Phosphorus-limited Daphnia: Intraspecific facilitation instead of competition

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Abstract

P-limited chemostat cultures of the green alga Scenedesmus acutus were inoculated with the herbivorous zooplankter Daphnia galeata after steady state had been reached. At low dilution rates (up to 0.5 d⁻¹) and consequently low P content of the food algae (P:C < 0.0011) Daphnia could not grow. At higher dilution rates (from 0.55 d⁻¹ upward) and higher P content of the food, Daphnia developed dense populations which were able to reduce algal biomass more than 10-fold. This reduction was accompanied by an increase in the P content of the algae which gave rise to increased birth rates of Daphnia. The birth rates of P-limited Daphnia depended on algal cell quotas in a saturating fashion which was mathematically similar to Droop's growth equation. During P limitation the birth rates of Daphnia were positively correlated to population density, which suggests intraspecific facilitation instead of competition.

During the last few years a fundamental difference in the elemental composition of phytoplankton and zooplankton has become evident. Phytoplankton have a variable concentration of biogenic elements in their biomass. The cellular content of limiting nutrients can best be described by Droop's (1973, 1983) growth equation. This equation predicts a decline of intracellular nutrient concentrations (cell quotas) with increasing nutrient limitation of reproductive rates. Minimal cell quotas of phosphorus, for example, can be about one order of magnitude smaller than saturating cell quotas (Sommer 1991). Zooplankton elemental composition, however, shows rather low physiological variability within species but some variability between species (Andersen and Hessen 1991; Hessen 1990; Hessen and Andersen 1991; Hessen and Lyche 1991). Among crustacean zooplankton, Daphnia spp. have P-rich tissue while copepods have relatively P-poor tissue. Atomic P:C ratios of ~0.012 in Daphnia are similar to the biomass composition of nutrient-saturated phytoplankton, whereas P:C ratios of ~0.005 in herbivorous copepods are similar to moderately P-limited phytoplankton.

The relative rigidity of zooplankton elemental composition has two consequences. First, in order to maintain their own stoichiometry, zooplankton should decrease the excretion of a limiting nutrient with decreasing concentration in the food. This assumption is the basis of several recent excretion models (Olsen and Østgaard 1985; Hessen and Andersen 1991; Sterner 1990). Second, even reduction or complete stoppage of excretion of a limiting element may become insufficient to assimilate enough of that element in relation to the amount of C retrievable from the food. Then growth and reproduction may become limited by a minor element instead of C or energy. Urabe and Watanabe (1992) calculated that the transition from C limitation to P limitation of Daphnia galeata takes place at P:C = 0.0046 under high food conditions and at P:C = 0.0026 under low food conditions.

If zooplankton can be limited by other elements than carbon, it would be tempting to expand the stoichiometric ratio approach of Tilman's (1982) competition theory from phyto- to zooplankton. There may be some doubts, however, whether zooplankton would compete in the classic sense for nutrient-limited algae. Low nutrient quotas in phytoplankton occur mainly during high levels of algal biomass. In such a situation zooplankton would not be limited by the concentration of the biomass-bound limiting nutrient in the water (e.g. algal P liter⁻¹) but by the P content of the available food biomass (e.g. P:C or P:biovolume). Any reduction of algal biomass by grazing would improve the nutritional status of algae be-
cause less algae would have to share the same flux of the limiting nutrient. According to a model by Sterner (1989) this improvement would even take place in the complete absence of nutrient release by zooplankton because the biomass effect is more important than the recycling effect. As long as there is no shift from P (or N or any other minor element) limitation to C limitation, more grazing would therefore imply less P limitation for zooplankton. In this case the inter- and intraspecific interactions between zooplankton would be positive instead of negative, i.e. facilitation instead of competition. This working hypothesis was tested by running P-limited chemostat cultures of a food alga (Scenedesmus acutus) at different dilution rates and inoculating them with D. galeata after attainment of steady state and thereafter following birth rates and growth rates of Daphnia and their relationships to food quality, food quantity, and Daphnia density.

**Methods**

*Scenedesmus acutus* was cultured in 10-liter chemostats at a 14:10 L/D cycle and a surface light intensity of 300 μEinst m⁻² s⁻¹ of PAR. The experimental temperature was 20°C. The medium consisted of sterile filtered water from Schönhsee which was enriched by K₂HPO₄ and NH₄NO₃ to reach final concentrations of 1.4 μM P and 70 μM N. Before inoculation by *Daphnia*, cultures were stirred continuously at 10 rpm by a motor-driven glass propeller that did not touch the walls or the bottom of the culture vessel. After inoculation with zooplankton, 10 min of stirring at the same speed were followed by a 20-min interval without stirring. After *Scenedesmus* had attained steady state, 100 *D. galeata* adults that had been prestarved for 3 d were inoculated into each chemostat. The experiments differed from each other by their dilution rates, ranging from 0.4 to 0.75 d⁻¹. In the following, the experiments are designated by a number (for the dilution rate) and in case of replication by a letter for the replicate.

Samples were taken at 2-3-d intervals. Samples for food C and food P were screened through 100-μm plankton gauze to exclude *Daphnia* from analysis. Thereafter samples for measuring POC (particulate organic C) were filtered onto glass-fiber filters and samples for measuring particulate P (PP) were filtered onto cellulose-nitrate filters from which P was extracted by the standard technique for total P. Samples of 1 liter were taken for counts of *Daphnia* individuals and eggs. At low population densities, the sample size was insufficient for precise estimates of population density, which resulted in considerable scatter around the general temporal trend. This scatter was removed after smoothing *Daphnia* counts and egg counts by calculating a 5-point running mean before calculating growth rates and birth rates.

Birth rates (b) were calculated according to the equation of Paloheimo (1974):

\[
b = \ln(1 + E/N)/D \quad (d^{-1})
\]

where \(E\) is the total number of eggs, \(N\) the number of animals, \(E/N\) the “egg ratio,” and \(D\) the development time of eggs. \(D\) is generally considered a function of temperature and was assumed to be 2.8 d at 20°C (Elster and Schwörbel 1970).

Population growth rates (r) were calculated as

\[
r = (\ln N_2 - \ln N_1)/(t_2 - t_1) \quad (d^{-1})
\]

where \(N_2\) is the number of individuals at time \(t_2\) and \(N_1\) the number of individuals at \(t_1\). Death rates (d) were calculated as

\[
d = b - r \quad (d^{-1}).
\]

The animals in the overflow container were also counted to test the loss of animals via the outflow.

**Results**

The chemostats needed 2–3 weeks until steady state was attained (not shown in Figs. 1 and 2). Data from the last sampling date before the inoculation of zooplankton were used to fit Droop’s (1973) equation of nutrient-limited growth to the data (nonlinear regression, Statgraphics):

\[
\mu = \mu_{max}(1 - q_0/q)
\]

where \(\mu\) is the reproductive rate (d⁻¹; equal to the dilution rate at steady state) and \(q\) the cell quota of the limiting nutrient (here P: C; atom/atom). \(\mu_{max}\) is the theoretical max-
Fig. 1. Time-course of algal POC, algal PP, and *Daphnia* density. The last sample before inoculation of animals is shown as the starting point. The dilution rate (d⁻¹) is indicated by the number in each graph.

The estimates for *S. acutus* are:

\[
\mu_{\text{max}} = 0.99 \text{ d}^{-1}; \quad q_0 = 0.0005 \text{ P:C};
\]

\[
r^2 = 0.96; \quad n = 7.
\]

The time-course of the experiments after zooplankton inoculation showed two completely different outcomes (Fig. 1). At dilution rates up to 0.5 d⁻¹ *Daphnia* could not grow and phytoplankton biomass remained high; at dilution rates >0.55 d⁻¹ *Daphnia* was able to grow and phytoplankton biomass declined. In three experiments (0.55A, 0.55B, 0.60) there was a slight indication for reciprocal oscillations between animal density and algal biomass (McCauley and Murdoch 1987). Experiment 0.75 was too short to detect such oscillations. In order to check the repeatability of the qualitative difference between low-dilution rate experiments and high-dilution rate experiments, I replicated the experiments at 0.50 and 0.55 d⁻¹.

In the experiments with low-dilution rates, phytoplankton showed practically no response to zooplankton addition. The nearly constant values of POC and PP resulted in a P quota of ~0.0009 P:C at a dilution rate of 0.4 d⁻¹ and of ~0.001 P:C at a dilution rate of 0.5 d⁻¹ (Fig. 2). In the experiments with higher dilution rates, algal POC dropped by more than an order of magnitude. A concomitant but much slower decrease in PP resulted in a nearly 10-fold increase in P quotas.

Animals were not exported at the dilution rate in any of the experiments. This result was due to the fact that animals concentrated near the bottom of the chemostat and remained below the surface from which the overflow was taken. This effect was strongest at low *Daphnia* densities. The abun-
dance maxima in the high-dilution rate cultures were $\sim 80$ liter$^{-1}$ and abundance minima later in the course of the experiments were always much higher than the inoculum density. In the low-dilution rate experiments, few *Daphnia* had any eggs at all, except for a few sporadic eggs during the first 2 weeks. In the experiments with high-dilution rates, egg ratios increased quite sharply after the first slight increases in the P quota and leveled off after further increase of the P quota (Fig. 2), indicating a saturating response. Midexperimental decreases in egg ratios corresponded to decreases in food carbon.

Cases of C limitation had to be excluded in order to analyze the relationships between birth rates and P quota in the food, which was easy because of strong negative correlations between the P quota and POC in all experiments with high-dilution rates ($r < -0.95$). Therefore, a plot of $b$ vs. POC concentrations showed a decrease at POC concentrations $>100$ $\mu$g atoms liter$^{-1}$ (Fig. 3). At concentrations below that, $b$ tended to increase with POC, thus indicating C limitation. After excluding these cases, a saturation response of the birth rate to the P quota in the food became apparent (Fig. 4). Among several saturation models with a threshold, an analog to Droop's (1973, 1983) two-parameter model showed the best fit:

$$b = b_{\text{max}}(1 - \frac{Q}{Q_0})$$

(5)

where $b_{\text{max}}$ is a theoretical maximum of the birth rate and $Q_0$ the minimal P quota in the food (as opposed to the minimal cell quota in the growing organism in Droop's original equation!). The parameter estimates in each experiment agreed relatively well among experiments (Table 1). Introducing time lags between P content of the food and birth rate did not improve the fit.
Table 1. Parameters of P-limited growth of *Daphnia galeata* according to Eq. 5 ($b_{max}$—maximal birth rate, $d^{-1}$; $Q_p$—minimal P quota in food, P: C by atoms).

<table>
<thead>
<tr>
<th>Exp.</th>
<th>$b_{max}$</th>
<th>$Q_p$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75</td>
<td>0.35</td>
<td>0.0012</td>
<td>0.61</td>
</tr>
<tr>
<td>0.60</td>
<td>0.33</td>
<td>0.0011</td>
<td>0.97</td>
</tr>
<tr>
<td>0.55A</td>
<td>0.33</td>
<td>0.0010</td>
<td>0.97</td>
</tr>
<tr>
<td>0.55B</td>
<td>0.32</td>
<td>0.00096</td>
<td>0.95</td>
</tr>
</tbody>
</table>

All demographic rates under study showed a density dependence (Fig. 5). Birth rates initially increased with population densities and leveled off thereafter with a slight indication of a decline at high densities in some experiments. Death rates also increased in a curvilinear mode with population densities. Usually >90% (and always >80%) of the mortality could be explained by export from the culture vessel. Therefore, physiological mortality played no important role. The positive density dependence of death rates is caused by the increasing proportion of animals near the overflow pipe at higher densities. The net growth rates showed an unimodal response to population densities with peaks at 16 (0.60) to 38 (0.75) animals liter$^{-1}$. The den-
Table 2. Density dependence of birth rates (\(b\)), net growth rates (\(r\)), and death rates (\(d\)) described by a polynomial of the general form \(y = a + bx + cx^2\), where \(x\) is the density in ind. liter\(^{-1}\). Density where rates are maximal—M.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>(y)</th>
<th>(a)</th>
<th>(b)</th>
<th>(c)</th>
<th>(r^2)</th>
<th>(P)</th>
<th>(M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75</td>
<td></td>
<td>-0.069</td>
<td>0.013</td>
<td>-0.00012</td>
<td>0.97</td>
<td>&lt;0.0001</td>
<td>54</td>
</tr>
<tr>
<td>0.60</td>
<td></td>
<td>0.021</td>
<td>0.0082</td>
<td>-0.000069</td>
<td>0.62</td>
<td>&lt;0.0001</td>
<td>59</td>
</tr>
<tr>
<td>0.55A</td>
<td></td>
<td>-0.033</td>
<td>0.0078</td>
<td>-0.000055</td>
<td>0.92</td>
<td>&lt;0.0001</td>
<td>71</td>
</tr>
<tr>
<td>0.55B</td>
<td></td>
<td>0.017</td>
<td>0.0009</td>
<td>-0.000065</td>
<td>0.94</td>
<td>&lt;0.0001</td>
<td>69</td>
</tr>
</tbody>
</table>

Density dependence of all rates could be described by a second-order polynomial with a positive linear and a negative quadratic term (Table 2).

There were several anomalously low C-limited values for \(b\) in experiment 0.60 which show up most clearly in Fig. 5. They correspond to the sharp midexperiment decline of \(Daphnia\) (Fig. 1) and a corresponding minimum of the egg ratio (Fig. 2) which cannot be explained by the available data.

**Discussion**

The density dependence of demographic rates differs markedly from the monotonously negative density dependence of growth rates as assumed in the logistic growth equation, for instance. Usually, negative density dependence is considered to be a consequence of interspecific competition. Conversely, a positive density dependence would suggest intraspecific facilitation. When judging the extent of intraspecific facilitation, it has to be considered that the density dependence of death rates was an experimental artifact. Even at the highest densities most of the observed loss was due to export via the overflow while physiological mortality was unimportant. Without artificial export the increase of death rates with density would have been much smaller, which would have displaced the peak of the growth rate vs. density curve to higher densities, i.e. into closer vicinity of the peak of the birth rate vs. density curve.

Apparently the transition from a positive to a negative density dependence is coupled with the transition from P limitation to C...
limitation of zooplankton growth. This conclusion is supported by the strong negative correlations between *Daphnia* density and food POC and the strong positive correlation between *Daphnia* density and the P quota of the food algae (Table 3). The regression equations in Table 3 can be used to calculate the POC levels corresponding to the *Daphnia* densities where birth rates were maximal (Table 2): the POC concentrations at this density were 103, 74, 86, and 87 pg atoms liter\(^{-1}\) in experiments 0.75, 0.60, 0.55A, and 0.55B, respectively. These values agree quite well with the cutoff limit of 100 µg atoms liter\(^{-1}\)—the value used to exclude C-limited birth rates from the analysis of P limitation. They are, however, much higher than the incipient limiting level for ingestion rates of ~22 µg atoms C liter\(^{-1}\) (Muck and Lampert 1984) which had been measured for the same species of *Daphnia* (then called *D. longispina*) and the same food alga. This discrepancy can tentatively be explained by the high detrital content of POC during the minima of food biomass as could be seen qualitatively by microscopic analysis. Larger detrital aggregates (>50 µm) were quite frequent and were probably not ingested but included in the chemical analysis of POC.

In conclusion, exploitative competition occurred only when the animals were limited by the quantity of available food, as expressed by the concentration of POC. There was no evidence for exploitative competition when the animals were limited by the quality of food, as expressed by the P quota. This case rather falls into the category of facilitation, because reduction of algal density by grazing increased the per capita P income of the algae and, therefore, improved their quality as a food source.

The second important result from my experiments is the drastic change in the qualitative outcome of experiments after small differences in the initial conditions. At a dilution rate of 0.50 d\(^{-1}\) the P quotas in the food of 0.00101 P : C (0.50A) and 0.00102 P : C (0.50B) at the day of *Daphnia* addition were insufficient to permit establishment of a grazer population. At a dilution rate of 0.55 d\(^{-1}\), only slightly higher P quotas of 0.00115 P : C (0.55A) and 0.00113 P : C (0.55B) permitted establishment of a grazer population. Of course, the growth of *Daphnia* was initially slow, but the increasing grazing pressure acted as a positive feedback loop by improving food quality and thus permitting increasing birth rates. After 1-1.5 months, *Daphnia* populations were dense enough to keep the algae at a biomass level more than 10-fold smaller than initially.

This situation resembles the clearwater phase in meso- to eutrophic lakes (Lampert 1988; Sommer et al. 1985) when, after the spring bloom of phytoplankton, high grazing pressure by cladocerans leads to collapse of algal biomass. A temporally extended version of this situation may be encountered in biomanipulated lakes where removal of fish reduces the mortality of zooplankton and permits a more or less permanent control of algal biomass by grazers (Shapiro and Wright 1984). It can be extrapolated from the above experiments that development of a clearwater phase or biomanipulation might fail if some factor delays development of grazers sufficiently long. Then the spring-bloom algae might continue their growth to the point where P limitation is strong enough to prevent development of grazers. Admittedly, P quotas of ~0.001 P : C are much smaller than P quotas usually found in natural waters (Sommer 1990), but the threshold quota for establishing a *Daphnia* population increases with increasing mortality.
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References


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