Effects of enhanced UV-B radiation on structure and function of phytoplankton communities

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Abstract

In a research project aimed at identifying the primary targets and effects of UV-B radiation on microalgae, it was concluded that most research on this subject did not meet the criteria necessary for a quantitative assessment for the effects of ozone related UV-B enhancement. Therefore a new experimental approach was developed. Experiments on long-term effects were carried out under simulated natural light conditions. A reduced growth rate accompanied by an increased cellular biomass and size proved to be the main effect of UV-B exposure. By immunofluoresent labeling and flow-cytometry, specific UV-B induced damage could be detected in the DNA of the studied organisms.

A global decrease in ozone concentration has been detected over the last 15 years. This depletion, which is most distinct during the Antarctic spring ("ozone hole"), raises concern about the negative effects of the resulting increase in UV-B (290-315 nm) radiation on aquatic ecosystems. To assess the impact of enhanced UV-B radiation on the aquatic environment most research is logically focused on the phytoplankton compartment. By virtue of its capacity to store solar energy (photosynthesis) phytoplankton forms the first level of the aquatic trophic structure. Furthermore, because of its photosynthetic activity and formation of calcite (coccolithophores), marine phytoplankton is an essential link in global carbon dioxide cycles. Currently, 40% of fossil fuel carbon dioxide is assumed to be stored in the oceans. Dimethylsulfide (DMS) production from marine phytoplankton blooms is the major source of cloud-condensation nuclei over the oceans. The amount of nuclei determines the albedo of clouds and thus the earth's radiation budget.

The role of phytoplankton raises two major questions: firstly, does enhanced UV-B radiation significantly affect climate feedback mechanisms and, secondly, what is the final impact on the



Figure 1. Schematic presentation of natural light conditions as experienced by algal cells (solid line = PAR-, dotted line = UV-B radiation). Left graph shows surface irradiances. Center graph shows how algae travel through a light gradient (light intensities as % of surface irradiance $[I_0]$). Right graph shows an example of the net effect of vertical transport on the light regime experienced by algae.

aquatic food chain. To answer both these questions, the primary targets and effects of enhanced UV-B radiation have to be defined. Research during the last decades has revealed overwhelming evidence for negative effects of enhanced UV-B radiation on algae. Effects on almost all metabolic processes have been described. However, the scientific merit of most findings is limited. The majority of UV-B effect studies on phytoplankton, both in the field and laboratory, has addressed short-term (hours) effects of acute exposure only. Furthermore, attention is directed mainly towards photosynthetic activity. Within a time frame of several hours neither DNA damage nor processes like induction of adaptation and repair mechanisms are likely to become manifest. As a matter of fact, the only prediction of the effects of enhanced UV-B radiation on Antarctic primary production is based on short-term photosynthesis experiments performed during the Antarctic spring [1]. Effects are rarely studied after a prolonged (several days) exposure period. Furthermore, most effects of UV-B radiation are measured in laboratory cultures under relatively high exposure rates. Both experimental time scales and appliedexposure levels hamper any extrapolation to field conditions. Nevertheless, field experiments indicate that even unaffected



Figure 2. Calculated relative attenuation of biologically effective UV-B radiation (Setlow DNA_{eff(300)}) for marine systems. The relative attenuation is given as the ratio to the attenuation of DNA effective UV-B radiation (K_{DNA}) to the attenuation of visible light (K_{PAR}) for four concentrations of humic acids. Concentrations are given as the absorption coefficient at 440 nm (g_{440}). Most open oceans have a g_{440} of about 0.02 and a chlorophyll-*a* concentration of about 1 mg.m⁻³.

levels of UV-B radiation form a natural stress on phytoplankton communities. It was roughly estimated that if no UV-B radiation was incident at the earth's surface, phytoplankton primary production would increase by about 12% [2]. The assessment of UV-B effects for climate change studies is also complicated by the temporal variation of the damaging radiation and additional effects of UV-A and visible light in determining the final effect level. Under natural conditions algal cells are rarely stratified in the water column. Generally, algae are transported up and down the water column by wind-induced vertical mixing of surface waters (Fig. 1). By travelling through the underwater light gradient, algae will experience a more or less fluctuating light regime. Vertical transport of 10 metres might take from about 0.5 h to more then 10 hours. This dynamics will to a large extent determine the final effect level. As algae need visible light (PAR) for growth and repair of UV-B damage, the ratio of the attenuation of UV-B to PAR determines the effective exposure. Detritus and algae can decrease the ratio in attenuation of about 6 as determined by pure water to a minimum of about 2.5 (Fig. 2). However, the concentration of humic acids is the critical factor in determining UV-B transmission under water. Humic acids selectively absorb UV radiation.



Figure 3. Schematic drawing of the culture system consisting of a flat rectangular culture vessel (CV), two Venetian blinds (VB) controlled via stepper motors (SM), a high-intensity PAR light source (HMI), a UV-B light source consisting of five fluorescent tubes (UV-12), a UV cut-off filter set (Schott WG305), a water-cooled blocking filter (Schott KG3) and a PC with interface (IF).



Figure 4. Steady-state cellular dry weight of *Selenastrum capricornutum* in continuous culture. Open circles refer to dry weights at the start of the light period and closed circles to those at the end of the light period. Bars represent the standard deviations (n = 3). Culture conditions: L:D = 12:12 h, PAR_{max} = \pm 550 µmol.m⁻².s⁻¹, t = 18°C; light regime is sinusoid as in Fig. 1.

We developed a special culture system, to simulate natural light dynamics as shown in Figs. 1 and 2. The system was characterized by a computer-controlled dynamic light system and the flat geometry of the culture vessel, enabling accurate dosimetry (Fig.3). Light intensities were regulated by angular slat displacements of the Venetian blinds. Continuous culture technique was applied to investigate long-term effects. The system and the quantification of the exposure levels have been described in full detail elsewhere [3].

In our experiments we aimed at long-term (several days) effects. Experiments performed so



Figure 5. Bivariate distribution of DNA content versus the amount of thymine dimers (both in relative units [r.u.]) in a population of *Cyclotella* sp. as detected by flow-cytometry. A: control, no UV-B exposure; B: exposed to 3 KJ.m⁻² UV-B (Setlow DNA_{eff(300)}). G1 and G2 (cells with a double amout of DNA) refer to the different cell phases.

far have given a conclusive picture of long-term effects of UV-B radiation. For several species tested we observed a decrease in growth rate coupled to an increase in cell weight and size after prolonged exposure ("steady-state" conditions) (Fig.4). This increase in cell size was caused by an increase in at least three major cell components (proteins, carbohydrates and pigments) [3]. These results do not match with the idea in which the photosystem is seen as the primary target. It does, however, fit a model in which DNA damage is the main target. Using a newly developed antibody labelling method [4], we were able to demonstrate the formation of thymine dimers in the DNA (Fig. 5). These dimers caused an arrest in the cell cycle (S phase) by inhibiting DNA replication.

Through our recent research we arrived at a point at which we understand the primary targets and effects of enhanced UV-B radiation. Like damage to the photosystem, DNA damage is also a crucial factor. Obviously, estimations based on short-term effects will have to be reevaluated. Furthermore, changes in energy transfer to higher trophic levels are to be expected. Algal grazing by herbivorous predators (zooplankton) is directly dependent on cell size. Sinking rates might also be affected, resulting in a proportional increase in vertical carbon fluxes from the surface layers of the water. Fairly accurate methods are available to estimate UV-B transmission in different water types (Fig.2). Actually, only now can we direct research to collecting data for an accurate assessment of the effects of enhanced UV-B radiation on phytoplankton communities on a global scale.

References

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