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Essential fatty acid content and the phosphorus to carbon ratio in cultured algae as indicators of food quality for *Daphnia*

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SUMMARY

1. A series of experiments examined growth of *Daphnia magna* on three algal diets (*Rhodomonas minuta*, *Scenedesmus acutus* and *Synechococcus* sp.) at varying physiological states [nitrogen and phosphorus (P) limitations] to test whether variation in algal fatty acid and/or elemental composition can predict *Daphnia* growth.

2. These algae differed widely in their essential fatty acid (EFA) composition while phosphorus (P) or nitrogen limitation had only a small influence on their ω3-polyunsaturated fatty acid (PUFA) content.

3. Individually, algal ω3-PUFA content explained 69% of the variation in the normalised growth of *D. magna*, while algal phosphorus content explained 11% of the variation. Quantitative models for *D. magna* growth used both algal ω3-PUFA content and algal C : P ratio as food quality indices. Together, algal ω3-PUFA content and C : P ratio explained 70% of the variation in the normalised growth rate of *D. magna*.

4. Our results indicate that EFA influenced algal food quality much more strongly than P. The EFA and mineral P impacts appear to be independent.

Keywords: *Daphnia*, essential fatty acids, food quality, growth model, phosphorus

Introduction

The importance of algal food quality for zooplankton production and dynamics, as well as overall ecosystems behaviour, has become increasingly recognised during the last 10 years. While many researchers agree that algal food quality is important (Sterner & Schulz, 1998), there is discussion about the most likely determinant of algal food quality in freshwater pelagic food webs (Brett, 1993; Hessen, 1993; Urabe & Watanabe, 1993; Müller-Navarra, 1995a; Gulati & DeMott, 1997; Brett, Müller-Navarra & Park, 2000). The two most studied and debated hypotheses are the mineral phosphorus (P) (Hessen, 1992; Urabe & Watanabe, 1992; Urabe, Classen & Sterner, 1997; DeMott, 1998; see Sterner & Schulz, 1998 for more references) and essential fatty acid (EFA) limitation hypotheses (Ahlgren *et al.*, 1990; Müller-Navarra, 1995a,b; Brett & Müller-Navarra, 1997; Müller-Navarra *et al.*, 2000). In addition, evidence also exists for nitrogen (protein) limitation (Checkley, 1985; Kilham *et al.*, 1997) and the importance of digestion resistance in determining food quality (Van Donk & Hessen, 1993, 1995; Van Donk *et al.*, 1997).

The mineral limitation hypothesis, and especially the P-limitation hypothesis, originated from the observation that *Daphnia* often have higher somatic phosphorus to carbon (P : C) ratios than their natural food (Hessen, 1992; Urabe & Watanabe, 1992; Sterner & Hessen, 1994). The P-limitation hypothesis states that *Daphnia* growth should be negatively affected by low phosphorus content in the seston and defines

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food quality as the seston phosphorus to carbon ratio (Urabe & Watanabe, 1992). This hypothesis is supported by a number of experiments showing that *Daphnia* fed with P-deficient algal monocultures (mainly green algae) and mixtures grew poorly (Urabe *et al*., 1997; Weers & Gulati, 1997; DeMott, 1998; DeMott, Gulati & Siewertsen, 1998; see Sterner & Schulz, 1998 for more references). The EFA limitation hypothesis is based on the fact that animals cannot synthesise polyunsaturated fatty acids (PUFA) *de novo*. In addition, the EFA hypothesis takes into account that the conversion rates from short chain ω3-polyunsaturated fatty acids (ω3-PUFA) to highly unsaturated fatty acids (HUFA) are low (Olsen, 1999). The EFA also play important roles in cell membrane physiology and hormone metabolism (Singer & Nicholson, 1972; Smith & Borgeat, 1985; Blomquist, Borgeson & Vundla, 1991). The PUFA are mainly produced by phytoplankton and aquatic animals obtain PUFA such as eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) directly from their diets (Ahlgren *et al*., 1996; Olsen, 1999). According to the EFA limitation hypothesis, food quality can be measured either as the absolute concentration of HUFA (and their precursors) when carbon is below saturating levels, or as the relative seston HUFA content (per mg C^{-1}) in systems in which carbon is well above the saturation level (Ahlgren *et al*., 1990; Müller-Navarra, 1995a,b; Brett & Müller-Navarra, 1997; Müller-Navarra *et al*., 2000).

Although both the mineral P and EFA hypotheses have been tested individually by several researchers, there is still much uncertainty and disagreement regarding the relative power of these two hypotheses to predict zooplankton growth. Several possibilities regarding the relative importance of EFA and P have been suggested as it is commonly believed that phosphorus limitation of algae can cause shifts in its EFA content (Shifrin & Chisholm, 1981; Müller-Navarra, 1995a; Weers & Gulati, 1997). One possibility is that algal food quality is merely correlated with its EFA content because both algal food quality and EFA content are influenced by P-limitation and algal food quality is directly determined by its phosphorus content (Weers & Gulati, 1997). A similar possibility is that algal food quality correlates with its P-content because P-limitation changes the biochemical composition, especially the EFA content, of the algae which in turn directly determines food quality (Müller-Navarra, 1995a; Ahlgren *et al*., 1998). It has also been suggested that EFA limitation is important only when algae are not P limited, while P-limitation is the primary or sole determinant of algal food quality when algae are P limited (Sundbom & Vrede, 1997; Boersma, 2000). An alternative possibility, which is seldom considered, is that both P and EFA can influence algal food quality for herbivorous zooplankton and these impacts are completely independent of each other. The present study will attempt to differentiate between these possibilities.

The objectives of this study were (1) to measure the growth of *Daphnia magna* Strauss using three different algal taxa under varying physiological states as food, (2) to elucidate the relative importance to growth of algal EFA and minerals in the food and (3) to develop an overall growth model which includes both biochemical and mineral components of algal food quality.

Methods

Culture of algae and Daphnia

Flow-through experiments were conducted using three algal species: *Rhodomonas minuta* Skuja, *Scenedesmus acutus* (Meyen) Chodat and *Synechococcus* sp. *Rhodomonas minuta* and *S. acutus* originated from the algal collection of the Max Planck Institute for Limnology in Plön, Germany. *Synechococcus* sp. was obtained from Dr William DeMott at the Indiana–Purdue University, Indiana, USA. We chose these alga taxa because of their large differences in fatty acid, especially PUFA, composition. In addition, by using the same strains of algae that have been used in previous studies with similar objectives, our results are comparable with those of prior studies. We define PUFA as polyunsaturated fatty acid molecules with a chain length of 18 or more carbon atoms. This covers most PUFA as we did not use diatoms that can have large amounts of PUFA molecules with a chain length of 16 carbon atoms. The fatty acid HUFA are defined as a subset of PUFA molecules with 20 or more carbon atoms. *Rhodomonas* is known to have high EPA content while *Synechococcus* has virtually no HUFA and very little PUFA (Ahlgren, Gustafsson & Boberg, 1992; Brett & Müller-Navarra, 1997; DeMott & Müller-Navarra, 1997). *Scenedesmus acutus* contains considerable amounts of short chain ω3-PUFA (18 : 3ω3 and 18 : 4ω3), but low amounts of ω3-HUFA (20 : 5ω3 and

22 : 6ω3) (Ahlgren *et al*., 1992; Müller-Navarra, 1995a). All algal species were cultured using the synthetic medium L16 (Lindström, 1983) modified with b_{12} and biotin vitamins (not for *S. acutus*) and earth extract. This medium is useful for culturing both algae and zooplankton as it has an ionic composition similar to that in many eutrophic lakes. The P- and N-deficient algae were grown in batch culture for 4–7 days by leaving phosphorus and nitrogen out of the growth medium. The clone of *D. magna* used in these experiments was isolated from a small pond near the University of California at Davis, USA and cultured for several years on *S. acutus* in L16 medium at 20 °C with a 16L : 8D h light cycle.

Flow-through experiments

We performed a series of flow-through experiments using the three algal species (*R. minuta*, *S. acutus* and *Synechococcus* sp.) at four different algal physiological states (nutrient saturated, P-limited, N-limited and senescent), with *D. magna* as the test zooplankter. These treatments were intended to create a wide range of algal biochemical and mineral composition by manipulating the culture environment. Therefore, each algal food was an independent treatment and not a homogeneous replicate within a 'treatment'. Each experiment had 12 different algal treatments (three species × four physiological states). This design was fully repeated six times except for one experiment which did not include *Synechococcus* sp. To take into account any changes in algal fatty acid and elemental (C, N and P) composition during each experiment, we measured those parameters at the beginning and end. The experiments were performed in a flow-through culture system to keep the food concentration constant (Lampert, Schmitt & Muck, 1988). Zooplankters were maintained in 250-mL chambers suspended in a temperature controlled water bath (20 ± 0.5 °C) placed in a temperature-controlled room (20 ± 0.5 °C). These chambers received a constant food supply from stirred reservoirs (2-L Erlenmeyer flasks, Corning Incorporated, Corning, NY, USA) with a multichannel peristaltic pump. The flow rate for each chamber was kept at 1.44 L day^{-1}. Each chamber had a 243 μm mesh screen at the bottom so that algae (but not the daphnids) could pass through. Food concentrations were kept well above the incipient limiting level (ILL) and ranged between 0.5 and 1.5 mg C L^{-1}. Algal food concentration was set using the relationship between absorbance at 800 nm and previously determined dry weights of each algal species. The calibration curve between absorbance and dry weight was determined separately for each experiment. Each flow-through chamber received six to eight 4-day-old *D. magna* which were born no more than 12 h apart and were maintained on *S. acutus* before use in the experiments. An aliquot of 20–30 juveniles was used for the initial biomass determinations.

After 3 days (standard deviation: 44 min) in the flow-through system, *D. magna* were collected from the chambers and washed in L16 media, without algae, for at least 30 min. They were then dried at 60 °C for 48 h before weighing. The somatic instantaneous growth rate of *D. magna* (g; day^{-1}) was calculated as the dry weight accrual during the experiment according to the following exponential equation:

$$g = [\mathrm{Ln}(W_t) - \mathrm{Ln}(W_0)]/t \quad (1)$$

where W_0 and W_t are the mean individual dry weights at the beginning and end of each experiment, respectively, and t is the duration of experiment in days. We measured *D. magna* weight to the nearest 1 μg with a Perkin–Elmer AD-6 microbalance (Perkin–Elmer Corporation, San Jose, CA, USA).

Analyses of algal biochemical and elemental composition

Two hundred and fifty millilitre of each algal food type was filtered onto a precombusted glass fibre filter (Whatman GF/C for *R. minuta* and *S. acutus*; Whatman GF/F for *Synechococcus* sp, Whatman International Ltd, Maidstone, UK). Filters with algae were kept at –80 °C until fatty acid extractions. We used 10 μL of 21 : 0 (1 mg mL^{-1}) as an internal standard that was added onto the freeze-dried filter immediately before extraction. Extraction and methylation were performed according to Kattner & Fricke (1986). Algal fatty acid composition was analysed using a gas chromatograph (Hewlett–Packard 6890, Agilent Technologies, Palo Alto, CA, USA). Individual fatty acid methyl esters (Sigma, St Louis, MO, USA) were dissolved into n-hexane and used as standards to determine retention times. Fatty acid quantities were calculated using the area ratios of a sample and the internal standard of known quantity. Response factors for the single fatty acids were tested with quantitative

mixes and the deviation from the internal standard used (21 : 0) was found to be smaller than 5%. As conversion of fatty acid molecules occurs on a stoichiometric basis rather than by weight, we expressed algal fatty acid concentrations in molar units. Particulate carbon and nitrogen content of seston was determined using a Perkin Elmer 2400 CHN Analyser. Algal particulate phosphorus content was analysed according to Solórzano & Sharp (1980).

Model fitting

In addition to the absolute growth rate of *D. magna*, growth rate in each experiment was also normalised to the averaged growth rate of *D. magna* fed with P sufficient *S. acutus* in the same experiment. In this way, growth rate can be compared by eliminating small overall growth rate differences between experiments that were the result of any variation in the preconditioning regime of test animals. Both growth rate and normalised growth rate were used for statistical analysis.

As *D. magna* growth saturates at a high concentration of algal EFA, we used an asymptotic model (Müller-Navarra, 1995b; Müller-Navarra *et al*., 2000) when fitting *D. magna* growth to algal EFA:

$$g = a * \{1 - \exp(-\mathrm{b} * \mathrm{X} + \mathrm{c})\} \quad (2)$$

where g is the growth rate or the normalised growth rate of *D. magna*, a is the maximum growth rate (i.e. the asymptote), X is algal fatty acid content and b and c are the curvature and intercept terms of the hyperbolic model. We used the Solver function in Microsoft Excel™ to obtain solutions for the asymptote, curvature and intercept terms while minimising Akaike's Information Criterion (AIC) and achieving a bias ~0.

At an algal C : P ratio greater than the 'critical threshold', above which the algal P supply is stoichiometrically under-representative, zooplankton growth rate should be linearly related to algal P content (Brett *et al*., 2000). According to the results of Brett *et al*. (2000), based on many experiments, a C : P ratio of 300 is a plausible critical threshold for *Daphnia* spp. This simple prediction can be expressed mathematically by the following expression:

$$\begin{aligned}&\text{Predicted growth reduction [P(C : P)]}\\ &\quad = 1 - \text{critical threshold/algal C : P}\\ &\quad = 1 - 300/\text{algal C : P}\end{aligned} \quad (3)$$

Thus, we used the Excel function min() to predict the normalised growth rate of *D. magna* from algal C : P ratio:

$$\begin{aligned}g_N &= \min\,[1, (1 - (1 - 300/\mathrm{C} : \mathrm{P}_{\mathrm{algae}})]\\ &= \min\,(1, 300/\mathrm{C} : \mathrm{P}_{\mathrm{algae}})\end{aligned} \quad (4)$$

where g_N is the normalised growth rate of *D. magna* and C : $\mathrm{P}_{\mathrm{algae}}$ is the observed C : P ratio in the algae. Note that min (1, 300/C : $\mathrm{P}_{\mathrm{algae}}$) means the lesser of 1 or the ratio of 300 to observed C : P ratio in the algae. We also used alternative critical thresholds (100–500) to fit the observed growth rate to the algal P content as a sensitivity analysis and found that a C : P ratio of 300 produced the highest fit between the observed growth rate and algal P content. Regression analyses were performed with StatView™II software.

To compare various candidate models for *D. magna* growth, AIC was used as a selection method. The AIC was calculated as:

$$\mathrm{AIC} = n\log(\hat{\sigma}^2) + 2K \quad (5)$$

where,

$$\hat{\sigma}^2 = \frac{\sum \hat{\varepsilon}_i^2}{n} \quad (6)$$

in which ε_i is an estimated residual for candidate models, n is the number of cases and K is the total number of estimated parameters plus 1 (for σ^2) (Burnham & Anderson, 1998).

Results

Algal fatty acids and elemental composition

Comparisons of the fatty acid composition of the three algae taxa under P saturated conditions exhibited distinctive differences in their total fatty acid (ΣFA), unsaturated fatty acid (UFA), total PUFA, total ω3-PUFA, ω3-HUFA and EPA content (Fig. 1). Total fatty acids (ΣFA) in *Synechococcus* sp. were about half that of *R. minuta* and *S. acutus*, largely because of a lower UFA content. However, the saturated fatty acid (SAFA) content was not particularly different amongst these three very different alga species.

Rhodomonas minuta had a low C : P ratio except for one case. That is, although this alga was cultured on P-deficient media, it did not respond with a very high

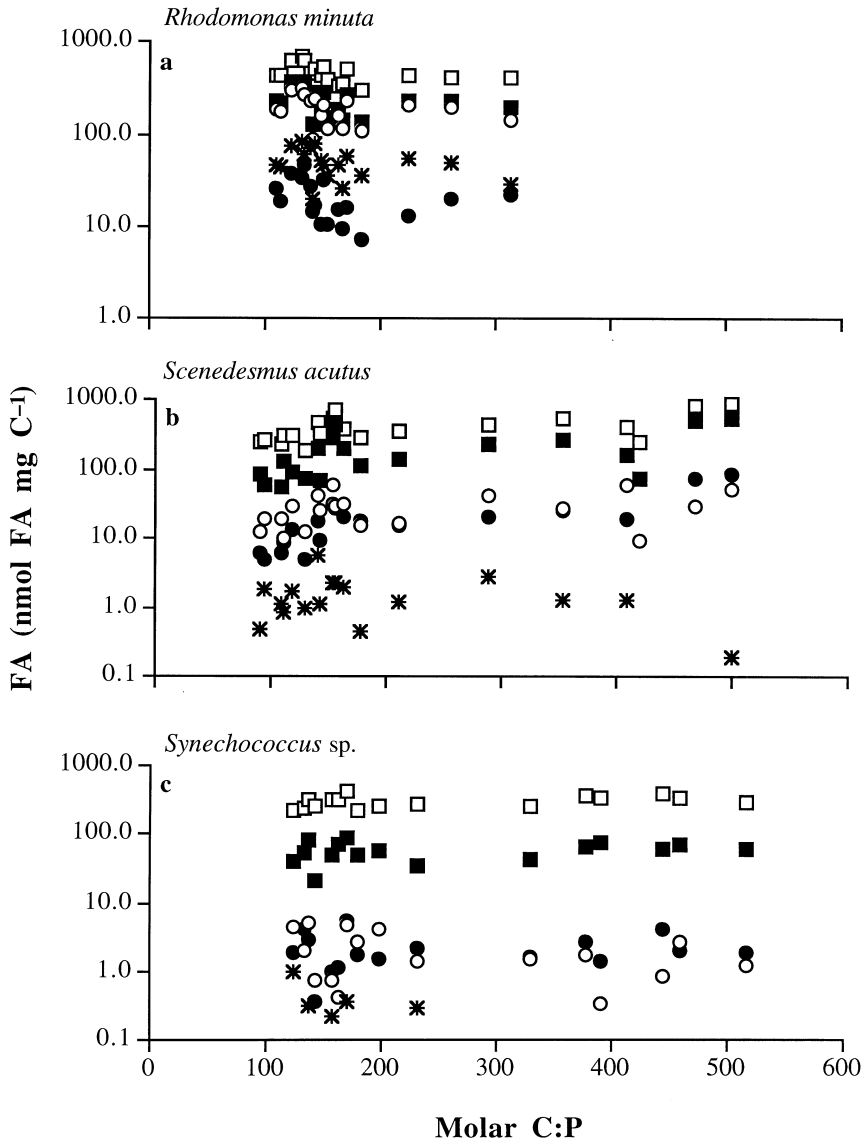

Fig. 1 Changes in the fatty acid moieties in relation to algal P-content for (a) *Rhodomonas minuta*, (b) *Scenedesmus acutus* and (c) *Synechococcus* sp. Please note the different fatty acid scale on panel (a). Each data point represents an average of fatty acids and C : P ratios for the beginning and end of each experiment. □: FA, ■: UFA, ●: ω6-PUFA, ○: ω3-PUFA and ✳: EPA.

C : P ratio. Total ω3-HUFA and, especially, EPA content decreased while ω6-PUFA content increased with moderate P deficiency (C : P > 250) in this algae species. However, these changes are inconclusive because there are few observations for strong P-limitation in *R. minuta*. *Scenedesmus acutus* showed the most dramatic changes in fatty acid composition with P-limitation. The ΣFA, UFA and PUFA content of *S. acutus* increased with P-limitation, while the ω3-HUFA, EPA and the ω3-HUFA/ω3-PUFA fraction decreased. Overall ω3-PUFA content did not change with P-limitation in *S. acutus* and *Synechococcus* sp. (Fig. 1). While P-limitation did not alter the UFA fraction in *Synechococcus* sp., UFA increased substantially with P-limitation in *S. acutus* because of an increase in 18 : 2ω6.

While *S. acutus* and *Synechococcus* sp. exhibited similar ranges in their C : P ratios, the observed ranges in C : N ratio amongst the three species were quite different (Fig. 2). The C : N ratio of *S. acutus* reached a maximum of 20, while *Synechococcus* sp. and *R. minuta* exhibited maximum C : N ratios of 15 and 11, respectively. No clear trends in algal fatty acid

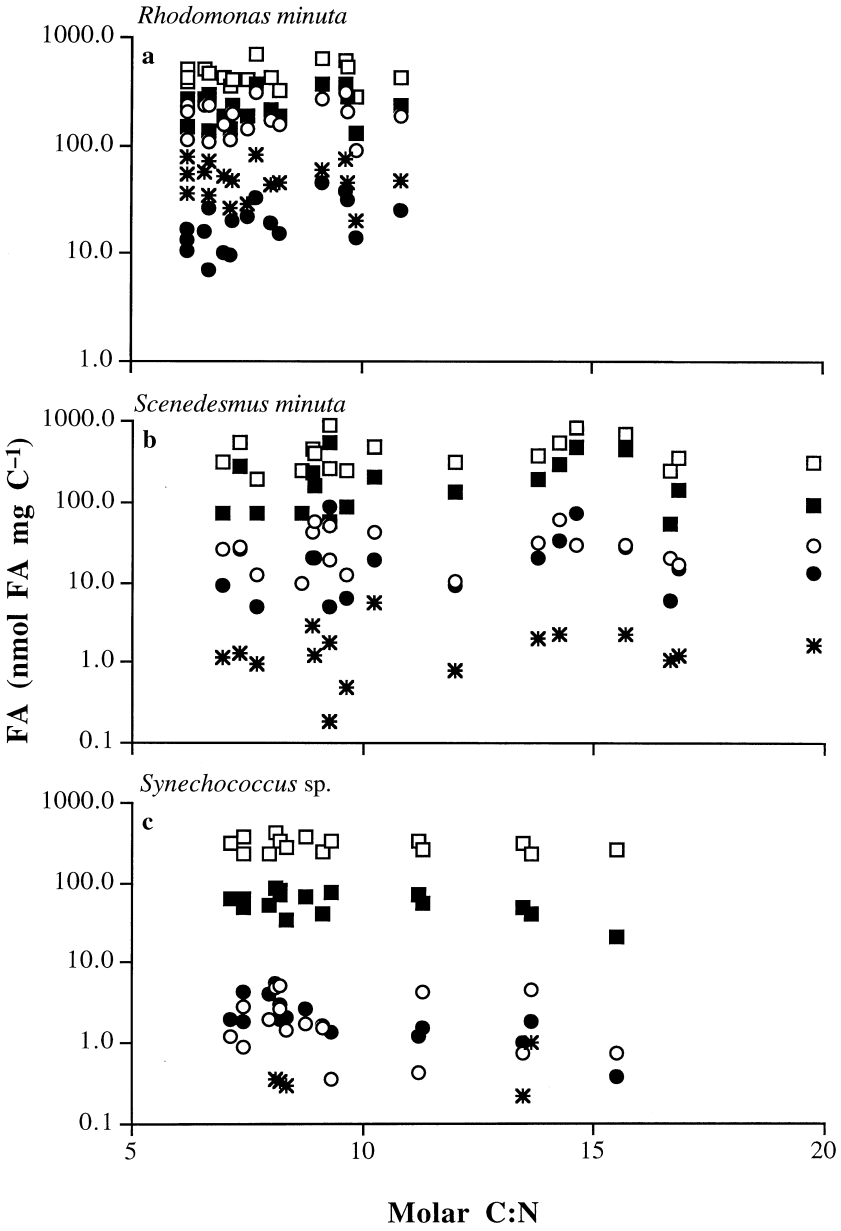

Fig. 2 Changes in the fatty acid moieties in relation to algal N content in (a) *Rhodomonas minutaI,* (b) *Scenedesmus acutus* and (c) *Synechococcus* sp. Please note different fatty acid scales on panel (a). Each data point represents an average of fatty acids and C : N ratios for the beginning and end of each experiment. □: FA, ■: UFA, ●: ω6-PUFA, ○: ω3-PUFA and ✳: EPA.

pattern were detected in relation to the algal C : N ratio, except for UFA content in *Synechococcus* sp., which decreased with N deficiency.

The source of variation in D. magna *growth rate*

To examine how much variation in *D. magna* growth rate can be explained by species affiliation and physiological status, we conducted a two-factor ANOVA with randomised block factor (different time) (Table 1). The ANOVA table revealed that species affiliation explained about 75% of the total variation in *D. magna* growth rate in our experiments. In contrast, physiological status and the interaction between physiological status and species affiliation explained only 1.7 and 3.6% of total variation in *D. magna* growth rate, respectively. These latter results were not significant at the 95%

Table 1 The ANOVA table for *Daphnia magna* daily growth rate as a response variable. Each batch of experiments conducted at the same time was considered as a block that had no interaction. Batch term was regarded as a random factor whose significance was not tested

Source of variation	d.f.	Sum of squares	Mean squares	*F*	*P*-value	Variance explained (%)
Batch	4	0.049	0.012			4.3
Species	2	0.851	0.426	92.04	0.000	74.6
Status	3	0.020	0.007	1.41	0.253	1.7
Species × status	6	0.041	0.007	1.49	0.207	3.6
Residuals	39	0.180	0.005			15.8
Total	54	1.142				

confidence level. The residual explained 15.8% of total variance in *D. magna* growth rate, while variance between algal batches contributed 4.3% to the total variance.

The impact of algal chemical composition on D. magna *growth rate*

The carbon content of the algal cultures used in these experiments varied from 0.5 to 1.5 mg C L^{-1}, thus excluding the possibility of carbon limitation for *D. magna* (Lampert, 1987). Accordingly, there was no relationship between *D. magna* growth rate and the algal carbon concentration (Fig. 3a). As all algae used can be filtered effectively by *D. magna*, differences in algal food biochemical composition appear to have been the main determinant of *D. magna* growth rate, contributing to the large scatter in Fig. 3(a). The molar C : N ratio of the algal cultures varied between 6 and 20, although no relationship was found between algal C : N ratio and *D. magna* growth (Fig. 3b).

Algal molar C : P ratio varied from 89 to 517, thus encompassing the expected critical C : P threshold of 300 (Urabe & Watanabe, 1992; Sterner, 1993; Urabe

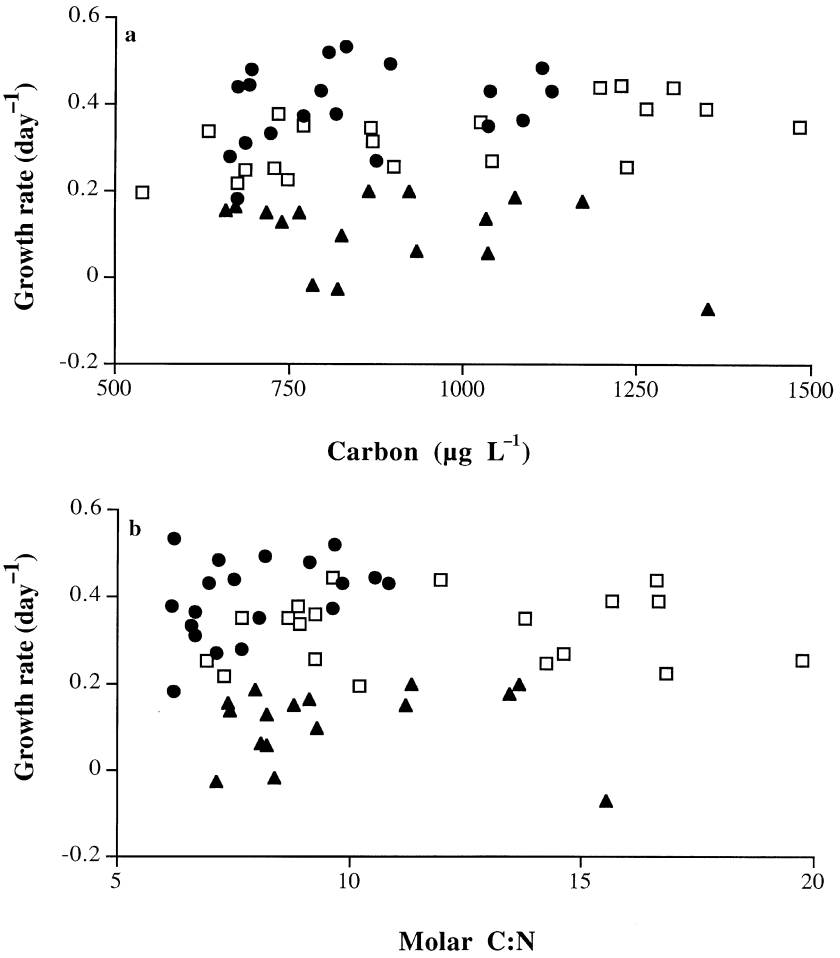

Fig. 3 (a) Algal carbon content and *Daphnia magna* growth rate and (b) algal molar C : N ratio and *D. magna* growth rate. Each data point represents an average of carbon and nitrogen amount and *D. magna* growth rate for the beginning and end value of each experiment. ●: *Rhodomonas minuta*, □: *Scenedesmus acutus* and ▲: *Synechococcus* sp.

et al., 1997). *Daphnia magna* growth rate tentatively decreased when C : P ratios exceeded 300 in *S. acutus* and *Synechococcus* sp. (Fig. 4). However, the effect of C : P on *D. magna* growth was relatively small compared with the effect of the different algal species on growth. In contrast to algal P content, algal EFA content exhibited a much stronger relationship with *D. magna* growth rate (Fig. 5; Table 2). Among all EFA tested, total ω3-PUFA showed the second lowest AIC, following PUFA, with *D. magna* growth rate while it showed the lowest AIC with normalised growth rate.

Thus, we chose algal ω3-PUFA content as a representative index for essential fatty acids. Algal ω3-PUFA content ranged from 0.4 to 583 nmol fatty acid mg C^{-1}. The highest ω3-PUFA value appeared to be an outlier as its leverage of 0.365 was much higher than the overall average leverage (0.036). However, the studentised deleted residual for the highest ω3-PUFA point was much lower (0.557) than the critical value in the *t*-distribution (2.01), indicating that the data point was not particularly influential to the fitted model. Therefore, the highest ω3-PUFA point was retained in subsequent statistical analyses.

We found that the asymptotic model explained 58% of the variation in the growth rates (69% for normalised growth rate) with ω3-PUFA content as the

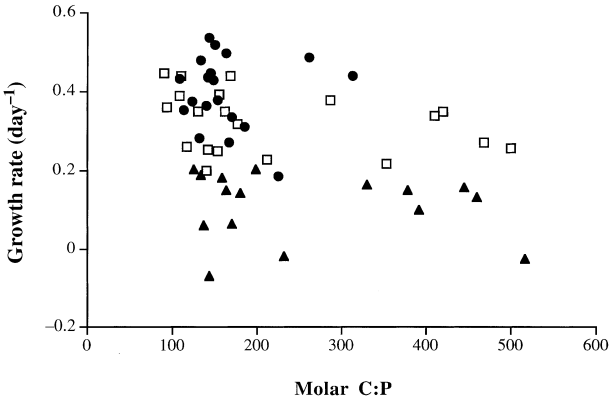

Fig. 4 Algal molar C : P ratios and *Daphnia magna* growth rate. Each data point represents an average of C : P ratio and *D. magna* growth for the beginning and end of each experiment. Legends are the same as Fig. 3.

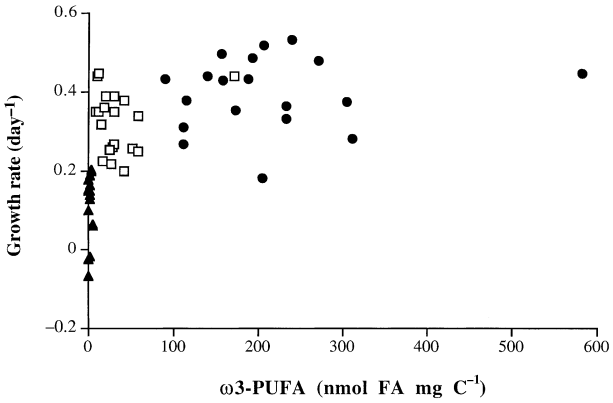

Fig. 5 The ω3-PUFA content and growth rates of *Daphnia magna*. Each data point represents an average of ω3-PUFA and *D. magna* growth rate for the beginning and end of each experiment. Legends are the same as Fig. 3.

Table 2 *Daphnia magna* growth rate and normalised growth rate regression model results for various algal fatty acid and P content parameters. AIC stands for Akaike's Information Criterion

Independent variable	Asymptote	Curvature	Intercept	Bias	r^2	*P*-value	AIC
Growth rate							
Hyperbolic (EPA)	0.390	0.807	–0.460	0.00	0.495	0.0001	–5.172
Hyperbolic (HUFA)	0.394	0.261	–0.367	0.00	0.445	0.0001	–2.932
Hyperbolic (PUFA)	0.365	0.942	–0.016	0.00	0.585	0.0001	–9.862
Hyperbolic (ω3-PUFA)	0.368	0.127	–0.149	0.00	0.584	0.0001	–9.813
Hyperbolic (ω3-PUFA) * P (C : P) [Equation 8]	0.374	0.127	–0.149	0.00	0.602	0.0001	–8.863
Normalised growth rate							
Hyperbolic (EPA)	1.161	0.994	–0.373	0.00	0.604	0.0001	40.554
Hyperbolic (HUFA)	1.171	0.334	–0.234	0.00	0.545	0.0001	43.869
Hyperbolic (PUFA)	1.107	0.076	–0.025	0.00	0.681	0.0001	35.337
Hyperbolic (ω3-PUFA)	1.120	0.101	–0.134	0.00	0.686	0.0001	35.004
P (C : P)				–5.22	0.114	0.0118	55.156
Hyperbolic (ω3-PUFA) * P (C : P) [Equation 7]	1.158	0.105	–0.152	0.00	0.699	0.0001	36.052

independent variable (Table 2). In contrast, when we assumed a critical threshold of 300, the P-limitation model explained 11% of the variation in normalised *D. magna* growth with relatively higher AIC values than the ω3-PUFA model. This was the best fit and the lowest AIC of the various critical thresholds tested (100–500) and a better fit than a linear model between *Daphnia* growth and algal C : P. As the C : P model with a threshold of 300 showed a large bias (Table 2), we minimised the bias by applying a correction factor to the growth depression portion (1–300/algal C : P). The correction factor (0.607) was calculated using the Excel Solver function with growth data from *Scenedesmus* fed *D. magna*. This optimisation did not affect the fit (r^2). As the ω3-PUFA and C : P terms appear to be independent, we combined the asymptotic model and the P-limitation model, explaining 60% of the variation in growth rates of *D. magna* (70% for normalised growth rate) (Table 2).

Multiple regression analyses without interaction terms gave a similar coefficient of determination ($r^2 = 0.593$ for growth rate and $r^2 = 0.694$ for normalised growth rate) for both EFA and C : P ratio (Table 3). The addition of an interaction term (ω3-PUFA * C : P ratio) to the multiple regression model did not substantially improve the model fit ($r^2 = 0.601$ for growth rate and $r^2 = 0.704$ for normalised growth rate), nor did it lower AIC significantly. Furthermore, plotting the residuals of the multiple regression model against the interaction term showed no systematic trends, indicating the interaction term was not necessary. Thus, the ω3-PUFA content explained virtually the same variation as did the combined model. However, this study did not include a large portion of cases of severely P-limited algae and this could have reduced the importance of the algal C : P ratio in these statistical analyses. The average C : P ratio for algae used in this study was 212 (median = 162). Therefore, we have chosen a conservative approach and have included the C : P ratio term in order to develop a more general model (combined model). Although the multiple regression models with an interaction term explained *D. magna* growth somewhat better than the combined model (but not in terms of AIC), we chose the latter as it is simpler. The final model obtained for this study was:

Table 3 Multiple regression results for *Daphnia magna* normalised growth rates using a saturation model of ω3-PUFA [Hyperbolic (ω3-PUFA)] and phosphorus limitation model of C : P ratio [P(C : P)] with and without interaction terms [Hyperbolic (ω3-PUFA)*P(C : P)]. Std. β stands for standardized β and AIC stands for Akaike's Information Criterion

Independent variable	Coefficient	*Std. β*	*t*	*P*-value	r^2/*partial* r^2	AIC
Growth rate						
Hyperbolic (ω3-PUFA) + P (C : P)				0.0001	0.593	–9.183
Hyperbolic (ω3-PUFA)	0.668	0.733	7.922	0.0001	0.547	
P (C : P)	0.125	0.105	1.132	0.263	0.023	
Intercept	–0.023					
Hyperbolic (ω3-PUFA) + P (C : P)+ Hyperbolic (ω3-PUFA)*P (C : P)				0.0001	0.601	–6.789
Hyperbolic (ω3-PUFA)	0.083	0.091	0.145	0.8851		
P (C : P)	–0.017	–0.014	0.096	0.9240		
Hyperbolic (ω3-PUFA)*P (C : P)	0.637	0.692	1.035	0.3037	0.022	
Intercept	0.103					
Normalised growth rate						
Hyperbolic (ω3-PUFA) + P (C : P)				0.0001	0.694	48.459
Hyperbolic (ω3-PUFA)	0.755	0.795	9.935	0.0001	0.655	
P (C : P)	0.393	0.112	1.397	0.1685	0.036	
Intercept	–0.166					
Hyperbolic (ω3-PUFA) + P (C : P)+ Hyperbolic (ω3-PUFA)*P (C : P)				0.0001	0.704	48.046
Hyperbolic (ω3-PUFA)	0.089	0.094	0.169	0.8664		
P (C : P)	–0.088	–0.025	0.186	0.8529		
Hyperbolic (ω3-PUFA)*P (C : P)	0.720	0.757	1.272	0.2091	0.031	
Intercept	0.265					

$$g_N = 1.158 \times [1 - \exp^{(-0.105 \times \omega 3 - PUFA - 0.152)}] \times \min[1, (1 - 0.607 \times (1 - 300/\mathrm{C} : \mathrm{P}_{\mathrm{algae}})] \quad (7)$$

where g_N is the normalised growth rate, ω3-PUFA is the ω3-PUFA content in algae (in nmol mg C^{-1}) and $C : P_{algae}$ is the algal molar C : P ratio. For actual growth rate:

$$g = 0.374 \times [1 - \exp^{(-0.127 \times \omega 3 - PUFA - 0.149)}] \times \min[1, (1 - 0.607 \times (1 - 300/\mathrm{C} : \mathrm{P}_{\mathrm{algae}})] \quad (8)$$

Discussion

According to some researchers, 'the phosphorus content of the algae was more important than the effect of the fatty acid composition' (Plath & Boersma, 2001), while others report simulation results suggesting factors other than phosphorus 'will be the primary determinants of algal food quality for most zooplankters in most lakes' (Brett *et al*., 2000). However, a lack of algal fatty acid data in most publications that study mineral limitation makes it difficult to examine directly the relative importance of those two hypotheses. In this study, we directly assessed the relative influences of EFA (using different algal species) and mineral content on algal food quality for *D. magna*.

Our results indicate that algal species affiliation and EFA content, especially the ω3-PUFA content, were better predictors of algal food quality for *D. magna* normalised growth ($r^2 = 0.69$; AIC = 35.004) than was algal P content ($r^2 = 0.11$; AIC = 55.156), although *D. magna* growth was related to both algal EFA and P content. The r^2 of *D. magna* growth vs. algal P content is weak compared with that reported by others (Sterner, 1993; Urabe & Sterner, 1996; DeMott *et al*., 1998). This may be partly because the large interspecific differences in algal food quality masked the P effect within algal species. If we apply the P-limitation model for the growth rates of *Daphnia* fed on *S. acutus* and *Synechococcus* sp. individually, the fits (r^2) increase to 0.25 and 0.50, respectively. This suggests that one can get a somewhat false impression of the importance of P-limitation if only one algal taxon is considered.

Several recent studies that found a positive correlation between seston EFA and phosphorus content argue that these food quality indices were interrelated in phytoplankton as an important class of lipids (phospholipids) is also rich in P (Ahlgren *et al*., 1997; Ahlgren *et al*., 1998). Another recent study reported that the phospholipid fraction of the seston was the best predictor of *Daphnia* growth ($r^2 = 0.50$) (De Lange & Arts, 1999). However, algal ω3-PUFA and P content were very weakly correlated ($r^2 = 0.002$ for C : P < 300; $r^2 = 0.127$ for C : P > 300) in the present study. These results do not necessarily support Ahlgren *et al*.'s (1997) hypothesis that both algal ω3-PUFA and EPA content are related to the P content in algae. Studies analysing both phospholipids and P in algae are rare; in daphnids the phospholipids are only a relatively small fraction (20–30%) of total P content (Vrede, Anderson & Hessen, 1999; D.C. Müller-Navarra, unpublished). Also, phospholipids are primarily structural lipids for membranes (Gurr & Harwood, 1991) and are believed to be fairly constant in zooplankton (Olsen, 1999). Thus, phospholipids may not be the main factor determining P metabolism in *Daphnia*. However, they may have a strong impact on lipid metabolism. For example, Farkas (1970) found that 40% of the lipids in *D. cucullata* SARS were phospholipids, in contrast to only 5% in *Eudiaptomus gracilis* SARS. Phosphorus bound to RNA may be more important in cladocerans, whereas P bound to phospholipids is more important in copepods (Carrillo, Reche & Cruz-Pizarro, 1996). In particular, RNA may constitute up to 10% of body mass in *D. magna* (McKee & Knowles, 1987).

Several researchers suggest that the low food quality of P-deficient algae might be because of an indirect effect via alterations in biochemical composition, such as reduced algal EPA (Müller-Navarra, 1995b) or linolenic acid content (Ahlgren *et al*., 1998). Studies showed that P-limitation reduced ω3-PUFA in the diatom species *Stephanodiscus hantzschii* Grunow var. *pusillus* and green algae *S. quadricauda* (Turpin) Brébisson (Ahlgren *et al*., 1998) and *Chlamydomonas reinhardtii* Dangeard (Weers & Gulati, 1997), while ω3-PUFA increased under P-limitation in *S. acutus* (Müller-Navarra, 1995a). In the present study, the ω3-PUFA content did not decrease with P-limitation (Fig. 1). Therefore, reduced *D. magna* growth rate with P-depleted algae in our experiments cannot be explained by the reduction in algal ω3-PUFA content. Evidence has accumulated that P-limitation is real and not an indirect effect of changes in algal fatty acid composition (Urabe *et al*., 1997; Weers & Gulati, 1997;

DeMott, 1998; Boersma, 2000). However, those studies have also shown that direct P deficiency explains from only 18–25% (Urabe *et al*., 1997; Boersma, 2000) to 67% (DeMott, 1998) of the growth reduction when related to P-sufficient *Scenedesmus*.

The present study does not support the finding that the algal ω3-PUFA content primarily influences food quality at low C : P ratio while the P content largely determines algal food quality at high algal C : P ratio (Sundbom & Vrede, 1997; Boersma, 2000). Our results suggest that, even under P-limitation, algal species affiliation and ω3-PUFA explained *D. magna* growth to a far greater degree than did the algal C : P ratio. However, when comparing our results with those of Sundbom & Vrede (1997), it has to be kept in mind that they used only one species of algae (*S. quadricauda*) in their experiments, although they altered the fatty acid spectrum by adding fatty acid emulsions.

In our study, the algal EFA content was uncoupled from the P content in determining algal food quality under P-limitation. However, this study unfortunately does not have enough data to demonstrate whether P-limitation and EFA limitation are acting independently. According to Liebig's Law of the Minimum, only one factor can be limiting at a certain time. This concept was applied with EFA to marine copepods using the stoichiometric approach (Anderson & Pond, 2000). Contrary to Liebig's Law, *D. magna* had a lower growth rate on P-depleted *Synechococcus* sp. food than with P-sufficient *Synechococcus* sp., indicating that P-depletion imposed an additional constraint on algal food quality beyond that imposed by the very low essential fatty acid content of this algae. The EFA and P limitation may be regarded as stressors for zooplankton. Accumulating studies on multiple stressors of zooplankton give a consensus that stressors are generally 'worse in combination than alone' (Cooney *et al*., 1983; Hanazato & Dodson, 1995; Folt *et al*., 1999). More detailed experiments are necessary to elucidate whether EFA and P act 'synergistically' or 'antagonistically' (Folt *et al*., 1999).

The present experimental study supports the outcome of a Monte-Carlo simulation which shows that algal taxonomy can explain four times more variation in *Daphnia* growth than did the seston C : P ratio (Brett *et al*., 2000). However, the relative importance of algal EFA content compared to the C : P ratio was greater than suggested by the simulation (Brett *et al*., 2000). The difference could be because of the fact that the average algal C : P ratio in the present study was lower (algal C : P = 212) than the mean of 276 observations of natural lake seston C : P ratios (average algal C : P = 292) reported by Brett *et al*. (2000). This means the algal C : P ratios in our experiments are somewhat biased against the P-limitation hypothesis. Inclusion of more cases with high C : P ratio algae would probably have increased the relative importance of P content to food quality in our study. However, our results cover the ecologically relevant range as Brett *et al*. (2000) showed that more than 90% of 276 observations had C : P ratios less than 600. Both the simulation and the present experimental study suggest that the algal species composition and algal EFA content are stronger predictors of *Daphnia* growth than algal phosphorus content.

Our study covers most of the realistic natural range of ω3-PUFA, with a range of 0.4–583 nmol FA mg C^{-1} (0.4–5.3 nmol FA mg C^{-1} for *Synechococcus* sp.). For example, ω3-PUFA levels were between 4 nmol FA mg C^{-1} (summer) and 63 nmol FA mg C^{-1} (spring) in Lake Berryessa (a large reservoir in California, USA; Park, 1999) while they were between 2.45 nmol FA mg C^{-1} in a hypereutrophic lake and 85 nmol FA mg C^{-1} in a oligotrophic lake (D.C. Müller-Navarra *et al*. unpublished). According to our combined model (equation 7 and 8), *D. magna* growth is predicted to be depressed if ω3-PUFA is below about 30 nmol FA mg C^{-1}. Therefore, under the natural conditions under which numerous algal species coexist, EFA limitation of *Daphnia* growth is highly likely except in oligotrophic lakes and during spring blooms of algae rich in EFA. However, our model is not complete because the present study does not include data for cases with very high C : P ratios (C : P > 600) and, especially, cases in which a high C : P ratio is coupled with a high ω3-PUFA content. Using the equation 8 obtained in the present study, we tested our model with data from independent studies (Weers & Gulati, 1997; D.C. Müller-Navarra, unpublished) which presented both fatty acid content and C : P ratios for cultured algal diets (Fig. 6). Overall, the observed growth rate of *D. galeata* Sars fed with green algae matched that predicted from our combined model. However, when considering the applicability of the model, it has to be kept in mind that here we measured growth of 4–7-day-old *D. magna* neonates raised in P-sufficient alga prior to the experiment. At this point we do not know to what

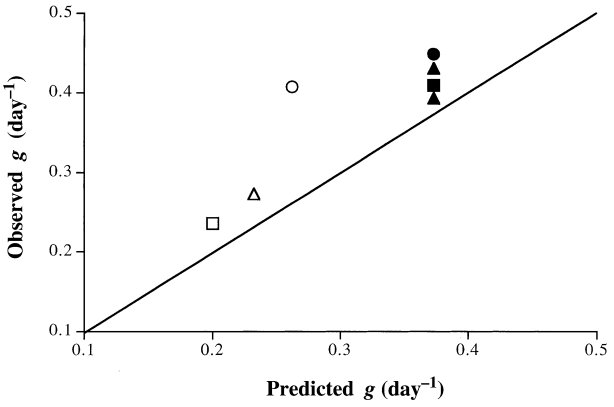

Fig. 6 Predicted growth rate of *Daphnia* from equations found in this study with ω3-PUFA content (nmol mg C^{-1}) and C : P ratio, vs. observed growth rate of *Daphnia galeata*. Data for P-saturated (C : P = 60) *Cyclotella meneghiniana* Kützing (●), P-deficient (C : P = 589) *C. meneghiniana* (○), P-saturated (C : P = 223) *Scenedesmus acutus* (■) and P-deficient (C : P = 1275) *S. acutus* (□) came from unpublished data for *D. galeata* by D.C. Müller-Navarra (see Müller-Navarra, (1995a) for experimental details). P-saturated (C : P = 64 and 81) *Chlamydomonas reinhardtii* (▲) and P-deficient (C : P = 796) *C. reinhardtii* (△) came from the *D. galeata* study of Weers & Gulati (1997). We placed a diagonal line on which observed values and predicted values were the same.

degree this pretreatment constrains the applicability of our model to daphnids of different physiological status. Although we could successfully apply our model to another *Daphnia* species (*D. galeata*), we cannot generalise from this whether it can be applied to all *Daphnia* or even to other cladoceran genera. Too little is known about physiological differences amongst the different cladocerans. Experiments with algae even more diverse in elemental (including higher C : P ratios) and biochemical (either as single species or as mixtures) composition and with several different *Daphnia* species should improve our model parameterisation. Both EFA and/or phosphorus limitation appear to be important in determining *Daphnia* growth in nature. For example, severe phosphorus limitation may occur because of high light : nutrient ratios (Urabe & Sterner, 1996; Sterner *et al*., 1997). Also, prolonged thermal stratification and phytoplankton blooms might suppress *Daphnia* growth. *Daphnia* growth would also be limited by dominance of low quality (low EFA content) phytoplankton species in the edible size range. Other variables such as size, nitrogen content, ingestibility, poor digestibility and toxicity of phytoplankton, probably interact with the effects of EFA and P content in determining overall food quality.

It is also possible that the fatty acid composition of the three different algal species might simply covary with some true (but unmeasured) limiting biochemical constituent of the algae, such as phospholipids, vitamins, trace elements or amino acids. However, we could show that algal taxonomy largely determined the essential fatty acid content, which was a much better predictor than algal elemental stoichiometry determined by nutrient availability. Our results indicate that EFA and P limitation influence algal food quality and these impacts appear to be independent of each other. Further research should attempt to apply these observations to natural seston.

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References

Ahlgren G., Goedkoop W., Markensten H., Sonesten L. & Boberg M. (1997) Seasonal variations in food quality for pelagic and benthic invertebrates in Lake Erken – the role of fatty acids. *Freshwater Biology*, **38**, 555–570.

Ahlgren G., Gustafsson I.-B. & Boberg M. (1992) Fatty acid content and chemical composition of freshwater microalgae. *Journal of Phycology*, **28**, 37–50.

Ahlgren G., Lundstedt L., Brett M.T. & Forsberg C. (1990) Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. *Journal of Plankton Research*, **12**, 809–818.

Ahlgren G., Sonesten L., Boberg M. & Gustafsson I.-B. (1996) Fatty acid content of some freshwater fish in lakes of different trophic levels – a bottom-up effect? *Ecology of Freshwater Fish*, **5**, 15–27.

Ahlgren G., Zeipel K. & Gustafsson I.-B. (1998) Phosphorus limitation effects on the fatty acid content and nutritional quality of a green alga and a diatom. *Verhandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie*, **26**, 1659–1664.

Anderson T.R. & Pond D.W. (2000) Stoichiometric theory extended to micronutrients: comparison of the role of

essential fatty acids, carbon, and nitrogen in the nutrition of marine copepods. *Limnology and Oceanography*, **45**, 1162–1167.

Blomquist G.J., Borgeson C.E. & Vundla M. (1991) Polyunsaturated fatty acids and eicosanoids in insects. *Insect Biochemistry*, **21**, 99–106.

Boersma M. (2000) The nutritional quality of P-limited algae for *Daphnia*. *Limnology and Oceanography*, **45**, 1157–1161.

Brett M.T. (1993) Comment on 'Possibility of N or P limitation for planktonic cladocerans: an experimental test' (Urabe and Watanabe) and 'Nutrient element limitation of zooplankton production' (Hessen). *Limnology and Oceanography*, **38**, 1333–1337.

Brett M.T. & Müller-Navarra D.C. (1997) The role of highly unsaturated fatty acids in aquatic food-web processes. *Freshwater Biology*, **38**, 483–499.

Brett M.T., Müller-Navarra D.C. & Park S.-K. (2000) Empirical analysis of the effect of phosphorus limitation on algal food quality for freshwater zooplankton. *Limnology and Oceanography*, **45**, 1564–1575.

Burnham K.P. & Anderson D.R. (1998) *Model Selection and Inference: a Practical Information-Theoretic Approach*. Springer, New York.

Carrillo P., Reche I. & Cruz-Pizarro L. (1996) Interspecific stoichiometric variability and the ratio of nitrogen to phosphorus resupplied by zooplankton. *Freshwater Biology*, **36**, 363–374.

Checkley D.M. *Jr* (1985) Nitrogen limitation of zooplankton production and its effect on the marine nitrogen cycle. *Archiv für Hydrobiologie Beiheft, Ergebnisse der Limnologie*, **21**, 103–113.

Cooney J.D., Beauchamp J.J. & Gehrs C.W. (1983) Effects of temperature and nutritional state on the acute toxicity of acridine to the calanoid copepod, *Diaptomus clavipes* Schacht. *Environmental Toxicology and Chemistry*, **2**, 431–439.

De Lange H.J. & Arts M.T. (1999) Seston composition and the potential for *Daphnia* growth. *Aquatic Ecology*, **33**, 387–398.

DeMott W.R. (1998) Utilization of a cyanobacterium and a phosphorus-deficient green alga as complementary resources by daphnids. *Ecology*, **79**, 2463–2481.

DeMott W.R., Gulati R.D. & Siewertsen K. (1998) Effects of phosphorus-deficient diets on the carbon and phosphorus balance of *Daphnia magna*. *Limnology and Oceanography*, **43**, 1147–1161.

DeMott W.R. & Müller-Navarra D.C. (1997) The importance of highly unsaturated fatty acids in zooplankton nutrition: evidence from experiments with *Daphnia*, a cyanobacterium and lipid emulsions. *Freshwater Biology*, **38**, 649–664.

Farkas T. (1970) Fats in fresh water crustaceans. I. Fatty acid composition of lipids obtained from *Eudiaptomus gracilis* and *Daphnia cucullata*. *Acta Biologica Academiae Scientiarum Hungaricae*, **21**, 225–233.

Folt C.L., Chen C.Y., Moore M.V. & Burnaford J. (1999) Synergism and antagonism among multiple stressors. *Limnology and Oceanography*, **44**, 864–877.

Gulati R.D. & DeMott W.R. (1997) The role of food quality for zooplankton: remarks on the state-of-the-art, perspectives and priorities. *Freshwater Biology*, **38**, 753–768.

Gurr M.I. & Harwood J.L. (1991) *Lipid Biochemistry: an Introduction*, 4th edn. Chapman & Hall, London.

Hanazato T. & Dodson D.I. (1995) Synergistic effects of low oxygen concentration, predator kairomone, and a pesticide on the cladoceran *Daphnia pulex*. *Limnology and Oceanography*, **40**, 700–709.

Hessen D.O. (1992) Nutrient element limitation of zooplankton production. *American Naturalist*, **140**, 799–814.

Hessen D.O. (1993) The role of mineral nutrients for zooplankton nutrition: reply to the comment by Brett. *Limnology and Oceanography*, **38**, 1340–1343.

Kattner G. & Fricke H.S.G. (1986) Simple gas-liquid chromatographic method for the simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. *Journal of Chromatography*, **361**, 263–268.

Kilham S.S., Kreeger D.A., Goulden C.E. & Lynn S.G. (1997) Effects of algal food quality on fecundity and population growth rates of *Daphnia*. *Freshwater Biology*, **38**, 639–647.

Lampert W. (1987) Feeding and nutrition in *Daphnia*. In: *Daphnia* (Eds R.H. Peters & R. De Bernardi), pp. 143–192. Istituto Italiano di Idrobiologia, Verbania, Pallanza.

Lampert W., Schmitt R.-D. & Muck P. (1988) Vertical migration of freshwater zooplankton: test of some hypotheses predicting a metabolic advantage. *Bulletin of Marine Science*, **43**, 620–640.

Lindström K. (1983) Selenium as a growth factor for plankton algae in laboratory experiments and in some Swedish lakes. *Hydrobiologia*, **101**, 35–48.

McKee M.J. & Knowles C.O. (1987) Levels of protein, RNA, DNA, glycogen and lipid during growth and development of *Daphnia magna* Straus (Crustacea: Cladocera). *Freshwater Biology*, **18**, 341–352.

Müller-Navarra D.C. (1995a) Biochemical versus mineral limitation in *Daphnia*. *Limnology and Oceanography*, **40**, 1209–1214.

Müller-Navarra D.C. (1995b) Evidence that a highly unsaturated fatty acid limits *Daphnia* growth in nature. *Archiv für Hydrobiologie*, **132**, 297–307.

Müller-Navarra D.C., Brett M.T., Liston A.M. & Goldman C.R. (2000) A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. *Nature*, **403**, 74–77.

Olsen Y. (1999) Lipids and essential fatty acids in aquatic food webs: what can freshwater ecologists learn from mariculture? In: *Lipids in Freshwater Ecosystems* (Eds M.T. Arts & B.C. Wainman), pp. 161–202. Springer-Verlag, New York.

Park S. (1999) *Essential Fatty Acid and Phosphorus Content as Food Quality Indices for Zooplankton Dynamics: Implications for Energy and Material Flows in Freshwater Pelagic Ecosystems.* PhD Dissertation. University of California, Davis.

Plath K. & Boersma M. (2001) Mineral limitation of zooplankton: stoichiometric constraints and optimal foraging. *Ecology*, **82**, 1260–1269.

Shifrin N.S. & Chisholm S.W. (1981) Phytoplankton lipids: interspecific differences and effects of nitrate, silicate and light-dark cycles. *Journal of Phycology*, **17**, 374–384.

Singer S.J. & Nicholson G.L. (1972) The fluid mosaic model of the structure of cell membranes. *Science*, **175**, 720–731.

Smith W.L. & Borgeat P. (1985) The Eicosanoids: prostaglandins, thromboxanes, leukotriens, and hydroeicosanoic acids. In: *Biochemistry of Lipids and Membranes* (Eds D.E. Vance & J.E. Vance), pp. 325–360. Benjamin/Cummings Publications. Co, Menlo Park, CA.

Solórzano L. & Sharp J.H. (1980) Determination of total phosphorus and particulate phosphorus in natural waters. *Limnology and Oceanography*, **25**, 56–760.

Sterner R.W. (1993) *Daphnia* growth on varying quality of *Scenedesmus*: mineral limitation of zooplankton. *Ecology*, **74**, 2351–2360.

Sterner R.W., Elser J.J., Fee E.J., Guildford S.J. & Chrzanowski T.H. (1997) The light: nutrient ratio in lakes: the balance of energy and materials affects ecosystem structure and process. *American Naturalist*, **150**, 663–684.

Sterner R.W. & Hessen D.O. (1994) Algal nutrient limitation and the nutrition of aquatic herbivores. *Annual Review of Ecology and Systematics*, **25**, 1–29.

Sterner R.W. & Schulz K. (1998) Zooplankton nutrition: recent progress and a reality check. *Aquatic Ecology*, **32**, 261–279.

Sundbom M. & Vrede T. (1997) Effects of fatty acid and phosphorus content of food on the growth, survival and reproduction of *Daphnia*. *Freshwater Biology*, **38**, 665–674.

Urabe J., Classen J. & Sterner R.W. (1997) Phosphorus limitation of *Daphnia* growth: is it real? *Limnology and Oceanography*, **42**, 1436–1443.

Urabe J. & Sterner R.W. (1996) Regulation of herbivore growth by the balance of light and nutrients. *Proceedings of National Academy of Sciences USA*, **93**, 8465–8469.

Urabe J. & Watanabe Y. (1992) Possibility of N or P limitation for planktonic cladocerans: an experimental test. *Limnology and Oceanography*, **37**, 244–251.

Urabe J. & Watanabe Y. (1993) Implications of seston elemental ratio in zooplankton ecology: reply to the comment by Brett. *Limnology and Oceanography*, **38**, 1337–1340.

Van Donk E. & Hessen D.O. (1993) Grazing resistance in nutrient-stressed phytoplankton. *Oecologia*, **93**, 508–511.

Van Donk E. & Hessen D.O. (1995) Reduced digestibility of UV-B stressed and nutrient-limited algae by *Daphnia magna*. *Hydrobiologia*, **307**, 147–151.

Van Donk E., Lürling M., Hessen D.O. & Lockhorst G.M. (1997) Altered cell wall morphology in nutrient-deficient phytoplankton and its impact on grazers. *Limnology and Oceanography*, **42**, 357–364.

Vrede T., Anderson T. & Hessen D.O. (1999) Phosphorus distribution in three crustacean zooplankton species. *Limnology and Oceanography*, **44**, 225–229.

Weers P.M.M. & Gulati R.D. (1997) Growth and reproduction of *Daphnia galeata* in response to changes in fatty acids, phosphorus, and nitrogen in *Chlamydomonas reinhardtii*. *Limnology and Oceanography*, **42**, 1584–1589.

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