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PAR and UV Effects on Vertical Migration and Photosynthesis in *Euglena gracilis*†

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ABSTRACT

Recently it was shown that the unicellular flagellate *Euglena gracilis* changes the sign of gravitaxis from negative to positive upon excessive radiation. This sign change persists in a cell culture for hours even if subsequently transferred to dim light. To test the ecological relevance of this behavior, a vertical column experiment was performed (max. depth 65 cm) to test distribution, photosynthetic efficiency and motility in different horizons of the column (surface, 20, 40 and 65 cm). One column was covered with a UV cut-off filter, which transmits photosynthetically active radiation (PAR) only, the other with a filter which transmits PAR and UV. The columns were irradiated with a solar simulator (PAR 162 W m^{-2}, UV-A 32.6 W m^{-2}, UV-B 1.9 W m^{-2}). The experiment was conducted for 10 days, normally with a light/dim light cycle of 12 h:12 h, but in some cases the light regime was changed (dim light instead of full radiation). Under irradiation the largest fraction of cells was found at the bottom of the column. The cell density decreased toward the surface. Photosynthetic efficiency, determined with a pulse amplitude modulated fluorometer, was negligible at the surface and increased toward the bottom. While the cell suspension showed a positive gravitaxis at the bottom, the cells in the 40 cm horizon were bimodally oriented (about the same percentage of cells swimming upward and downward, respectively). At 20 cm and at the surface the cells showed negative gravitaxis. Positive gravitaxis was more pronounced in the UV + PAR samples. At the surface and in the 20 and 40 cm horizons photosynthetic efficiency was better in the PAR-only samples than in the PAR + UV samples. At the bottom photosynthetic efficiency was similar in both light treatments. The data suggest that high light reverses gravitaxis of the cells, so that they move downward in the water column. At the bottom the light intensity is lower (attenuation of the water column and self shading of the cells) and the cells recover. After recovery the cells swim upward again until the negative gravitaxis is reversed again.

INTRODUCTION

Euglena gracilis is a unicellular freshwater flagellate. As it is a photosynthetic organism it needs to expose its chloroplasts to light. However, cellular targets, such as photosynthesis, motility and proteins are affected by intensive solar radiation (1–4). This means that the cells have to find positions in their habitat, in which solar radiation is not too high, but delivering sufficient light for photosynthesis. The regulation of the optimal position is achieved by a combination of a negative gravitaxis (cells swim upward in the absence of other stimuli) and positive (at low irradiances) or negative (at high irradiances) phototaxis. Additionally, the cells react to changes (increase or decrease) in ambient illumination with tumbling photophobic responses (5). Light perception in *Euglena* was found to be regulated by a perception mechanism based on a novel photoreceptor. The photoreceptor is a photoactivated adenylyl cyclase, which produces cAMP upon blue light illumination (6,7). Similar perception mechanisms were found in other organisms (8). Gravitaxis is also based on a physiological mechanism, partially aided by a passive alignment in the water column (9). Physiological experiments as well as detailed movement analysis strongly suggest the involvement of mechano-sensitive ion channels in a signal transduction chain of gravitaxis (9–11). The whole cell body acts as a statocyst, activating these channels when the cells deviate from a vertical swimming direction (12). Recently, it was found that negative gravitaxis is converted into a positive one upon environmental stress, such as high irradiance (13). There is evidence that this sign change is mediated by reactive oxygen species (ROS), probably hydrogen peroxide (14). Induced positive gravitaxis can persist for several hours even when the cells are transferred to dim light after high light exposure. The aim of this work was to investigate the importance of the gravitactic sign change in *E. gracilis* when exposed to ultraviolet solar radiation in a natural habitat.

MATERIALS AND METHODS

Organisms and growth conditions. The flagellate *E. gracilis* Z was obtained from the algal culture collection at the University of Göttingen (15). The cells were grown for about 10 days in a mineral medium as described earlier (16,17) in 3000 mL Erlenmeyer flasks at about 20°C under continuous light of about 18 W m^{-2} from mixed cool white and warm tone fluorescent lamps.

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Exposure to artificial radiation. When *E. gracilis* was in the exponential growth phase, 1.5 L of the culture (10^6 cells mL^{-1}) was diluted with 5 L of mineral medium. The cell suspension was thoroughly mixed and filled into two 70 cm long Plexiglas columns with an inner diameter of 7.2 cm (each column about 2.6 L of cell suspension). The column was covered on the sides from top to bottom with aluminum foil. The top of one of the columns was covered with a 395 nm filter (transmits photosynthetically active radiation [PAR] only), the other with a 295 nm filter (transmits PAR + UV; both filters from Digefra, Munich, Germany). The upper edge of the column was covered to avoid light guidance via the Plexiglas material into the column. The samples were placed in a temperature-controlled chamber at 20°C and exposed to a light source (Sol 1200; Dr. Hönle, Martinsried, Germany), which produces a spectrum similar to the solar spectrum (18). The irradiances reaching the surface of the columns were: PAR 162 W m^{-2}, UV-A 32.6 W m^{-2} and UV-B 1.9 W m^{-2} (water surface in the Plexiglas column). The experiment was conducted for 10 days. With some exceptions a daylight/dim light cycle of 12 h:12 h (6:00 A.M. to 6:00 P.M.) was achieved (dim light: about 10 W m^{-2} from white fluorescent lamps). Additionally, dim light samples were used as controls.

Sample treatment. Samples (about 5 mL each) were drawn by means of a syringe with a tube attached at defined depths (surface, 20, 40 cm and bottom, which was at 65 cm) of the water columns shortly before measurement (not more than 20 min between drawing samples and measurement). The removed fluid was replaced by fresh medium. The drawn samples were subjected to motility and pulse amplitude modulated (PAM) analysis.

Motility analysis. Samples of cells were transferred into a custom-made cuvette (0.1 mm depth and 20 mm diameter) made from stainless steel with glass windows (Daimler-Benz Aerospace, Bremen, Germany). Motion analysis was performed with the cell tracking system WinTrack 2000 (19). The system is based on a video A/D flash converter (Meteor, Matrox, Canada) connected to a PCI slot of an IBM-compatible computer which digitizes the analog video images from a CCD camera mounted on a horizontally oriented microscope.

The digitized images are transferred to the computer memory. Objects are detected by brightness differences between cells and background. The movement vectors of all motile cells on the screen are determined by subsequent analysis of five consecutive video frames (movement vectors of the objects from frame 1 to frame 5). From thousands of individual cell tracks movement and cell form parameters were calculated, from which the parameter "upward swimming cells" is presented in this study. This parameter describes the fraction of cells swimming ±30° vertically upward. Additionally, the software provides circular histograms of the movement directions of all tracked cells, which provide a good impression of the direction distribution within the population.

As the cuvette has a defined depth, it was also possible to count the cells by means of the software, as the volume of the observed frame was exactly known. The number of cells per frame was extrapolated to cell number per mL. At least 10 measurements were performed to calculate one data point (data are presented as mean value and standard deviation).

Image analysis was performed in darkness to avoid any phototactic or photophobic effect on the orientation of the cells. To exclude the evaluation of immotile cells, which sediment in the vertical cuvette, the software accepted only cells with a swimming speed faster than the sedimentation velocity (about 20 $\mu m\ s^{-1}$). In all experiments the movement of the cells was visually monitored by the experimenters on screen in order to avoid any mistakes of data acquisition of the obtained cell tracks by the software.

PAM analysis. Photosynthetic efficiency of the cells was determined by means of a PAM fluorometer (Water-PAM; Walz, Effeltrich, Germany). Cell samples were measured immediately after being drawn from the columns (no dark adaptation). The time between sampling and measurement was not more than 5 min.

The photosynthetic yield was determined according to the following equation (20,21): Yield $= (F'_m - F_t)/F'_m$, with F'_m representing the maximal fluorescence achieved after saturation of the photosynthetic electron transport chain (light pulse, 5400 mol photons $m^{-2}\ s^{-1}$) and F_t as temporary fluorescence.

Eight measurements were performed for each sample. Data are presented as mean values with standard deviation.

Additionally, light curves (PAR *vs* yield) were recorded at following illuminations 0, 65, 96, 144, 226, 327, 482, 737 and 1101 μmol photons $m^{-2}\ s^{-1}$. The distance between subsequent light steps was 10 s. The electron transport rate (ETR) was calculated according to the following formula: ETR = yield × PAR × factor, where the factor was 0.41 (software preset).

Statistical analysis. Statistical significance was tested with the Student's *t*-test and ANOVA.

RESULTS AND DISCUSSION

Samples under illumination

The largest fraction of cells was at the bottom of the columns (in the UV + PAR as well as in the PAR-only columns), while the cell number was significantly ($P < 0.001$ in all comparisons) decreasing toward the surface, as shown for a representative day (Fig. 1). At the surface and at the 20 cm level the cell density was in a range between 10^5 and $2–3 \times 10^5$ cells mL^{-1}, at the 40 cm level between 3×10^5 and 4.5×10^5 cells mL^{-1} were counted, while at the bottom more than 6×10^6 cells mL^{-1} were detected (these data are from the sixth experimental day; the cell number has increased since the start of the experiment). The results were similar for all days in the course of the experiment. Upon longer exposure to radiation (with and without UV) the cells showed a typical movement behavior (measured in the darkness): at the surface and in the 20 cm level the cell culture showed an upward movement (more pronounced at the surface), mostly a bimodal movement in the 40 cm layer, while at the bottom the orientation was preferably directed downward (Figs. 2 and 3). This pattern was similar at all experimental days. The data presented show the movement behavior of cells shortly after taking the samples, but a spot check analysis of samples after more than 1 h storage in darkness also gave similar results (data not shown). Although the cells at the bottom swam preferably downward, always a small fraction of cells was swimming upward. Due to the high number of cells at the bottom level, this proportionally small number of cells (about 10–30%) is sufficient to deliver enough cells to the other horizons of the column. Ten percent of upward swimming cells

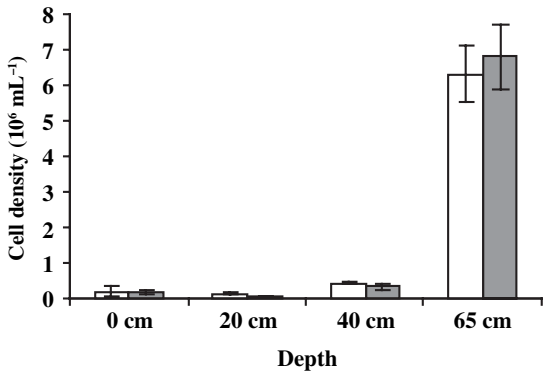

Figure 1. Distribution of *Euglena gracilis* cells in vertical water columns during intensive radiation from above as a function of depth (in cm). Light bars: PAR-treated cells; gray bars: UV + PAR-treated cells. Each bar is calculated from at least 10 single measurements. Error bars: standard deviation. The data show the results of a representative experimental day.

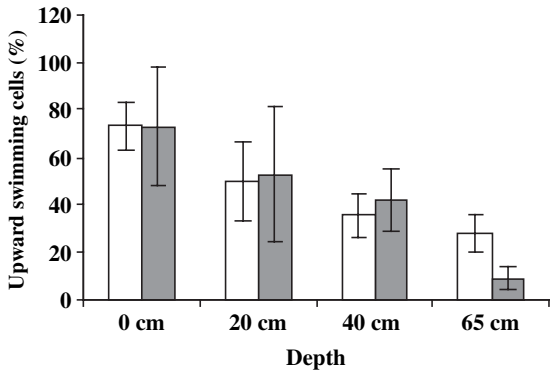

Figure 2. Movement direction of *Euglena gracilis* cultures in vertical water columns under high irradiance from above as a function of depth (in cm). Light bars: PAR-treated samples; gray bars: UV + PAR samples. "Upward swimming cells": fraction of cells swimming upward (±30°). Each bar is calculated from at least 10 single measurements. Error bars: standard deviation.

from the bottom level translates to about 6×10^5 cells swimming towards the 40 cm level. At the 40 cm level about 40% of the cells swam upward, which is equal to about 2.5×10^5 cells mL^{-1}. The theoretical cell numbers calculated from the movement parameters are close to the measured cell numbers. The data indicate steady-state equilibrium between upward and downward-oriented cells, which is far to the side of downward swimming. The reason for this is that when once positive gravitaxis is induced, it is stable for a relatively long time (13). It is very likely that high light radiation reverses the gravitaxis of *E. gracilis* from negative to positive, because positive gravitaxis only occurs under light, not under dim light conditions. The cells swim downward and are then protected from the light due to attenuation and self shading. In addition, downward swimming is most likely partially also supported by negative phototaxis. Photometric measurements of samples of different depth indicate a strong absorption of light toward the bottom of the column. The transmission of PAR was in a range of 70% per cm medium at the surface and decreased into a range of less than 20% per cm at the bottom layer, resulting in a huge absorbance with increasing depth in the column (decrease of light at least to a factor 10^{-5}). We assume that the cells recover in the shaded conditions at the bottom and swim upward again. In previous investigations it was found that the sign change in gravitaxis is probably triggered by ROS, most likely hydrogen peroxide (14). The evolution of ROS is higher under UV (22), which possibly explains the stronger response of the UV-treated cells (fraction of downward swimming cells higher at the bottom). The amount of upward swimming cells at the surface is unexpectedly high. This might be due to a fraction of cells which are incapable of converting gravitaxis and continue to swim upward despite high light exposure (or perhaps due to severity of the high light effects).

The photosynthetic efficiency of irradiated cells was very low at the surface and at the 20 cm level in the PAR as well as in the UV-treated samples ($P < 0.001$, compared with 40 cm or bottom level, respectively, no statistical difference between 40 cm and bottom), but relatively high in deeper horizons (Fig. 4). There the photosynthetic efficiency was close to the dim light control, which was in the range of 0.25 (the photosynthetic yield of the *E. gracilis* Z-strain is relatively low compared to that of other algae or higher plants). Inhibition of photosynthesis was more pronounced in the UV-treated samples ($P < 0.001$ in all comparisons), which can also be seen from light curves (Fig. 5). UV was found to inhibit photosynthesis very effectively (23,24). The data show that self shading and the attenuation in a vertical water column protects cells from the impact of UV. It is very likely that cells near the surface recovered from the light-induced decrease in photosynthetic efficiency, when they swam downward toward the bottom layer.

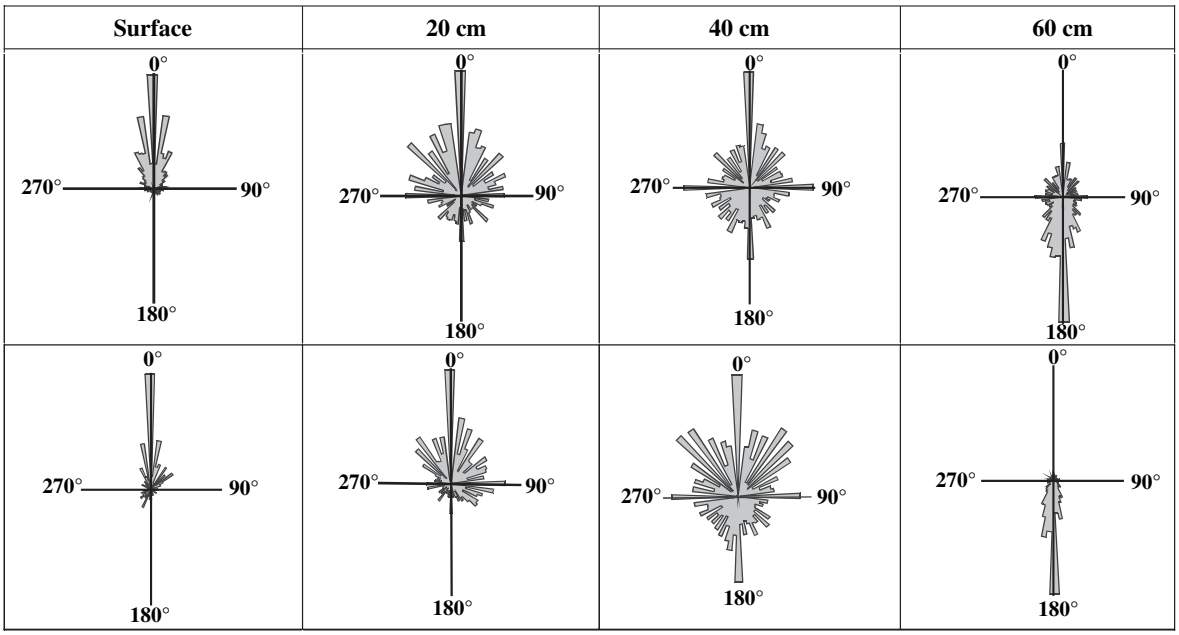

Figure 3. Circular histograms of the movement direction of *Euglena gracilis* cell cultures in vertical columns under high irradiance as a function of depth (in cm). Upper row: PAR-treated samples; lower row: UV + PAR-treated samples.

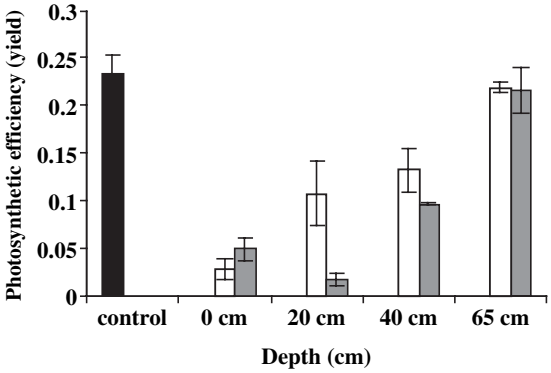

Figure 4. *Euglena gracilis*: Dependence of photosynthetic efficiency on depth inside vertical water columns. Control: dim light control; light bars: PAR-treated cells; gray bars: UV + PAR-treated cells. Each bar is calculated from eight single measurements. Error bars: standard deviation.

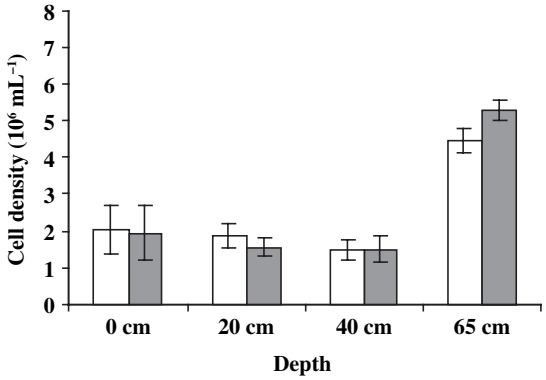

Figure 6. Cell concentration of *Euglena gracilis* in vertical water columns as a function of depth, after 12 h recovery in dim light. Light bars: PAR-treated cells; gray bars: UV + PAR-treated cells. Each bar is calculated from at least 10 single measurements. Error bars: standard deviation.

Samples under reduced light conditions

At reduced light intensities (30% of total radiation, data not shown) or under dim light conditions more cells were found near the surface compared to light conditions. At the bottom level the cell densities (at the sixth experimental day) were about 4–5 $\times$ 10^6 cells mL^{-1}, while at the other horizons the cell density was in the range of 1.5–2 $\times$ 10^6 cells mL^{-1} (Fig. 6). These numbers show the degree of cell distribution, not the total number of cells in the column. The cells showed negative gravitactic orientation at all horizons (UV- as well as PAR-treated cells, Figs. 7 and 8). This was similar for all experimental days. Although the percentage of upward swimming cells at the surface is obviously higher under high light, the total number of upward swimming cells is much higher in dim light, because of the much higher cell density at the surface in dim light (about 10^6 cells mL^{-1} in dim light *vs* 2 $\times$ 10^4 cells mL^{-1} upon irradiation). Also under dim light the largest fraction of cells was near the bottom and the cell density decreased toward the surface. This means that the high cell density at the bottom horizon is not solely due to excessive radiation, because also under dim light the bottom fraction is the largest. However the cells showed negative gravitaxis (upward swimming) at all horizons, so that the accumulation of cells at the bottom cannot be explained by active cell movement. Most likely this phenomenon is based on physical and fluid dynamic properties in combination with the negative gravitactic behavior (bioconvection) and can be found in all negative gravitactic microorganisms, investigated in a variety of theoretical and experimental studies (25,26). A high concentration of cells in a volume element of the medium increases the specific density of this volume and consequently this dense pocket of the medium sinks downward. The sedimentation speed of the medium cell cluster is faster than the upward movement of the cells.

The photosynthetic efficiency was relatively high at all horizons, but was lower ($P < 0.001$ compared to the other horizons) in the bottom layer (Fig. 9). Probably the percentage

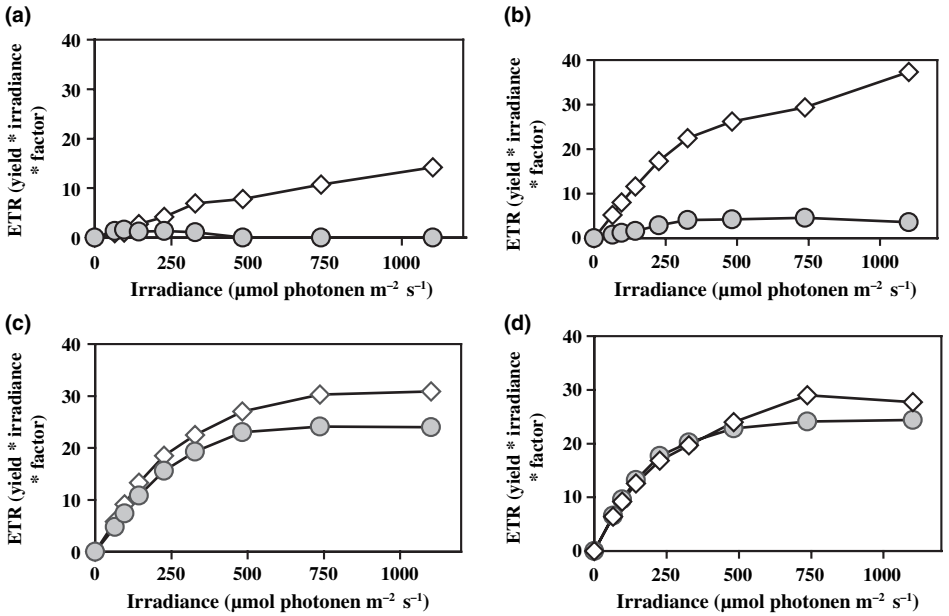

Figure 5. Representative light curves of *Euglena gracilis* cultures in vertical water columns as a function of depth. Diamonds: PAR-treated samples; circles: UV + PAR. A light curve shows the kinetic of photosynthetic efficiency upon increasing radiation. (a) Surface, (b) depth 20 cm, (c) depth 40 cm, (d) depth 65 cm. ETR, electron transport rate: yield $\times$ irradiation [μmol photons s^{-1} m^{-2} ($=\mu$E) $\times$ factor 0.41].

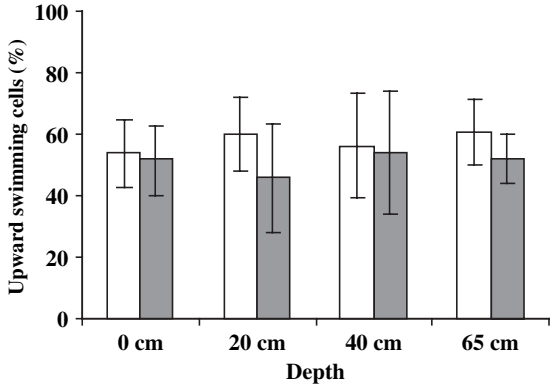

Figure 7. Movement direction of *Euglena gracilis* cultures in vertical water columns after recovery in dim light (12 h). Light bars: PAR-treated samples; gray bars: UV + PAR samples. "Upward swimming cells": fraction of cells swimming upward (±30°). Each bar is calculated from at least 10 single measurements. Error bars: standard deviation.

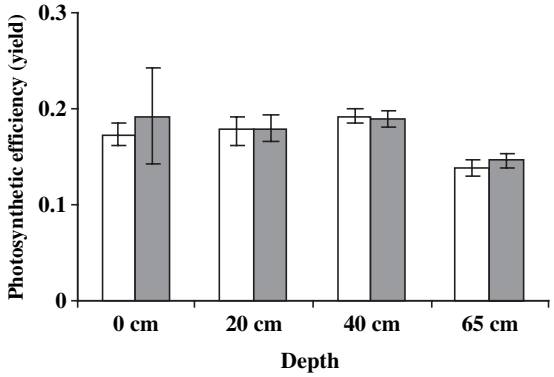

Figure 9. Photosynthetic yield of *Euglena gracilis* cultures at different depths in vertical water columns after dim light recovery (12 h). Light bars: PAR-treated samples; gray bars: UV + PAR samples. Each bar is calculated from eight single measurements. Error bars: standard deviation.

of light-impaired cells is higher at the bottom, because un-affected cells moved toward the surface, while cells in which motility or gravitaxis was affected preferably accumulated at the bottom. This was also observed in vertical migration experiments with other species, e.g. *Dunaliella salina* and *Gymnodinium chlorophorum* (D.-P. Häder, unpublished).

The observations under dim light show that *E. gracilis* cells are able to recover after high light exposure. Negative gravitaxis reconstitutes after prolonged dim light exposure. Self shading and attenuation in deeper parts of a vertical water column produce dim light conditions, in which cells recover from excessive radiation effects perceived at the surface.

CONCLUSION

The data indicate that excessive radiation converts gravitaxis of *E. gracilis* from negative to positive. Previous work has indicated that this is possibly caused by ROS. As ROS are a byproduct of photosynthesis as well as photochemistry, they could be an effective gravitaxis-reversing signal in both PAR and UV + PAR irradiance treatments. Due to the absorption properties of the medium, ROS induction is only pronounced near the surface. The cells swim downward (possibly additionally supported by negative phototaxis) and are protected from exposure due to attenuation and self shading. They recover in the shaded conditions and swim upward and the cycle starts again. As the gravitactic sign change is persistent for a longer period, the number of cycles will be limited for a given cell during the course of a day. Moreover only few cells seem to cycle between the bottom and the surface at the same time. Possibly some cells are impaired by radiation so that they lose the capability of gravitactic sign change. The high fraction of upward swimming cells detected in the course of the experiment provides some support for this hypothesis.

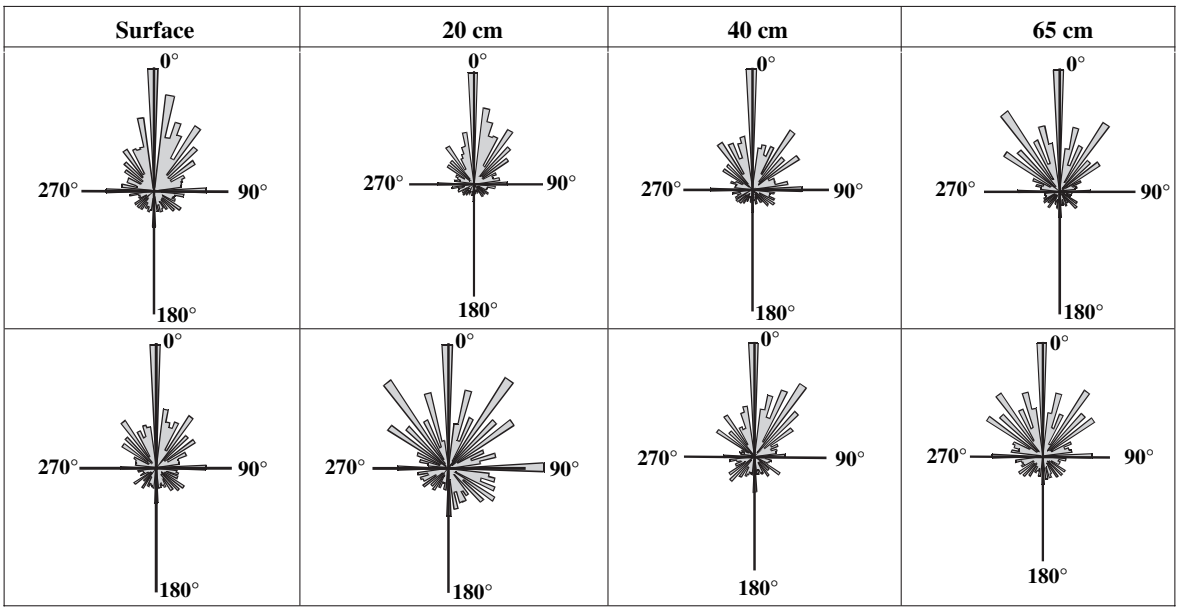

Figure 8. Circular histograms of the movement direction of *Euglena gracilis* cell cultures in vertical columns after dim light recovery (12 h) in dependency of the depth. Upper row: PAR-treated samples; lower row: UV + PAR-treated samples.

Probably the cells are using the mechanism of gravitactic sign change to avoid prolonged high irradiance exposure (both PAR and UV), because *E. gracilis* was found to be very sensitive against UV radiation, which affects various cellular targets, such as DNA, proteins, the apparatus of motility and others (1,3,27–30). The question is why the cells show persistent gravitactic sign change upon high irradiation and do not adjust the position in the water column by means of a combination of phototaxis and gravitaxis as described in the Introduction. Gravitactic sign change is neither dependent on the photoreceptor nor on the chloroplasts, because gravitaxis can also be converted by strong radiation in colorless and chloroplast-free mutants or the heterotrophic relative of *E. gracilis*, *Astasia longa* (13). As the photoreceptor is destroyed upon longer UV exposure (30), which prevents an orientation by means of phototaxis, the cells can still escape from deleterious light conditions by simply swimming downward.

We conclude that under moderate light conditions, *E. gracilis* orients itself in the water column by a combination of phototaxis and gravitaxis (31). Upon excessive radiation, triggered possibly by ROS, the cells switch to positive gravitaxis and swim downward, where they are protected from irradiation and recover.

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