Hydrobiologia **307**: 147–151, 1995.
P. Larsson & L.J. Weider (eds), Cladocera as Model Organisms in Biology.
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Reduced digestibility of UV-B stressed and nutrient-limited algae by Daphnia magna

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Key words: Daphnia, digestibility, grazing, nutrient limitation, phytoplankton, UV-B

Abstract

Daphnia magna was fed the green alga *Selenastrum capricornutum*, cultured under four different growth conditions: (1) phosphorus limitation, (2) nitrogen limitation, (3) UV-B irradiation, and (4) no nutrient limitation, no UV-B irradiation. Contrary to non-limited algal cells, nutrient-limited cells were not efficiently assimilated. Especially, P-limited cells passed through the gut mostly intact, while N-limited cells were partly assimilated. Also, algae exposed to moderate doses of UV-B radiation (0.3 mW cm^{-2} of UV_{312}) were less efficiently assimilated after being grazed. Digestibility of the algae decreased with increased UV-B exposure time. Nutrient-limited and UV-B stressed algal cells increased in volume and became granular in appearance. These changes in the algal cells, combined with changed cell wall properties, most probably reduced their digestibility.

Introduction

There are numerous studies on the effects of nutrient-limitation (e.g. Hecky & Kilham, 1988) and UV-B radiation (e.g. Steemann-Nielsen, 1964; Lorenzen, 1979; Worrest, 1982; Cullen & Lesser, 1991) on phytoplankton growth and productivity. Both nutrient limitation and UV-B radiation may induce a delay in algal cell division. Furthermore, current levels of solar UV-B radiation are known to inhibit other metabolic processes in algal cells such as nutrient uptake and flagellate motility (Döhler, 1985; Häder & Häder, 1988). Nutrient limitation and UV-B stress may also change the cell morphology and structure of the algae. An increase in average cell size has been observed both for UV-B irradiated algal cells (Karentz *et al.*, 1991; Veen, 1991) and for nutrient limited cells (Mitchell *et al.*, 1992; Van Donk & Hessen, 1993). These changes in stressed algal cells may also influence their digestibility for zooplankton. Recently Van Donk & Hessen (1993) demonstrated that P-limited green algae (*Scenedesmus subspicatus* and *Selenastrum capricornutum*) passed largely intact through the gut of *Daphnia* and were thus protected from heavy grazing losses. The reduced digestibility of P-limited cells was attributed to structural and morphological changes in the P-limited cells. Other studies have demonstrated decreased growth and reproduction in *Daphnia* if fed P-limited algae even when food was abundant (Hessen, 1990; Watanabe, 1990; Sommer, 1992; Sterner, 1993). So, apparently P-limitation of the phytoplankton can alter trophic interactions, reducing the transfer of energy to zooplankton.

In order to analyse whether reduced digestibility is also present among algae subject to other stress factors than P-limitation, we conducted a series of experiments with *D. magna* feeding on N-limited and UV-B irradiated cells of the green alga *Selenastrum capricornutum*. Also, a replicate of the grazing experiment with P-limited cells, as described in Van Donk & Hessen (1993), was carried out.

Materials and methods

The green alga *Selenastrum capricornutum* NIVA CHL 10, used in the experiments, was obtained from the culture collection of the Norwegian Institute for Water Research (Skulberg and Skulberg, 1990). An inoculum of the axenic algal culture was incubated in the inorganic nutrient medium Z8 20% at 20 °C (Skulberg & Skulberg, 1990). Illumination was provided by cool-white fluorescent tubes at 70 μE m^{-2} s^{-1} using a 14:10 h LD cycle.

To obtain P- and N-limited algal cells, exponentially growing cells were inoculated into flasks containing a phosphorus-free and nitrogen-free medium, respectively. The cells entered stationary state after five days (P-limitation) and three days (N-limitation).

To obtain UV-stressed algae, cells harvested during exponential growth, were incubated in complete medium in flat-bottom beakers and exposed to UV_{312}-radiation. A Vilber-Lourmat VL-115M, 15 Watts Lamp, with peak irradiance at 312 nm was used as the UV source. Additional white light illumination was given as described above. A gradient of UV-B doses was achieved by exposing the algae to UV_{312} during various periods (2, 4, 8, 12, 20, 25 and 48 h). We used a standard intensity of 0.3 mW cm^{-2}, or 0.018 J cm^{-2} min^{-1}, corresponding to maximum, mid-summer irradiance as measured near Oslo (Hessen & Van Donk, 1994). These exposure periods represented doses of 2.2, 4.3, 8.6, 13.0, 21.6, 27.0 and 51.8 J cm^{-2} UV_{312} respectively. Light intensity was measured with a Vilber-Lourmat VLX-3W Radiometer, with peak sensitivity at 312 nm. The UV_{312}-dose recorded by this instrument is somewhat higher than those measured with the commonly used LI-COR spectro-radiometer, since the former integrates some radiation on both sides of 312 nm.

The grazing experiments were carried out with the zooplankter *Daphnia magna*, obtained from a laboratory culture grown on *S. capricornutum* for more than two years. In the first two experiments, feeding by *D. magna* on the green alga *S. capricornutum* cultured under P- and N-limited conditions, was studied. In the third experiment *D. magna* was fed UV-B stressed algae. A control experiment was carried out with non-limited, non-irradiated algal cells.

All experiments were conducted in the dark in 0.5 l Erlenmeyer flasks and six daphnids (length 2.8–3.3 mm, mean dry weight 160 μg ind^{-1}) were incubated in 400 ml algal suspensions. For each treatment, algae without zooplankters were also incubated in the dark to control whether algal numbers changed due to factors other than grazing.

Table 1. Disappearance rates of the green alga *Selenastrum capricornutum* due to grazing by *Daphnia magna* (15 ind l^{-1}) grazing on P-limited (Exp. I), N-limited algae (Exp. II) and UV-B stressed algae (Exp. III) (h = hours of exposure to 0.018 J cm^{-2} min^{-1} UV_{312}). C:N:P ratios and cell volumes of the cells are measured at the start of the experiment. In the controls, algal cells were non-limited and not stressed by UV-B. (Standard errors are given in parentheses). An asterisk (*) indicates that the values differ significantly ($p<0.05$) from the controls

Treatment	C:N:P in algae (atomic ratios)	Cell volume (μm^3)	Disappearance rate (day^{-1})
Control	67:9:1	31.5 (±1.8)	1.35 (±.08)
Exp. I			
P-lim	714:71:1	58.6 (±2.7)*	0.22 (±.03)*
Exp. II			
N-lim	55:2:1	39.4 (±4.3)*	0.94 (±.18)*
Exp. III			
UV_{312}			
2h		33.6 (±1.3)	1.33 (±.04)
4h		26.7 (±2.3)*	1.48 (±.22)
8h		36.6 (±3.4)*	1.25 (±.14)
12h		42.2 (±1.6)*	1.06 (±.21)*
20h		53.7 (±2.2)*	0.62 (±.03)*
25h		56.2 (±2.1)*	0.47 (±.08)*
48h		64.2 (±3.5)*	0.32 (±.03)*

The initial algal cell concentration in all experiments was *c.* 2 × 10^5 cells ml^{-1}. The mean volume of the algal cells was measured at the start of the grazing experiments by an electronic particle counter (Coulter Counter). Volumes of stressed and non-stressed algal cells were statistically compared by applying the non-parametric Mann-Whitney U test (Fowler & Cohen, 1993). The critical probability value was set at $p<0.05$. The grazing experiments lasted for 2–3 days. Flasks were shaken manually four times a day. At the start of Exp. I and II the C, N and P contents of both non- and nutrient-limited algae were analyzed. C and N contents of algae, filtered onto acid-washed pre-ignited GF/F-filters, were measured on a Carlo-Erba CHN 1106 elemental analyzer, and P content of the total samples was measured after peroxo-disulfate digestion.

Algae were counted at intervals of four to eight hours during the three days of incubation (*Daphnia* introduced at t = 0). Samples were preserved with Lugol's solution. Algae were counted microscopically

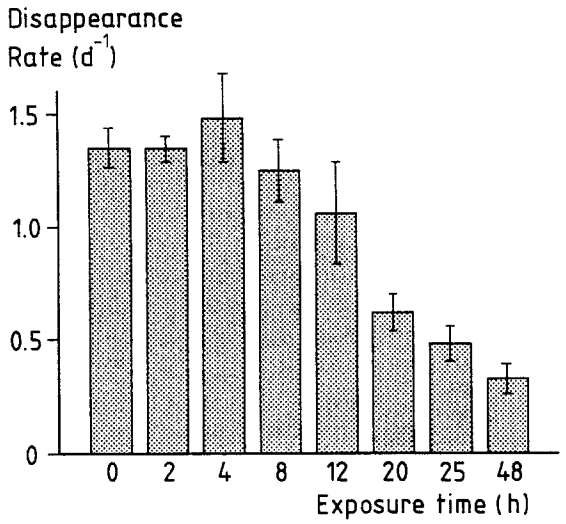

Fig. 1. Mean disappearance rates (day^{-1}) of the green alga *Selenastrum capricornutum* caused by grazing of *Daphnia magna*. The algae were first exposed to UV_{312}-radiation (0.018 J cm^{-2} min^{-1}) during different periods. Standard error bars are given.

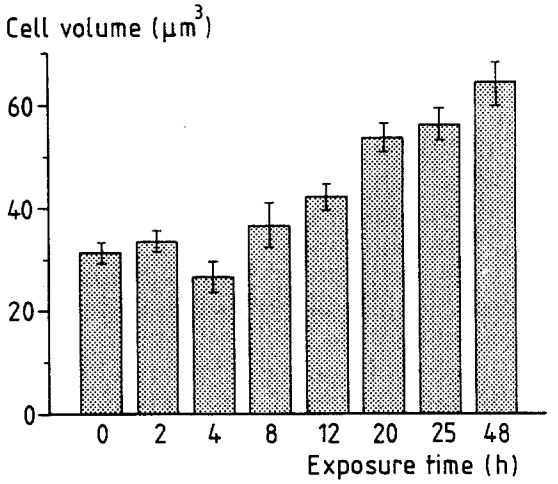

Fig. 2. Mean cell volumes of the green alga *Selenastrum capricornutum* after exposure to UV_{312}-radiation (0.018 J cm^{-2} min^{-1}) during different periods. Standard error bars are given.

and disappearance rates of the algae were calculated from the slope of the regression between the decrease in cell numbers (ln(cells/ml)) and time (days). Because measurements for replicate flasks were similar compared with other sources of variation, the replicates were pooled. The calculated disappearance rates of the algae were not true clearance rates because a large proportion of the algae in the nutrient-deficient and UV-irradiated treatments were grazed, but not digested. The disappearance rates measured in Exp. I, II and III were compared statistically with the rate in the controls by applying a t-test (critical probability value of $p<0.05$) on the regression lines (Fowler & Cohen, 1993).

The egestion products of the daphnids were analysed during each experiment. Animals were placed individually on slides under cover slips. After defecation, rectum contents of *Daphnia* were dispersed and examined microscopically (Porter, 1975).

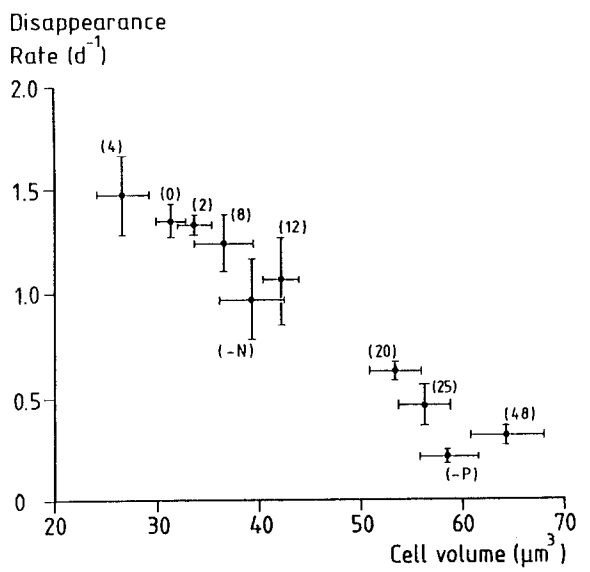

Fig. 3. Mean disappearance rates (day^{-1}) of the green alga *Selenastrum capricornutum* grazed by *Daphnia magna* as a function of mean algal cell volumes, measured during nutrient-limitation (−P = P-limitation, −N = N-limitation) and UV-B stress (2–48 h = hours of exposure to UV_{312}-radiation of 0.018 J cm^{-2} min^{-1}). Standard error bars are given.

Results

The algal disappearance rates determined for *D. magna* feeding on P- and N-limited algal cells in experiments I and II and UV-B irradiated cells in experiment III are given (Table 1). Further, the C:N:P ratios and mean volumes of the algal cells are given, as well as disappearance rate for non-limited and nonirradiated algae in the control experiment. Algae incubated in the dark without *Daphnia* demonstrated no significant changes in cell numbers during the three days of incubation.

A significant lowered algal disappearance rate (80–85% reduction relative to the control) was observed in experiment I when *D. magna* was feeding on P-limited

S. capricornutum. *Daphnia* feeding on N-limited algae in experiment II demonstrated a significant reduction in disappearance rate of approximately 30% (Table 1).

In experiment III when *Daphnia* were grazing on UV-B stressed algae, disappearance rates were found to be significantly reduced only for algae irradiated for 12 h or more (>13.0 J cm^{-2} UV_{312}). Algae stressed by UV-B for 48 h (51.8 J cm^{-2} UV_{312}) demonstrated a reduction of approximately 75% relative to the control (Table 1 and Fig. 1).

Both nutrient limited and UV-B stressed algae increased their cell volumes. Under the light microscope, the large cells had a granular appearance with internal, spherically-shaped clear areas. The largest cell volumes were found for P-limited algae and 48 h UV-B irradiated algae (approximately twice the normal cell volume; Table 1). A significant increase in cell volume was found for all algae irradiated for 8 h or more (>8.6 J cm^{-2} UV_{312}). A significant decrease in cell volume was measured only for algae irradiated during 4 h (Table 1; Fig. 2). A negative relationship was found between algal cell volumes and disappearance rates (Fig. 3).

Fecal analyses revealed that the algal cells with a larger volume were normally grazed, but a high number of these cells passed undamaged through the daphnid gut, probably because of reduced digestibility. In contrast, nonlimited and nonirradiated cells were assimilated efficiently.

Discussion

Cell volume of both nutrient-limited and UV-B stressed cells increased and under the light microscope, these cells had a granular appearance. Such changes in algal cells have also been observed in other studies where algae were cultured under nutrient limitation (Mitchell *et al.*, 1992; Sterner *et al.*, 1993; Van Donk & Hessen, 1993) or UV-B stress (Karentz *et al.*, 1991; Veen, 1991). Karentz *et al.* (1991) and Veen (1991) stated that an increase in cell dry weight of UV-B stressed algae reflects a storage of proteins and carbohydrates due to delayed cell division. Myklestad (1977) and Søndergaard & Schierup (1982) observed the release of photosynthetic products by algae growing under nutrient-stress.

Our results indicate that digestibility of algae by *Daphnia* may be severely influenced by the physiological state of the algae. Contrary to nonlimited and nonirradiated algal cells, nutrient limited and UV-B irradiated cells were assimilated with very low efficiency. Specifically, P-limited and UV-B stressed algae passed largely intact through the gut.

Van Donk & Hessen (1993) found a doubling of cell volume for P-limited cells of *Selenastrum capricornutum* and a considerable reduction in the assmilation rate by *D. pulex* (approximately 75%) and *D. magna* (approximately 50%). In these experiments digestibility of the P-limited cells decreased while feeding rates were not influenced. A decrease in clearance and feeding rates was measured by Sterner *et al.* (1993) for *D. pulex* grazing on severely P- and N-limited green alga *Scenedesmus acutus*. To our knowledge no previous grazing experiments with UV-B stressed algae have been carried out.

Preliminary results (Van Donk *et al.*, in prep.) from our experiments with the green alga *Chlamydomonas reinhardtii*, cultured under P- and N-limited conditions, support the results presented here. Transmission electron microscopic photographs of nutrient-limited *Chlamydomonas* revealed intracellular storage of photosynthetic products, considerable thickening of the cell wall mainly consisting of glycoproteins, and accumulation of mucous carbon compounds around the cell wall. The same grazing experiments, executed with a mutant clone of *Chlamydomonas reinhardtii* lacking a cell wall, showed, however, efficient assimilation of non-limited as well as nutrient-limited cells. Although the structure of the nutrient-limited cells changed due to storage of photosythetic products, the volumes of the mutant cells did not increase significantly. These results indicate that changes in and around the cell wall are most likely responsible for the decrease in digestibility. One might speculate whether UV-stress inhibits P-uptake in algae, giving rise to the same physiological properties as those observed under real P-deficiency. Döhler & Biermann (1987) measured a reduced ^{15}N-nitrate uptake for two marine diatoms under UV-B irradiation.

Summarizing, we conclude that UV-B stressed and nutrient-limited phytoplankton strongly increased their resistance to digestibility, most probably due to both mucous secretion and changes in the cell wall structure. Thus, at times when phytoplankton growth rates are reduced by these stress factors, also grazing pressure on these phytoplankters would be reduced. These grazing experiments were only conducted with stressed green algae. Studies on other edible algae are necessary to assess if these effects form a common phenomenon among phytoplankton populations. Sever-

al studies report differential sensitivity of freshwater assemblages to inhibition by UV-B radiation (e.g. Gala & Giesy, 1991; Döhler, 1985). Reduced digestibility of stressed algal cells, if also present among natural populations, may significantly alter trophic interactions and reduce the transfer of energy between primary producers and consumers in aquatic ecosystems.

Acknowledgements

We would like to thank the staff at the Norwegian Institute for Water Research, Oslo, for the use of algal cultures and laboratory facilities and Dr R. D. Gulati and Prof Dr W. J. Wolff for their constructive comments on earlier versions of the manuscript.

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