



Food quantity and quality regulation of trophic transfer between primary producers and a keystone grazer (*Daphnia*) in pelagic freshwater food webs

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The transfer of energy and nutrients from plants to animals is a key process in all ecosystems. In lakes, inefficient transfer of primary producer derived energy can result in low animal growth rates, accumulation of nuisance phytoplankton blooms and dissipation of energy from the ecosystem. Most research on carbon transfer efficiency in pelagic food webs has focused on either food quantity or food quality, with the latter considered separately as either elemental stoichiometry or biochemical composition. The natural occurrence and magnitude of these types of growth limitations and their combined effects on *Daphnia*, a keystone grazer in pelagic freshwater ecosystems, are largely unknown. Our empirical models predict that the strength and nature of food quantity and quality limitation varies greatly with lake trophic state (total phosphorus, TP) and that *Daphnia* growth rates and thus energy and nutrient transfer efficiency are highest in lakes with intermediate trophic status (TP 10–25 $\mu\text{g l}^{-1}$). We predict that food availability place the greatest constraint on *Daphnia* growth in nutrient poor lakes (TP $\leq 4 \mu\text{g l}^{-1}$). Phosphorus limitation of *Daphnia* growth increased with decreasing TP, but the overall effect was never predicted to be the dominant constraining factor. Eicosapentaenoic acid (EPA, 20:5 ω 3) limitation was predicted to occur in both nutrient poor and nutrient rich lakes and placed the primary constraint on food quality in the most productive lakes. Two contrasting EPA-models gave different results on the magnitude of EPA-limitation, implying that additional food quality factors decrease *Daphnia* growth at high TP. In conclusion, the model predicts that *Daphnia* growth should peak in mesotrophic lakes, food quantity will place the greatest constraint on growth in oligotrophic lakes and EPA will primarily limit growth in eutrophic lakes.

The transfer of carbon across trophic levels in food webs is a central function in ecosystems and has been the subject of numerous studies since the pioneering work of Lindeman (1942). Animals that feed on plants or detritus play a critical role in the transfer of carbon, energy and nutrients in all food webs (Cebrian 2004), and the trophic transfer efficiency across the plant–herbivore interface is recognized to be highly dependent on food quantity and food quality (Sterner and Hessen 1994, Müller-Navarra and Lampert 1996, Brett and Müller-Navarra 1997). *Daphnia* is a keystone genus in pelagic freshwater ecosystems, providing a major link in the energy flow between primary producers and

secondary consumers in these food webs. When both food quantity and food quality are favourable, *Daphnia* gross growth efficiency is high and this makes more primary producer carbon available thereby promoting the growth of secondary consumers such as planktivorous fish. When food quantity and/or food quality limits *Daphnia* growth, carbon is transferred at lower rates into zooplankton biomass, and a substantial carbon pool can accumulate at the primary producer trophic level instead of being transferred up through the food web. This accumulation of primary producer biomass is associated with a wide range of negative conditions including poor water clarity, nuisance phytoplankton

blooms, hypoxia, and high dissolved organic carbon concentrations. Low transfer efficiency can also be associated with large respiratory losses from the consumers when they feed on nutritionally imbalanced food (Darchambeau et al. 2003, Hessen et al. 2004). Low energy transfer will also lead to reduced standing stocks of secondary consumers, such as fish.

When food conditions are optimal for *Daphnia* they are able to grow fast and build up a large population biomass and therefore exert greater top-down control over primary producer biomass and water clarity. Better food resource conditions also result in higher zooplankton reproduction rates, which in turn make the zooplankton more resilient to over-exploitation by upper trophic levels.

The growth of *Daphnia* is regulated by several factors, the most basic of which is the overall availability of food. Particulate carbon (partC) provides a good approximation of *Daphnia* resource availability, and at low food concentrations, insufficient carbon is available for investment in somatic growth and reproduction (Lynch 1989). Thus, *Daphnia* growth decreases gradually at food concentrations below $1 \text{ mg partC l}^{-1}$, and this decrease is particularly strong below approximately $0.4 \text{ mg partC l}^{-1}$ (McCauley et al. 1990, Müller-Navarra and Lampert 1996). Food quality can limit herbivore growth when the nutrient:carbon ratio for a required nutrient in the food is too low compared to the same ratio in the consumer (Sterner and Hessen 1994). A diet with a suboptimal nutrient content results in an excess intake of carbon, which has to be disposed of via defecation, excretion or respiration (Darchambeau et al. 2003, Anderson et al. 2005). This will result in low gross growth efficiencies in the herbivores. Consequently, food quality is a critical factor in determining the efficiency of energy and nutrient transfer in ecosystems.

In pelagic food webs most of the food quality research has focused on either elemental stoichiometry or polyunsaturated fatty acid composition. Whereas the negative effects of resource phosphorus to carbon ratios on *Daphnia* growth have been extensively documented (Hessen 1992, Sterner 1993, DeMott et al. 1998, Andersen et al. 2004) our understanding of essential fatty acid effects on animal nutrition is still evolving. However, the seston content of eicosapentaenoic acid, 20:5 ω 3 (EPA), has been shown to be important for *Daphnia* nutrition in several studies (Ahlgren et al. 1990, Brett and Müller-Navarra 1997, Ravet et al. 2003). Although both food quantity and food quality parameters have been shown to be important regulators of herbivore growth, it is still not clear where these factors place the greatest constraints on carbon transfer in natural environments.

In this study we use empirical models to combine and quantify the effects of food quantity and food

quality (seston P- and EPA-content) on *Daphnia* growth across a lake trophic status gradient. Our objective was to first find out how the food quantity and quality variables vary with lake trophic state and then predict the depression of *Daphnia* maximum growth potential as a function of seston quantity and quality across a wide gradient of lake trophic states representing ultraoligotrophic to hypereutrophic conditions. Expected depression of *Daphnia* growth rates relative to maximum growth potential were used as a proxy for depression of trophic transfer efficiency. This study is the first to predict the combined effects of food quantity and critical food quality variables within a probabilistic framework. It also places regulation of *Daphnia* growth into an environmental context by predicting the influences of seston quantity and quality as a function of lake trophic state.

Material and methods

Overall study design

We compiled data to assess the relationships between lake trophic state (total phosphorus concentration, TP, $\mu\text{g l}^{-1}$) and food quantity (particulate carbon, partC, mg l^{-1}), seston P-content (particulate phosphorus:carbon ratio, P:C, molar ratio), and the relative seston EPA-content (sestonic EPA:C ratio, $\mu\text{g mg}^{-1}$). Based on these associations we used a Monte Carlo approach to generate distributions of these food quantity and quality factors at specific TP levels. These distributions were used to predict the relative growth rate depression (RGD) of *Daphnia* maximum growth using published associations between *Daphnia* growth and partC, P:C, and EPA:C, respectively. Thus, we were able to connect lake TP concentrations with predicted depressions of *Daphnia* growth in a probabilistic manner across a wide range of lake trophic states.

Data compilation

Data on lake TP and sestonic PartC, P:C, and EPA:C were collected from published and unpublished studies in which TP measurements could be matched to at least one of the food quantity or food quality factors during the same sampling occasion (Table A1). The seston data was generally collected from the epilimnion during a May to October sampling season. One value per lake and year was included in our analyses; multiple sampling values collected during a single year were averaged. The large majority of observations (>90%) came from temperate and subarctic lakes in North America and Europe which merely reflects where most limnological research is conducted. However, data from arctic regions as well as Asian and African lakes were

also included. Only one study reporting the relationship between lake TP concentration and seston EPA:C content was found (Müller-Navarra et al. 2004). In addition, data on TP and EPA:C from 17 temperate and sub-alpine lakes were used (Persson and Vrede, unpubl.). These data come from the 12 lakes described in Persson and Vrede (2006), as well as from four additional oligotrophic lakes in the same region and the mesotrophic Lake Erken. All samples were taken and analysed as described in Persson and Vrede (2006).

Modelling the relationships between TP and food quantity and food quality

The statistical software package JMP IN 5.1 (SAS Inst.) was used to generate regression equations between TP and partC, P:C, and EPA:C. Data were log₁₀-transformed prior to analysis. We acknowledge that spurious correlation (Brett 2004) could distort the R² value for the P:C regression. A recent statistical analysis of seston composition (Hessen et al. 2003), which is not prone to spurious correlations (since they compared independent terms), showed that low P:C ratios were associated with high relative contributions of non-autotrophic carbon to the partC pool in oligotrophic lakes where low P:C ratios were most likely to occur. TP was also identified as the main driver of sestonic C:P ratios in a recent study focusing on this subject (Hessen 2006).

Using the regression equations reported in the legend to Fig. 1 and their associated standard error of the estimate (SEE) we simulated 1000 estimates of partC, P:C, and EPA:C, respectively, at each of nine chosen TP levels. The TP concentrations we used for the simulations were 2.5, 4, 6.3, 10, 16, 25, 40, 63 and 100 µg l⁻¹. These represent a wide range of ecologically relevant lake trophic states from ultraoligotrophic to hypertrophic conditions, spanning a wide range of variation in the food quantity and food quality variables considered, while still being confined within the limits of the TP concentrations observed in the compiled data set. The TP concentrations are distributed approximately log-normally, and the higher resolution in the low TP end can be justified because the range 0–25 µg l⁻¹ encompasses the large majority of lakes (87%; Fig A1). The simulations were done by first generating normally distributed random numbers with a mean of 0 and a standard deviation equal to the SEE from each of the three regression models. For this we used the random number generator in Microsoft Excel, with a unique random seed for each food variable. To the random numbers we subsequently added the point prediction values at each TP concentration, using the regression equations in the legend of Fig. 1. Thus our predictions for partC, P:C, and EPA:C were normally distributed around the regression formula point pre-

dictions. The food parameter values were assumed to be normally distributed around the trend line, and the variability around the mean was assumed to be homogenous along the TP gradient. These simulated numbers were then retransformed to linear scale. Since the retransformation of log₁₀ models back into linear space introduces a bias (Sprugel 1983), our regression model predictions were corrected by dividing the retransformed values with a correction factor (CF; Eq. 1), where SEE is the standard error of the regression model estimate (Fig. 1), before the data were entered into the *Daphnia* growth models

$$CF = 10^{2.303 \times SEE^2/2} \quad (\text{Sprugel 1983}) \quad (1)$$

For example, for partC we first generated nine columns with 1000 values in each with the mean zero, and with the standard deviation 0.237. For the first column, representing log₁₀ partC at TP 2.5 µg l⁻¹, we then added the log₁₀ value of partC that the correlation predicted at TP 2.5 µg l⁻¹. And for the second column, for TP 4 µg l⁻¹, the predicted log₁₀ value of partC at TP 4 µg l⁻¹ was added, and so on. Each of these simulated numbers were then retransformed and corrected for bias as described above. The retransformed numbers, which estimate the food variables at each of the nine TP levels, were subsequently entered into growth models that predicted *Daphnia* growth depression as a function of food quantity and food quality.

Modelling *Daphnia* growth response

Using the output from the modelling of the food quantity and food quality variables as functions of lake trophic state (i.e. 1000 numbers for each food variable at each TP), we calculated the depression of *Daphnia* growth relative to their maximum growth potential (Relative growth depression, RGD) as a function of the food variables (Eq. 2–5) and the associated uncertainties of these equations. Each TP level thus gave estimates of each food variable, and the food variables in turn gave estimates of *Daphnia* RGD.

$$RGD_{\text{partC}} = e^{-4.3(\text{partC}-0.014)} \quad (2)$$

(Müller – Navarra and Lampert 1996)

$$RGD_{\text{P:C}} = 1 - \text{P:C}/0.00333 \quad (3)$$

(Brett et al. 2000)

$$RGD_{\text{EPA:C}} = e^{-0.47(\text{EPA:C}-0.081)} \quad (4)$$

(Müller – Navarra et al. 2000)

$$RGD_{\text{EPA:C}} = e^{-1.87(\text{EPA:C}+0.213)} \quad (5)$$

(Ravet, Persson and Brett, unpubl.)

RGD is related to relative growth rate (RGR) as $RGD = 1 - RGR$. All equations thus give effects on

Daphnia somatic growth in terms of a fraction of the maximum growth potential that is lost due to food quantity or quality deficiency. PartC was entered as mg C l⁻¹, P:C as molar ratios and EPA:C as µg EPA mg C⁻¹. The uncertainties of Eq. 2–5 were incorporated into our calculations by adding random numbers with a mean of 0 and a standard deviation equal to the SEE of the models (Eq. 2–5) to the point predictions of the RGDs. For each combination of TP and food quality variable, 1000 random numbers were generated as above. The SEE for Eq. 2–5 were 0.017 for RGD_{partC} (Eq. 2), 0.20 for RGD_{P:C} (Eq. 3), 0.079 for RGD_{EPA:C} (Eq. 4), and 0.056 for RGD_{EPA:C} (Eq. 5). For example, if the expected seston P:C ratio was 0.0011, then the RGD_{P:C} would be 0.67 ± 0.20. We got the SEE of Eq. 2–5 by fitting the given equations to the datapoints of the cited studies. However, the SEE for Eq. 3 was based on an unpublished analysis using the data from Brett et al. (2000).

In order to avoid biologically unrealistic values, the results from Eq. 3 were set to zero for P:C ratios above 0.00333, and resulting negative values of RGD_{partC} and RGD_{EPA:C} using Eq. 5 were set to zero. It should also be noted that Eq. 2–5 cannot be applied at any arbitrary values of the independent variables, but only within the empirically observed range of variation (Fig. 1). For example, RGD would be > 1 for EPA:C ratios smaller than 0.014, which is not biologically reasonable. However, such low EPA:C ratios were not observed, and consequently never used in the calculations.

Equation 4, *Daphnia* growth based on EPA-concentration from Müller-Navarra et al. (2000), was refit to an asymptotic maximum growth rate of 0.55 d⁻¹. This can be justified by empirical data showing that observed maximum specific growth rates of *Daphnia* spp. fed high quality food often lie within the range 0.5–0.6 d⁻¹, (Müller-Navarra and Lampert 1996, Ravet et al. 2003, Brett et al. 2006). Using this lower growth rate reduces the predicted influence of EPA on RGD. Equation 5 is based on *Daphnia* feeding on EPA supplemented cultured algae, providing an alternative to Eq. 4 that is based on *Daphnia* fed field collected seston. It should be noted that the main difference between Eq. 4 and Eq. 5 is that Eq. 4 comprises all components of food quality that differ between the cyanobacteria-dominated and the diatom-dominated periods in that study, whereas Eq. 5 only includes manipulations of the EPA-content of the food (keeping all other components constant). Since other food quality variables, e.g. sterols (von Elert and Wolffrom 2001, von Elert et al. 2003) and amino acids (Laabir et al. 1999), may be correlated with dietary EPA-content, this is an important distinction between these studies, and the difference in results between these equations

may be interpreted as the contribution of unknown factors (e.g. sterols and amino acids) to RGD.

How the effects of the growth limiting variables combine are largely unknown and we therefore present predictions based on assumptions representing worst and best case scenarios. Thus, we calculated the total RGD as a function of partC, P:C ratio, and EPA:C content of the food either as a multiplicative interaction (worst case scenario; Eq. 6) or a max function (best case scenario; Eq. 7):

$$\text{RGD}_{\text{tot}} = 1 - (1 - \text{RGD}_{\text{partC}}) \times (1 - \text{RGD}_{\text{P:C}}) \times (1 - \text{RGD}_{\text{EPA:C}}) \quad (6)$$

$$\text{RGD}_{\text{tot}} = \max(\text{RGD}_{\text{partC}}; \text{RGD}_{\text{P:C}}; \text{RGD}_{\text{EPA:C}}) \quad (7)$$

where the max function returns the maximum value of RGD_{partC}, RGD_{P:C}, and RGD_{EPA:C}.

The biological meaning of the multiplicative model is that food quantity and food quality can, at the same time constrain animal growth. The food quantity being the primary resource which can be used with different efficiency depending on its quality (expressed as the concentration of that element or compound in the biomass of the food resource). Implicit in Eq. 6 is that food quality is important also when food quantity is low (Boersma and Kreuzer 2002, Acharya et al. 2004), and that EPA:C is of nutritional importance even when phytoplankton P:C ratios are less than 0.0033, i.e. at C:P ratios greater than 300 (Ravet and Brett 2006). It can be argued that RGD_{tot} approaches 1 if a large number of food factors are used in a multiplicative model, but this is not a problem when only using a few factors, especially if these factors are not related in the same way to TP.

In contrast to the multiplicative model, the best case scenario is modelled as a max model that treats the resources as essential and non-substitutable, and only the most limiting factor can affect growth. The biological meaning of this is that if food resources are abundant, there is only one food quality factor that can limit *Daphnia* growth at the time. Likewise, at low food quantities (at or close to the starvation point), the energy in the food is almost completely used for maintenance metabolism whereas dietary P and EPA are not needed for biosynthesis of new biomass.

Equation 6 may yield an overestimation of RGD_{tot} since it assumes multiplicative factor interactions. Equation 7 yields a more conservative estimate of RGD_{tot} since only the factor having the largest predicted influence will affect the growth rate, no factor interactions. The two models we use can be expected to be endpoints among biologically reasonable models, and we believe that the true RGD_{tot} can be found within the range of these two models.

Results

Seston partC and P:C ratio were significantly positively correlated to TP (Fig. 1a-b). The EPA:C ratio of seston showed a significant unimodal relationship with TP, with the highest EPA:C ratios at approximately TP $10 \mu\text{g l}^{-1}$ (Fig. 1c). Low partC in oligotrophic systems coincided with a large RGD of *Daphnia*, and there was a 0.52 ± 0.19 (mean \pm SD) depression of the potential growth due to low food quantity at TP $2.5 \mu\text{g l}^{-1}$ (Fig. 2a). Growth depression due to food availability decreased with increasing TP and was virtually absent at TP 63 and $100 \mu\text{g l}^{-1}$. The RGD due to P:C was highest at TP $2.5 \mu\text{g l}^{-1}$ (0.17 ± 0.23), declined to 0.09 ± 0.18 at TP $6.3 \mu\text{g l}^{-1}$, and decreased to even lower levels at higher TP (Fig. 2b). When *Daphnia* growth was calculated based on the EPA-content of field seston (Eq. 4) the growth depression due to low EPA:C was at a minimum of 0.43 ± 0.18 at TP $10 \mu\text{g l}^{-1}$, and gradually increased with decreasing TP to 0.56 ± 0.17 at TP $2.5 \mu\text{g l}^{-1}$ and with increasing TP to 0.75 ± 0.15 at TP $100 \mu\text{g l}^{-1}$ (Fig. 2c). The EPA:C model based on EPA supplementation experiments (Eq. 5) predicted *Daphnia* RGD with a maximum of 0.19 ± 0.11 at TP $100 \mu\text{g l}^{-1}$, a minimum RGD of 0.05 ± 0.06 at TP $10 \mu\text{g l}^{-1}$, and intermediate RGD (0.08 ± 0.07) at TP $2.5 \mu\text{g l}^{-1}$ (Fig. 2d).

The combined growth depression, RGD_{tot} was calculated both using Eq. 4 and 5, and both with the multiplicative (Eq. 6) and the max (Eq. 7) model (Fig. 3). When using Eq. 4 for the EPA-limitation, *Daphnia* RGD_{tot} were lowest at TP 16 and $25 \mu\text{g l}^{-1}$, reaching on average 0.53 ± 0.18 at these TP concentrations when the multiplicative factor interaction (Eq. 6) was used (Fig. 3a). When no factor interaction was included and only the most dominating factor was in effect at each TP (i.e. using Eq. 7) the minimum RGD_{tot} of *Daphnia* were 0.46 ± 0.16 and 0.45 ± 0.17 , at TP 10 and $16 \mu\text{g l}^{-1}$ (Fig. 3b). When the EPA-limitation of Eq. 5 was used with the multiplicative Eq. 6, the minimum *Daphnia* RGD_{tot} of 0.14 ± 0.13 and 0.13 ± 0.11 were found at TP 25 and $40 \mu\text{g l}^{-1}$ respectively (Fig. 3c). And with no interaction RGD_{tot}

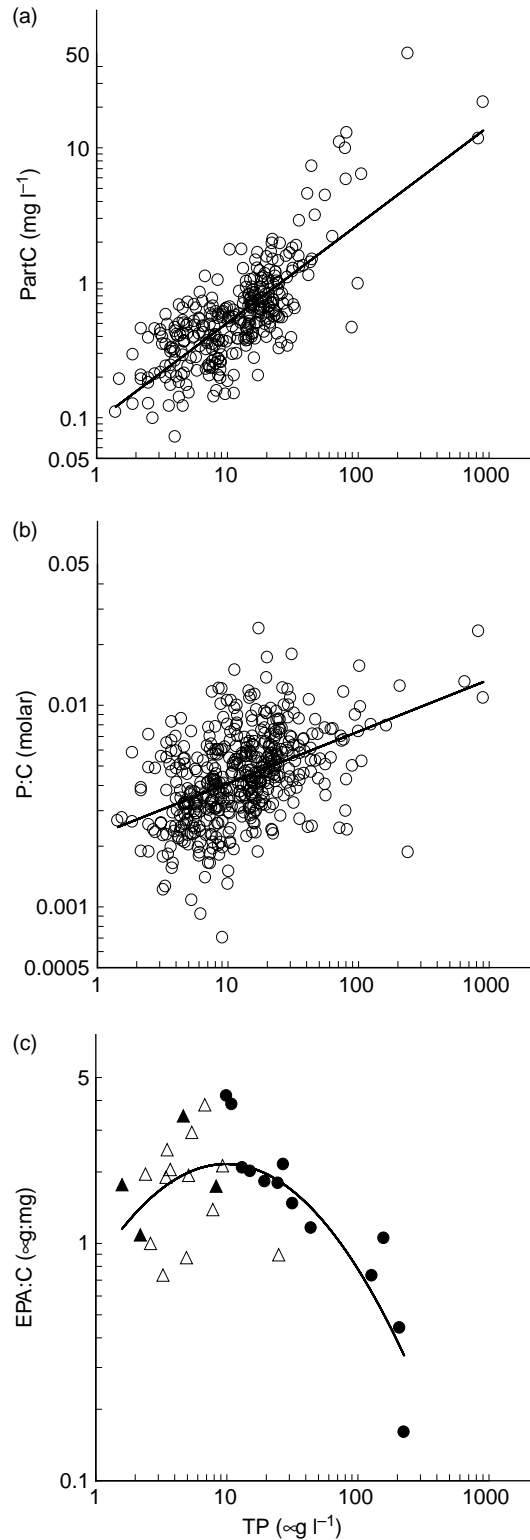
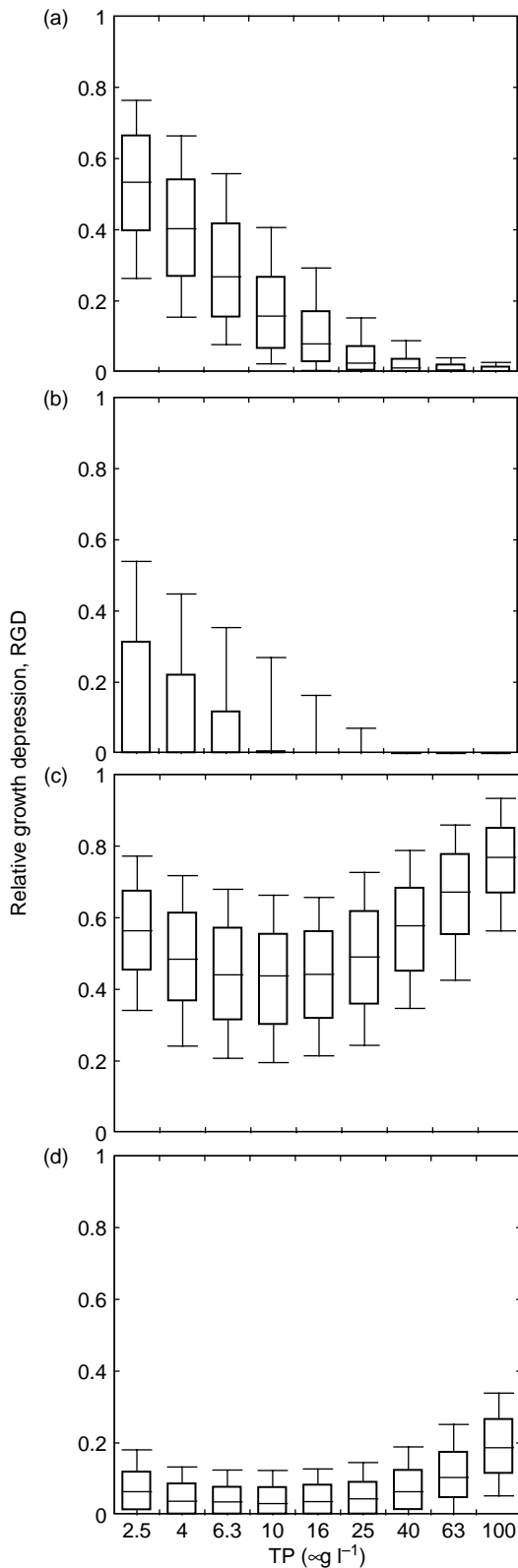


Fig. 1. Regressions between total phosphorus (TP) and seston variables. (a) For particulate carbon, $\log_{10}(\text{partC}) = 0.72 \times \log_{10}(\text{TP}) - 1.02$; $n = 314$; $p < 0.0001$; $R^2 = 0.59$, standard error 0.237. (b) For P:C ratio, $\log_{10}(\text{P:C}) = 0.26 \times \log_{10}(\text{TP}) - 2.64$; $n = 485$; $p < 0.0001$; $R^2 = 0.21$, SE 0.198. (c) For EPA:C ratio, $\log_{10}(\text{EPA:C}) = 0.40 - 0.061 \times \log_{10}(\text{TP}) - 0.44 \times [\log_{10}(\text{TP}) - 1.1]^2$; $n = 30$; $p < 0.0001$; $R^2 = 0.58$, SE 0.199. Observations presented by \bullet are from Müller-Navarra et al. (2004), and those by Δ or \blacktriangle are from Persson and Vrede (unpubl.), empty symbols were $40 \mu\text{m}$ prefiltered.



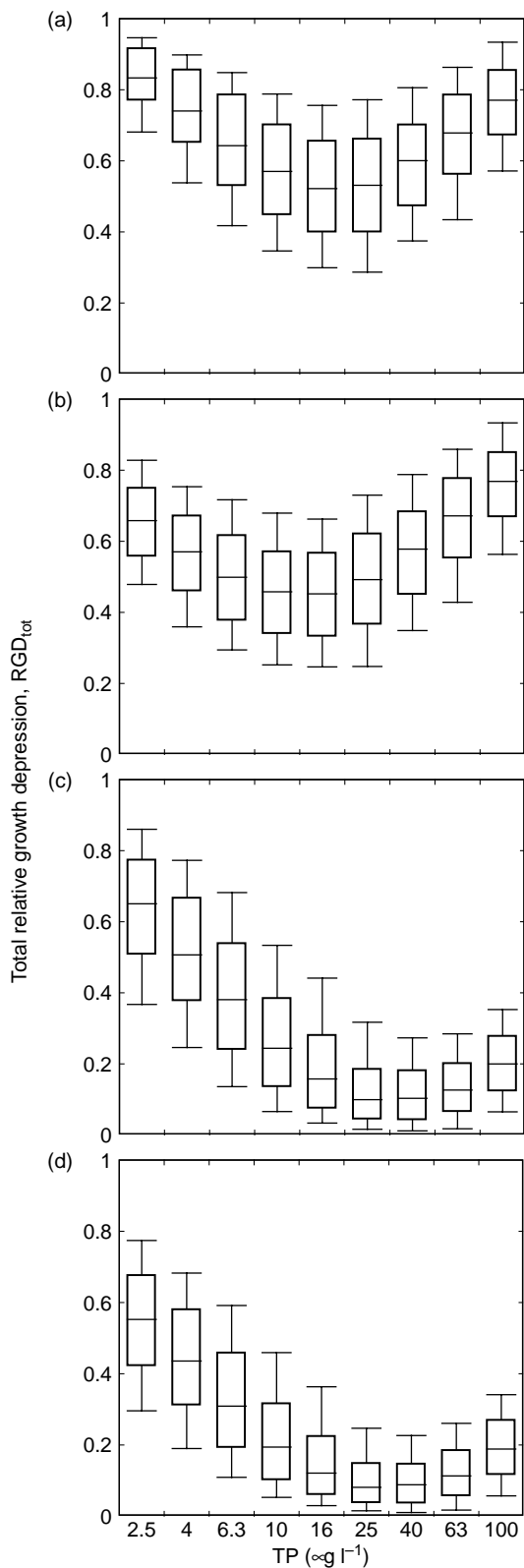
the minimums were 0.11 ± 0.11 at both TP 25 and $40 \mu\text{g l}^{-1}$ (Fig. 3d).

Discussion

Our results predict that the trophic transfer of energy and nutrients across the plant–animal interface in planktonic systems will on average be most efficient in lakes of intermediate nutrient content. In nutrient poor lakes we predict that low food quantity exerts the greatest constraint on herbivore production, while in nutrient rich lakes essential fatty acid limitation exerts the greatest constraint. Furthermore, *Daphnia* growth ought to be constrained by food quantity and/or quality across the entire range of lake trophic states, i.e. the maximum growth potential is not expected to be reached at any TP. PartC, P:C and EPA:C all covaried significantly with lake TP concentration, implying that both food availability and food quality vary over the investigated TP range.

Apart from TP, there are several other environmental factors such as mixing depth (Diehl et al. 2002), light intensity (Urabe and Sterner 1996), UV light intensity (Leu et al. 2006) and trophic cascades (Carpenter et al. 1985) that also influence the quantity and quality of seston, either directly or indirectly. The point predictions of food quality and food quantity variation along the TP gradient therefore does not give the whole picture of factors constraining secondary production. However, the effects of these factors contribute to the scatter in the compiled data in Fig. 1 and are therefore indirectly included in the error terms of our model simulations. There may also be a considerable within-year variation in food quality and food quantity that is not captured by our model. Both food quantity and food quality has been shown to vary seasonally (Müller-Navarra and Lampert 1996), and there may thus be periods during the year when our model predictions either systematically under- or over-estimate the degree of food quantity or food quality limitation in lakes with specific trophic states. For example, the clear-water phase in early summer is a period with comparatively low food quantity, but presumably high food quality in terms of fatty acids because the phytoplankton community is dominated by

Fig. 2. The depression of *Daphnia* relative growth rate across a gradient of total phosphorus. (a) For particulate carbon. (b) For the seston phosphorus to carbon ratio. (c) For the field seston concentration of EPA (eicosapentaenoic acid, 20:5 ω 3), using Eq. 4. (d) For the concentration of EPA in supplemented phytoplankton cultures, using Eq. 5. The boxed line is the median observation, the margins of the boxes indicate the 25th and 75th percentiles, and the whiskers indicate the 10th and 90th percentiles.



taxa with known high concentrations of e.g. EPA. Likewise, in eutrophic lakes, the food quality can be expected to be much higher during the spring bloom when diatoms dominate compared with the late summer situation when cyanobacteria and/or green algae typically dominate (Sommer et al. 1986). It should therefore be kept in mind that our model predictions give a quantitative picture of the seasonal average influence of food quality and food quality over a wide range in trophic states.

This is the first study that tries to quantify the effect of P-limitation on *Daphnia* growth across a wide spectrum in lake TP concentrations. The predicted effect of P-limitation was smaller than expected from previous research (Hessen 1992, Gulati and DeMott 1997, Sterner and Elser 2002). However, often this research has concentrated on the effects of low P:C ratios on various aspects of *Daphnia* growth by forcing the P:C ratio of cultured algae to levels that, judging from this compilation, are rare in natural lake seston. Both correlative and experimental studies have found P-limitation of *Daphnia* in natural environments (Hessen 1992) and there are also eutrophic lakes in which P-limitation is an important regulating factor, at least during parts of the season (DeMott and Gulati 1999). But the large scale trend of our study is that, on average, the biggest effects of low P:C should be found in lakes of very low TP.

This study adds observations from extremely nutrient poor lakes to the previously published covariation between TP and EPA:C (Müller-Navarra et al. 2004). These new data expand our knowledge by indicating that relative EPA availability is highest in mesotrophic and moderately oligotrophic lakes, but that it decreases in both more oligotrophic and more eutrophic lakes. Both P and EPA were less concentrated in the seston of oligotrophic lakes (Fig. 1b-c). This could be due to a dilution of living phytoplankton with detrital carbon of autochthonous or allochthonous origin (Hessen et al. 2003). Alternatively, low nutrient:C ratios in phytoplankton can be associated with high water clarity (Urabe and Sterner 1996, Sterner et al. 1997).

The *Daphnia* growth rate depressions calculated using Eq. 4 (Fig. 2c) were based on experiments with natural seston dominated by diatoms or cyanobacteria (Müller-Navarra et al. 2000). It is reasonable to assume

Fig. 3. The total relative growth rate depression of *Daphnia* across the gradient of total phosphorus. (a) According to the field seston model for EPA:C growth depression, using the multiplicative interaction between factors, Eq. 6. (b) According to the field seston model for EPA:C growth depression using the major limiting factor, Eq. 7. (c) According to the EPA addition model based on Ravet, Persson and Brett (unpubl.) for EPA:C depression from Eq. 6. (d) According to the EPA addition model based on Ravet, Persson and Brett (unpubl.) for EPA:C depression, from Eq. 7.

that food quality factors other than EPA:C differed between the diatoms and the cyanobacteria and that the study therefore overestimated the growth depressing effects due to EPA. The large differences between the results from using field seston (Fig. 2c) and supplemented lab cultures (Fig. 2d) points out that factors other than EPA have large effects on secondary production, such as availability of other fatty acids, dietary sterols (von Elert and Wolffrom 2001, von Elert et al. 2003) and amino acids (Laabir et al. 1999), toxicity (Lampert 1981), or digestibility (Porter 1975). These factors may have caused additional differences in food quality among the samples reported on in Müller-Navarra et al. (2000). A developing field of research is the effect of dietary sterols on secondary production (von Elert and Wolffrom 2001, von Elert et al. 2003). The negative effects on secondary production due to sterol deficiency can be expected to be particularly strong when cyanobacteria dominate (von Elert et al. 2003). Cyanobacteria make up an increasing percentage of seston biomass as TP increases (Downing et al. 2001) and it is, therefore, likely that the negative effects of sterol deficiencies will increase with higher TP. The results from Eq. 4 might have exaggerated the $RGD_{EPA:C}$ at low TP since cyanobacteria, and e.g. sterol deficiency, is unlikely to occur in these lake types. There is at present very little information available regarding relationships between sestonic sterol content and lake TP, which would have been necessary to include sterol effects in this study.

When using Eq. 4 to represent EPA-limitation the maximum *Daphnia* growth rates were predicted to occur around lake TP 10–25 $\mu\text{g l}^{-1}$, whereas when using Eq. 5, this maximum was predicted to occur at 25–40 $\mu\text{g l}^{-1}$. Factors that negatively affect *Daphnia* growth tend to increase as lakes become more eutrophic, however these other factors are not taken into account in Eq. 5 since this is solely based on an EPA-response, as discussed above. If these were incorporated into the results of Eq. 5 more growth reduction would probably occur at higher TP and the result would be more similar to the maximum predicted *Daphnia* growth rates occurring at lake TP 10–25 $\mu\text{g l}^{-1}$. The trophic transfer efficiency maximum based on our model is therefore most likely found in this latter interval.

Equation 2–5 were based on the somatic growth of juvenile *Daphnia*, this measure has been shown to be closely related to population growth rate when food quantity was varied (Lampert and Trubetskova 1996), but there could be differences when considering the effects of P- and EPA-limitation. The concentration of EPA was higher in *Daphnia* eggs than in *Daphnia* somatic tissue in the study by Becker and Boersma (2005), implying that the EPA required for egg production might be higher.

The *Daphnia* growth formulas were based on different *Daphnia* species: Eq. 2 on *D. galeata*, Eq. 3 on averages for many experiments and *Daphnia* taxa, Eq. 4 on *D. magna*, and Eq. 5 on *D. pulex*. Preferably all equations would have been based on averages of many studies like Eq. 3 to minimize species effects. Large-bodied *Daphnia* species have lower starvation thresholds for food quantity than small-bodied (Gliwicz 1990), and most likely the same is true for the shape of their functional response curves (Eq. 2). The large *D. magna* can be expected to be less affected by food quantity limitation than what is predicted by this model since Eq. 2 was based on results on *D. galeata*, a smaller species. Stoichiometric theory predicts that fast growing, P-rich, organisms will be more vulnerable to P-limitation than those with slower growth and lower P-content (Sternner and Elser 2002). But in a comparison of ten *Daphnia* taxa no such relationship was found (DeMott and Pape 2005), making it hard to estimate the effects of P-limitation on different *Daphnia* species. There may also be differences amongst *Daphnia* species in their growth response to different food constraints which reflect local adaptations to prevailing environmental conditions. There is thus latitude for differences among systems, and also seasonal differences in terms of the effect of varying food quantity and food quality, but to our knowledge there are no studies that have addressed these issues.

This study provides a step towards better understanding of when food quantity and/or food quality are of importance but future studies are required to empirically test both the underlying assumptions of the models and the model predictions. First, the models need to be tested. It is not clear whether Eq. 6 (which assumes an interaction between the factors), Eq. 7 (which assumes that resources are essential and non-substitutable) or another potential model (that assume e.g. that resources are substitutable and/or non-essential) is the best descriptor of how food quality factors interact. The empirical support for any of these models is at present weak or absent, and there is a need for empirical tests of these alternative models. However, we would like to emphasise that Eq. 6 and Eq. 7 yield similar predictions in terms of at which TP secondary production is most constrained. Second, to test the model predictions regarding limiting agents and magnitude in RGD there is a need for simultaneous food quantity and food quality measurements in situ complemented with growth experiments with natural seston and factorial manipulations of food quantity and food quality (i.e. P- and EPA-content of food).

In conclusion, our models predict that the strength and nature of food quantity and quality limitation of *Daphnia* growth varies with lake trophic state, and that some combination of food quantity and/or quality limitation should be expected in nearly all lakes. Food

availability was predicted to be the dominant constraint on *Daphnia* growth in the most nutrient poor conditions and EPA-limitation the dominant constraint in the most productive lakes. Furthermore, *Daphnia* growth rates are expected to be highest in lakes with intermediate trophic status. Our model thus make predictions of patterns that can be tested in empirical investigations, and together with potential future results we believe that this study holds promise to enhance our understanding of how carbon transfer efficiency is linked to lake trophic status, and thus improve our ability to predict effects of environmental changes on ecosystem processes and biogeochemical cycles.

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Appendices

The following supplementary material is available online at www.oikos.ekol.lu.se as O15639:

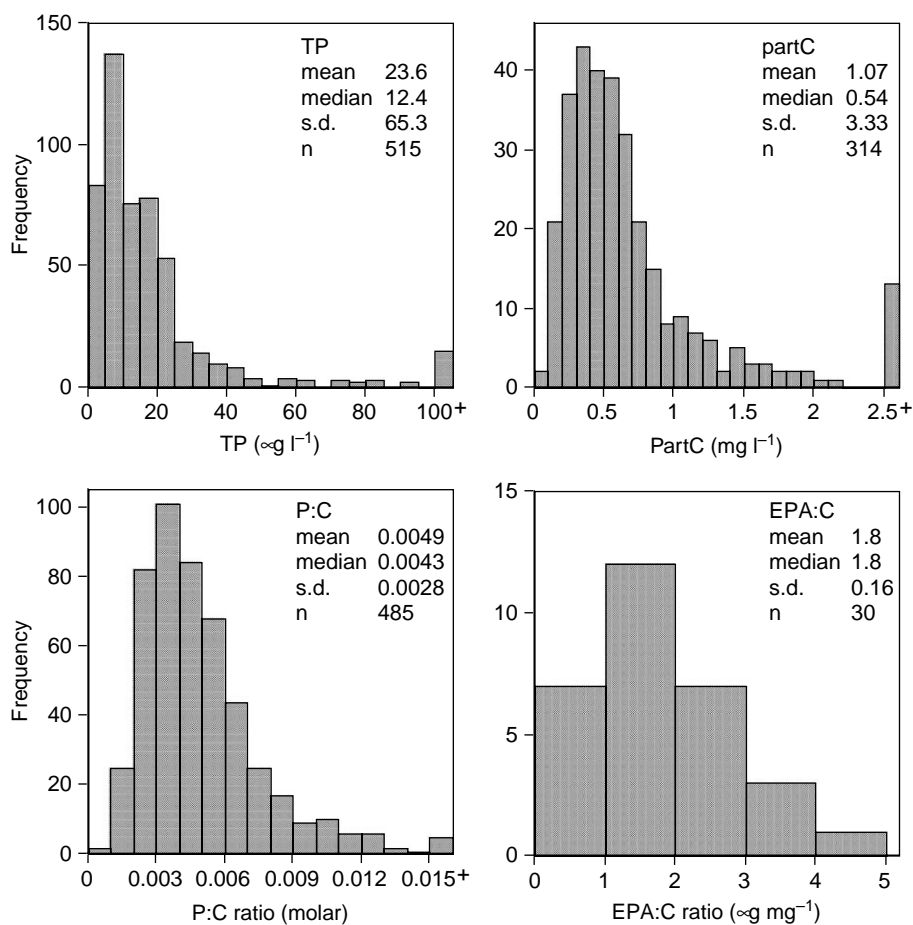


Fig. A1. Frequency histograms summarizing the dataset of total phosphorus (TP), particulate carbon (partC), phosphorus:carbon ratios (P:C), and EPA:C ratios. All unique TP observations were included in the TP distribution graph and 30 TP observations from the EPA:C dataset were added ($n = 485 + 30$). n number of observations.

Table A1. Number of samples and sources of the compiled data of total phosphorus (TP), particulate carbon (partC), and P:C ratios.

TP	PartC	P:C	Sampling season	Source
1		1	April	Aizaki, M. & Otsuki, A. Japan. J. Limnol., 48, 99–106 (1987)
2	2	2	Entire year	Blomqvist, P., Bell, R.T., Olofsson, H., Stensdotter, U. & Vrede, K. Can. J. Fish. Aquat. Sci., 52, 551–565 (1995)
5		5	Entire year	Brett, M.T., unpublished
9	9	9	April–October	Broberg, O. Hydrobiologia, 150, 11–24 (1987)
1		1	Entire year	De Haan, H. & De Boer, T. Freshw. Biol., 16, 661–672 (1986)
3	3	3	May–October	DeMott, W.R. & Gulati, R.D. Limnol. Oceanogr., 44, 1557–1564 (1999)
1		1	Summer	Elser, J.J. & George, N.B. J. Plankton Res., 15, 977–992 (1993)
2	2	2	Summer	Elser, J.J., Chrzanowski, T.H., Sterner, R.W. & Mills, K.M. Ecoscience, 5, 120–136 (1998)

Table A1 (Continued)

TP	PartC	P:C	Sampling season	Source
118	118	118	May–October	Fee E.J. <i>et al.</i> Can. Tech. Rep. Fish. Aquat. Sci., 1662, 1–39 (1989)
9		9	June–September	Guildford, S.J. <i>et al.</i> Can. J. Fish. Aquat. Sci., 51, 2769–2786 (1994)
2		2	Not available	Guildford, S.J. & Hecky, R.E. Limnol. Oceanogr., 45, 1213–1223 (2000)
1	1	1	May–November	Halfon, E.J. Great Lakes Res., 10, 299–306 (1984)
51	51	51	Ice-free season	Hecky, R.E., Campbell, P. & Hendzel, L.L. Limnol. Oceanogr., 38, 709–724 (1993)
125		125	May–September	Hessen, D. O. & the Norwegian Institute of Water Research, unpublished
5		5	April–December	Hochstädter, S. Freshw. Biol., 44, 453–63 (2000)
9	9	9	April–November	Johengen, T.H., Nalepa, T.F., Fahnenstiel, G.L. & Goudy, G. J. Great Lakes Res., 21, 449–464 (1995)
11	11	11	August	Jones, J. unpublished
30	30	30	July & September	Kunz, T.J. & Diehl, S. Freshw. Biol., 48, 1050–1063 (2003)
13	13	13	May–October	Lindström, E. unpublished
1	1	1	Entire year	Mukanomeje, R., Plisnier, P.D., Descy, J.P. & Massaut, L. Hydrobiologia, 257, 107–120 (1993)
3	3	3	Late monsoon – dry season	Nakanishi, M., Watanabe, M.M., Terashima, A., Sako, Y. & Konda, T. Japan. J. Limnol., 49, 71–86 (1988)
1	1	1	June–September	Ojala, A., Kokkonen, S. & Kairesalo, T. Aquat. Sci., 65, 287–296 (2003)
6	6	6	April–September	Persson, J. <i>et al.</i> , unpublished
20	20	20	April–October	Persson, J. & Vrede, T. unpublished
1	1	1	Entire year	Reuter, J.E., Rhodes, M.E., Lebo, M.E., Kotzman, M. & Goldman, C.R. Hydrobiologia, 267, 179–189 (1993)
19	19	19	March & summer	Shortreed, K.S. & Stockner, J.G. Can. J. Fish. Aquat. Sci., 43, 110–134 (1986)
2		2	Entire year	Sterner, R.W. Limnol. Oceanogr., 39, 545–550 (1994)
16		16	July–August	Sterner, R.W., Elser, J.J., Fee, E.J., Guildford, S.J. & Chrzanowski, T.H. Am. Nat., 150, 663–684 (1997)
1		1	May–October	Tezuka, Y. Japan. J. Limnol., 46, 239–246 (1985)
2	2	2	June–August	Vanni, M.J., Layne, C.D. & Arnott, S.E. Ecology, 78, 1–20 (1997)
3		3	Summer & Autumn	Villar-Argaiz, M., Medina-Sánchez, J.M. & Cruz-Pizarro, L. Freshw. Biol., 46, 1017–1034 (2001)
10	10	10	July–October	Vrede, K. unpublished
2	2	2	April–October	Vrede, T., unpublished
Σ	485	314	485	