 3- to 7-day-old animals for each taxon.

feeding on P-sufficient resources (1.52–1.22%) is moderate and is consistent with the notion that *Daphnia* are high P species. Declines in P-content and growth with P-deficient diets seem characteristic of daphniids but varied considerably among taxa. Thus, our data set seems suitable for answering two interrelated questions: (1) Is stoichiometric theory useful in predicting the distribution of *Daphnia* species across habitats that consistently differ in mortality risk, growth rate selection and resource stoichiometry? and (2) Does stoichiometric theory help explain the evolutionary diversification of this important grazer? Our results should not be considered a general test of the growth rate hypothesis, which is well-supported by comparisons among more diverse species that show much greater ranges in both P-content and maximal growth rate (e.g., Main et al. 1997; Elser et al. 2003).

Relationship between *Daphnia* P-content and habitat preference

The strongest habitat pattern was the tendency for small-bodied taxa from shallow lakes to have higher P-content than larger taxa from stratified lakes and ponds. Even during summer, the seston C:P ratio of the shallow lakes was <300, while the seston ratio of the surface waters of deep, stratified lakes was >300 (DeMott and Tessier 2002; DeMott et al. 2004a). Thus, the lower P-content of taxa from deep lakes could be adaptive in their low P environment. On the other hand, our data are also consistent with the hypothesis that *Daphnia* P varies with body size between species. We did not expect a relationship between *Daphnia* P-content body size based on previous studies (reviewed by Sterner and Hessen 1994), nor does such a relationship hold in comparisons among cladoceran families. For example, bosminiids, which are very small-bodied cladocerans have consider-

ably lower P-content than larger-bodied *Daphnia* (Sterner and Hessen 1994).

Ponds and deep, stratified lakes show the greatest contrast in food quality (Tessier and Woodruff 2002b) and an order-of-magnitude difference in mortality risk (Dudycha 2001, 2004). Fishless ponds in Michigan often dry out during summer and thus, like arctic ponds, provide a shorter growing season and less stable environment than deep lakes (Dudycha 2004). Consistent with this pattern, taxa from ponds showed tendencies toward higher maximal growth rates and greater sensitivity to P-limitation although neither of these comparisons was statistically significant. However, our results show very similar P-content in taxa from ponds and deep lakes, and thus *Daphnia* P-content does not correspond to the strong differences in food quality and mortality risk between these habitats.

We also found very similar P-content within species complexes (i.e., *D. pulex/pulicaria* and their hybrid; *D. mendotae/dentifera* and their hybrid). These similarities may reflect phylogenetic constraints. Interestingly, DeMott et al. (2004b) found evidence that a shallow lake population of *D. dentifera* had a higher P-content than a conspecific deep lake population when both were grown on a P-sufficient diet. Since the shallow lake population experienced higher birth and mortality rates and P-sufficient resources, this difference in P-content is consistent with the predictions of stoichiometric theory. Consistent with our laboratory results, studies on field-collected animals confirm that taxa from shallow lakes, including *D. parvula* and *D. dubia* have higher P-content than *D. pulicaria* from deep lakes (DeMott et al. 2004b).

Variation in *Daphnia* P-content and the growth rate hypothesis

The growth rate hypothesis predicts that taxa with higher P-content should have higher concentrations of RNA and higher maximal growth rates with P-sufficient diets (Elser et al. 1996, 2000). Contrary to this prediction, our interspecific comparisons found a nonsignificant negative relationship between *Daphnia* P-content and growth on the P-sufficient diet. This contrary result is due, in part, to fast-growing, large-bodied taxa with low P-contents from the *D. pulex* group. Main et al. (1997), a previous study supporting the growth rate hypothesis, included cladoceran species representing a much broader range in P-content and also considered ontogenetic change within *Daphnia* species in their analysis. Thus, our results suggest that one or more assumptions of the growth rate hypothesis are incorrect for comparisons between *Daphnia* species. One assumption that could readily be tested is that P-content as percent of mass is a consistent predictor of interspecific differences in RNA content. Very recently, Acharya et al. (2004) reported differences between two *Daphnia* species in the ratio of P-content to RNA content. A second basic assumption is that RNA concentration limits the maximal growth rate. Another factor, such as the maximum

assimilation rate might limit the maximal growth rate. Although these assumptions of stoichiometric models may apply over a broad range in body P, our results suggest that one or both do not apply over the moderate range in P-content found among *Daphnia* species.

As mentioned earlier, our experimental design may have been biased against finding high growth rates in small-bodied species. This is because our experiments extended to the first adult instar when growth slows (DeMott 2003). While restricting experiments to the juvenile stage could allow the small and intermediate size taxa to reduce the growth rate differences with larger species, it is unlikely that the low P members of the *D. pulex* group have the lowest juvenile growth rates. This conclusion is supported by experiments measuring juvenile growth with natural seston of varying quality using many of the same species and clones (Tessier and Woodruff 2002b). The three members of the *D. pulex* group exhibited higher growth rates than intermediate-size taxa from deep lakes (e.g., *D. mendotae* and its hybrid) but small species (e.g., *D. ambigua* and *D. dubia*) had juvenile growth rates similar to those of the *D. pulex* group.

Variation in *Daphnia* P-content and sensitivity to P-limited growth

Stoichiometric models predict that sensitivity to P-limited growth should scale directly with a consumer's P-content. These mass balance models are simply predicting that a consumer's requirement for an element are proportional to the percent composition of its body tissues (Sterner and Elser 2002). Again, our results do not support this prediction and a key comparison is between *D. pulicaria* and the *D. pulex × pulicaria* hybrid. Although these two closely related taxa showed essentially identical patterns of P-content, *D. pulicaria* was the least sensitive of the ten taxa to P-limitation and the hybrid was the most sensitive. The fact that these results are based on four separate experiments with *D. pulicaria* and three experiments with the hybrid increases confidence in the results. Moreover, since these taxa are very similar in body size, size-related biases can be ruled out. Besides P-requirements, other factors, including P assimilation efficiency and P excretion rate could influence the sensitivity of a consumer to P limitation (DeMott et al. 1998).

Consistent with our results, DeMott (1998) found that *D. pulicaria* from a Czech reservoir was the least sensitive to P-limitation of five European daphniids, including *D. pulex*. Also consistent with our results, Elser et al. (2000) found that *D. pulicaria* from lakes was less sensitive to P-limitation than *D. pulex* from ponds. Unlike our laboratory results, Elser et al. (2000) found a higher P-content in *D. pulex* than in *D. pulicaria*. However, since Elser et al. (2000) measured the P-content of field-collected animals, the differences they measured could have been caused by lower P diets in Wisconsin lakes compared to arctic ponds. DeMott and Tessier (2002) found that seston from the deep lakes occupied by *D. pulicaria* was strongly P-

deficient during summer (molar C:P ratio 300–600). Thus, our results suggest that the low sensitivity of this species to P-limitation is consistent with adaptation to P-deficient resources that is unrelated to *Daphnia* P-content. However, growth assays with natural seston and the same hybrid *D. pulex × pulicaria* clone revealed only weak and sporadic evidence for P-limited growth, despite seston C:P ratios from 300 to 600 in the surface waters of stratified lakes (DeMott and Tessier 2002; DeMott et al. 2004a).

We defined growth rate and growth differentials solely in terms of mass. However, *Daphnia* feeding on P-deficient diets show declines in both growth and P-content. In experiments that measured growth and P-content on each of 4 successive days, DeMott (2003) found that growth of *Daphnia* on P-deficient algae declined as P-content declined. Our experiments and those of nearly all previous studies started with neonates born to mothers fed P-sufficient diets. This approach underestimates the long-term impact of P-limitation, especially since mothers feeding on P-deficient resources produce low P neonates (DeMott et al. 1998; Boersma and Kreutzer 2002). P-deficient green algae show declines in essential fatty acids (reviewed by Brett and Müller-Navarra 1997) and decreases in digestibility (Van Donk et al. 1997). Thus, some part of the sensitivity to P-limitation that we measured could be due to these factors.

Variation in P homeostasis in *Daphnia*

Homeostasis is the tendency of an organism to maintain constant internal conditions in the face of environmental change. This is the first study of which we are aware that tested for variation between zooplankton species in P homeostasis and we found considerable variation among daphniids. Variation in *Daphnia* P-content will be easier to detect in taxa that exhibit weaker homeostasis. Previous evidence of declines in P-content in zooplankton has come mainly from studies of *D. magna* feeding on P-deficient laboratory diets (DeMott et al. 1998; Plath and Boersma 2001; Boersma and Kreutzer 2002; DeMott 2003). Interestingly, *D. magna* showed the weakest homeostasis and strongest declines in P-content of the ten taxa studied. Surprisingly, early laboratory experiments that supported the strict homeostasis model were also conducted with *D. magna* (Hessen 1990). Although we cannot exclude possible clonal variation in P homeostasis in *D. magna*, a narrow range in the algal C:P ratio probably explains the lack of variation in *Daphnia* P in Hessen's (1990) study. Stoichiometric models have generally assumed strict homeostasis and thus, have not made predictions about the possible significance of variation in homeostasis between taxa.

In conclusion, our results show that taxa from ponds and deep lakes have a narrow, overlapping range in P-content, despite the contrasting patterns of mortality risk and food richness found in these two habitats (Dudchya 2001; Tessier and Woodruff 2002b). Moreover, the two key predictions of stoichiometric theory are not supported

by comparisons between *Daphnia* taxa. Across the moderate range of P-content shown by these species, unidentified factors other than P-content seem to determine differences in maximal growth rates and sensitivity to P-limited growth. Instead of a positive relationship between *Daphnia* P-content and maximal growth rate, *Daphnia* P-content was inversely related to body size. Relationships with body size and/or adaptations to specific habitats and environmental conditions may underlie differences in maximal growth and sensitivity to P-limited growth between *Daphnia* species.

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