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Effects of stoichiometric dietary mixing on *Daphnia* growth and reproduction

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Abstract. Herbivores often encounter nutritional deficiencies in their diets because of low nutrient content of plant biomass. Consumption of various diet items with different nutrient contents can potentially alleviate these nutritional deficiencies. However, most laboratory studies and modeling of herbivorous animals have been done with diets in which all food has uniform nutrient content. It is not clear whether heterogeneous versus uniform food of equal overall nutrient content is of equivalent nutritional value. We tested the effects of dietary mixing on performance of a model organism, *Daphnia*. We fed two species of *Daphnia* (*D. galeata*, *D. pulicaria*) with diets of equivalent bulk stoichiometric food quality (C:P) and studied whether they would produce equivalent performance when C:P was uniform among cells or when the diet involved a mixture of high C:P and low C:P cells. *Daphnia* were fed saturating and limiting concentrations of a uniform food of moderate C:P (UNI) or mixtures (MIX) of high C:P (LOP) and low C:P (HIP) algae prepared to match C:P in UNI. *Daphnia* were also fed HIP and LOP algae separately. Juvenile growth rate and adult fecundity were measured. *D. galeata* performance in UNI and MIX treatments did not differ, indicating that partitioning of C and P among particles did not affect dietary quality. Similarly, *D. pulicaria*'s performance was similar in the MIX and UNI treatments but only at low food abundance. In the high food treatment, both growth and reproduction were higher in the MIX treatment, indicating some benefit of a more heterogeneous diet. The mechanisms for this improvement are unclear. Also, food quality affected growth and reproduction even at low food levels for both *D. pulicaria* and *D. galeata*. Our results indicate that some species of zooplankton can benefit from stoichiometric heterogeneity on diet.

Keywords: Bulk-seston, mixed-uniform-diet, nutrient, carbon:phosphorus ratio, zooplankton

Introduction

Herbivores often face nutritional challenges due to the gross chemical imbalances between plant matter and animal tissue (White 1993, Begon et al 1990, Plath and Boersma 2001, Sterner and Hessen 1994). This is true for energetics (available calories per gram) as well as for specific elements (e.g. C:P or C:N ratios, Sterner 1993). For example, in terrestrial systems, C:nutrient ratios of foliage are normally 5 to 10 times higher than the average C:nutrient ratio of herbivorous insects while seston C:nutrient ratios in freshwater systems are 2 to 5 times higher than body C:nutrient ratios for filter-feeding zooplankton (Elser et al 2000a). These nutritional challenges may contribute to the prevalence of feeding specializations among herbivores (Futuyama & Gould 1979; Strong et al 1984) in which insects focus their feeding on plant tissues, species or time frames for which food is rich in food nutrient content. Stoichiometric imbalance may also be an important force driving changes in the elemental composition of herbivore biomass itself (Fagan et al. 2003, Jaenike & Markow 2002).

While variation in C: nutrient ratios in lake seston is generally narrower than for foliage in terrestrial systems (Elser et al 2000a), recent work makes it clear that algal nutrient content is an important factor affecting zooplankton production (Sterner and Hessen 1994; Gulati and DeMott 1997; Sterner and Schulz 1998). While food quality is a complex and multivariate parameter, one important aspect of food quality appears to be the degree of stoichiometric mismatch between the elemental composition of the diet and the elemental composition of the consumer's body (Urabe and Watanabe 1992; Sterner and Hessen 1994; Elser et al. 1996, 2000b; Hessen 1997; DeMott et al. 1998), as high

C:nutrient ratios in algae consistently reduce the growth and reproduction of herbivorous zooplankton (Sterner and Hessen 1994; Urabe et al. 1997; Sterner and Shultz 1998). Furthermore, direct manipulation of food P content under lab (Urabe et al. 1997, Boersma 2000) and field conditions (Elser et al 2001) demonstrate that P can be directly limiting to growth when food C:P ratios are high. However, most laboratory studies to date have used unialgal chemostat cultures raised under different conditions of nutrient limitation so that algal biomass in different treatments contrasts in C:nutrient ratio but all cells in the population are chemically uniform. Field studies extrapolating from this laboratory work have compared the C:nutrient ratios of suspended seston in lakes to elemental ratios deemed unsuitable from these unialgal studies (e.g. Elser and Hassett 1994; Elser et al. 2000a). However, lake seston is composed of a wide array of particles, including many different taxa and a variety of particle sizes (Knisely and Geller 1986). Therefore zooplankton food base is very heterogeneous and likely includes a mixture of particles, some of which have low C:nutrient ratios (e.g. bacterial cells) while some have high C:nutrient ratios (large nutrient-limited algae, detritus). Indeed, algae may be the most biochemically diverse biota in nature with differences in cell wall, storage products (protein, certain types of fatty acids), and pigment types among major algal taxa (Sterner 1993). There are a variety of ways that zooplankton might respond to a heterogeneous diet, including food selection (Koehl 1984; Knisely and Geller 1986; Butler et al. 1989). However, generalist filter feeders such as *Daphnia* likely have limited abilities to discriminate among specific particles in their diet (DeMott 1986, 1988; Butler et al. 1989). Differential assimilation of energy (C) and nutrient elements from particles that contrast in C:nutrient ratio is another possibility. That is, it is conceivable that

phosphorus contained in P-rich particles might be more readily available for assimilation compared to the P content of the aggregate particles as a whole. To date these issues have not been well-studied, but they have important implications regarding the suitability of directly applying stoichiometric insights from laboratory studies to field conditions.

The possibility of differential assimilation of P-rich particles also potentially complicates stoichiometric modeling of zooplankton-phytoplankton interactions. While most models of zooplankton growth treat the diet as a homogenous entity (e.g. in terms of energy or C currencies), new families of stoichiometric models of zooplankton growth have appeared (Andersen 1997; Loladze et al. 2000; Muller et al. 2001). While capturing some degree of stoichiometric realism by adding a second currency, these models currently depict the phytoplankton as a single biomass pool that can vary in its abundance and its C:nutrient ratio. This assumption makes the models analytically tractable. However, because we do not know the degree to which animal growth is sensitive to the packaging of potentially limiting nutrient among high C:nutrient particles and low C:nutrient particles, we do not know how much realism these models have sacrificed by treating the phytoplankton as a single homogeneous entity.

Finally, attention has also been turned recently to potential interactions between food quality and food quantity (Sterner 1997), as the potential role of nutrient limitation due to stoichiometric imbalance at low food quantity is not clear (Sterner and Elser 2002). For example, comparing *Daphnia* response to high C:P and low C:P food, Sterner and Robinson (1994) found no food quality effects on growth at very low concentrations

of food. The same was found in a study using rotifers (Rothhaupt 1995). These findings point to a high degree of P conservation in the animals such that at low food concentrations the primary limitation reflects energy (C) balance necessary to maintain basal metabolism. Contrary to these findings, Boersma and Kreutzer (2002) reported that even at low food levels not only energy is of importance, but mineral content of the food as well. Thus, further studies illuminating interactions between food quantity and food quality are needed.

In this paper we examine the effects of diets that are uniform vs. heterogeneous in quality on the growth and reproduction of two species of *Daphnia*. Here, quality is gauged in terms of stoichiometric ratios, with low quality food items having high C:P ratio relative to the requirements of the consumer. At a given C:P of the total algal biomass, is *Daphnia*'s performance on a mixture of low C:P and high C:P cells the same as its performance on algal cells of uniform C:P? Furthermore, do these responses differ for abundant food (food present at saturating concentrations) vs. low food abundance where the total food supply is insufficient to meet requirements for regular maintenance and maximal body growth? If the answer to the former question is "yes," then it suggests that studies applying physiological insights from laboratory with uniform diets can readily be applied in field assessments of food quality conditions of *Daphnia* despite heterogeneity among particles. An affirmative answer also would indicate that mathematical simplifications involving single phytoplankton biomass compartments are not unduly artificial. However, if the answer is "no", then field studies may need a more sophisticated approach for assessing stoichiometric food quality of lake seston and

mathematical models may need to become somewhat more complex to capture impacts of diet heterogeneity. The answer to the second question will help clarify the current uncertainty about the relative importance of food quality at low food abundance, also an important issue in applying stoichiometric frameworks to various field and laboratory settings and in developing appropriate theoretical frameworks in food web models.

Methods

Cultures and Chemostats: Laboratory experiments were conducted using *Daphnia galeata* and *Daphnia pulicaria*, collected at Lake Biwa, Japan, and established in stock cultures at Arizona State University (Tempe, Arizona, USA).

The green alga *Scenedesmus acutus* was cultured in chemostats under three conditions of P supply. P-rich *Scenedesmus* (“HIP,” hereafter) was cultured in an artificial medium (COMBO) developed by Kilham et al. (1998), modified to contain 1000 μM N and 50 μM P (molar N:P ratio 20) at a dilution rate of 1.0 day^{-1} .

Scenedesmus with moderate P content presented as the uniform diet (“UNI,” hereafter) was cultured in COMBO with 500 μM N and 12.5 μM P (N:P 40) at a dilution rate of 0.25 day^{-1} . Low P *Scenedesmus* (“LOP,” hereafter) was cultured in COMBO with 500 μM N and 5 μM P (N:P 100) at a dilution rate of 0.12 day^{-1} . Chemostats were allowed to equilibrate for two weeks to stabilize food quality before the start of experiments. The concentrations and C:N:P ratios of algae from the chemostats and in the MIX food preparations were monitored every other day throughout the experiment. Algal samples collected on filters were dried in an oven at 60° C and held in a dessicator until analysis

for C and N content (using Perkin-Elmer model 2400 elemental analyzer) and P content (using persulfate oxidation followed by the acid molybdate technique; APHA 1992).

Scenedesmus in these three cultures thus produced had biomass C:P ratios of 110.2 ± 7.3 (HIP; mean ± 1 s.e. of values of algae measured every two days during the study), 456 ± 20.7 (UNI) and 933.7 ± 23.6 (LOP). A fourth type of food ("MIX," hereafter; C:P = 458 ± 19.7) was prepared by mixing appropriate amounts of HIP and LOP, to match the C:P of algae produced in the UNI chemostats. C:N ratios of all the cultures were in a narrow range of 6 to 10 (molar ratio).

Growth experiments: Laboratory experiments were conducted on a large number of neonates of *Daphnia galeata* and *Daphnia pulicaria* of approximately the same age, size and condition. To collect a large number of cohorts, a few animals from stock cultures were isolated and grown individually on high food (>1.5 mg C/L) for a few days until they began reproduction. From this stock about 80-100 third clutch neonates were separated and grown individually in 250-ml jars with sufficient, high quality food until they began reproduction. These animals were then transferred to clean jars at regular intervals and neonates (<24 h old) from the third and subsequent clutches of these animals were used for the experiments.

Individual growth and reproduction: The main experiments examined effects of the four diets (HIP, UNI, MIX, LOP) at two food levels on the growth rate of individual *Daphnia*. Because of practical limitations of the culturing apparatus and the handling of larger number of animals, separate sets of experiments were performed using *Daphnia galeata*

and *Daphnia pulicaria*; for each species, treatments for low and high food were also performed separately. Growth rate was estimated by measuring the rate of change of animal body area using video image analysis. The advantage of estimating growth by image analysis is that the growth rate can be measured on an individual basis while keeping the animals alive for subsequent growth and reproduction. Thus, growth rates and reproduction could be assessed for the same animal. The initial body area of animals (at < 24 hours) was measured by taking lateral images of each animal and then calculating its area using Image-Pro Express®, Version 4 (Media Cybernetics Inc.) software. After image capture, all the animals were then transferred to 70-ml jars (individually) capped and placed on a plankton wheel. Approximately 90% of the animals were in good condition after image capture; injured animals were discarded and replaced immediately with the healthy ones. Since animals were handled individually, mortality of animals during the actual experiments was less than 2%. For each species, there was a total of 8 treatments: four diet type treatments (HIP, UNI, MIX and LOP *Scenedesmus*) supplied at low (0.25 mg C L^{-1}) and high (1.5 mg C L^{-1}) food levels. There were 12 replicates for each treatment combination.

Food and media were replenished every 24 h by pipette-transferring of the *Daphnia* from each jar. All growth experiments were conducted at 25°C in the laboratory. Final measurement of body size was made after 72 hours using the same image capture approach as the initial measurements. A 72-h growth period was used in order to avoid complications due to the onset of reproduction and diversion of resources to egg production; microscopic examination indicated the animals in the HIP treatment

began ovarian maturation at around 96 h. After the final image capture, animals were transferred back to the jars for continued feeding and quantification of their reproductive output. Each day during media and food transfer, animals were checked and newly born neonates were transferred and counted for the first three clutches. In general, the experiments lasted for about 10-14 days for animals receiving HIP food and 14-30 days for those in the LOP treatment. Initial and final body area measurements were converted to body dry mass (see below) for calculation of growth rate (μ : in units of day^{-1}) as:

$$\mu = \ln(\text{final weight} / \text{initial weight}) / 3$$

Weight-area regression: Simultaneous with the experiments just described, a second experiment was performed to establish the relationship between body area (from image analysis) and body mass for each treatment. This was necessary because food quantity and food quality can affect the mass per unit length (and presumably area) in *Daphnia* (Duncan 1985; Hessen 1989; DeMott et al. 1998). From the large cohort of *Daphnia* neonates, 8-10 animals each were placed in replicate 500-mL jars containing food of the appropriate food quality and quantity combination. A few animals (six replicates of 25 to 30 each) were randomly removed for initial measurements. These animals were photographed and placed immediately on pre-weighed filters for dry weight measurement. Animals remaining in the 500-ml jars were transferred to new food and media every 24 hours by filtering each bottle through a cylinder with Nitex mesh at the bottom. During incubation, jars were kept gently agitated to prevent settling of food. After 72 hours all the animals (four replicates of 15-20 each for all food type) were photographed and placed onto pre-weighed filters. The dry weight of the samples and the

combined body area measurements were used to create a regression between animal body area and dry weight for use in calculating growth rate for animals raised in the main part of the experiment (described above).

Results

Food C:P ratio for all three HIP, UNI and LOP *Scenedesmus* chemostats remained fairly consistent throughout the experiments and the C:P ratios of the UNI and mix cultures were nearly identical. Bulk C:P ratios in the mixed and UNI treatments were above 300 ensuring P limitation of the *Daphnia* (Urabe and Watanabe 1992). Animals in the LOP food treatment suffered modest (~ 20%) mortality during the course of the experiments, primarily after about 7-8 days but in general survivorship was good across all treatments.

Regressions of dry weight and body area were generally tight for both the *Daphnia* species (Table 1). Analysis of covariance on dry mass (with body area as co-variant) did not show significant difference between the two species (ANCOVA, $P=0.46$). However, the weight-area regression was significantly affected by food concentration ($P<0.001$) as well as food quality ($P<0.001$).

Analysis of variance indicated that growth and fecundity differed for the two species, for different food types (HIP, UNI, MIX, LOP), and for different food concentrations (high vs. low) and that there were various significant interaction effects (Table 2). In general, animals grew most rapidly and produced larger broods on P-rich HIP food but grew most

slowly and produced smaller clutches on P-deficient LOP food (Figures 1 and 2). In addition, growth rates and reproduction in low food treatments were generally lower than with high food abundance (Figures 1 and 2). To determine the effect of dietary mixing of high C:P and low C:P particles, performance on MIX and UNI diets should be compared. *Daphnia galeata* growth and reproduction were identical on MIX and UNI diets at both high and low food levels (Figures 1C,D, and 2 C,D; Scheffe's tests for growth, $P = 0.94$ for high food and $P = 0.99$ for low food, and for reproduction $P = 0.07$ for high food and $P = 0.98$ for low food). Similarly growth and reproduction of *D. pulicaria* on UNI and MIX foods were statistically indistinguishable in the low food abundance treatment (Figures 1B and 2B; Scheffe's tests for growth and reproduction $P > 0.88$). However, at high food abundance, *Daphnia pulicaria* juveniles grew more rapidly and reproduced more robustly on the MIX diet relative to the UNI diet (Figures 2A and 2B; $P < 0.0003$ in Scheffe's tests). Thus, there was a statistically significant 3-way interaction ($P < 0.02$) in the overall analysis of variance (food type x food level x species); the two species differed in how they responded to the combinations of food type and food quality presented.

Considering the effects of food quality, growth rates and reproduction on UNI and MIX diets of intermediate C:P generally fell between HIP and LOP food. The effect of food quality on *Daphnia* growth and especially reproduction was consistently stronger, on an absolute basis, in the high food concentration treatments (Figures 1 and 2; "quality effect" in Table 2). Similarly, the effect of food quantity for a given food ("quantity effect" in Table 2) was consistently larger for animals feeding on HIP vs. LOP food.

Thus, there was a significant type x treatment interaction (Table 2; $P < 0.0001$) for fecundity; this interaction was also marginally insignificant for growth rate (Table 2; $P < 0.06$). In sum, the data show that while the impact of food quality was larger for animals receiving high food levels, algal food quality still affected the growth and reproduction of both species at both high and low food concentrations.

Discussion

Heterogeneous vs. Homogeneous Diet: Our results present a situation in which the glass may be considered 3/4 full or 1/4 empty. In three of the four situations we considered (*D. galeata* under high and low food; *D. pulicaria* under low food), animals performed equally on foods of identical C:P but containing a mixture of low and high C:P cells (MIX) or a uniform assemblage (UNI) (Fig 1B,C,D). This suggests that the contrast between physiological studies using unialgal cultures of uniform chemical condition and field studies in which diverse particles with contrasting elemental composition may not unduly compromise a relatively straightforward field assessment of stoichiometric food quality using bulk seston measurements. While more information will be gained via studies that examine size-fractionated data (e.g. Elser et al. 1995) or even data in which elemental composition of individual particles is considered, such measures may not be necessary to gain an overall assessment of the stoichiometric quality of food available for generalist filter feeders such as *Daphnia*. Furthermore, these results suggest that stoichiometric models that depict phytoplankton as a single compartment (but with varying nutrient content) are not entirely unreasonable, considering the benefit of increased tractability this assumption provides. It is important to note that our result is

based on single algal species with varying nutrient content, therefore applicability of these conclusions to situations where the food involves multiple species of algae that might differ in nutrient content needs further investigation.

On the other hand, 1/4 of the glass was empty: at high food concentrations, *D. pulicaria* grew more rapidly and had higher reproductive output when given access to particles with low C:P ratio (Fig. 1A), despite the fact that both the UNI and MIX diets had identical bulk C:P ratio. These results are potentially important, as *D. pulicaria* is a widespread species and often implicated as a keystone species in lake food webs due to its major role as a grazer in stratified lakes when planktivore pressure is reduced (Johnson and Kitchell 1996; Tessier et al. 2001). The mechanism of *D. pulicaria*'s improved performance on MIX (or reduced performance on UNI) cannot be discerned from our data. A variety of possibilities exist. One possibility is that this species is indeed capable of discriminating among low C:P and high C:P cells and thus was able to achieve a more P-rich diet in the MIX treatment. However, work by W. R. DeMott (Demott 1982, 1986, 1988) has shown that, in contrast to copepods and *Bosmina*, *Daphnia magna* was not able to select between dead and living cells, *Daphnia magna* and *Daphnia galeata* were unable to discriminate between algae-flavored and non-flavored spheres, and *Daphnia rosea* could not select between bacteria and algae. To the extent that these studies are applicable to *D. pulicaria* they suggest that selective feeding on the low C:P cells in the MIX diet was unlikely to have produced our results. There are however, various other reports that suggest that *Daphnia* can show at least some differential grazing capability, but this varies from species to species. Knisely and Geller (1986) found that *Daphnia*

hyalina ingested filamentous cyanobacteria but *Daphnia galeata* did not. Similarly, Butler et al. (1989) reported that both *Daphnia rosea* (possibly via differential retention on filters) and *Diaptomus kenai* (possibly via chemo-detection) were able to discriminate between algal cells differing in nutritional status. It is known that selective feeding by copepods and *Bosmina* decreases when edible phytoplankton is scarce (DeMott 1988). One possible explanation for our results is that *D. pulicaria* was able to selectively feed when food was in excess but was non-selective when food was scarce; more work is needed to test this hypothesis. We can not rule out another possibility, in which P-limited algae may vary in the abundance of important biochemical constituents such as unsaturated fatty acids and sterols (Ahlgren et al., 1990; Muller-Navarra, 1995a, b, von Elert 2002) and thus a higher growth rate on the MIX diet may have reflected the contribution of important biochemical constituents by the HIP cells in the mixture. This can only be evaluated by comparing the biochemical composition of our mixed versus uniform diets but unfortunately we do not have such data. However, studies of Urabe & Watanabe (1992) and Sterner (1993) showed that *Daphnia* feeding on P-limited *Scenedesmus* in this range of biomass C:P ratio do suffer direct mineral nutrition of their growth rate. It may also be the case that our results reflect differences in digestibility of P-limited cells due to thickening of the cell wall (Van Donk & Hessen, 1993; Lurling & Van Donk 1997). Our results are inconclusive on this question because we did not measure ingestion rates and assimilation efficiencies. It can be also argued that the increased growth on MIX (relative to UNI) at high food concentration could also have arisen if *D. pulicaria* had higher digestive or assimilation efficiencies for limiting P when P was present in P-rich particles. Only detailed ingestion and assimilation studies would

be able to determine the mechanism by which *D. pulicaria* was able to achieve a higher growth rate on MIX vs. UNI diets at high food abundance.

Impact of food quality at low food abundance: For *D. pulicaria*, the difference in average growth rates for HIP relative to LOP food for high food concentration was 0.29 day^{-1} , similar to a reduction of 0.27 day^{-1} at low food (Table 3). Likewise for *D. galeata*, the differences in growth rates for HIP relative to LOP were 0.22 and 0.19 day^{-1} at high and low food concentrations. Similarly, Boersma and Kreutzer (2002) recently reported that food nutrient content influenced the growth rate of *Daphnia magna* even at very low food levels. Similar to our study diets, they showed that *Daphnia magna*'s growth rate reduction from high P to low P *Scenedesmus* was $\sim 0.30 \text{ day}^{-1}$ in both high and low food treatments (Table 3). Their growth data for *D. magna* are similar to our data for *D. galeata* and *D. pulicaria* but the results of both studies are contrary to Sterner and Robinson's (1994) report of where there were no differences in growth rates for *Daphnia magna* individuals fed low concentrations of P-limited and P-sufficient algae. It is difficult to pinpoint the reason for differences in the results of Sterner and Robinson and those that we and Boersma and Kreutzer obtained. Several factors could be involved, such as differences in the ontogenetic stages considered. Sterner and Robinson evaluated growth rates from day two to day five whereas we and Boersma and Kreutzer studied growth from within 12 h of birth to day three. It is known that *Daphnia* has its highest rates of juvenile growth during its first 24 h of life (Gorokhova and Kyle 2002). Perhaps the impacts of food quality are particularly acute during these early stages and can be felt even at low food abundances in early juvenile stages that were not monitored

by Sterner and Robinson (1994). It is, however, also possible that the initial growth depression in Sterner and Robinson's experiment must be negated in the next few days (4th and 5th day), because of a potential time lag in growth. Whatever the basis of the discrepancy among existing studies, our data and those of Boersma and Kreutzer (2002) seem to suggest that *Daphnia* cannot reduce its P loss to zero when the food is P deficient. Thus, the idea that only energy content of the food is important at very low levels of food may not hold; *Daphnia pulicaria* at least can still suffer P limitation at low food concentration. More work with additional *Daphnia* species and other groups of zooplankton is needed to better understand the impact of low quality food at low abundance.

Conclusions

We found that the effects of uniform vs. heterogeneous diets were minimal at both high and low food concentration for *D. galeata* but that effects were significant for *D. pulicaria* when the food was abundant. Thus, the effects of stoichiometric heterogeneity in the diet of a filter feeder seem to vary as a function of species and food availability. We also found that there was a significant impact of food nutrient content even at low food levels, contrary to previous proposals in stoichiometric theory. The results therefore suggest that, at least for certain species, field studies will need a more sophisticated approach for assessing stoichiometric food quality of lake seston and mathematical models may need to incorporate impacts of dietary heterogeneity.

Our findings provide motivation for further studies of how feeding strategies by filter-feeding herbivores can overcome the imbalance in C: nutrient ratios they routinely confront. We need to better understand the degree to which filter-feeding herbivores can switch from passive to selective feeding in response to heterogeneity in nutrient content among food items and whether they can employ differential digestion to adjust their relative assimilation of multiple nutrients from different diet items to better match their body requirements. As is often the case, more data of this type for additional species, as well as for other groups and systems, will be important in a final assessment of the importance of incorporating dietary heterogeneity in stoichiometric analysis.

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Table 1. Regression coefficients of dry weight (μg) and body size (image analysis pixel area determination) for *Daphnia pulicaria* and *Daphnia galeata* at high and low food concentrations, and LOP, UNI, MIX and HIP treatments.

	<i>D. pulicaria</i>				<i>D. galeata</i>			
	High food		Low food		High food		Low food	
	Slope	R ²	Slope	R ²	Slope	R ²	Sope	R ²
LOP	1.17E-04	0.82	1.00E-04	0.99	1.34E-04	0.93	1.31E-04	0.97
UNI	1.43E-04	0.90	1.25E-04	0.91	1.79E-04	0.88	1.39E-04	0.99
MIX	1.57E-04	0.98	1.26E-04	0.99	1.76E-04	0.98	1.61E-04	0.97
HIP	1.71E-04	0.99	1.53E-04	0.99	2.26E-04	0.91	1.83E-04	0.99

Table 2. Results of analysis of variance (ANOVA) for growth rate and reproduction data.

(Species-*D. pulicaria*, *D. galeata*; Food type-High, Low; Treatment-LOP, UNI, MIX,

HIP). Initial sample size: 12*4*2*2 (replicates*treatment*food type* species) =192.

Dependent Variable: Growth Rate, N: 179, Multiple R: 0.932, Squared multiple

R: 0.868. (Mortality at the end of final growth measurement ~7%)

Dependent Variable: Average Clutch Size, N: 155, Multiple R: 0.978, Squared

multiple R: 0.956. (Mortality at the end of 3rd clutch ~20%)

	DF	Growth Rate		Reproduction	
		F-Value	P-Value	F-Value	P-Value
Species	1	279.2	<0.0001	18.5	<0.0001
Food type	1	466.7	<0.0001	570.1	<0.0001
Treatment	3	87.9	<0.0001	397.2	<0.0001
Species*Type	1	10.7	0.0013	0.2	0.6962
Species*Treatment	3	1.9	0.1199	31.0	<0.0001
Type*Treatment	3	2.5	0.0567	122.6	<0.0001
Species*Type*Treatment	3	3.3	0.0209	32.4	<0.0001

Table 3. Average growth rates (d^{-1}) and clutch sizes (eggs female $^{-1}$) for *Daphnia* fed variations combinations of HIP and LOP *Scenedesmus* at high and low food concentrations. The reductions in growth or reproduction due to feeding on HIP vs. LOP food at a given food concentration (quality effect) and due to feeding on high vs. low concentrations of food for a given food quality (quantity effect) are given. Clutch sizes are averaged over 1st three clutches. *Daphnia magna* data from Boersma & Kreutzer (2002).

Parameters	Species	Food Level mg C/L	HIP		LOP		Absolute Reduction	
			Mean	S.E.	Mean	S.E.	Quality effect (HIP-LOP)	Quantity effect (HI-LOW)
Growth Rate (per day)	<i>Daphnia pulicaria</i>	1.5	0.575	0.022	0.285	0.025	0.290	0.195
		0.25	0.380	0.014	0.113	0.022	0.268	0.173
	<i>Daphnia galeata</i>	1.5	0.496	0.023	0.277	0.027	0.219	0.233
		0.25	0.263	0.021	0.072	0.018	0.190	0.205
	<i>Daphnia magna</i>	1.0	0.469	0.045	0.140	0.021	0.328	0.316
		0.1	0.153	0.012	-0.139	0.037	0.292	0.280
Reproduction (Nos./clutch)	<i>Daphnia pulicaria</i>	1.5	14.47	1.065	2.33	0.570	12.13	7.52
		0.25	6.95	0.593	2.00	0.387	4.95	0.33
	<i>Daphnia galeata</i>	1.5	12.90	0.442	2.20	0.351	10.70	7.33
		0.25	5.57	0.416	1.73	0.380	3.83	0.47

Figure 1. Average growth rates (μ day⁻¹) of *Daphnia pulicaria* at uniform food of high C:P (Hip), moderate C:P (Uni), low C:P (Lop) and mixture of Hip and Lop (Mix) in (A) high and (B) low food treatments, and of *Daphnia galeata* in (C) high and (D) low food treatments. Error bars indicate standard errors. (Statistics: Scheffe's comparison test)

Figure 2. Average clutch size (1st three clutches) of *Daphnia pulicaria* fed uniform food of high C:P (Hip), moderate C:P (Uni), low C:P (Lop) and mixture of Hip and Lop (Mix) in (A) high and (B) low food treatments, and of *Daphnia galeata* in (C) high and (D) low food treatments. Error bars indicate standard errors. (Statistics: Scheffe's comparison test)





