

# Effects of algal food quality on fecundity and population growth rates of *Daphnia*

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## SUMMARY

1. Food quality was at least as important as food quantity for both fecundity and population growth responses of the cladoceran *Daphnia pulicaria* fed the green alga *Ankistrodesmus falcatus* grown under N limitation, P limitation, or non-limited condition.
2. The fecundity of *D. pulicaria* was reduced under conditions of low food quality (low N or low P) compared with that for animals fed control non-limited algae regardless of ration size. The reduced fecundity of *D. pulicaria* fed P-limited food could be partially alleviated by increasing the ration (hence, compensation), but such was not the case for animals fed N-limited food.
3. Population growth rates of *D. pulicaria* ( $r_{\max}$ ) were significantly reduced under conditions of low-quality food for both N-limited and P-limited algae. Population growth rates were unaffected by ration size, indicating no compensation.

## Introduction

There is a rich literature which demonstrates that the biochemical composition of algal food affects the production responses of animal herbivores. This literature provides a very mixed message about the exact nature of this interaction between algae and zooplankton. There are two general problems that make interpretations of feeding studies difficult: (i) the large intraspecific and interspecific variation in the biochemical composition of live diets grown under resource limitation; and (ii) the large variation in responses of herbivores to diets of differing quality.

A variety of algal cellular constituents are important determinants of food quality for zooplankton: C, N, P, lipids, essential fatty acids (FA), protein and essential amino acids. There are likely to be taxon-specific differences in responses to limiting resources in both algae and zooplankton that confound any generalizations (Sturner & Hessen, 1994). The observed variations among studies in biochemical composition of algae is discussed in Kilham *et al.* (1997). Moreover, the food quality of algae varies interspecifically. Growing cells

at intermediate growth rates using continuous or semi-continuous cultures reduces this variation and produces cells that are growing at rates similar to natural algae (Sommer, 1989).

The variation in herbivore responses among previous studies partly results from using algae of undefined physiological condition ('stationary phase'), varying experimental conditions (inadequately defined medium), and from complex and perhaps variable food requirements of crustaceans, which, for example, are unable to synthesize many essential amino acids and FA (Ahlgren *et al.*, 1990; Goulden & Place, 1990; Harrison, 1990).

Recently, there has been an increasing number of papers suggesting that the seston elemental ratio of C:N:P is a major factor affecting zooplankton responses to food quality (Hessen, 1992; Sommer, 1992; Sturner, Elser & Hessen, 1992; Urabe & Watanabe, 1992; Sturner & Hessen, 1994). This idea was challenged by Brett (1993) who argued that elemental N limitation was very unlikely, and energy, protein content and FA

should be considered instead. In response, Hessen (1993) and Urabe & Watanabe (1993) stated that there is no basic incompatibility between these points of view even if N and C are consumed as organic compounds. Phosphorus, however, is largely assimilated in inorganic form in the gut (Hessen, 1993). One assertion by Brett (1993) that '... animals can compensate for low quality by simply eating more food' (p. 1335), was challenged by Hessen (1993), and was tested explicitly in this study.

The objective of this study was directly to measure the fecundity and population responses of *Daphnia pulicaria* (Forbes) to a green alga, *Ankistrodesmus falcatus* (Braun), grown under P or N limitation in steady state at 15% of maximum growth rate, or growing without limitation (at maximal growth rate,  $\mu_{\max}$ ). To test the hypothesis that Cladocera can compensate for poor food quality by eating more food, food quality was examined for effects of a range of rations (severely limiting to *ad libitum*).

## Materials and methods

### Culture conditions

The green alga *A. falcatus* (clone from Academy of Natural Sciences; Goulden, Henry & Tessier, 1982) was used as the food in all experiments. Algae were grown at 15% of maximum growth rate ( $\mu_{\max} = 1.34 \text{ day}^{-1}$ ) under N or P limitation in semi-continuous cultures, or were grown at maximum growth rate with no limitation. Growth conditions and biochemical characterizations are detailed in Kilham *et al.* (1997).

The cladoceran *D. pulicaria* was used (clone isolated by C.E. Goulden from Lake Waynewood, PA). Stock cultures of animals were maintained in 1-l flasks in full COMBO medium (S.S. Kilham, D.A. Kreeger, C.E. Goulden & L. Herrera, personal communication) which was changed once each week. These animals were fed a ration of  $> 1.6 \times 10^7 \mu\text{m}^3 \text{ ml}^{-1}$  of non-limited *A. falcatus* three times each week.

All cultures of algae and zooplankton were maintained in a culture chamber with a 14 : 10 h light : dark cycle at  $20 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$ . Fecundity experiments were performed in the chamber at low light. Population experiments were performed at  $22 \text{ }^\circ\text{C}$ , with low levels of light on a 16 : 8h light : dark cycle. COMBO medium was used in all experiments and was modified for low-nutrient experiments (see Kilham *et al.*, 1997). This

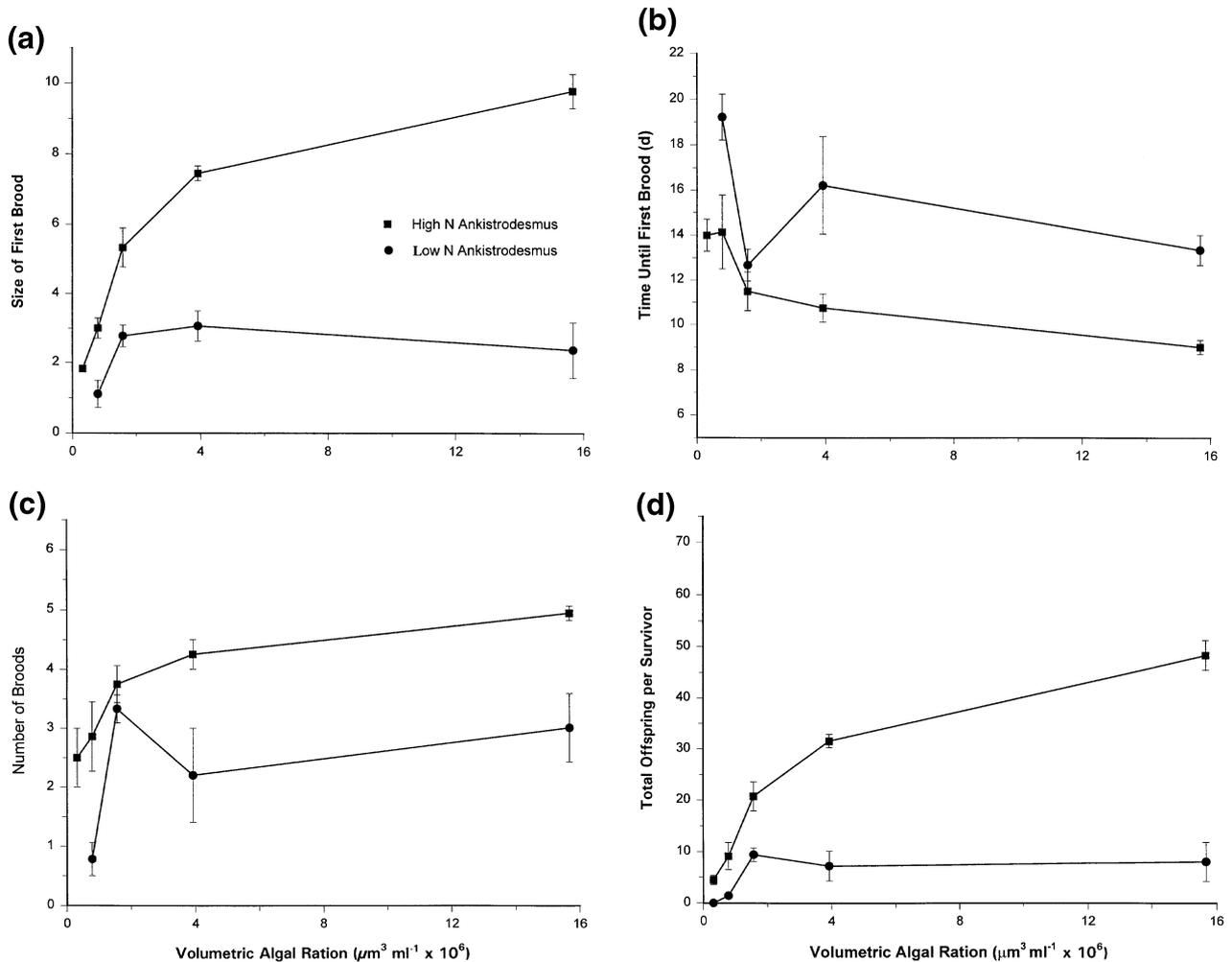
medium was shown to support excellent reproduction of *D. pulicaria* (S.S. Kilham, D.A. Kreeger, C.E. Goulden & L. Herrera, personal communication).

Algal cells can change size when grown in nutrient-limited conditions. To distinguish qualitative from quantitative effects of dietary treatments on consumers, it is important to carefully equilibrate algal rations based on biovolume and not cell number (Kreeger & Langdon, 1993). Algal rations were therefore determined on a volumetric basis ( $\mu\text{m}^3 \text{ ml}^{-1}$ ) using a Coulter Counter (model ZB1) with Channelyser (model 256). There was an average  $40 \mu\text{g}$  ash-free dry weight (AFDW)  $\mu\text{m}^{-3} \times 10^8$  [the highest ration (E, see below) was  $6.4 \mu\text{g ml}^{-1}$ ] for algae grown under all conditions.

### Fecundity experiments:

Gravid adult female *D. pulicaria* (fifteen to twenty) were separated 24 h before the start of the experiment and fed non-limited, exponentially growing algae. Juvenile *D. pulicaria* produced by these females ( $< 24$  h old) were individually placed in separate beakers with 60 ml of appropriate medium and algal food. Three times each week (Monday, Wednesday, Friday) the animals were transferred to fresh medium. The *D. pulicaria* were fed four times a week: on Sunday and after each medium change. For the first 6 days of the experiments, every beaker was checked five times a day, and any animals caught in the surface film were submerged. This apparently did not affect survival, which was generally excellent. Beakers were checked daily for newly produced juveniles, and any offspring were removed and counted. Fecundity experiments ran for 21 days.

Two experiments were conducted separately to measure the food value of low-N (N : P = 1) and low-P (N : P = 200) -reared algae. In the low N fecundity experiment there were eleven dietary treatments. These treatments consisted of five ration levels of N-limited algae (low-N COMBO) and five levels for non-limited algae (full COMBO; N : P = 20). To test whether zooplankton themselves were affected by the N concentration in the medium, the eleventh diet consisted of non-limited algae (highest ration, grown in full COMBO) delivered to zooplankton held in low-N COMBO (no effects were seen). The rations were as follows: ration A =  $0.3 \times 10^6 \mu\text{m}^3 \text{ ml}^{-1}$ ; ration B =  $0.8 \times 10^6 \mu\text{m}^3 \text{ ml}^{-1}$ ; ration C =  $1.6 \times 10^6 \mu\text{m}^3 \text{ ml}^{-1}$ ; ration D =  $4.0 \times 10^6 \mu\text{m}^3 \text{ ml}^{-1}$ ; ration E =  $1.6 \times 10^7$



**Fig. 1** Reproduction of *Daphnia pulicaria* feeding on five different ratios of *Ankistrodesmus falcatus* grown at 15% of  $\mu_{\text{max}}$  under N limitation (low N; circles) or non-limited control cells growing at  $\mu_{\text{max}}$  (high N; squares). (a) Size of first brood as a function of ration size. (b) Day of first brood as a function of ration size. (c) Number of broods per female over 21 days as a function of ration size. (d) Total offspring per survivor over 21 days as a function of ration size.

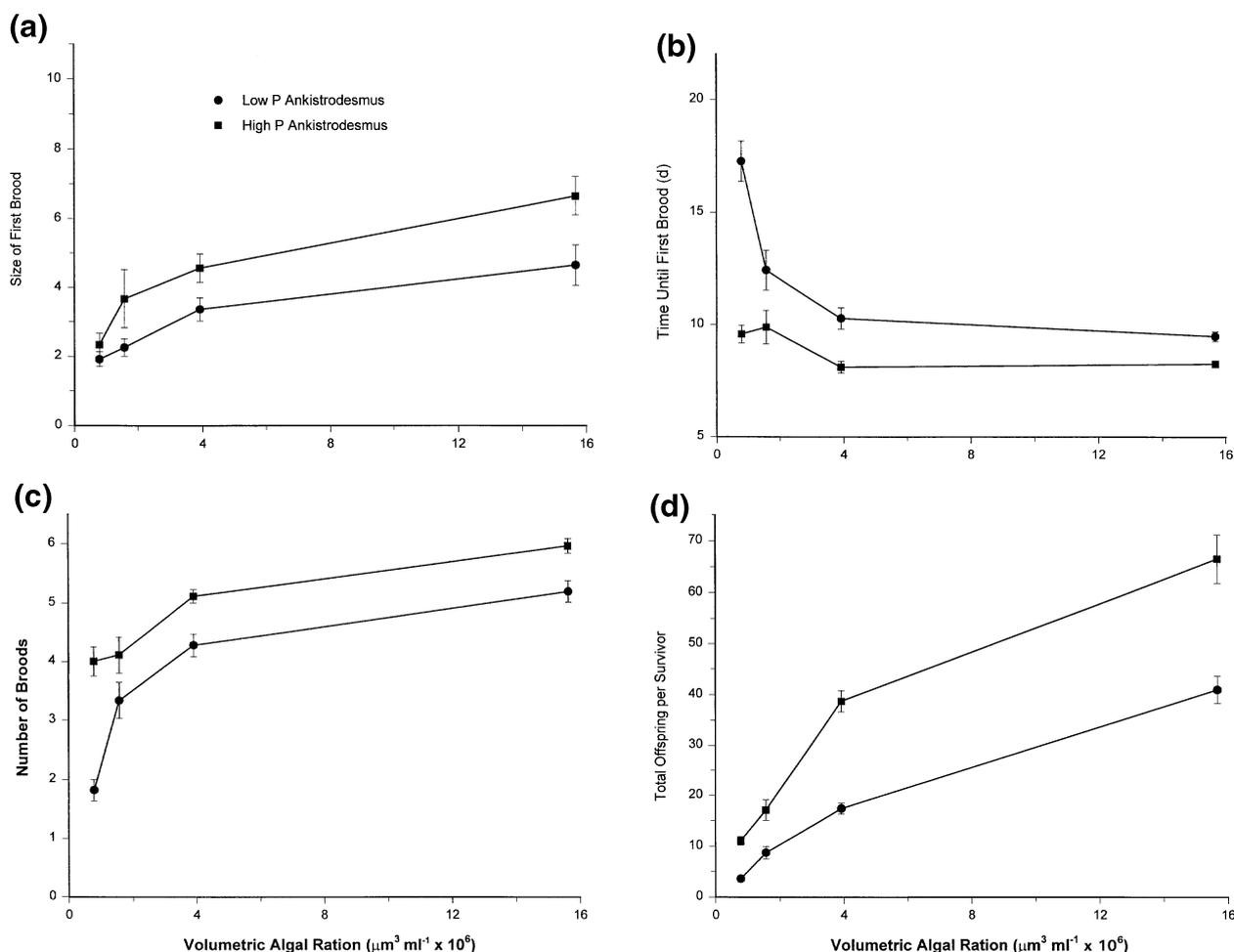
$\mu\text{m}^3 \text{ml}^{-1}$ . There were twelve replicates for each of eleven treatments (132 beakers), each containing a single animal.

In the Low-P fecundity experiment, zooplankton were fed four rations (rations B, C, D, E as above) of P-limited algae (low P) or non-limited algae. The animals did not reproduce in the lowest ration in the low-N experiment so it was removed from the design of the low-P experiment. As in the low-N experiment, an additional treatment was included with full COMBO algae (ration E) added to zooplankton in low-P COMBO. No effects were observed. There were twelve replicates of each of the nine treatments (108 beakers), each containing a single animal.

#### Population experiments

To determine whether the population growth rate ( $r_{\text{max}}$ ) of *D. pulicaria* was affected by food quality, the  $r_{\text{max}}$  of animals fed N-limited, P-limited or non-limited algae were compared. To discern quantitative effects from qualitative effects, algae were delivered to zooplankton at two different rations: ration C =  $1.6 \times 10^6 \mu\text{m}^3 \text{ml}^{-1}$ ; and ration E =  $1.6 \times 10^7 \mu\text{m}^3 \text{ml}^{-1}$ .

In the low-N population experiment, forty to fifty gravid females were separated 24 h before the start of the experiment and fed full COMBO algae. Five juveniles (< 24 h old) were then placed in each of a series of 1.3-l polycarbonate roller bottles with the appropriate medium and food ration (low-N or full



**Fig. 2** Reproduction of *Daphnia pulicaria* feeding on four different ratios of *Ankistrodesmus falcatus* grown at 15% of  $\mu_{\text{max}}$  under P limitation (low P; circles) or non-limited control cells growing at  $\mu_{\text{max}}$  (high P; squares). (a) Size of first brood as a function of ration size. (b) Day of first brood as a function of ration size. (c) Number of broods per female over 21 days as a function of ration size. (d) Total offspring per survivor over 21 days as a function of ration size.

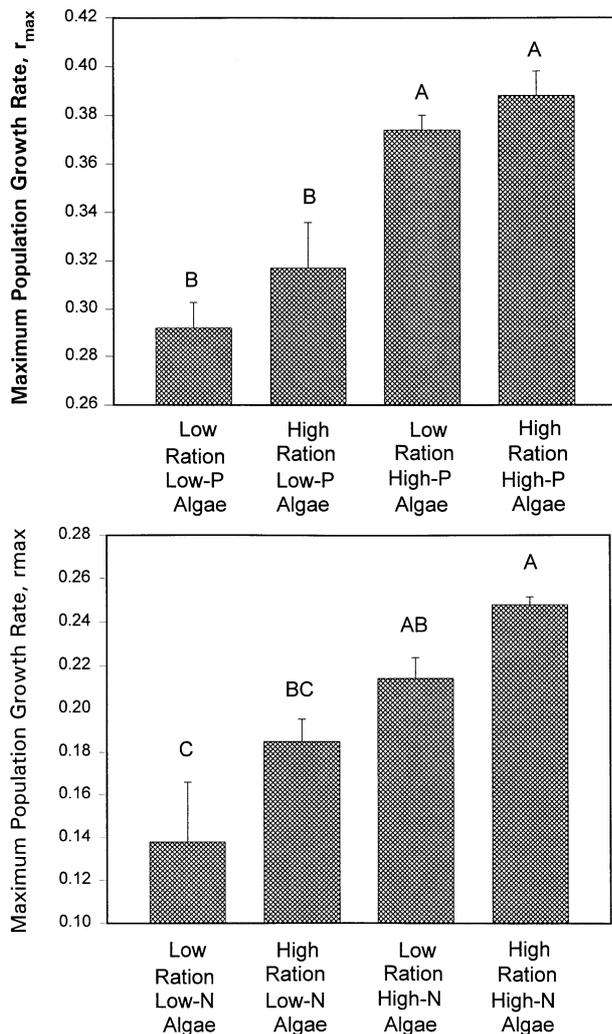
COMBO algae, and low or high ration levels). These bottles were placed on plankton wheels or roller tables and rotated at 1 r.p.m. There were eighteen replicates of each of four treatments (seventy-two bottles). Every 2 days animals were transferred to new bottles with fresh algal food. Each experiment ran for 12 days. Three replicate bottles of each treatment were sacrificed at each medium change, and the animals counted. The  $r_{\text{max}}$  was determined by the equation:

$$[\ln(n_{\text{DAYX}}) - \ln(n_{\text{DAY0}})] / \text{time (days)}$$

The same design as for low N was used in the low-P population experiment, except that forty to fifty gravid females were separated 3 days before the start of the experiment into either low-P algae in low-P COMBO, or into non-limited algae in full COMBO

medium. As before, adult females were removed after 24 h, but the juveniles remained in the flasks for an additional 2 days before separation into roller bottles. Four *D. pulicaria* juveniles (48–72 h old) were placed into each roller bottle with appropriate medium and algal food.

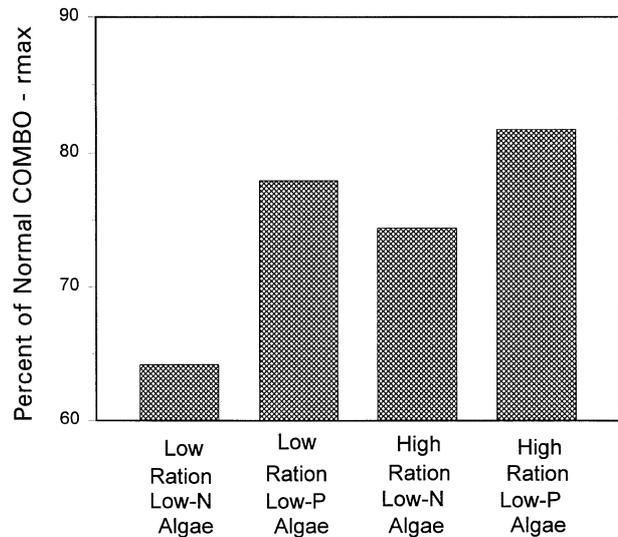
Statistical analyses used were two-way ANOVA procedures (Sokal & Rohlf, 1969) applied to examine the main effects of food quantity and food quality, and their possible interaction, on zooplankton performance. For data presentation purposes, one-way ANOVAs with LSD multiple range analyses were also performed on *Daphnia* performance data to portray significant ( $P = 0.05$ ) differences between treatments (denoted on figures with different letters associated with bars).



**Fig. 3** Population growth rates ( $r_{\max}$ ) of *Daphnia pulex* feeding on low or high rations of *Ankistrodesmus falcatus* grown at 15% of  $\mu_{\max}$  under low P or low N, or non-limited control cells growing at  $\mu_{\max}$  (high N, high P). Data are for the highest growth rates ( $r_{\max}$ ) measured over the period of population growth (see Table 1).

## Results

The fecundity responses of *D. pulex* cultured in either low-N algae or algae grown in complete medium (high N) at five ration levels are shown in Fig. 1. There was poor survivorship and no reproduction by animals fed the lowest ration of low-N algae. The size of first brood (Fig. 1a) was significantly higher in the high-N algae compared with the low-N algae at all food levels. The effect of algal food quality on the day of first brood (Fig. 1b) and on the number of broods per female (Fig. 1c) was significantly different at all but one ration level. Low-N algal food significantly decreased



**Fig. 4** Comparisons of the  $r_{\max}$  values for *Daphnia pulex* fed on low-nutrient algae as a percentage of the  $r_{\max}$  values for animals fed non-limited control cells growing at  $\mu_{\max}$ . All  $r_{\max}$ 's are significantly lower than the controls for the animals fed on nutrient-limited algae.

fecundity of *D. pulex* at all ration levels (Fig. 1d). Importantly, fecundity by animals fed the highest rations of low-N algae did not approach the fecundity achieved by animals fed any but the lowest ration of non-limited algae. There was, therefore, no indication of compensation at higher ration levels.

The fecundity of *D. pulex* cultured in low-P algae compared with those fed non-limited algae (high P) at four ration levels are shown in Fig. 2. The size of first brood (Fig. 2a) at all but the lowest ration level was significantly lower in the low-P food as compared with the high-P food. The day of first brood (Fig. 2b) and the number of broods per female (Fig. 2c) were significantly lower in low-P food at all ration levels. There appeared to be partial compensation at higher ration levels for both of these responses, however, the fecundity of *D. pulex* (Fig. 2d) was significantly lower at all food rations for the low-P food. The fecundity of animals fed the highest ration of low-P algae (ration E) was as high as for animals fed a lower ration of high-P food (ration D), indicating partial compensation.

The population growth rates ( $r_{\max}$ ) were significantly affected by food quality, but unaffected by food quantity. Population growth rates of *D. pulex* growing on low-N, low-P or on non-limited algae (high N, high P) at low and high ration levels are illustrated in Figs 3 and 4. Because  $r_{\max}$  peaked and

**Table 1** Mean population growth rates calculated for the period from start of experiment to day 8, 10 or 12. Each treatment had three replicates for each day. Values used for  $r_{\max}$  in Figs 3 and 4 are in bold type

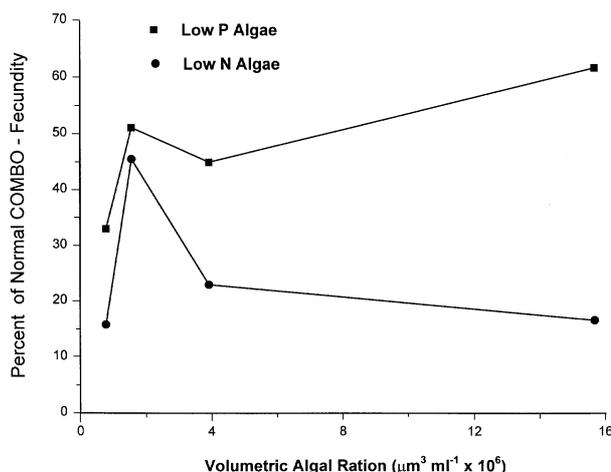
Day	Low ration	High ration	Low ration	High ration
	Low P	Low P	High P	High P
8	0.21	<b>0.32</b>	<b>0.37</b>	<b>0.39</b>
10	<b>0.29</b>	0.29	0.33	0.33
12	0.26	0.30	0.31	0.31
	Low N	Low N	High N	High N
8	0.11	0.15	0.16	0.23
10	<b>0.14</b>	0.17	0.20	<b>0.25</b>
12	0.12	<b>0.18</b>	<b>0.21</b>	0.20

began to decline before the end of the two experiments (Table 1), it was suspected that density dependence was achieved, especially in the case of low-P algae, and for the high rations of algae grown in complete medium in both experiments. Because of this,  $r_{\max}$  values reported in Figs 3 and 4 were calculated during the period of density-independent growth for each treatment (greatest  $r_{\max}$  per treatment in Table 1). Statistical comparisons of these  $r_{\max}$  values were sound because  $r_{\max}$  was calculated from three independent population samples harvested every 2 days during the experiment.

For the experiment in which animals were fed low-N or high-N algae, the two-way ANOVA analyses indicated a strong significant ( $P = 0.0023$ ) association between  $r_{\max}$  and algal type. The ration size was not significantly ( $P > 0.05$ ) associated with  $r_{\max}$ , and ration and algal type did not interact significantly ( $P > 0.05$ ). Similarly,  $r_{\max}$  of daphnids in the phosphorus experiment was significantly affected by algal type (low P or high P,  $P = 0.0003$ ), but  $r_{\max}$  was unaffected by ration size ( $P > 0.05$ ) and ration did not interact with algal type ( $P > 0.05$ ).

## Discussion

Food quality was as important as food quantity in determining the fecundity responses of animals grown on nutrient-limited algae. The fecundity of animals fed low-N algae remained low at all ration levels, with no increase at the highest food ration (Fig. 1d). The conclusion is that food quality is of paramount importance in determining the fecundity of animals fed N-limited food and that there was no compensation for



**Fig. 5** Comparisons of fecundity of *Daphnia pulex* fed nutrient-limited algae as a percentage of fecundity in control cultures fed non-limited cells growing at  $\mu_{\max}$ .

poor quality, low-N food by simply eating more food. In the case of animals fed P-limited algae, fecundities did increase with increasing ration but the slopes diverged between low-P and high-P treatments (Fig. 2d). Fecundity at the highest ration level of P-limited food was similar to that for animals fed a lower ration of high quality, non-limited algae (Fig. 2d), indicating partial compensation for poor quality food by higher total consumption. Comparisons of *Daphnia* fecundity responses under N and P limitation as a percentage of that in high quality food indicated that the animals could not compensate for the effect of low-N algae, but limited compensation occurred for low-P algae (Fig. 5). In all cases, N-limited algae were poorer food than P-limited algae.

This finding differs from results of Sterner (1993), who reported that *D. obtusa* growth correlated best with the P content of algal food. Until microencapsulated diets are developed, which permit defined manipulation of specific dietary constituents, conclusions will be limited to such correlational evidence. However, this can be problematic; for example, Müller-Navarra (1995) recently demonstrated how seemingly P-limited *Daphnia* could actually be limited biochemically by essential FA that covaried with phosphorus content.

The partial compensation observed in the present study for animals fed P-limited algae could have been affected by the experimental design. The low-nutrient medium used for the animal fecundity experiments had the concentration of the influent low-P algal

**Table 2** Effects of food quality parameters on  $r_{\max}$ . To distinguish the significance of each parameter from intrinsic variability between the two experiments, multiple regressions (two variables: experiment number, algal parameter) were used to obtain the statistics below. (In all cases  $n = 24$ ; \* =  $P < 0.05$ ; \*\* =  $P < 0.01$ ; NS = not significant; POM, particulate organic matter; CHO, carbohydrate; TG, triglyceride; PL, phospholipid; C : P, carbon : phosphorus ratio.) Algal components are from Kilham *et al.* (1997)

Algal parameter	Slope	P-value	$r^2$	Durban-Watson
POM ( $\mu\text{g } \mu\text{m}^{-3}$ )	-0.0315	0.47 (NS)	0.71	1.358
POM ( $\mu\text{g cell}^{-1}$ )	-0.00533	0.77 (NS)	0.68	1.358
Cell volume ( $\mu\text{m}^3$ )	-0.00033	0.78 (NS)	0.68	1.310
Cell protein ( $\mu\text{g } \mu\text{m}^{-3}$ )	0.130	0.06 (NS)	0.85	2.125
Cell protein ( $\mu\text{g cell}^{-1}$ )	0.061	0.19 (NS)	0.78	1.740
Protein content (%POM)	0.0034	0.005**	0.94	3.255
Cell CHO ( $\mu\text{g } \mu\text{m}^{-3}$ )	-0.0267	0.007**	0.93	2.839
Cell CHO ( $\mu\text{g cell}^{-1}$ )	-0.0139	0.078 (NS)	0.84	1.766
CHO content (%POM)	-0.00944	0.009**	0.93	2.925
Cell lipid ( $\mu\text{g } \mu\text{m}^{-3}$ )	-0.0799	0.15 (NS)	0.80	1.595
Cell lipid ( $\mu\text{g cell}^{-1}$ )	-0.0198	0.49 (NS)	0.71	1.766
Lipid content (%POM)	-0.00517	0.004**	0.95	3.347
Protein : lipid ratio	0.210	0.005**	0.94	3.247
Protein : CHO ratio	0.0327	0.006**	0.94	3.199
Protein : (lipid + CHO)	0.112	0.004**	0.94	3.316
Phosphorus ( $\mu\text{mol } \mu\text{m}^{-3}$ )	0.0358	0.10 (NS)	0.82	1.691
Phosphorus ( $\mu\text{mol cell}^{-1}$ )	0.0366	0.027*	0.89	2.114
C : P ratio	-0.00003	0.10 (NS)	0.82	1.705
TG : PL ratio	-0.276	0.25 (NS)	0.76	1.679

medium, which was  $5 \mu\text{M P}$ . The algae may have been able to take up additional P from this medium. The animal experiments were performed under very low light, so the algae were unlikely to be able to grow or substantially alter their biochemistry. P uptake could, however, contribute to the inconsistencies between present results and those of Sterner (1993).

Animals fed high quality, non-limited algae showed steadily increasing fecundities with increasing ration sizes (Figs 1d and 2d) as expected (Tessier & Goulden, 1987). Animals fed P-limited algae also had increasing fecundity with increasing ration (Fig. 2d). In contrast, as the ration of N-limited algae was increased, daphniid fecundity remained constant above a threshold ration. Sterner & Robinson (1994) found that as ration was increased above the maintenance requirements for carbon, the potential for non-energetic nutrient limitation increased. The present results are consistent with this result. However, all of the rations in the present study were above the maintenance level.

Fecundities in the control treatments differed somewhat between the two experiments, especially at the two highest ration levels (Figs 1 and 2). Subtle differences in inherent reproductive capacity are known over time (Goulden, Henry & Tessier, 1982; Tessier & Goulden, 1987), and in the present study differences

were seen between the experiments in the fecundity and  $r_{\max}$  of the *D. pulicaria* fed non-limited cells growing at  $\mu_{\max}$  (control cultures).

The low-N algae had a greater negative effect than low-P algae on *D. pulicaria* fecundity (Fig. 5). One aspect of food quality that correlated strongly with fecundity was the protein : lipid ratio, which was lowest (0.36) for N-limited cells, somewhat higher (0.44) in P-limited cells and highest (1.14) in the non-limited growing cells (Kilham *et al.*, 1997). Protein : lipid ratio of food might, therefore, be a good proxy for food quality.

The population growth rate experiments showed the same basic trends as those seen in the fecundity experiments, namely that food quality was as important as food quantity. Low-N food was inferior to low-P food, and both food types were significantly lower than when high quality, non-limited algae were used as food. One difference was that there was no significant effect of ration size in either experiment (Figs 3 and 4), which indicated that there was no compensation of food quantity for food quality in the population growth rates.

The results clearly demonstrate that *Daphnia* reproduction can be severely lowered by suboptimal food quality. This result supports the growing body of

evidence for the importance of food quality. However, literature reports are equivocal on which nutrient is most limiting for freshwater zooplankton. A multiple regression analysis of the data on effects of food quality parameters on  $r_{\max}$  (Table 2) was also unclear about which qualitative aspects were limiting. The traditional approach to studying nutrient/food quality limitation is to focus on the absolute quantity of a particular constituent. However, because animals could not compensate for inferior food quality by simply ingesting more food, this suggests that the biochemical *balance* of nutrients is more important than the absolute abundance of any one constituent; for example, the dietary protein content, protein : lipid ratio, protein : carbohydrate ratio and protein : (lipid + carbohydrate) ratio were all positively associated with *Daphnia*  $r_{\max}$  ( $P < 0.01$ ; multiple regression with factors experiment number and food quality parameters) and lipid content and carbohydrate content were negatively associated with  $r_{\max}$  ( $P < 0.01$ ). In contrast to these parameters reflecting the relative balance of dietary constituents, absolute abundances of nutrients in the diet were poor indicators of food quality; for example, cellular protein, lipid, carbohydrate, etc. were not significantly related to  $r_{\max}$  ( $P > 0.05$ ). Furthermore, the elemental C : P ratio, as well as cellular P and lipid class composition, were not correlated with growth rate. Thus *D. pulicaria* appeared to favour a nitrogen-rich or protein-rich diet.

The present study has shown that the cladoceran *D. pulicaria* is sensitive to diets of nutrient-limited algae, and it responds to these changes in food quality by having reduced fecundity and a lower population growth rate. Animals fed N-limited algae were unable to compensate for these quality effects by higher food quantity. Zooplankton fed P-limited food demonstrated a limited ability to compensate with fecundity, but their  $r_{\max}$  remained lower at all rations compared to non-limited food.

Recent studies (Van Donk *et al.*, 1997) have shown that the cell walls of the green alga *Chlamodomonas reinhardtii* were much thicker under P limitation than under N limitation, and that P-limited cells were not as well digested by *D. pulex* and *D. magna* as algae growing under non-limited conditions. This study made no direct measurements of cell wall effects, but the P-limited *A. falcatus* promoted better fecundity and growth rates of *D. pulicaria* than the N-limited cells, which was different from the trends observed by Van Donk *et al.* (1997)

and Van Donk & Hessen (1993). Chen & Folt (1993) showed that two freshwater copepods feeding on non-limited cells of *C. reinhardtii* had very low fecundities and survivorship, but much higher clearance and ingestion rates, compared with feeding on *Cryptomonas erosa*. The animals actually ingested much more C, N and protein of the green alga than of the cryptomonad, but were apparently unable to assimilate it, which may also have been because of thicker cell walls of the green alga decreasing food quality.

Although animal production was positively correlated with dietary protein content in this study, this does not necessarily counter reports that the other nutrients are important limiting agents (phosphorus: Sterner, 1993). The specific limiting nutrient might vary spatially and temporally as algal species composition shifts and the abundance of dissolved nutrients for producers changes (Kreeger *et al.*, 1997). Furthermore, the specific nutritional requirements of zooplankton can vary over their life cycle (Roman, 1991), although Andersen & Hessen (1995) emphasized that this variation was small. However, the present results show that qualitative measures of the relative balance of dietary constituents such as the protein : lipid ratio can be better indicators of food quality (positively related to growth rates and fecundity) than specific nutrients (absolute amounts of an element or biochemical; Andersen & Hessen, 1995). This is consistent with the growing realization that foraging models are often based on dietary protein : energy ratios and not on energy or protein *per se* (Bowen, Lutz & Ahlgren, 1995).

It is not surprising that biochemical indices of food quality have greater predictive power than elemental indices. Using only elemental concentrations fails adequately to consider the various biochemical forms, and hence bioavailability, of a given element (Andersen, 1994; Andersen & Hessen, 1995). Thus, protein : energy ratios might be more useful than C : N ratios, although this generalization is complicated because the dietary importance of protein is multifaceted (amino-N, essential amino acids), and protein can be used for both anabolism (growth, biosynthesis) and catabolism (energy). A full understanding of nutritional limitation in zooplankton cannot be achieved without obtaining further information on the various roles of specific biochemical fractions, the variable digestibilities of the biochemical fractions, and the varying nutritional requirements of the animals; all of which can influence nutritional limitation (Kreeger *et al.*, 1995).

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