

Grazing resistance in nutrient-stressed phytoplankton

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Abstract. Grazing experiments were performed with the zooplankters *Daphnia pulex* and *Daphnia magna*, feeding on phosphorus-saturated and phosphorus-limited cells of two green algae (*Scenedesmus subspicatus* and *Selenastrum capricornutum*). P-limited algal cells passed largely intact through the gut and were thus spared from heavy grazing pressure. P-saturated algal cells, in contrast, were efficiently assimilated. Structural and morphological changes in the P-limited cells most probably reduced their digestibility. This phenomenon may be an important factor in zooplankton production and competition, and may serve as an example of a highly efficient strategy of P-limited algae to resist heavy grazing pressure.

Key words: Grazing – Phosphorus limitation – *Daphnia* – Selective digestibility

Recent experiments have demonstrated lower growth and reproduction in *Daphnia* when fed P-limited algae even when food was abundant. Hessen (1990) observed lower growth in *Daphnia pulex* after it had been grazing on the green alga *Selenastrum capricornutum* when the atomic C:P ratio of this alga increased from 67 to 167. Watanabe (1990) reported reduced growth in *D. similis* fed *Chlamydomonas reinhardtii* when the C:P ratio increased from 105 to 416, and likewise Sterner and Smith (1992) reported a general decrease in growth and egg production when feeding *D. pulex* with highly P-deprived *Scenedesmus acutus* (C:P = 2400). The data of Lehman and Naumoski (1985) also indicate lower egg-production in *D. pulex* when shifting from P-saturated to P-deficient food. Since *Daphnia* have high P:C requirements for somatic growth, the reduced growth may be interpreted as a strict P-limitation of the grazer (Hessen and Andersen 1992; Hessen 1992; Urabe and Watanabe 1992), but may also be caused by lower content of essential nutrients in the P-limited algae (cf. Ahlgren et al. 1990),

increased gut passage time and/or decreased assimilation efficiency. If the assimilation efficiency was reduced to a level where viable cells pass through the gut, this could serve as an example of a highly efficient strategy for grazing resistance under nutrient stress. This could also provide an explanation for the many different values reported for assimilation and growth efficiency of *Daphnia* feeding on the same algal species (see Lampert 1987).

In order to investigate the mechanisms behind the reduced success of *Daphnia* feeding on nutrient-stressed algae, a series of experiments were conducted with *D. pulex* and *D. magna* feeding on P-saturated and P-limited cells of two algal species.

Methods

The green algae *Selenastrum capricornutum* NIVA CHL 1 and *Scenedesmus subspicatus* NIVA CHL 55, were selected for the grazing experiments. The species are kept in cultures at the culture collection of the Norwegian Institute for Water Research (Skulberg and Skulberg 1990). The zooplankters *Daphnia pulex* and *Daphnia magna* were also taken from a laboratory monoculture, where they had been grown on *Selenastrum capricornutum* for more than 2 years. Inoculum phytoplankton cultures were incubated in the inorganic nutrient medium Z8 20% at 20° C (Skulberg and Skulberg 1990). Illumination was provided by cool-white fluorescent tubes at 70 $\mu\text{E m}^{-2} \text{s}^{-1}$ operating on a 14:10 h LD cycle. Cells growing exponentially with maximum rate were used in the grazing experiments with P-saturated cells. To obtain P-limited algal cells, some of these exponentially growing cells were inoculated into flasks containing a phosphorus-free medium. The cells reached a steady population level after 5 days. The grazing experiments were carried out in 1-l Erlenmeyer flasks into which P-saturated and P-limited cells were inoculated in 500 ml culture medium with a low P concentration (0.5 $\mu\text{g P l}^{-1}$). Before inoculation, the algal cells were washed by centrifuging in the low-P medium. The initial algal cell concentration was c. $5 \cdot 10^4$ cells ml^{-1} .

In the first experiment the feeding of *D. pulex* on the green alga *S. capricornutum* under P-saturated and P-limited conditions was studied. In the second experiment *D. pulex* was fed *Sc. subspicatus*, along with a replicate of the first experiment. The third experiment was performed with *D. magna* grazing on P-saturated and P-limited *S. capricornutum*. At the start of experiments I and II 30 similar-sized individuals of *D. pulex* (length 2.0–2.5 mm, weight 34–67 $\mu\text{g C}$)

were added to each algal monoculture of 500 ml (P-saturated and P-limited cells). For the grazing experiments with *D. magna* (experiment III) 15 individuals (length 3.1–3.7 mm, mean weight 142–197 $\mu\text{g C}$) were incubated in 500 ml algal suspension. Before incubation the animals were washed to remove surface contamination. The grazing experiments lasted 2–3 days and were carried out in triplicate (experiment I) or in duplicate (experiments II and III). Control flasks were without animals. Flasks were shaken manually four times a day. At the start of the experiment the C, N and P contents of *S. capricornutum* were analyzed. C and N contents were measured on a Carlo-Erba CHN 1106 elemental analyzer and P content on the total samples after peroxy-disulfate digestion. Zooplankton dry weight was measured at the start and end of the experiment. Samples for algal counts were taken at $t=0, 20, 28, 43, 70$ h in experiment I and at $t=0, 4, 7, 20, 24, 28, 31, 48$ h in experiments II and III (*Daphnia* introduced at $t=0$). Samples were preserved with Lugol's solution. Algae were counted microscopically and least-squares regressions of $\ln(\text{cells ml}^{-1})$ versus time (days) were calculated. Because measurements for replicate flasks were close compared to other sources of variation, replicates were pooled. The slopes of the regressions for the controls (algal growth rates) added to the disappearance rates in the grazing experiments gave the gross disappearance rates. Release of P in the grazing flasks would not enhance algal growth relative to the control for the P-saturated algae which had a surplus of intracellular P. For the P-limited algae, release of P by zooplankton was extremely low (Van Donk, Faafeng, Hessen, Källqvist, submitted) and would only have minor effects on algal growth rate. The disappearance rates were not true clearance rates since a high proportion of algae in the P-deficient treatments were not digested. The faeces of the daphnids were analysed during each experiment. Animals were placed individually in a drop of water on slides under cover slips. After defecation, rectum contents of *Daphnia* were dispersed and examined microscopically (Porter 1975).

Results

The algal disappearance rates determined for *D. pulex* feeding on P-limited algal cells in experiments I and II were greatly reduced compared with those on P-saturated cells (Figs. 1 and 2, Table 1). A reduction of c. 70–75% of the gross disappearance rate was observed for *S. capricornutum* and *Sc. subspicatus* grazed by *D. pulex*. In experiment III *D. magna* feeding on P-limited *S. capricornutum* demonstrated a reduction in rate of c. 50% (Table 1). After 2 days, mean weight of *D. pulex* feeding on P-limited cells had decreased by 37% (*S. capricornutum*) and 29% (*Sc. subspicatus*) relative to P-saturated cells. Feeding rate was tested by adding suspensions of mono-dispersed, fluorescent beads to flasks with similar densities of P-saturated and P-limited algae, but no difference in gut filling time (c. 10–12 min) was found in the two treatments. Faeces analyses revealed that the P-limited algal cells passed for the most part undamaged through the guts of the daphnids (Fig. 3a), probably because of reduced digestibility. P-saturated algal cells, in contrast, were efficiently assimilated (Fig. 3b). Under the light microscope P-limited *S. capricornutum* and *Sc. subspicatus* had a granular appearance with internal, spherically-shaped clear areas. Further P-limited *S. capricornutum* increased in volume from 31 to 64 μm^3 cell $^{-1}$ and became supercoiled (Fig. 4). Apparently, these changes in the algal cells due to P limitation could serve as a morphological constraint on efficient assimilation.

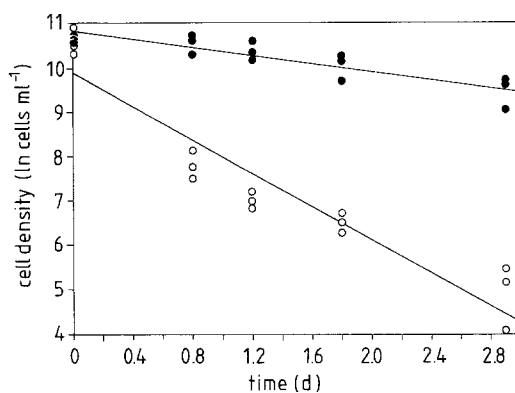


Fig. 1. Cell density of *Selenastrum capricornutum* (● P-limited; ○ P-saturated) after incubation with 30 similar-sized individuals of *Daphnia pulex* (length 2.0–2.5 mm) for 3 days in 500 ml culture medium with a low P-concentration ($0.5 \mu\text{g P l}^{-1}$). The experiments were carried out in triplicate

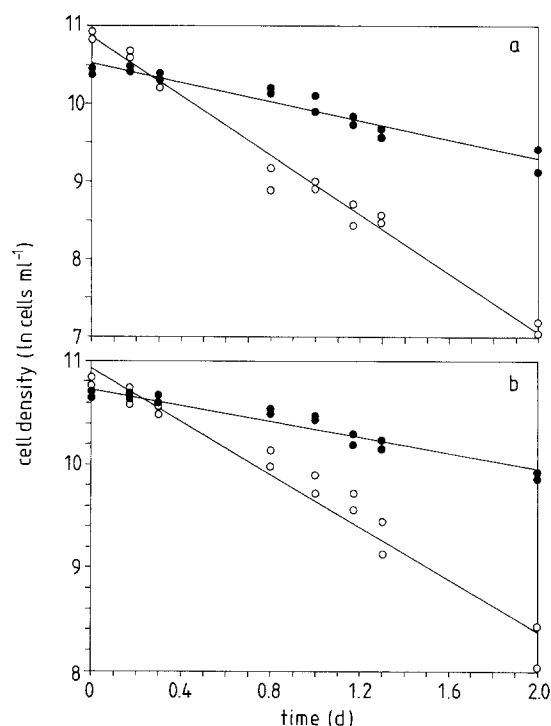


Fig. 2. a Cell density of *Selenastrum capricornutum* (● P-limited; ○ P-saturated) and b of *Scenedesmus subspicatus* (● P-limited; ○ P-saturated) after incubation with 30 similar-sized individuals of *Daphnia pulex* (mean length 2.0–2.5 mm) for 2 days in 500 ml culture medium with a low P concentration ($0.5 \mu\text{g P l}^{-1}$). The experiments were carried out in duplicate

Discussion

It is well recognized that various algal species have different resistance to grazing and therefore also under nutrient-saturated conditions provide food of highly differing quality for zooplankton. Cyanobacteria are in general a poor food source for zooplankton grazers like *Daphnia* (Arnold 1971; Lampert 1982, 1987) in part due to morphological properties and toxin production. Fur-

Table 1. Disappearance rates (D.r.) of algae (SE in parentheses) as measured in three experiments with zoo plankters *Daphnia pulex* (60 ind./l in experiments I and II) and *Daphnia magna* (30 ind./l

in experiment III) grazing on P-saturated and P-limited algae (Sel = *Selenastrum capricornutum*; Scen = *Scenedesmus subspicatus*)

Treatment	C : N : P in algae (atomic ratios)	D.r. (d ⁻¹)	G.r. (d ⁻¹)	Gross D.r. (d ⁻¹)
<i>Exp I</i>				
Sel (P-sat.)	159 : 21 : 1	-1.90 (±0.20)	0.65 (±0.09)	-2.55 (±0.22)
Sel (P-lim.)	2032 : 48 : 1	-0.43 (±0.06)	0.18 (±0.07)	-0.61 (±0.09)
<i>Exp II</i>				
Sel (P-sat.)	159 : 14 : 1	-2.10 (±0.07)	0.75 (±0.11)	-2.85 (±0.13)
Sel (P-lim.)	1032 : 46 : 1	-0.62 (±0.04)	0.24 (±0.08)	-0.86 (±0.09)
Scen (P-sat.)	-	-1.30 (±0.08)	0.76 (±0.07)	-2.06 (±0.11)
Scen (P-lim.)	-	-0.40 (±0.03)	0.18 (±0.03)	-0.58 (±0.13)
<i>Exp III</i>				
Sel (P-sat.)	159 : 14 : 1	-3.51 (±0.18)	0.75 (±0.11)	-5.26 (±0.21)
Sel (P-lim.)	1032 : 46 : 1	-2.37 (±0.15)	0.24 (±0.08)	-2.61 (±0.17)

Gross D.r. = Dis. rate corrected for growth rate of algae (G.r.) as measured in controls without grazers

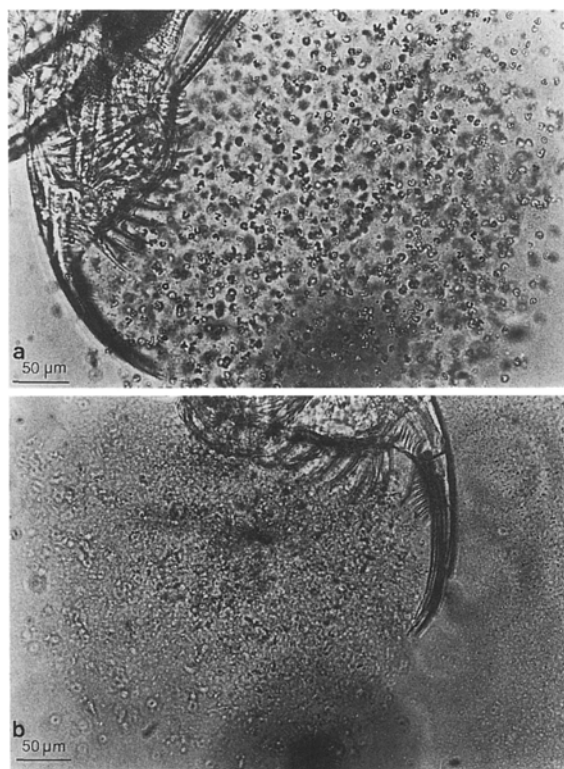


Fig. 3a, b. Light microscopic photographs of faeces of *Daphnia pulex* after grazing on **a** P-limited and **b** P-saturated *Selenastrum capricornutum*

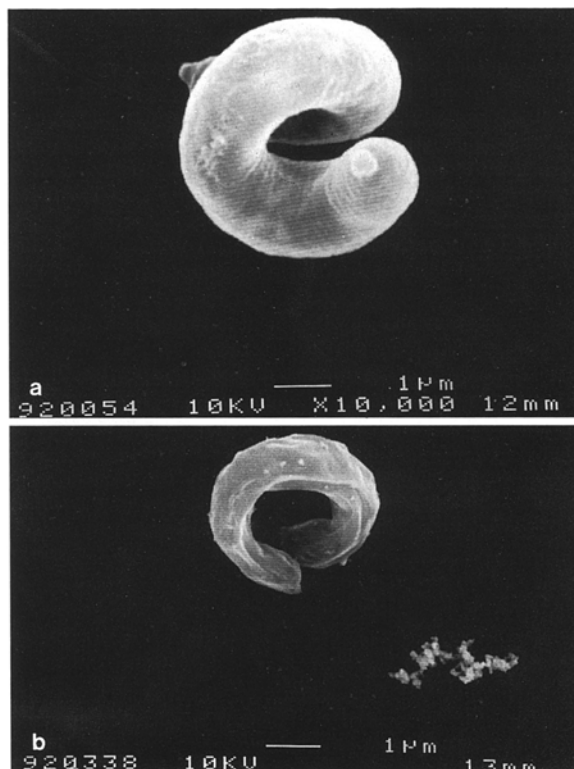


Fig. 4a, b. Scanning electron microscopic photographs of **a** P-limited and **b** P-saturated *Selenastrum capricornutum*

thermore, some green algae and cyanobacteria surrounded by gelatinous sheaths may avoid assimilation and pass undamaged through the guts of zooplankton (Porter 1973; Lampert 1982). Perhaps also mucous carbon compounds, which algae may accumulate extracellularly under nutrient deficiency (Myklestad 1977; Søndergaard and Schierup 1982), serve as a barrier to digestive enzymes. In our experiments P-limited algal cells differed

from P-saturated cells by the presence of starch granulae under the light microscope, analogous to the findings of Sterner and Smith (1992) for *Scenedesmus acutus* under P- and N-limitation. Further P-limited *S. capricornutum* clearly demonstrated morphological changes (Fig. 4).

We suggest that both mucus secretion and cell structural/morphological changes may be important mechanisms for nutrient-stressed phytoplankton, strongly in-

creasing their resistance to grazers. While high growth rates may compensate to some extent for grazing losses at high nutrient availability, some defence mechanisms would obviously be beneficial during periods when pools of dissolved nutrients are depleted (end of spring bloom) and zooplankton biomass and grazing pressure are high. If such properties are common among phytoplankton, they could also provide an explanation for the many different values reported for zooplankton assimilation and growth when feeding on the same algal species (Lampert 1987).

Such grazing resistance would also have important feedback effects on the zooplankton community. Some zooplankters, such as calanoids, may distinguish between nutritionally high-quality and poor-quality algae (DeMott 1986), and actively discriminate against the latter. These calanoids would thus be competitively favoured in situations with a high proportion of such undigestible algae. The cladoceran *Daphnia*, however, has no such ability (Hessen 1985; DeMott 1986), and would thus ingest all similar-sized algae with equal efficiency. The "strategy" we report here would thus be a way for slow-growing nutrient-limited phytoplankton to resist heavy grazing pressure from *Daphnia* and related non-selective filter-feeders.

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