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Loss of flagella in the green alga *Chlamydomonas reinhardtii* due to *in situ* UV-exposure*

ELLEN VAN DONK[1] and DAG O. HESSEN[2]

[1]Agricultural University Wageningen, Department of Water Quality Management and Aquatic Ecology, P.O. Box 8080, 6700 DD Wageningen, The Netherlands.
[2]University of Oslo, Biological Institute, Div. Limnology, P.O. Box 1027, Blindern, 0316 Oslo, Norway.

SUMMARY: In this preliminary study the flagellated, planktonic green alga *Chlamydomonas reinhardtii* was exposed to natural UV doses in an oligotrophic alpine lake and a mesotrophic lowland lake, both situated in Norway. The algae, which were incubated in glass, quartz or mylar-covered flasks at different depths, showed a pronounced loss of flagella with increasing UV radiation. Apparently, UV-A was as detrimental as UV-B for the flagella. The potential role of *C. reinhardtii* as a bio-dosimeter for UV stress, and potential food chain effects are discussed.

Key words: Phytoplankton, flagella, UV, *Daphnia*, *Chlamydomonas*, bio-dosimeter.

INTRODUCTION

Phytoplankton in the upper euphotic zone of oceans and lakes exhibit variable degrees of photoinhibition during periods of peak irradiance. The fraction of total surface photoinhibition that can be attributed to the narrow waveband of ultraviolet-B radiation (290 to 320 nm) has recently been the topic of much research due to the increase in surface UV-B resulting from stratospheric ozone depletion (*e.g.* Kerr and McElroy, 1993). UV-B radiation reduces photosynthesis, growth, survival, nutrient uptake and photosynthetic pigment concentrations (*e.g.* Steemann-Nielsen, 1964; Lorenzen, 1979; Worrest, 1982; Cullen and Lesser, 1991; Döhler, 1985), affects motility and phototactic orientation (*e.g.* Häder, 1985; Häder and Häder, 1988; Ekelund, 1993) and increases mutagenesis in DNA and protein damage (*e.g.* Karentz *et al.*, 1991).

Increased UV-stress may also have an impact on phytoplankton species composition. Various algal species seem to have different susceptibility to UV radiation (Worrest, 1982; Gala and Giesy, 1991). Smaller species of diatoms were found to be more susceptable to UV-B than larger ones (Karentz *et al.*, 1991). Flagellated algae, important primary producers of biomass in freshwater habitats, seem more susceptible than non-motile algae (Häder and Häder, 1988; Häder, 1993).

Recently performed laboratory experiments have indicated a strong susceptibility for UV-B in the green flagellate alga *Chlamydomonas reinhardtii*, with the number of flagella, P-uptake and growth rates as sensitive parameters (Hessen *et al.*, 1995). A strong relation was found between loss or withdrawal of flagella and UV-exposure. Reduction of P-uptake was well correlated with inactivation of fla-

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gella and algal growth inhibition. A problem with such laboratory studies is to apply realistic spectral distributions. In this preliminary study we attempt to evaluate these UV effects on *Chlamydomonas* under natural light conditions in lakes, and thus further assess the potential use of this alga as a UV dosimeter. We screened a range of light regimes in an oligotrophic alpine lake and in a mesotrophic lowland lake.

MATERIAL AND METHODS

Cells from a dense culture of *Chlamydomonas reinhardtii* (NIVA-CHL 13) were diluted in 50% Z8 medium (Skulberg and Skulberg, 1990), before *in situ* exposure. Three types of flasks were used: 150 ml quartz flasks, the same bottles wrapped in Mylar (single layer) and 150 ml glass bottles. While quartz flasks are transparent to UV-B and UV-A, the Mylar filter blocks below 320 nm (transparent to UV-A but not to UV-B), and glass bottles block all UV-B and most UV-A.

The incubations were performed at two localities: a small, shallow (mean depth 3 meters) oligotrophic alpine lake and a mesotrophic lowland lake (Lake Trollvann). The alpine locality was situated at Finse, ca. 300 km west of Oslo, S.E. Norway, at 1200 m altitude. Mid-summer chlorophyll-*a* levels for this lake are less than 2 µg l^{-1}. Lake Trollvann is located on the outskirts of Oslo (300 m above sea level), with mid-summer chlorophyll-*a* concentrations of around 6-8 µg l^{-1}. This lake is slightly influenced by humic substances. The flasks were exposed horizontally under a buoy in the center of each lake. Incubations were carried out from 6 to 8 July 1994 at depths 0.1 and 0.5 m in the alpine locality (water temperature 6°C) and from 22 to 24 June 1994 at depths 0.5, 1.0 and 2.0 m in the lowland lake (water temperature 18 °C). Initial cell concentration in the flasks incubated in the alpine lake was 4 x 10^5 cells ml^{-1} and in the lowland lake 1.5 x 10^5 cells ml^{-1}. In both localities the experiments started at 9 a.m. and in the alpine locality, where a considerable light stress was anticipated, samples at 0.1 m were subsequently taken at 1 p.m. (after 4 h) and 6 p.m (after 9 h) in the afternoon, while at 0.5 m only at 6 p.m. the first day (after 9 h) and the two following days (after 33 and 57 h). In the lowland lake, with expected low underwater UV-levels, samples were taken after 24 and 48 h. Both incubations were performed in bright sun, and care was taken to carry out sampling in dim light (under cover) to avoid light shock of the algae at the surface. Subsamples of 5 ml were fixed with lugol to count number of cells under a light microscope and to examine cell shape and flagella.

Light levels of the two localities were measured by a LICOR 1800 underwater spectroradiometer with a 2 nm resolution from 300 to 840 nm.

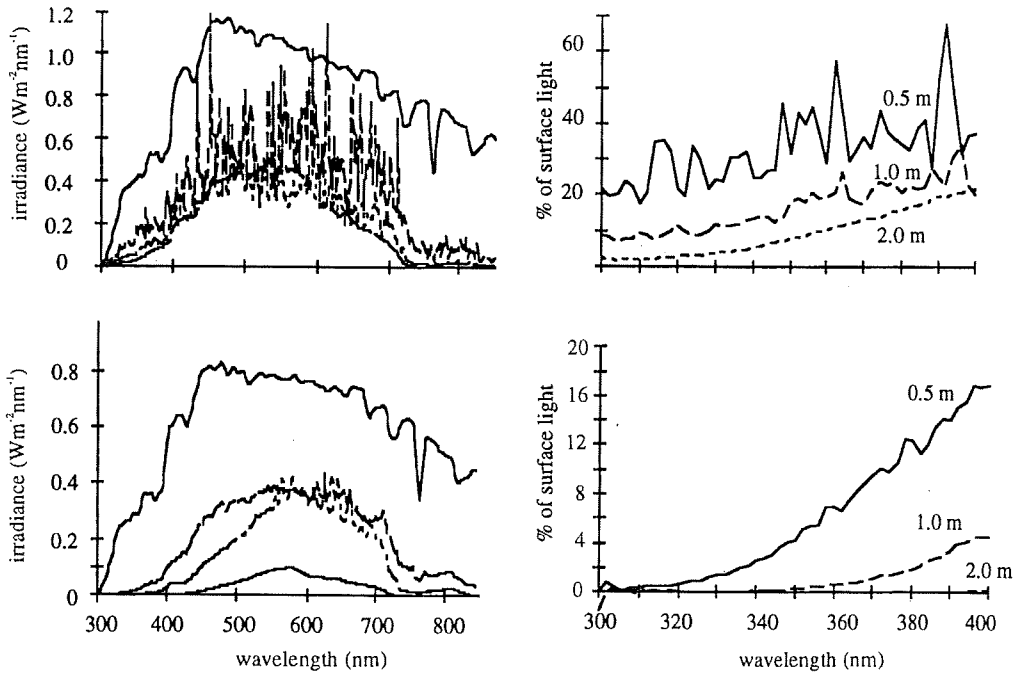

FIG. 1. – Maximum light intensity and spectral distribution at surface (air), 0.5 m, 1 m and 2 m (top to bottom, left panel) in the clear alpine lake (upper) and the mesotrophic lowland lake (lower) during the incubation experiments. Right panel: percentage remaining light of wavelengths 300-400 nm at 0.5 m, 1 m and 2 m relative to surface (air) for the Alpine lake (upper) and the lowland lake (lower). Note different scales on left panel.

RESULTS

Irradiance

The underwater light field of the two localities reflected different productivity and absorption properties (Fig. 1). Total irradiance was somewhat higher at the alpine site, but relative absorption at 0.5, 1.0 and 2.0 m clearly confirmed the higher UV-exposure of the algae in the alpine lake. At 0.5 m in this locality, 20-30% of surface (air) light remained over the range from 300 to 400 nm. At 1 m, *ca.* 10% of surface light remained in the UV-B region, increasing up to more than 20% towards 400 nm. A smoother curve was measured at 2 m, where less than 3% remained in the UV-B, but again increasing sharply in the UV-A, up to > 15% of surface light. This is in contrast to the lowland lake, where almost no UV-B was detectable even at 0.5 m, and a maximum of only 16% at 400 nm.

Loss of flagella

At the start of the experiments between 86% (lowland lake) and 98% (alpine lake) of the cells had two flagella and only resp. 12% and 2% cells with no flagella were recorded (Figs. 2 and 3).

In the alpine locality a strong response was observed upon radiation in the quartz flasks (Fig. 2). After 9 h less than 10% had two intact flagella

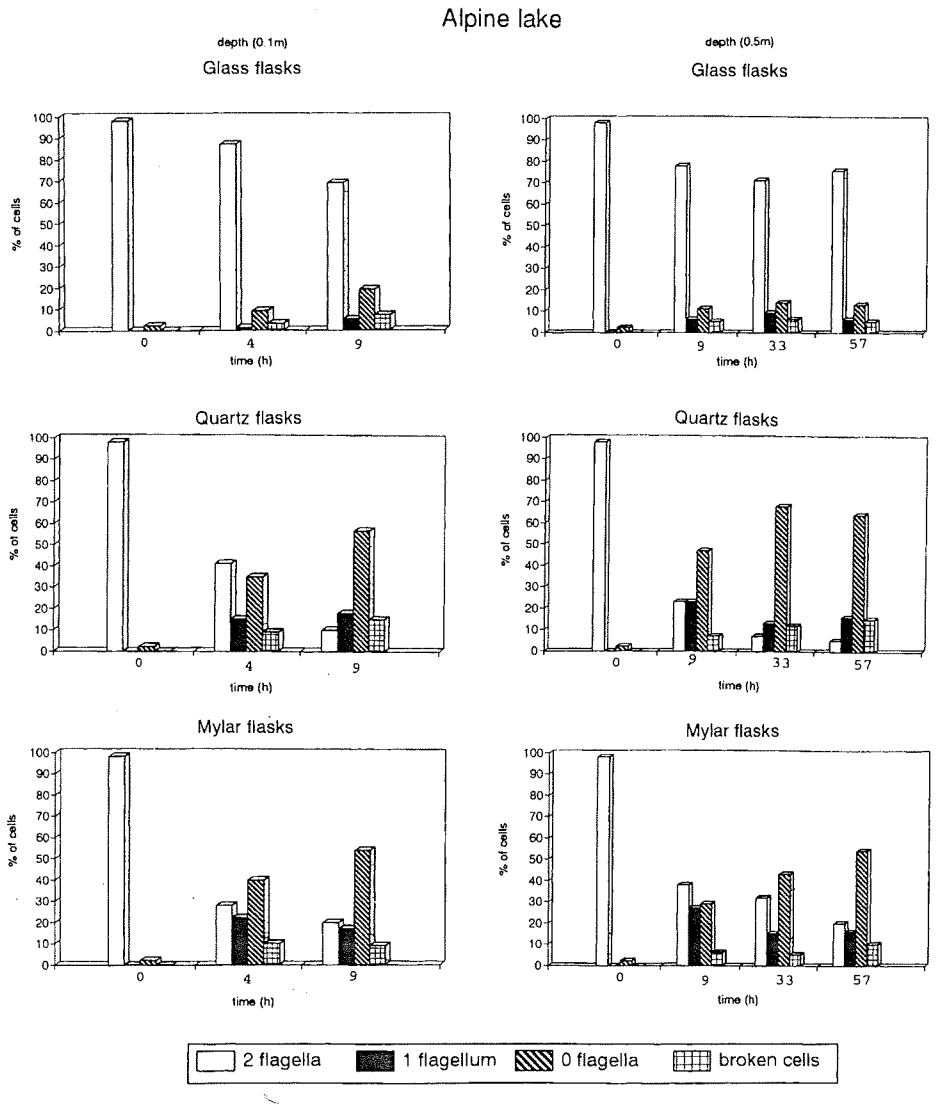

FIG. 2. – Percentages of *Chlamydomonas* cells with two, one, and zero flagella and percentages of cells broken present in glass, quartz and mylar flasks after 4 and 9 h incubation at a depth of 0.1 m, and after 9, 33 and 57 h incubation at a depth of 0.5 m in the alpine locality. The experiment started 6 July 1994 at 9 a.m. (t=0).

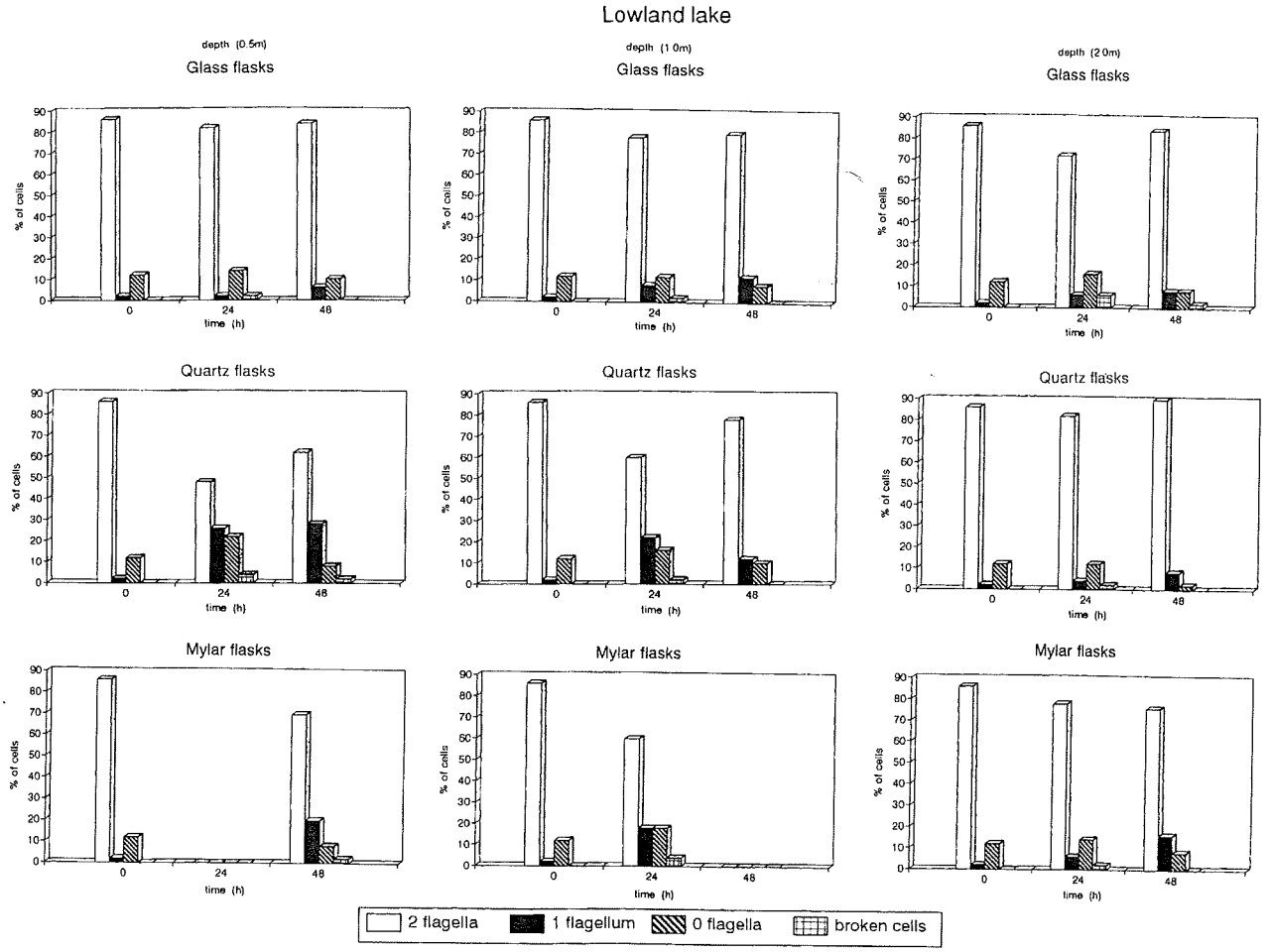

FIG. 3. – Percentages of *Chlamydomonas* cells with two, one, and zero flagella and percentages of cells broken after 24 and 48 h incubation in glass, quartz and mylar flasks at a depth of 0.5 m, 1.0 m and 2.0 m in the mesotrophic lowland lake. The experiment started 22 June 1994 at 9 a.m. (t=0).

at 0.1 m, and also at 0.5 m only 7% of the cells were intact after 33 h. As much as 15% of the cells were dead with cytoplasma leakage after 9 h at 0.1 m and one day later (33 h) at 0.5 m. Also the Mylar flasks gave a high percentage of harmed (broken) cells, indicating a considerable UV-A stress (Fig. 2). There was, however, a large difference in pigment bleaching between algae incubated in quartz and Mylar flasks. At both depths, exposure in quartz flasks revealed very pale cells with no sign of pigment recovery even after two days of indoor post-exposure incubation (unpubl. data). This is in contrast to the Mylar and glass bottles, where the color remained during exposure, and where an increased color and cell density was recorded during two days after exposure. In the glass flasks 69% of the cells were still with two flagella after 9 h incubation at 0.1 m and 76% at 0.5 m after 57 h (Fig. 2)

In the lowland lake two samples were lost (Mylar flasks at 0.5 m 24 h and at 1 m 48 h; see Fig. 3). Nevertheless, the data confirmed a trend with an increasing proportion of cells with one or no flagella in the quartz flasks relative to the glass flasks, and a decreasing effect with depth, to apparently no effects at 2 m (Fig. 3). After two days of incubation in the quartz flasks at 0.5 m *ca.* 40% of the cells had lost one or two flagella, against only 10% after two days at 2 m. The effects were slightly lower in the Mylar treatments, but also here pronounced effects were found (Fig. 3).

Growth rates

Negative growth rates (decrease in cell numbers) were found for *Chlamydomonas* incubated in the alpine lake (Table 1), and even the glass flasks yielded high, negative growth rates. Algae incubated at

TABLE 1. – The growth rates (μ, day^{-1}) of *Chlamydomonas reinhardtii* in glass, quartz and mylar flasks, as measured after incubation in an oligotrophic alpine lake and a mesotrophic, humic lowland lake (Trollvann) at different depths. (SE are given in parentheses). x = no growth rates measured due to lost samples.

	Growth rates μ (day^{-1})		
Depth (m)	GLASS	QUARTZ	MYLAR
	Alpine Lake		
0.1	- 2.86 (0.80)	- 2.93 (0.51)	- 3.76 (0.95)
0.5	- 0.68 (0.32)	- 0.99 (0.26)	- 0.78 (0.40)
	Lowland Lake		
0.5	0.45 (0.13)	0.25 (0.04)	x
1.0	0.15 (0.03)	0.21 (0.11)	x
2.0	0.05 (0.01)	0.06 (0.02)	0.05 (0.04)

0.5 m were less effected than those incubated at 0.1 m. In the lowland lake, algae were growing in all flasks. The highest growth rate was measured in the glass flasks at 0.5 m (0.45±0.13), algae incubated at the same depth in the quartz flasks showed a significantly lower growth rate (0.25±0.04). In all flasks incubated in the lowland lake growth rates of *Chlamydomonas* decreased with increasing depth, reflecting limitation of PAR-light.

DISCUSSION

The *in situ* incubated *Chlamydomonas* showed a pronounced loss of flagella with increasing UV-radiation. This corresponds well with recently performed laboratory experiments, where a strong relation was found between loss or withdrawal of flagella and UV-exposure (Hessen *et al.*, 1995). The ultimate cause for this reaction is not clear, probably it may reflect a stress situation with preparation for encystment. The exposure experiments demonstrated essentially the same effect under natural light regimes as was found with low doses of artificial UV-B (lamps with peak intensity at 312 nm). A pronounced flagellar loss was detected already at an UV-B dose of 0.6 kJ m^{-2} (Hessen *et al.*, 1995). These *in situ* experiments similarily revealed effects at very low ambient UV intensity, emphasizing the potential role of *C. reinhardtii* as a susceptible and biologically relevant dosimeter. However, these experiments were only preliminary and the susceptibility of *Chlamydomonas* to UV-B vs. UV-A needs a further evaluation. While the laboratory effects were clearly related to UV-B, the field data give strong evidence for a detrimental UV-A effect as well. These results are in line with the findings for the marine alga *Phaeocystis pouchetii*, of which especially the flagellate stage in the life cycle suffered mortality as a result of natural UV-A exposure (Davidson and Marchant, 1994). The negative growth rates even in glass flasks in the alpine locality could partly be due to low temperature (6°C), but could as well be attributed to light stress from the near UV-region. While the effects of various wavelengths at different intensities calls for a further attention, it is evident that both UV-A (field data) and UV-B (laboratory exp.) at moderate doses may induce profound effects on flagellar status in *C. reinhardtii*.

The flagella of *Chlamydomonas* play an important role in the cell recognition and the mating process (e.g. Musgrave, 1993). Further are flagella fundamental for photo-orientation and motility. Häder (1985, 1986) found for both freshwater green flagellates and marine dinoflagellates a loss of orientation abilities and reduced motility at increasing UV-doses. Häder (1993) demonstrated that UV-radiation affected both photoreceptor proteins and tubulin bands involved in motility. Loss of flagella has not been reported in these papers. Flagella may also excrete enzymes facilitating uptake of nutrients. Recently, a direct uptake of inorganic P by the flagella of *Chlamydomonas* was observed (A. Musgrave, pers. comm). Hessen *et al.* (1995) demonstrated for *C. reinhardtii* a decrease in phosphate uptake well correlated with flagellar loss. Nutrient-starvation may subsequently change cell morphology and cell wall properties of algae (increase in cell size due to storage of proteins and carbohydrates, thickening of the cell wall) (Van Donk and Hessen, 1993). 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 (unpubl. data).

In several studies an increase in average cell size has been observed after long-term UV-exposure (Karentz *et al.*, 1991; Veen, 1991; Van Donk and Hessen, 1995). These effects were comparable with the morphological changes found for algae growing under nutrient limitation (Mitchell *et al.*, 1992; Van Donk and Hessen, 1993). Karentz *et al.* (1991) and Veen (1991) stated that increase in cell dry weight of UV-B stressed algae reflects a storage of proteins and carbohydrates due to delayed cell division. One might speculate whether UV-stress in general inhi-

bits nutrient uptake in algae, which after long-term exposure may give rise to the same physiological properties as those observed under direct nutrient deficiency.

These changes in stressed algal cells may influence the functioning of the zooplankton community. Nutrient limited algae are known to be poor food for zooplankton (e.g. Hessen, 1992; Sterner *et al.*, 1993). Recently, Van Donk and Hessen (1995) found that the green alga *Selenastrum capricornutum* stressed by nutrient limitation or UV-B radiation was less efficiently assimilated after being grazed by *Daphnia magna*. The stressed algae passed largely intact through the gut and were thus protected from heavy grazing pressure. Reduced digestibility of stressed algal cells, if also present among natural populations, may significantly alter trophic interactions and reduce transfer of energy between primary producers and consumers in aquatic ecosystems.

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REFERENCES

Cullen, J.J. and M.P. Lesser. – 1991. Inhibition of photosynthesis by ultraviolet radiation as a function of dose and dosage rate: results for a marine diatom. *Mar. Biol.*, 111: 183-190.

Davidson, A.T. and H.J. Marchant. – 1994. Comparative impact of *in situ* UV exposure on productivity, growth and survival of antartic *Phaeocystis* and diatoms. *Proc. NIPR Symp. Polar Biol.*, 7: 53-69.

Döhler, G. – 1985. Effect of UV-B radiation (290-320 nm) on the nitrogen metabolism of several diatoms. *J. Plant. Physiol.*, 118: 391-400.

Ekelund, N.G.A. – 1993. The effect of UV-B radiation and humic substances on growth and motility of the flagellate, *Euglena gracilis*. *J. Plankton Res.*, 15: 715-722.

Gala, W.R. and J.P. Giesy. – 1991. Effects of ultraviolet radiation on the primary production of natural phytoplankton assemblages in lake Michigan. *Ecotoxicology and Environmental Safety*, 22: 345-361.

Häder, D.P. – 1985. Effects of UV-B on motility and photobehaviour in the green flagellate, *Euglena gracilis*. *Arch. Microbiol.*, 141: 159-163.

Häder, D.P. – 1986. The effect of enhanced solar UV-B radiation on motile microorganisms. In: R.C. Worrest and M.M. Caldwell [Eds.] *Stratospheric Ozone Reduction, Solar Ultraviolet Radiation and Plant Life*, pp. 223-233. Springer Verlag, Berlin, Heidelberg, New York.

Häder, D.P. – 1993. Risks of enhanced solar ultraviolet radiation for aquatic ecosystems. In: F.E. Round and D.J. Chapman [Eds.] *Progress in Phycological Research*, Vol.9., pp 1-45. Biopress Ltd., Bristol.

Häder, D.P. and M. Häder. – 1988. Inhibition of motility and phototaxis in the green flagellate, *Euglena gracilis*, by UV-B radiation. *Arch. Microbiol.*, 150: 20-25.

Hessen, D.O. – 1992. Nutrient element limitation of zooplankton production. *Am. Nat.*, 140: 799-814.

Hessen, D.O., Van Donk, E. and T. Andersen – 1995. Growth responses, P-uptake and loss of flagella in *Chlamydomonas reinhardtii* exposed to UV-B. *J. Plankton Res.*, 17: 17-27.

Karentz, D., Cleaver, J.E. and D.L. Mitchell. – 1991. Cell survival characteristics and molecular responses of antartic phytoplankton to ultraviolet-B radiation. *J. Phycol.*, 27: 326-341.

Kerr, J.B. and C.T. McElroy. – 1993. Evidence for large upward trends of ultraviolet radiation linked to ozone depletion. *Science*, 262: 1032-1034.

Lorentzen, C.J. – 1979. Ultraviolet radiation and phytoplankton photosynthesis. *Limnol. Oceanogr.*, 24: 1117-1120.

Mitchell, S.F., F.R. Trainor, P.H. Rich and C. E. Goulden. – 1992. Growth of *Daphnia magna* in the laboratory in relation to the nutritional state of its food species, *Chlamydomonas reinhardtii*. *J. Plankton Res.*, 14: 379-391.

Musgrave, A. – 1993. Mating in *Chlamydomonas*. In: F.E. Round and D.J. Chapman [Eds.] *Progress in Phycological Research*, Vol.9., pp 193-225. Biopress Ltd., Bristol.

Skulberg, O.M. and R. Skulberg. – 1990. Research with algal cultures-NIVA's Culture Collection of Algae. NIVA-Report ISBN 82-551743-6.

Steemann-Nielsen, E. – 1964. On a complication in marine productivity work due to the influence of ultraviolet light. *J.Cons. Inst. Explor. Mer.*, 29: 130-135.

Sterner, R.W., D.D. Hagemeier, W.L. Smith and R.F. Smith. – 1993. Phytoplankton nutrient limitation and food quality for *Daphnia*. *Limnol. Oceanogr.*, 38: 857-871.

Van Donk, E. and D.O. Hessen. – 1993. Grazing resistance in nutrient stressed phytoplankton. *Oecologia*, 93: 508-511.

Van Donk, E and D.O. Hessen. – 1995. Reduced digestibility of UV-B stressed and nutrient-limited algae by *Daphnia magna*. *Hydrobiologia*, 307: 147-151.

Veen, A. – 1991. Experimental analysis of phytoplankton production as affected by enhanced UV-B radiation. In: *Annual Scientific Report Institute of Public Health and Environmental Protection*, Bilthoven ISBN 90-6960-012-9.

Worrest, R.C. – 1982. Review of literature concerning the impact of UV-B radiation upon marine organisms. In: J. Calkins [Ed.] *The Role of Solar Ultraviolet in Marine Ecosystems*, pp. 309-346. Plenum, New York.