LIGHT, NUTRIENTS, AND P:C RATIOS IN ALGAE: GRAZER PERFORMANCE RELATED TO FOOD QUALITY AND QUANTITY

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Abstract. Continuous cultures of the green algae Selenastrum capricornutum were grown at different concentrations of total phosphorus (1–50 μmol P/L) and different light levels (10–200 μmol quanta·m⁻²·s⁻¹). Growth yields in terms of C, N, and P were positively correlated to total P level. Total biomasses in terms of C and N, but not P, were also positively correlated to light level. The cell quotas of C were positively correlated with light, whereas cell quotas of P were negatively correlated with light. The resulting elemental ratios gave a quite consistent pattern, where high light caused significant reductions in both N:C and P:C ratios, as well as strong reductions in chlorophyll to carbon ratios. The increase in P:C ratio with increasing total P and decreasing light can be interpreted as an adaptive response to self-shading. In that case, our results indicate that light adaptation not only involves a cost in terms of increased chlorophyll synthesis, but also in terms of increased P demands. This provides new insight not only into the physiological regulation of C and P uptake in algae, but it could also explain deviations from the Redfield ratio.

The growth of juvenile Daphnia magna fed S. capricornutum from the different light and phosphorus treatments was studied in a series of short-term assays (7 d) covering a gradient of food concentrations. The response of Daphnia growth rate along this quantity (0.5–5.0 mg C/L) and quality (0.5–12 μg atomic P/[mg atomic C]²) gradient gave a close fit to a double hyperbola model. Changes in elemental ratios of the algae were reflected in the growth rate of Daphnia, such that up to 40% reduction in its growth rate could be attributed to increased C:P ratios. This study demonstrates that the physiological responses of phototrophs in terms of chlorophyll content and elemental composition depend strongly on ambient light and nutrient regimes. It also confirms that these patterns can yield contrasting responses on herbivore growth responses along the food quantity and quality axes.

Key words: Daphnia; food quality; grazer; green algae (Selenastrum capricornutum); light; nutrients; photosynthesis.

INTRODUCTION

Aquatic ecosystem productivity is governed primarily by the inputs of light and nutrients, both in absolute and relative terms. While the quantitative yield of primary production is related to the quantity of photosynthetic active radiation (PAR) and key nutrients, recent studies demonstrate that the qualitative output in terms of elemental ratios may be determined by the balance of light and nutrients (Urabe and Sterner 1996, Sterner et al. 1997). At high light levels, photosynthetic carbon fixation is kept high, and unless the total supply of inorganic phosphorus is also high, the uptake of C and P may be desynchronized, causing decreasing P:C ratios. This may be manifested as an accumulation of C-rich storage compounds such as lipids or starch in algal cells (Berman-Frank and Dubinsky 1999). Conversely, P:C ratios tend to be high under low light and abundant P (Urabe and Sterner 1996).

Algae have a large capacity for acclimating their photosynthetic machinery to different light conditions. Increasing the investment into light-harvesting pigments, as reflected by the chlorophyll to carbon ratio, is a well-documented response to light limitation across a wide variety of algal taxa (Geider 1987). Moreover, there is a growing body of evidence indicating that photoadaptation to low light conditions also carries a cost in terms of increased N demands, mainly due to the high protein content of photosynthetic machinery (Raven 1984). While there is evidence that the synthesis of chlorophyll, and thus the capacity for light adaptation, may be constrained by N limitation (Prezlin and Matlick 1983), there appear to be no data considering any corresponding effect of P limitation.

Primary producers may exhibit a highly flexible cell stoichiometry with P:C ratios spanning more than an order of magnitude (Andersen 1997, DeMott et al. 1998). Metazoan herbivores, in comparison, have a much tighter regulation of their stoichiometry, with zooplankton species like Daphnia spp. having far higher P:C ratios than that normally encountered in their food (Andersen and Hessen 1991, Sterner and Hessen 1994). When the P:C ratio of the algal food decreases beyond the threshold for balanced net intake of C relative to P, growth efficiency in terms of C will decline since an increasing share of gross ingested C cannot

A number of experiments have verified that the growth of zooplankton herbivores, notably P-demanding species like Daphnia spp., indeed may be constrained at sufficiently low food P:C ratios (Urabe and Watanabe 1992, Sterner 1993, Urabe et al. 1997, DeMott et al. 1998). The existence of actual thresholds for P limitation is disputable, however. Based on theoretical and empirical evidence, P:C ratios over the range from 3–7 μg atomic P/[mg atomic C]−1 (atomic ratio C:P = 150–300) have been proposed as thresholds for the onset of P limitation in Daphnia (Hessen 1992, Urabe and Watanabe 1992, Sterner 1993). More recent experiments by DeMott et al. (1998) point to a threshold for reduced growth that exceeds 10 μg atomic P/[mg atomic C]−1 for juvenile Daphnia (C:P atomic ratio <100). This will not be a fixed limit, however, but will depend on the grazers’ assimilation of elements from the food particles and on the grazers’ own somatic stoichiometry, which is not entirely fixed. The food quantity also may in itself be a determinant of thresholds for P limitation, since when the grazer encounters food concentrations where net C intake approaches maintenance metabolism, the C:P threshold for potential P limitation will rise to infinity (Sterner and Robinson 1994).

The bottom line of the P limitation argument is that high growth rates increase the demand for RNA (Hessen 1990, Elser et al. 2000b), and particularly ribosomal RNA. P deficiency thus translates into lower growth rates (DeMott et al. 1998). The evolutionary explanation for such a mismatch may, from the autotrophs’ perspective, be seen as an adaptation to cause lower grazer fitness (at the expense of its own growth, however; cf. White 1993), while the grazers’ dilemma would be the trade-off between high protein synthesis and the risk of direct mineral limitation.

Under conditions with light limitation and P saturation, algal biomass will be low, but the algal cells will contain high P:C ratio (i.e., high food quality for herbivores). Following a transition towards light saturation while maintaining P deficiency, algal biomass will increase and finally reach the carrying capacity, but the P:C ratio of the cells will decrease. This shift in elemental stoichiometry could cause a trade-off scenario for grazers along the same gradient of increased light and decreased P (Urabe and Sterner 1996). The herbivores’ growth rate would be predicted to increase initially due to increased biomass of high quality algae, and to reach a peak growth rate at the optimum light: nutrient balance, before finally declining due to decreasing food P:C (i.e., decreased quality).

The relative importance of food quality vs. food quantity limitation will be of vital importance for assessments of ecosystem productivity. While food quantity limitation is assumed to be more important than food quality limitation in most localities, the latter may pose additional constraints not only for zooplankton secondary production, but also for a wide diversity of terrestrial grazers ranging from insects to mammals (McNaughton 1990, White 1993, Elser et al. 2000a). The P:C or N:C ratios of phototrophs are commonly far below those of their grazers in both terrestrial and aquatic ecosystems (Elser et al. 2000a), and this stoichiometric mismatch may have profound effects on energy flow and community structure. For example, Hessen and Faafeng (2000) found a mean sestonic P:C ratio of 4.5 μg atomic P/[mg atomic C]−1 in 110 Norwegian lakes, and estimated that P limitation could potentially constrain growth rates of P-demanding taxa like Daphnia spp. in a majority of the surveyed lakes.

If food quality can be governed by large-scale patterns of light and nutrients, this raises a set of intriguing issues related to both the within-lake variability of food quality that occurs over season and depth, and the between-lake variability that exist with respect to productivity and light penetration. It also poses far-reaching questions regarding the control of carbon sequestration, storage, and export in ecosystems. In the present account we focus on the steady state responses of algae grown under light and nutrient limitation in continuous culture, and their subsequent short- and intermediate-term effects on herbivore grazing and growth rates.

**Experimental setup**

The experiments were performed in a continuous, two-step, culture system, consisting of a primary phototroph chamber and a secondary herbivore chamber. The primary step was a 2-L glass vessel receiving a nominal light intensity (I) of 70 μmol quanta·m−2·s−1 from 25-W blue-white fluorescent tubes. Light gradients were implemented by varying the distances from the light sources, and by wrapping culture vessels in neutral density filters. For the first test, experiments were run in duplicates over a four-step gradient of light (I) from 23 to 200 μmol quanta·m−2·s−1. Since the highest light level (200 μmol quanta·m−2·s−1) tended to give chlorotic and unstable cultures, the remaining experiments were run in triplicates under only two light regimes: 70 μmol quanta·m−2·s−1 (light saturation) and 10 μmol quanta·m−2·s−1 (light limitation). The primary producer was the green algae Selenastrum capricornutum grown in COMBO medium (Kilham et al. 1998). A total P gradient was achieved by reducing P in the medium, yielding nominal P concentrations of 1, 1.8, 2.5, 5.0, 10, and 50 μmol/L. For all levels, actual concentrations were monitored by routine analysis of total P in the reservoir tanks. The full COMBO medium has 1000 μmol N/L and 50 μmol P/L and this N:P ratio of 20 (atomic : atomic) is close to the optimal N:P ratio for Selenastrum capricornutum of 22 (Rhee and Gotham 1980). At 50 μmol P/L, we assume no nutrient
limitation, while for all treatments with 10 μmol P/L and less (i.e., N:P ≥ 100), there will be an increasing degree of P limitation. Peristaltic pumps supplied medium at a flow corresponding to a dilution rate of 0.42 d⁻¹ in the phototroph step. Timer-controlled magnetic stirrers mixed the primary chambers. Stirring was found preferable to air bubbling because air bubbles tended to cause supersaturation problems in the downstream Daphnia cultures.

Selenastrum cultures were allowed to run for 2 wk before the start of the Daphnia growth experiments, in order to obtain a stable level of cell numbers, cell volumes, and particulate C, N, and P. Algae from all cultures, i.e., from all light and nutrient treatments, were diluted to standardized particulate carbon concentrations of 0.5, 1.0, and 1.5 mg/L before being fed to the Daphnia cultures. For the 1.0, 1.8, and 10 μmol P/L treatments, an additional high food concentration treatment level (5 mg C/L) was also run. Individual growth rates of Daphnia were followed in COMBO medium (without N and P to avoid changes in algal stoichiometry) for the first 7 d after hatching under all experimental protocols. Neonates, <24 h old, obtained from the same well-fed stock culture, were placed individually in 20-mL vials and fed standardized food concentrations. Somatic growth rates were measured on five parallel vials for each food concentration and each combination of light and P treatments. Growth rates were based on weight estimates obtained by a nondestructive image analysis technique, which will be described in full detail elsewhere (Fierro et al. 2002). Comparison of measured dry masses and volumes obtained by image analysis gave a close fit over the entire gradient from neonates to senescent individuals ($r^2 = 0.96, n = 60$).

Previous studies have suggested that the increased cell wall thickness in nutrient-limited Selenastrum could induce a grazing resistance (Van Donk and Hessen 1993, Van Donk et al. 1997). The reduced assimilation efficiency of thick-walled cells will be perceived as reduced clearance rates in short-term grazing experiments, since ingested algae would be defecated as whole cells, and interpreted as noningested cells. Since a considerable share of nutrient-stressed algae may pass intact thorough the gut, this would also translate to reduced growth rates of the grazers. Two grazing experiments with different sized Daphnia on different food qualities (C:P ratios) were performed to check for this possibility. Both grazing experiments used algae grown in 1.8 μmol P/L medium at low and high light intensity, yielding P:C ratios of 2.5 and 1.0 μg atomic P[mg atomic C]⁻¹, diluted to a fixed food concentration (5 mg C/L). The first experiment used four different age groups (2, 4, 8, and 25 d old) of Daphnia raised in high quality food (high P:C). The second experiment used a single cohort of animals (starting from <24-h-old neonates), raised in the same food qualities as used in the grazing assays. Food and media were replenished every day, while individual growth rates were followed over this period. Grazing rates were measured after 2, 4, and 8 d. Each grazing experiment was run in triplicate with 30 Daphnia per 200 mL beaker, against beakers with zero grazers as controls. Ingestion and clearance rates were estimate by flow cytometric (FCM) analysis of cell numbers and autofluorescence properties. Samples for FCM were collected after 1 and 10 min, and then every 30 min over a 6-h period.

**Analytical protocols**

The phototroph growth chambers were sampled at least twice a week. Subsamples of 100 mL were filtered on pre-ignited (500°C, 2 h) GF/F filters and analyzed for particulate C and N on a Carlo-erba CHN 1106 elemental analyzer (CE Elantech, Lakewood, New Jersey, USA). Particulate P was measured on corresponding filters placed in 15 mL distilled water that was acidified (150 μL of 4.0 mol/L H₂SO₄), added peroxidisulfate (0.15 g K₂S₂O₈), and autoclaved (121°C, 1 h). The particulate fraction of P was then analyzed spectrophotometrically by the standard ammonium-molybdate method. Total P and dissolved P were analyzed correspondingly on unfiltered and GF/F filtered water samples. The freshwater food web literature offers a confusing variety of units for reporting elemental ratios, e.g., as both C:P or P:C ratios, on both weight or molar basis. There are arguments in favor of all alternatives, but for compositional ratios it appears logical to put the major element in the denominator. Thus, throughout this paper, ratios are expressed as P:C, μg atomic P[μg atomic C]⁻¹. Due to convention, concentrations of P are expressed in molar units, while bio-masses and yields are expressed in mass units.

Algal cell numbers and size distributions were analyzed by flow cytometry. Samples of 1.0 mL were collected in Eppendorf vials, preserved with 20 μL 50% glutaraldehyde (1% final concentration), and stored in a refrigerator until analysis. FCM analyses were performed on a Skatron Argus 100 flow cytometer (Skatron A/S, Oslo, Norway) equipped with high-power mercury arc lamp (103 W) and a 100-μm nozzle. The instrument was modified with gas-pressure-controlled sheath flow delivery and a syringe-based sample injection system, which minimized sample cross-contamination. Monodisperse latex beads (Polysciences No. 23517, Europa GmbH, Eppelheim, Germany; mean diameter 1.0 μm, CV = 1.27%) were used for instrumental alignment and calibration. By using autofluorescence as trigger channel, egested cells would be excluded from FCM counts (cell autofluorescence was severely reduced by passage through the animal gut). Triggering on forward scatter and gating on fluorescence enabled differentiation between bacteria and Selenastrum in the FCM counts. Even under low light and high P, the share of bacteria gave a minor contribution (<5%) to total biomass. For determinations of
chlorophyll $a$. 25-mL subsamples were filtered in the dark on GF/F filters and stored at $-25^\circ$C in the dark before analysis. After testing with both acetone (90%) and methanol (100%) extraction, the latter method was chosen due to its higher extraction efficiency.

RESULTS

**Effects on autotrophs**

The effects of different light levels on phytoplankton yield in terms of particulate C, N, and P as well as chlorophyll $a$ were quite pronounced (Fig. 1). In low light, algal biomass increased threefold across the total P gradient (from $\sim$4 to 12 mg C/L), while the high light treatment at the same total P level typically doubled the carbon biomass yield. The low yield in the 10 $\mu$mol P/L treatment could have been caused by substantial growth on the growth chamber walls under high light. Particulate N yield resembled the pattern for particulate C with a less pronounced effect of light, while the yield of particulate P increased more than tenfold over the total P gradient for both light regimes. Cell quotas of C showed low variability and ranged from 20 to 60 pg/cell in all $\leq$10 $\mu$mol P/L treatments, but exceeded 100 pg/cell at the highest total P and light levels (Fig. 2). This contrasts with the cell quotas of P, which increased from near 0.1 pg/cell at low ambient P to more than 3 pg/cell at 50 $\mu$mol P/L (Fig. 3). The different responses of cell quotas of C and P along the P gradient at the two light regimes were manifested as a consistent pattern in cell P:C ratios. In full-strength COMBO medium (50 $\mu$mol P/L), no changes in Selenastrum P:C were detected over a gradient from 23 to 200 $\mu$mol quanta-m$^{-2}$s$^{-1}$ (Fig. 4). P:C was close to 12 $\mu$g atomic P/[mg atomic C]$^{-1}$, with no significant differences between treatments. When ambient P was reduced, there was a successive decrease in the P:C ratio to a level of 2.5±1.5 $\mu$g atomic P/[mg atomic C]$^{-1}$ at low light, and $<0.8$ $\mu$g atomic P/[mg atomic C]$^{-1}$ at high light.

Responses of phototroph biomass yield and composition to the experimental treatment variables were analyzed statistically by regression analysis. Dependent variables included biomass yields (as particulate C, N, and P, chlorophyll $a$, and cell abundance), cell quotas (as C, N, P per cell), and cell composition ratios (N:C, P:C, chlorophyll:C). As both independent variables spanned more than an order of magnitude, we chose to log10 transform all variables (both dependent and independent). All regression models used the same full factorial set of dependent variables, including two single effect terms and a multiplicative interaction term. All yield-type response variables (particulate C, N, P, chlorophyll $a$, and cell abundance) showed significant positive relationships with total P level (Table 1). Yields of particulate C, N, and cell number—but not yields of particulate P and chlorophyll $a$—also increased significantly with light level. It is noticeable that the regression slopes of the total P effect are generally close to 1, while the light effect slopes are substantially $<1$. This implies close to linear proportionality to total P and sublinear proportionality to light in...
Fig. 2. Cell quotas of carbon for six levels of total phosphorus. Shaded bars represent low light treatments; open bars are high light treatments. Each bar represents the mean ± 1 SD for a minimum of six separate measurements over a 2-wk period. The first (50 μmol P/L) treatment was designed differently from the others, with duplicate runs for the four light levels indicated above (as μmol quanta·m⁻²·s⁻¹), instead of triplicate runs at two light levels.

The consistent pattern of signs and magnitudes of the regression slopes (Table 1) strongly suggests that the interaction term may be interpreted as the modulating effect of total P on the response to light conditions. Thus, a given increment in light level appears to give a larger biomass yield increase at low than at high total P. It is reasonable to attribute this to the increased self-shading effects with increasing carrying capacity, and the corresponding cellular adaptive responses to compensate for this. This interpretation is supported by the pattern of regression slopes for the cell-composition-type variables in Table 1 (chlorophyll:C, N:C, P:C), which all have negative light effects and positive interaction terms. In other words, the carbon specific cell content of chlorophyll, N, or P can be increased by either decreasing the light at constant total P, or by increasing total P at constant light. The overall similarity between the adaptive response to self shading and light limitation in terms of P:C is roughly the same as for chlorophyll:C and N:C. The regression models for the cell quota-type variables (C/cell, N/cell, P/cell) all have nonsignificant total P effects and significant positive interaction effects, while the light effects show different patterns (positive for C/cell, nonsignificant for N/cell, and negative for P/cell).

The strong interaction between the total P and light level on the size distributions of Selenastrum cells is also clearly by the flow cytometric analyses. The forward scatter signal in flow cytometry is generally found to be closely related to cell size, although it is also
Fig. 3. Cell quotas of phosphorus for six levels of total phosphorus. Shaded bars represent low light treatments; open bars are high light treatments. Each bar represents the mean ± 1 SD for a minimum of six separate measurements over a 2-wk period.

known to be influenced by other factors like cell shape, texture, and refractive index (Shapiro 1995). The forward-scatter cytograms (Fig. 5) show no effect of light level on cell size or shape at the highest total P level (50 μmol/L). At total P concentrations of 5 μmol/L and below, there was both a gradual increase in mean forward scatter signal with decreasing total P, and an increasing deviation between the high and low light treatments. Cell size distributions were almost identical among the three replicated cultures for all total P and light treatments.

Effect on grazers

The additive effect of high light and low P yielded a substantial increase in cell size, but the cells would still be within a grazable range for all size categories of *Daphnia*. There could, however, be more qualitative changes associated with the increased volume, such as changed cell wall properties and biochemical composition that would affect the next trophic level. The two separate grazing assays demonstrated two different aspects (Fig. 6). If the animals were raised on an optimal (low C:P) diet, and then offered algae with different C:P ratios, there were no difference in clearance rates either in 2, 4, or 8-d-old animals. A separate grazing assay was also performed with large (25-d-old) animals on the same food, and also this yielded almost identical clearance rates (52±55 mL-individual⁻¹-d⁻¹). If, however, the animals were raised on low and high C:P diets, respectively, the individuals fed high C:P (low quality) food had reduced clearance rates. This is most obviously a consequence of lower growth rates (Fig. 6) and thus reduced per capita clearance rates due to reduced age-specific size.

For algae grown in full COMBO medium (50 μmol P/L), there was no difference between light treatments on *Daphnia* growth rates (Fig. 7), which ranged from 0.22 d⁻¹ at 0.5 mg C/L to 0.28 d⁻¹ at 1.5 mg C/L.
**TABLE 1.** Linear regression models for response variables as functions of light \((I)\) and phosphorus \((P)\).

<table>
<thead>
<tr>
<th>Response</th>
<th>(R^2)</th>
<th>(F)</th>
<th>(df)</th>
<th>(P)</th>
<th>(\log(P))</th>
<th>(\log(I))</th>
<th>(\log(P) \times \log(I))</th>
<th>(\log(P))</th>
<th>(\log(I))</th>
<th>(\log(P) \times \log(I))</th>
</tr>
</thead>
<tbody>
<tr>
<td>log(POC)</td>
<td>0.24</td>
<td>30.60</td>
<td>3, 288</td>
<td>(&lt;0.0001)</td>
<td>0.64</td>
<td>0.35</td>
<td>-0.32</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
</tr>
<tr>
<td>log(PON)</td>
<td>0.49</td>
<td>89.36</td>
<td>3, 283</td>
<td>(&lt;0.0001)</td>
<td>0.74</td>
<td>0.21</td>
<td>-0.23</td>
<td>(&lt;0.0001)</td>
<td>0.0012</td>
<td>(&lt;0.0001)</td>
</tr>
<tr>
<td>log(POP)</td>
<td>0.80</td>
<td>371.95</td>
<td>3, 272</td>
<td>(&lt;0.0001)</td>
<td>1.06</td>
<td>-0.01</td>
<td>-0.17</td>
<td>(&lt;0.0001)</td>
<td>0.9218</td>
<td>0.0029</td>
</tr>
<tr>
<td>log(Chl)</td>
<td>0.42</td>
<td>33.97</td>
<td>3, 141</td>
<td>(&lt;0.0001)</td>
<td>0.47</td>
<td>-0.05</td>
<td>-0.19</td>
<td>(&lt;0.0001)</td>
<td>0.4320</td>
<td>0.0003</td>
</tr>
<tr>
<td>log(cells)</td>
<td>0.14</td>
<td>14.37</td>
<td>3, 264</td>
<td>(&lt;0.0001)</td>
<td>0.87</td>
<td>0.30</td>
<td>-0.54</td>
<td>(&lt;0.0001)</td>
<td>0.0021</td>
<td>(&lt;0.0001)</td>
</tr>
<tr>
<td>log(chl:C)</td>
<td>0.50</td>
<td>46.09</td>
<td>3, 140</td>
<td>(&lt;0.0001)</td>
<td>-0.06</td>
<td>-0.42</td>
<td>0.12</td>
<td>0.3860</td>
<td>(&lt;0.0001)</td>
<td>0.0072</td>
</tr>
<tr>
<td>log(N:C)</td>
<td>0.57</td>
<td>122.22</td>
<td>3, 281</td>
<td>(&lt;0.0001)</td>
<td>0.08</td>
<td>-0.15</td>
<td>0.09</td>
<td>0.1072</td>
<td>(&lt;0.0001)</td>
<td>0.0015</td>
</tr>
<tr>
<td>log(P:C)</td>
<td>0.99</td>
<td>833.43</td>
<td>3, 270</td>
<td>(&lt;0.0001)</td>
<td>0.37</td>
<td>-0.41</td>
<td>0.17</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
</tr>
<tr>
<td>log(C/cell)</td>
<td>0.29</td>
<td>56.80</td>
<td>3, 262</td>
<td>(&lt;0.0001)</td>
<td>-0.15</td>
<td>0.15</td>
<td>0.16</td>
<td>(&lt;0.0001)</td>
<td>0.0001</td>
<td>0.0009</td>
</tr>
<tr>
<td>log(N/cell)</td>
<td>0.56</td>
<td>111.03</td>
<td>3, 257</td>
<td>(&lt;0.0001)</td>
<td>-0.14</td>
<td>-0.04</td>
<td>0.29</td>
<td>0.1526</td>
<td>0.5229</td>
<td>(&lt;0.0001)</td>
</tr>
<tr>
<td>log(P/cell)</td>
<td>0.86</td>
<td>513.90</td>
<td>3, 258</td>
<td>(&lt;0.0001)</td>
<td>0.15</td>
<td>-0.33</td>
<td>0.38</td>
<td>0.0797</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
</tr>
</tbody>
</table>

**Notes:** All variables are log transformed. All models are full factorial including single-effect terms \(\log(I)\) and \(\log(P)\), as well as an interaction effect term \(\log(I) \times \log(P)\). All underlined whole-model \(F\) ratios and regression slopes are significant at the 1% level, or better.
lower total P, where higher and more light dependent P:C ratios were induced, this was also reflected in zooplankton growth rate. At 5.0 μmol P/L, growth ranged from 0.17 d⁻¹ at 0.5 mg C/L, to 0.25 d⁻¹ at 1.5 mg C/L. The tenfold increase in algal biomass from 0.5 to 5 mg C/L increased zooplankton growth rates by 40–50%, the same order of magnitude as for the effect of C:P alone.

Growth rate as function of food concentration of *Daphnia* is commonly described by a rectangular hy-
We propose here that the Holling type-II food quantity effect on *Daphnia* growth rate may be extended with a multiplicative, rectangular, hyperbolical term representing the food quality effect. If $g \left( \text{d}^{-1} \right)$ denotes the specific growth rate, $C$ the food concentration (mg C/L), and $Q$ the P:C ratio of food (mg atomic P/mg atomic C), then the functional relationship becomes

$$\begin{align*}
g = g' \frac{C}{C + C' Q + Q'}
\end{align*}$$

where $g'$ is the asymptotic growth rate, and $C'$ and $Q'$ are the half-saturation parameters for food concentration and P:C ratio, respectively. The double hyperbola model showed a reasonably good fit to the observed growth rates (Fig. 8), with model parameters fitted by nonlinear least squares all being significantly different from zero ($g' = 0.37 \pm 0.02 \text{ d}^{-1}$, $C' = 0.53 \pm 0.07$ mg C/L, $Q' = 0.97 \pm 0.14$ mg atomic P/mg atomic C). It should be stressed, however, that the general validity of this model is not tested outside the range of food concentrations from 500 to 5000 µg C/L.

**DISCUSSION**

According to standard theory for nutrient-limited chemostat cultures (e.g., DeAngelis 1992), one would expect algal cellular nutrient:C ratios to be a function of dilution rate, but not total nutrient concentrations in the medium (which would only affect steady state biomass yield). Changes in steady state cell composition with dilution rate can be explained mechanistically as changes in the allocation between the major intracellular compartments relating to structure, storage, and biosynthesis (Shuter 1979, Kooijman 1993). The almost 40-fold variation in P:C ratio found in our cultures, despite the fact that all cultures were run at the same dilution rate, thus must be attributed to the effects of light limitation and photoadaptation rather than to changes in specific growth rate. Furthermore, since the effects of light level were different between cultures with high and low total P, we are considering both the direct effects of treatment-imposed light limitation and the indirect effects of biomass-induced self-shading. The consistently high P:C ratios at all light levels in the 50 µmol/L total P treatment, probably indicate that these cultures were generally P saturated and light limited (although additional effects of CO2 limitation cannot be ruled out). In all the lower total P treatments (≤10 µmol/L), there was probably some degree of co-limitation by both light and phosphorus, and also indications of considerable interaction between these two limiting factors. The interaction effects may partly be interpreted as biomass-dependent self-shading increasing the degree of light limitation, and partly as photoadaptation being constrained by P limitation.

Our results clearly support the mutual role of light and nutrients as determinants of phytoplankton P:C ra-
Fig. 7. Growth rates for *Daphnia* (1–8 d old) raised on a gradient of food quantities and qualities. Each bar represents the mean ± 1 SD of three replicates measuring five individual growth rates of single *Daphnia* raised on food at different combinations of light and P treatments at four different food concentrations (0.5, 1.0, 1.5, and 5.0 mg C/L). The individual growth rates were obtained by the image analysis technique.

Fig. 8. *Daphnia* growth rate as a function of food quality (P:C ratio) and quantity (mg C/L), fitted to a double hyperbola model. The mesh grid is the model prediction surface, while observations are represented by ball-and-stick symbols where the stick is the projection of the observation onto the model prediction surface.
reducing the dilution rate from 1.0 d^{-1}, by increasing the light from 10 to 70 μmol quanta. Urabe and Sterner (1996) similarly recorded a range in algal P:C from ~1 μg atomic P/[mg atomic C]^{-1} at low P (1-10 μmol/L) and high light to >10 at high P and low light. A corresponding range of elemental ratios has been reported in previous experiments with P-starved and P-saturated freshwater chlorophytes in batch cultures (van Donk and Hessen 1993, Van Donk et al. 1997) and continuous cultures (DeMott et al. 1998). The latter authors obtained “P-sufficient” Scenedesmus at 100 mmol P/L, yielding a P:C ratio of 12.5 μg atomic P/[mg atomic C]^{-1}. By reducing the dilution rate from 1.0 d^{-1} to 0.15 d^{-1}, and reducing medium P to 6 mmol P/L, P:C ratio over a range of 1.2-1 μg atomic P/[mg atomic C]^{-1} was produced. Lake seston is of heterogeneous origin, of which detritus with high P:C frequently make up a considerable share (cf. Sterner and Hessen 1994). Thus lake seston stoichiometry may not necessarily mimic the elemental composition of its algal component. Nevertheless, the span of C:P ratios in lake seston corresponds well with that achieved in algal cultures (cf. Heckey et al. 1993, Elser and Hassett 1994, Sterner et al. 1997, Hessen and Faafeng 2000).

The range of light and nutrients applied in these experiments is well within the limits of natural variation within (light) and between (nutrients) lakes. While the low-light treatment was 14% of full light in our experiments, the compensation depths where photosynthesis balances respiration is commonly assumed to be 1% of surface radiation. Phytoplankton within a lake may experience the full range of light exposure both on a diurnal time scale and on other time scales due to mixing regimes. The concentration of total phosphorus (TP) will also be one of the main determinants of the underwater light climate in lakes, since high levels of chlorophyll a will effectively attenuate photosynthetic active radiation (PAR). This does not imply a covariation between high TP and low light, however, since cell quotas of P may be depleted under situations with low supply and high phytoplankton biomass.

Algal cells may respond to increased light levels in various ways, and the decreased P:C could be interpreted as a decoupling of C and P uptake that would be accentuated by low P (cf. Urabe and Sterner 1996). While the final outcome of an increased light: nutrient ratio in terms of increased C:P seems consistent, the responses in terms of C and P uptake and metabolism may be more subtle. Apparently the algal cell quota of C increases under high light, consistent with the theory of storage of C-rich compounds resulting from “excess” C generated by C fixation at a high rate. The cell quota of P did not, however, show any consistent pattern, although for all runs above 1 μmol P/L, the cell P quota tended to decrease under high light. This was reversed at 50 μmol P/L, where cell quotas of both C and P increased, yielded uniform (and high) cellular P:C ratios.

An increased cell size in P-limited green algae has previously been demonstrated, and seems to be linked to the storage of excess C as starch granules (van Donk et al. 1997). For Selenastrum (NIVA CHL 1, the same clone as used in these experiments) a doubling in cell volume occurred upon transition from P saturation to P limitation (van Donk and Hessen 1993, van Donk et al. 1997). This morphological change was supported by the forward scatter cytograms (representing a combined signal of optical density and particle size).

One very intriguing outcome of these experiments was the apparent dependency of cell-specific chlorophyll not only on light, but also on ambient P. Judging from the decreasing chlorophyll a: carbon ratio over the gradient of ambient P, chlorophyll a synthesis may in fact be directly constrained by the availability of P. Under low light, there is a need for more light-harvesting machinery, and apparently the synthesis of the membrane-rich chloroplasts could be constrained not only by N (Prezelin and Matlick 1983), but also by the availability of P for the phospholipid synthesis. This may induce several feedback effects both on total light harvesting and C fixation that may in turn affect cell biochemistry and stoichiometry.

One basic question is whether or not such induced effects on algal cell nutrient quotas should be regarded as transient or persistent. This may hardly be sorted out from batch culture experiments. The fact that cell quotas and P:C ratios remained stable over a >3-wk run under all treatments in these experiments suggest that these cellular responses may be quite persistent under given scenarios. Data from a wide range of lakes do also suggest some inherent properties of seston stoichiometry accredited to nutrients, light, and mixing regimes across lakes (Sterner et al. 1997).

The relevance of these elemental changes for food web production hinges upon the potential for P limitation in zooplankton. The cladoceran family Daphnia is most relevant in this regard, not only because it has high P:C ratios (and thus high specific requirements for P), but also because of their prominent position in the food webs of many lakes. There are, however, no fixed thresholds of P:C for the onset of P limitation in Daphnia. Hessen (1992) estimated a threshold near 8 μg atomic P/[mg atomic C]^{-1} while Urabe and Watanabe (1992) applied a threshold for onset of P limitation at 3 μg atomic P/[mg atomic C]^{-1}. Based a set of very thorough experiments, DeMott et al. (1998) found onset P limitation at P:C-ratios as high as 11 μg atomic P/[mg atomic C]^{-1}. Urabe and Sterner (1996) found a strong negative effect on biomass accumulation in juvenile Daphnia when P:C decreased from ~4 to 2 μg atomic P/[mg atomic C]^{-1}, in accordance with their proposed threshold of 3 μg atomic P/[mg atomic C]^{-1}. These differences in proposed thresholds for onset of
P limitation are by no means trivial. A threshold of 11 μg atomic P [mg atomic C]^{−1} would mean that phytoplankton in a majority of lakes would offer suboptimal quality with respect to P requirements for Daphnia growth. Hessen and Faafeng (2000) estimated that Daphnia in 88% of out of 110 Norwegian lakes would theoretically be P limited judged from sestonic P:C ratios when applying the high threshold of 11 μg atomic P [mg atomic C]^{−1}, but even if the threshold was reduced to 3 μg atomic P [mg atomic C]^{−1}, some 40% of the Daphnia populations would theoretically face P limitation. This does not imply that food-quality constraints are more important than quantity constraints, however. Rather, P limitation may be superimposed on C limitation, providing an additional tax on the net uptake of C by the animals.

The Holling type-II response to food quality would indicate no pronounced threshold, but an increasing effect with decreased P:C ratios in food. Since the grazers to some extent may adjust their own somatic demands for P relative to C, i.e., they are not strictly homeostatic (DeMott et al. 1998), there will be no fixed threshold that will apply to all situations. The response of food C:P ratios will depend also on the assimilation of C relative to P. A high assimilation of P relative to C will raise the threshold and vice versa. Finally, the food quantity itself could influence the potential onset of P limitation (Sterner and Robinson 1994, Sterner 1997). As food quantity in terms of C approaches the threshold for positive net growth, an increasing share of the assimilated C will be used for maintenance metabolism, and at zero net growth the somatic need for P will approach zero. The model of food quantity vs. food quality limitations (Fig. 8) may thus not be valid for food concentrations below the observed range of food concentrations (500–5000 μg C/L), and particularly not when approaching the threshold carbon concentration for net growth in Daphnia (~50 μg C/L).

The effects of lowered P:C in the diet on Daphnia growth has been repeatedly demonstrated in laboratory experiments (Urabe and Watanabe 1992, Sterner 1993, DeMott et al. 1998) as well as in field studies (Sterner et al. 1997), and the potential for direct P limitation has been verified experimentally (Urabe et al. 1997). Although alternative explanations for reduced growth of Daphnia on P-deficient food have been advocated, such as deficiencies in the supply of polyunsaturated fatty acids (Müller-Navarra 1995, Brett and Müller-Navarra 1997), the potential role of these may be overruled by strict P deficiency under low P:C ratios in the food (Sundbom and Vrede 1997, Boersma 2000). The effect of increased algal cell-wall thickness and grazing resistance under strong P deficiency has also been demonstrated for batch cultures of green algae like Selenastrum and Chlamydomonas (Van Donk and Hessen 1994, Van Donk et al. 1997). The removal of algal cells from cultures by Daphnia grazing was strongly reduced when the algae was strongly P limited, simply due to passage of intact cells through the gut. Corresponding effects have not been reported in continuous cultures, however (Sterner 1993, DeMott et al. 1998). Our grazing experiments over a range of Daphnia ages at different P and light treatments indicated neither reduced grazing rates or gut passage of intact algae. The lower clearance rates found for animals raised on low P diet reflected simply lower growth rates of these animals compared with control. This may illustrate the potential for overall lower community grazing in P-limited systems due to food quality depression among herbivores.

The study demonstrates a quite subtle regulation of cellular levels of nutrients and chlorophyll in planktonic algae that is related to ambient light and nutrient levels. While high light causes elevated cell quotas of C, there is an apparent increased demand for P that may be seen as an adaptation to self shading and increased cellular levels of chlorophyll. This adaptation was reflected as increased cell quotas of P with reduced light. These responses may have profound effects on cellular stoichiometry, nutrient allocation, and carbon sequestration of autotrophs, and these effects in turn can propagate up the food chain. Clearly, a short-term result of these responses on high-P-demanding grazers could be reduced herbivore growth rates that in turn could translate into reduced total grazing rates when sestonic P:C ratios are low. This sequence of events would typically occur during in the late phase of algal blooms containing high biomasses of nutrient deficient cells. These food quality effects will be superimposed on the food quantity effects, but the relative importance of these factors over a seasonal cycle is not settled, however. These findings could also have strong implications for our understanding of carbon sequestration in aquatic ecosystems. They also provide new insights (and questions), not only of the physiological regulation of C and P uptake and demands in algae, but also could explain systematic deviations from the Redfield ratio, a cornerstone in plankton ecology and aquatic C models.

While these aspects of elemental regulation in autotrophs and the subsequent effects on grazers have been explored primarily in freshwater habitats, we believe that these findings could be of general validity. In particular the elemental mismatch between autotrophs and herbivores also seems to appear also in marine and terrestrial ecosystems (White 1993, Elser et al. 2000a). Since autotroph production and biomass is commonly considered to be N limited in these systems, the vegetation and consumer N:C ratios would presumably be more important than the P:C ratios studied in freshwater systems. Yet the role of P limitation both for autotroph regulation of photosynthesis and success of grazers calls for further scrutiny also in these systems.

**Literature Cited**


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