Photoorientation, Motility and Pigmentation in a Freshwater Peridinium Affected by Ultraviolet Radiation

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Abstract. The effects of ultraviolet radiation on orientation, motility and pigmentation were measured in the freshwater dinoflagellate, *Peridinium gatunense*. Histograms of the positive phototactically oriented cells showed a decrease in the degree of orientation even after short exposure times. Quantification of the orientation revealed a significant inhibition after 2 h exposure. Similarly, the percentages of motile cells and the mean linear velocity of the cells was strongly affected. UV radiation also caused a massive bleaching of the cellular pigments as shown by absorption spectra determined after increasing exposure times. The ecological consequences of these effects are discussed.

Key words: Dinoflagellates — Image analysis — Motility — *Peridinium gatunense* — Phototaxis — Pigment bleaching — Ultraviolet radiation

Abbreviations:
DCMU: 3-(3', 4' dichlorophenyl)1,1-dimethylurea
DBMIB: 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone

Introduction

The freshwater dinoflagellate, *Peridinium gatunense* produces large algal blooms in Lake Kinneret (Israel). The populations appear in early spring and dominate the phytoplankton populations; they represent 95 % or more of the total biomass and produce up to 300 g of biomass per square meter water surface (Serruya 1978). In June the algal blooms disappear, which may be due to nutrient depletion or to nonpermissive temperatures; another possible factor is increased UV-B radiationlevels.

A number of flagellates has been shown to be affected by the UV component of solar radiation (Häder and Häder 1988a, b; 1989). Motility and photoorientation are strongly inhibited even after short exposure to solar

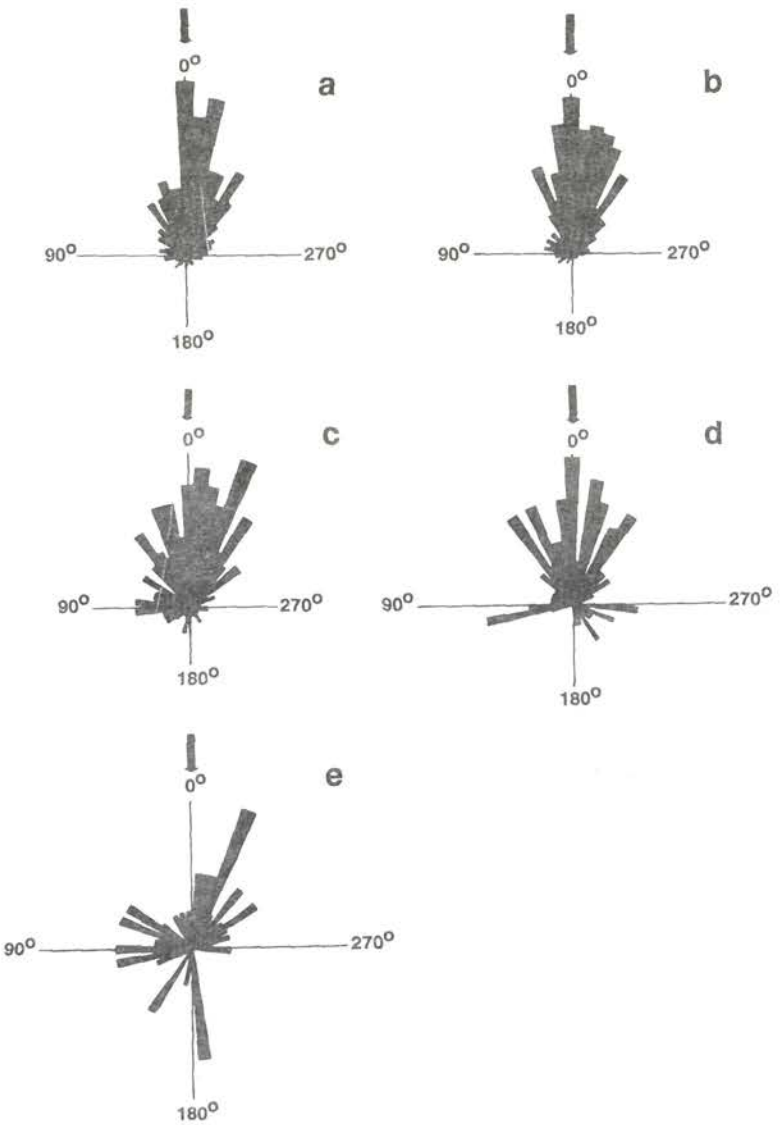

Fig. 1. Circular histograms of the movement direction of *Peridinium gatunense* cells; a test light stimulus of 10 klx after an exposure to ultraviolet radiation for 0 min (*a*), 30 min (*b*), 60 min (*c*), 105 min (*d*) and 120 min (*e*). More than 300 tracks were determined for each histogram and binned in 64 sectors. The arrows indicate the direction of the light stimulus.

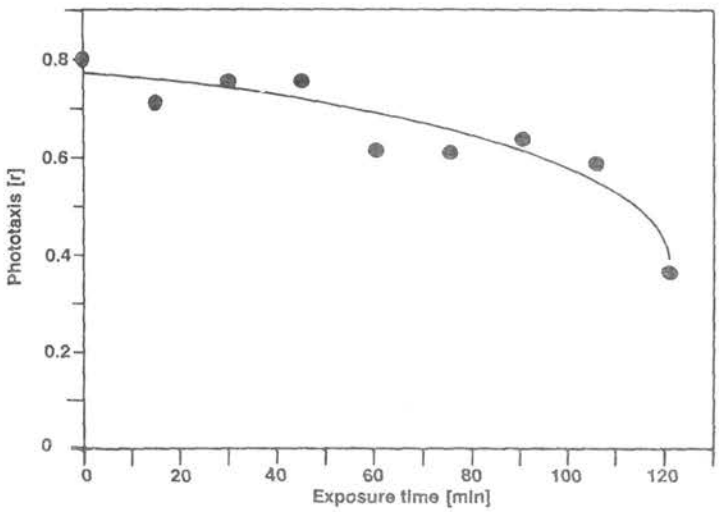

Fig. 2. Degree of phototactic orientation (Rayleigh test, ordinate) of *Peridinium gatunense* to a test light of 10 klx impinging laterally from 0° in dependence on the ultraviolet radiation exposure time (abscissa, min).

radiation. DNA is unlikely to be the target for the UV-B inhibition of motility, and photodynamic reactions do not seem to play a role in the UV-B inhibition in some organisms either (Häder et al. 1986; Häder and Häder 1988a, b). Rather, a direct effect on intrinsic components of the photoreceptor and motor organelles can be assumed.

In the water column many flagellates orient by using a number of external factors, such as gravity (Briegleb et al. 1986; Kessler 1985, 1986), chemical (MacNab 1985; Berg 1985) and thermal (Mizuno et al. 1984; Poff 1985) gradients, or the magnetic field of the earth (Frankel 1984; Esquivel and de Barros 1986). *Peridinium gatunense* utilizes light as a major clue to optimize its position in its habitat. The cells possess two different phototactic strategies (Liu et al. 1990): At low fluence rates the cells show a positive phototaxis, which brings the population toward the surface. At irradiances above 20 klx the cells show a prominent transversal phototaxis (diaphototaxis) which has also been observed in other flagellates (Rhiel et al. 1988a, b).The action spectrum ranges from 550 nm to beyond 700 nm. The photoreceptor pigments have not been identified as yet, but the involvement of the photosynthetic pigments can be ruled out since there is no activity in the Soret band of the chlorophylls *a* and *c*. In addition,

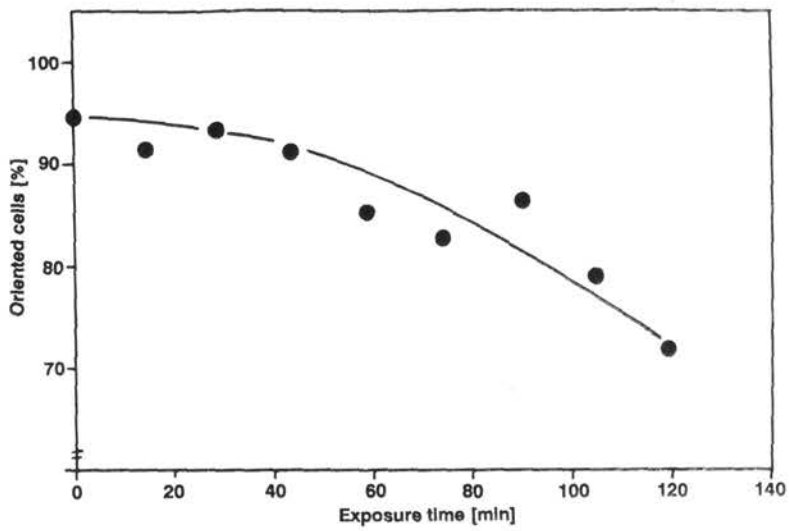

Fig. 3. Percentages of cells (ordinate, %) moving in the two upward quadrants in dependence on the ultraviolet radiation exposure time (abscissa, min).

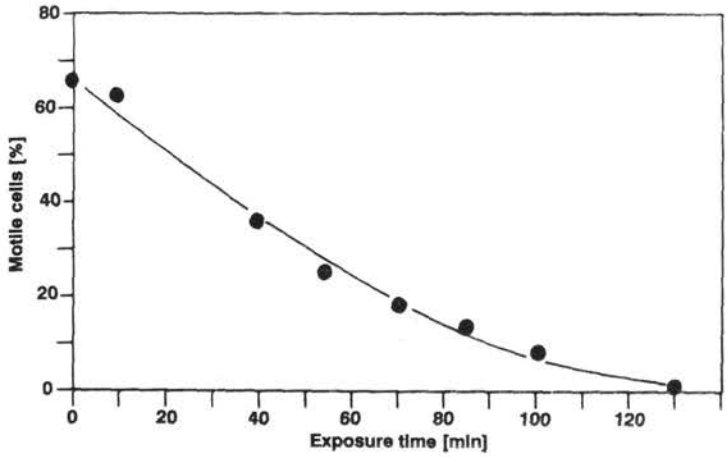

Fig. 4. Effect of ultraviolet radiation on the percentage of motile cells of *Peridinium gatunense* (ordinate, %) in dependence on the exposure time (abscissa, min).

the inhibitors of the photosynthetic electron transport chain, DCMU and DBMIB, do not affect photoorientation in *Peridinium gatunense*.

In contrast to this freshwater species, marine dinoflagellates, which form red tides in the North and the Baltic Seas (Tangen 1977; Spector 1984), have been found to use only positive phototaxis (Halldal 1958; Hand et al. 1967; Forward 1974a; Ekelund and Häder 1988). In Gyrodinium the photoreceptor is thought to be located in a structure at the basis of the flagella. In lateral light, a stigma-like structure (Dodge and Crawford 1969) periodically casts a shadow on the photoreceptor, since the cells show helical rotation during forward locomotion (Hand 1970; Hand and Schmidt 1975).

Gyrodinium also differs from the freshwater *Peridinium gatunense* since the action spectrum peaks in the near UV and the blue spectral regions. A second, photochromic pigment, whose red form absorbs at 620 nm and whose dark red form absorbs near 700 nm, is thought to modulate this activity (Forward 1974b, 1977).

The aim of this paper is to characterize and quantify the effects of ultraviolet radiation on motility, photoorientation and pigment composition in the freshwater alga, *Peridinium gatunense*, since a possible decrease in the population size of this phytoplankton organism due to solar ultraviolet radiation could have drastic effects on both the fishery industry in Lake Kinneret and the fresh water supply in Israel.

Materials and Methods

The freshwater dinoflagellate, *Peridinium gatunense* Nygaard (formerly *P. cinctum fa. westii*), obtained from Dr. Lindström, was isolated from Lake Kinneret (Lindström and Rodhe 1978) and grown in a medium described recently (Lindström 1983), in a temperature-controlled room under mixed fluorescent lamps (14 W m^{-2}) without shaking. Aliquots of a culture in logarithmic growth were inoculated into 40 ml of fresh medium contained in 100 ml Erlenmeyer flasks.

Samples were harvested after twenty days of growth and exposed to ultraviolet radiation in an open glass container (50 × 50 × 18 mm) covered with a 13 % UV-B transmitting neutral density filter of the reflective type (ESCO Products, Inc.). The cuvette was filled with a 3 mm layer of the cell suspension and placed under an inverted transilluminator (Bachofer, Reutlingen, FRG). The produced radiation is higher than solar radiation below 300 nm and lower than solar radiation above 300 nm. The spectral distribution and the fluence rates were determined with an Optronics (Optronics, Orlando, Florida, model 742) double monochromator spectroradiometer.

Photoorientation of the cells was determined in a glass cuvette (75 × 8 × 0.17 mm^3 inner dimensions) placed on the stage of a conventional light microscope (Olympus, BH-2, objective magnification 2.5 ×). A CCD video camera (Philips LDH 0600), mounted on top of the microscope, recorded the images of the moving cells in dark field to enhance the contrast. An infrared cut-off filter was inserted to avoid disturbance of the orientation of the organisms by the monitoring beam (RG 735, Schott & Gen., Mainz, FRG); neither induced the infrared measuring beam any photosynthetic oxygen production to which cells outside the light field could respond aerotactically.

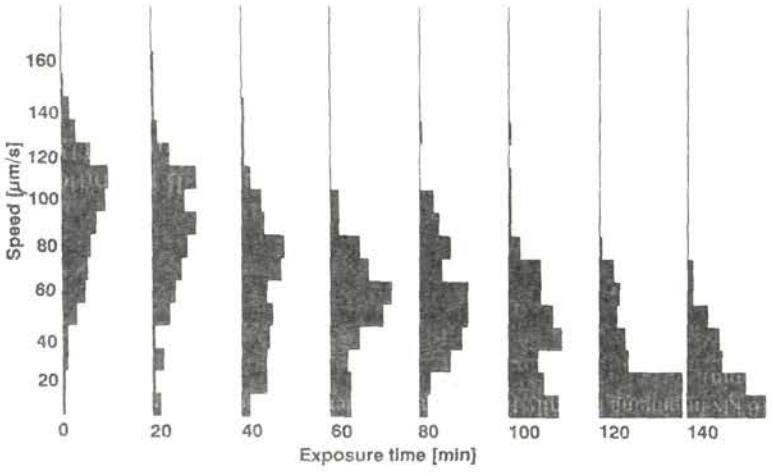

Fig. 5. Effect of the exposure time (abscissa, min) to ultraviolet radiation on the speed of movement (ordinate, $\mu m\ s^{-1}$) of *Peridinium gatunense*.

The video image was digitized (PIP-1024B, Quebec, Canada) with a spatial resolution of 512 × 512 pixels at 256 possible grey levels each. An IBM AT compatible microcomputer (Tatung 7000, Taipei, Taiwan) was used to access the video image. The program was written in Assembly Language and allowed to find and to follow in real time a randomly selected microorganism for a predefined period of time using algorithms described previously (Häder and Lebert 1985). The raw data (angular deviation from the light direction were stored in a disk file for subsequent statistical and mathematical analyses. The precision of orientation was quantified using the Rayleigh test (Batschelet 1981; Mardia 1972) and quadrant summation. Circular histograms were calculated by binning individual cells' tracks in 64 sectors. The phototactically active light was produced by a 250 W slide projector (Prado, Leitz, Wetzlar, FRG) equipped with a 250 W quartz halogen bulb.

Motility of the cells was determined with the same hardware as described above. The position of individual cells was determined in subsequent video frames, the distance travelled was calculated and the time consumed was read from the built-in hardware clock of the computer.

Absorption spectra were determined in cell suspensions stabilized in 0.5 % agar prepared with growth medium. A quartz spectrophotometer cuvette (optical path length 10 mm, 2 mm thick) was filled to the top, closed with a lid and sealed with vaseline and parafilm. The cuvette was then exposed to ultraviolet radiation and spectra were determined at regular time intervals. The decrease in the absorption was then calculated by substracting the initial spectrum (before UV exposure) from all subsequently determined spectra.

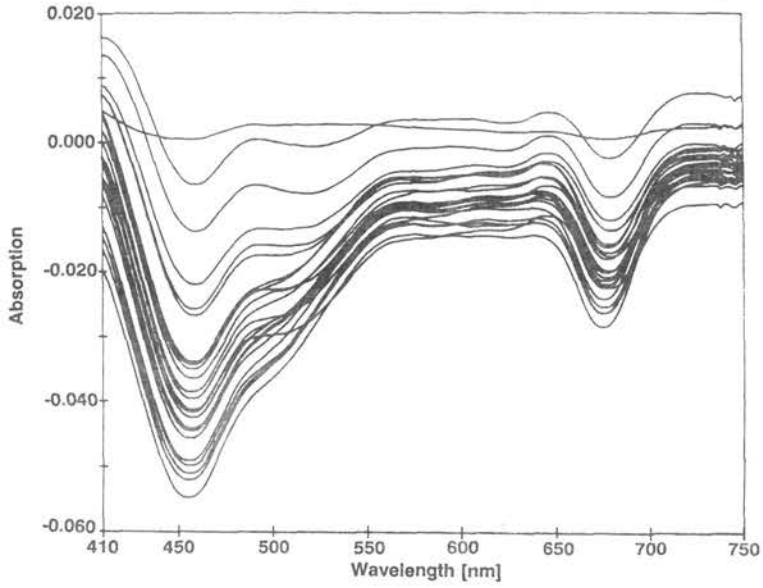

Fig. 6. Absorption spectra of *Peridinium gatunense* determined after increasing times of exposure to ultraviolet radiation (from top to bottom: 14, 43, 72, 93, 116, 142, 162, 185, 207, 232, 258, 280, 303, 326, 347, 371, 405, 444, 466, 488, 511, 539, 613, 633, 658, 680, 708, 781, 827 and 875 h).

Results

The cells of *Peridinium gatunense* showed a pronounced positive phototactic orientation in a test light beam of 10 klx before exposure to ultraviolet radiation (Fig. 1*a*). After 30 min exposure there was a small but noticeable reduction in the degree of orientation (Fig. 1*b*). After 60 min (Fig. 1*c*) and especially after 105 min (Fig. 1*d*), the degree of orientation decreased steadily. When exposed for 120 min (Fig. 1*e*) the orientation was almost totally lost. The decreasing orientation was quantified using the Rayleigh test (Fig. 2): there is a steady decline in the $\bar{r}$-value with increasing exposure time and a drastic drop at 120 min of exposure. Calculating the percentages of cells moving in the upward directed quadrants, however, demonstrated that even when the histograms showed an almost random behaviour most cells moved in the half toward the light source (Fig. 3).

The percentages of motile cells in the population decreased immediately after the onset of ultraviolet radiation (Fig. 4). After 130 min hardly any motile cell could be found in the population. Ultraviolet radiation also affected the swimming speed of *Peridinium gatunense*. Before the exposure to ultraviolet

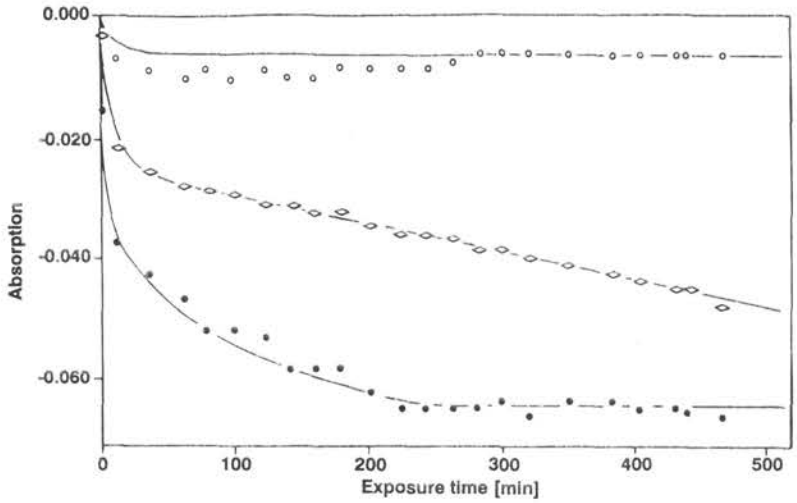

Fig. 7. Decrease in absorption (abscissa, absorbance) at key wavelengths, reflecting the major photosynthetic pigments of *Peridinium gatunense*, due to UV bleaching after increasing exposure times (abscissa, min): 450 nm (closed circles), 520 nm (open circles), 680 nm (diamonds).

radiation the cells moved with velocities which varied widely and had a mean value of approximately 110 μm s (Fig. 5). The histograms showed a steady decline in the linear speed of movement immediately after the onset of the ultraviolet radiation, and after 140 min of exposure most cells were immotile.

When a cell suspension was kept under ultraviolet radiation an obvious bleaching could be detected even by visual inspection after short exposure times. The absorption spectra determined at regular time intervals showed a gradual decline in the concentration of all photosynthetic pigments, including the carotenoids and the chlorophylls *a* and *c* (Fig. 6). This bleaching was quantified by calculating the absorption at distinctive wavelengths indicative of specific pigments and plotting in dependence on exposure time. Obviously, not all pigments were bleached with the same kinetics (Fig. 7). The carotenoids measured at 520 nm had a half-life of about 10 h whereas the red absorbing band had a half-life of about 23.3 h. However, absorption still decreased at the end of the experiment, so the half-life can be even longer than estimated. The maximum at 450 nm has a rather short half-life of 6.7 h which indicates that the Soret band of chlorophyll *a* is superimposed by a second pigment with a much shorter half-life.

Discussion

The inhibition of photoorientation and motility in the freshwater dinoflagellate, *Peridinium gatunense*, cannot be attributed to visible light or overheating since the radiation source used for these experiments emits only negligible amounts of visible or infrared radiation. Thus, unlike with solar radiation the effects are exclusively due to ultraviolet radiation.

The mechanisms of ultraviolet inhibition of photoorientation and motility are not known. However, DNA does not seem to be the primary ultraviolet target in some microorganisms studied so far since inhibition of motility in cyanobacteria and green flagellates was not found to be relieved by a photorepair mechanism. Also photodynamic effects could be ruled out as the possible inhibition mechanism since quenchers and scavengers of free radicals and singlet oxygen production were not capable of abolishing the inhibition (Häder et al. 1986; Häder and Häder 1988b, 1989). Therefore, it could be speculated that intrinsic components of the photoreceptor array and the propulsive apparatus of the cell, respectively, could be the target for ultraviolet radiation. This hypothesis is supported by the bleaching effect of ultraviolet radiation on pigmentation, which commences immediately after exposure.

Judging from the transmission curves of various bodies of water (Jerlov 1970) the cells might be exposed to considerable UV-B fluence rates at the level where the cells move during the daytime. Since the cells do not seem to orient with respect to the ultraviolet radiation component in solar radiation a significant increase in the UV-B radiation due to a partial ozone layer destruction could affect them adversely.

Ultraviolet radiation affects both the percentage of motile cells and the average individual swimming speed within even short exposure times. In addition, the precision of photoorientation is inhibited in motile cells. Both effects impair the survival of the populations since they hamper the adaptation of the cells in their natural habitat with constantly changing conditions. Measurements in the water column are required to show whether under current UV-B conditions hazardous radiation levels occur, e.g., during summer when the populations disappear from the phytoplankton communities in the lake.

Acknowledgements. This work was supported by a grant from the Bundesminister für Forschung und Technologie (project KBF 57) to D.-P. Häder. The authors gratefully acknowledge the skilful technical assistance of H. Vieten, R. Krzyzanowsky, J. Schäfer, K. Schmidt and B. Vollertsen.

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Final version accepted January 9, 1990