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Orientation of the Photosynthetic Flagellate, *Peridinium gatunense*, in Hypergravity

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Abstract. The photosynthetic freshwater flagellate, *Peridinium gatunense*, uses both positive phototaxis and negative gravitaxis to move upwards in the water column. At higher fluence rates approaching those at the surface of their habitat, the cells tend to become unoriented and thus stop their upward movement. Orientation and motility of *Peridinium gatunense* has been studied in the slow rotating centrifuge microscope (NIZEMI), which allows observation of swimming behavior during centrifugation acceleration between 1 *g* and 5*g*. The movement vectors were analyzed by real time image analysis capable of tracking many cells simultaneously. At 1 *g* the orientation was not very precise, but the degree of orientation increased significantly at higher acceleration forces up to about 3 *g*. Most cells were capable of swimming even against an acceleration vector of 3.8 *g*; at higher acceleration forces the cells were not able to cope with the centrifugal force. The linear velocity of cells swimming against 1 *g* was about 20% lower than that of cells moving in other directions. The velocity decreased even more in cells swimming against higher acceleration forces.

The freshwater dinoflagellate, *Peridinium gatunense*, produces large populations in Lake Kinneret (Israel), while marine dinoflagellates form red tides in, e.g., the North and the Baltic Seas [65, 68]. The algal blooms appear in early spring and outcompete all other phytoplankton populations. They produce up to 300 g of biomass per square meter of surface water and represent 95% or more of the total biomass production in the lake [63]. The algal blooms disappear in summer; this may be partially owing to nutrient depletion or nonpermissive temperatures; another possible factor is increased UV-B radiation levels [38, 44], which have also been shown to affect other flagellate populations [33–35] and gliding microorganisms [29, 40].

Many microorganisms orient in the water column by using a number of external factors [32, 57, 69] such as gravity [13, 47, 48], chemical [5, 53] and thermal [55, 58] gradients, or the magnetic field of the earth [18, 20]. *Peridinium gatunense* utilizes both light and gravity as major clues to optimize its position in the microhabitat. The cells possess two different phototactic strategies [52]: at low fluence rates the flagellates orient with a positive phototaxis, which brings the population closer to the surface. At irradiances above 20 klx the cells show a pronounced transversal phototaxis (diaphototaxis), which has also been observed in other flagellates [59, 60]. The action spectrum for phototaxis extends from 550 nm to beyond 700 nm, but the photoreceptor pigments have not been identified as yet. However, 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*. Furthermore, the inhibitors of the photosynthetic electron transport chain, DCMU and DBMIB, do not affect photoorientation in *Peridinium gatunense*.

The second important external factor for orientation, gravitaxis, has the advantage of being available also in the absence of light. It has been observed in microorganisms for more than a century [1, 46, 62, 68]. In addition to a few other species [s. review 4, 6–12, 15, 64, 71], mainly ciliates [19, 45, 66] and flagellates have been studied [16, 47, 48, 60]. Neither the gravireceptor organelle nor the sensory transduction chain for the stimulus transmission has been identified yet. It has been suggested that graviorientation in flagellates is passively brought about by an asymmetric mass distribution within the cell; the alternative is an active physiological perception [14,

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49, 67]. Roberts [61] assumes a hydrodynamic interaction between the medium and the cell to be the reason for gravitactic orientation in the ciliate *Paramecium*, while a model by Winet and Jahn [70] tries to explain gravitaxis by a nonequilibrium ratio between sedimentation and rotation during forward movement. However, buoyancy, which has been assumed by Fukui and Asai [24] to be the mechanism for negative gravitaxis, could be ruled out by Taneda et al. [67], who also excluded an effect of the hydrostatic pressure [66]. The experimental analysis of gravitactic orientation has been hindered by the difficulty to access the ranges both above and below 1 *g*. The aim of this paper is to quantify and characterize the orientation and velocity distribution during 1 *g* and hypergravity conditions in the freshwater alga, *Peridinium gatunense*.

Materials and Methods

Organisms and experiment preparation. The freshwater dinoflagellate, *Peridinium gatunense* Nygaard (formerly *P. cinctum* fa. *westii*), was obtained from Dr. Lindström and originally isolated from Lake Kinneret [51]. It was grown in a medium described recently [50], in a temperature-controlled room under mixed fluorescent lamps (14 W m^{-2}) in standing cultures.

For experiments, 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 20 days of growth, and all experiments were carried out with the cells in their original growth medium. Cell suspensions were transferred into a rotor compressor microchamber (developed by Dr. Briegleb, DLR, Köln, Germany), which was mounted on the object stage of the microscope within the NIZEMI (see below).

Construction of the NIZEMI. The slow rotating centrifuge microscope (NIZEMI) was developed and constructed by Dornier (Friedrichshafen, Germany) on behalf of the Deutsche Versuchsanstalt für Luft- und Raumfahrt (DLR, Köln) and the Federal Minister for Research and Technology (BMFT, Bonn, Germany). The NIZEMI consists of a Zeiss (Oberkochen, Germany) Axioplan light microscope accommodated horizontally on a circular rotating table driven by an electric motor (Fig. 1). In order to balance the load during operation, to minimize the excess heat production from the lamp and to manipulate the movement of the specimen on the object stage by remote control via a joystick, considerable modifications were necessary. The image of the moving organisms was recorded by a CCD camera (Aqua TV HR 600, Aqua TV, Kempten, Germany) and displayed on a video screen (AVT-1220, AVT Horn, Aalen, Germany). The additional macro unit, which allows the analysis of larger objects under increased acceleration forces, was not used for this purpose.

Image analysis of organism tracks. The video sequence from the NIZEMI was either analyzed on-line during the experiment or recorded on a VHS video recorder (Mitsubishi HS 3600 E). The

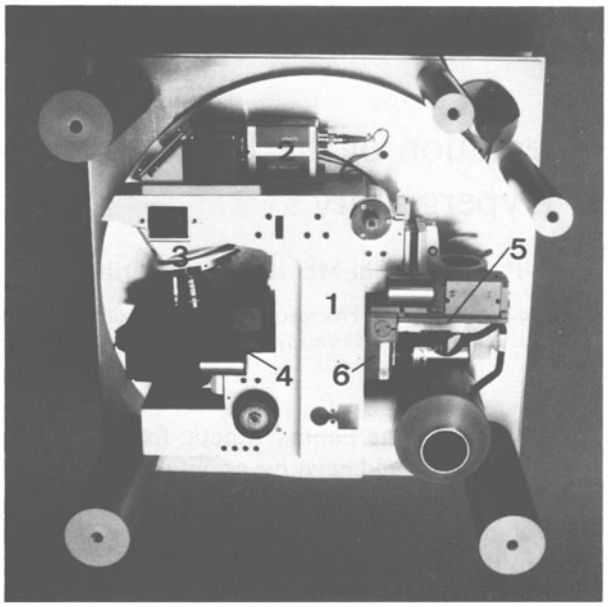

Fig. 1. Principal schematic diagram of the NIZEMI showing the main components of the micro and macro observation unit. 1 = microscope; 2 = micro CCD camera; 3 = objective holder; 4 = micro stage; 5 = macro stage; 6 = macro CCD camera.

image was digitized at a spatial resolution of 512 × 512 pixels with 256 possible gray levels each using a Matrox digitizer card (PIP-1024, Quebec, Canada), plugged into an IBM AT compatible microcomputer (Tatung 7000, Taipei, Taiwan). The digitized image was manipulated by on board look-up tables (LUTs, [31]) before being stored in a dedicated video memory.

The computer had access to the video memory and could manipulate and analyze pixels on a random basis. The image was displayed on an analog monitor in pseudocolor graphics with a second set of LUTs. The tracking program was developed in the computer language C which handles the input and output routines as well as the mathematical analysis of the data [39]. All time-consuming procedures such as manipulation of image pixels were written in 80286 ASSEMBLY language. The algorithm starts by taking four snapshots from the video sequence at 80-ms intervals. Then the first image is scanned sequentially line by line until an object is found that differs by a predefined threshold from the background.

The outline of the cell is analyzed with the chain code algorithm [21], which is very fast and allows a drastic reduction in the amount of information. Further procedures have been developed to estimate the circumference, area, and centroid [22, 23]. The centroids and areas of all organisms found in the first frame are stored in an array. Subsequently, the positions of all organisms are determined in the next frame (80 ms later) with the stored centroids as starting points. This process is repeated for the third and fourth digitized image, writing the centroids into a second array. When two organisms meet, the direction of movement is no longer defined; consequently, a 50% increase in the area is used as an indication to discard an organism. Upper and lower limits for the area allow cells to be distinguished from debris or noise in the image. The system analyzes up to 500 movement vectors per minute and stores the deviation angles from the stimu-

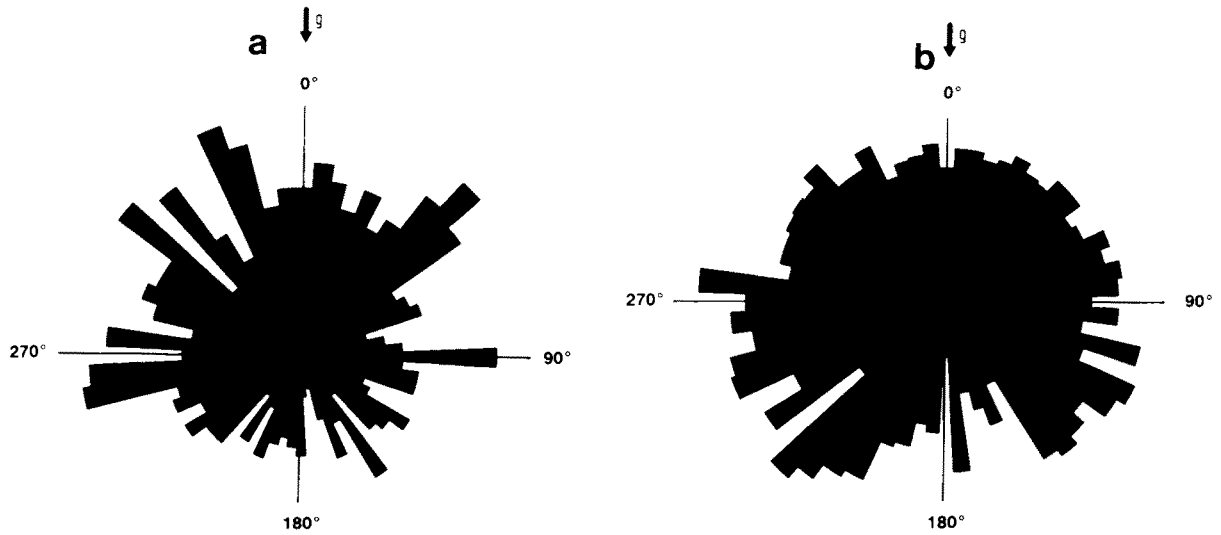

Fig. 2. Circular histograms of the movement vectors (a) and velocity distribution (b) of *Peridinium gatunense* in a vertical cuvette in darkness; 1000 tracks have been analyzed.

lation direction, the gravity, or acceleration vector (defined as 0°) in a disk file. The individual speed of movement of the organisms can be calculated, since the distances they have moved are known as well as the time elapsed between the first and final frame.

From these raw data, circular histograms of the direction distribution as well as the velocity distribution are calculated by subsequent programs written in a higher level language (Turbo Pascal, Borland) [27]. In addition, Rayleigh tests are performed to determine the directedness of the moving organisms [2, 3, 54], and the direction of movement is calculated by Fast Fourier Analysis, which also allows smoothing of the histograms [38].

Results

The gravitactic orientation of *Peridinium gatunense* was studied at 1 *g* in a vertical cuvette with 50 × 50 × 1 mm^3 inner dimensions (Fig. 2a). The organisms showed a moderate gravitactic orientation with an r-value of about 0.19. The velocity histogram indicates that cells swimming against the gravity vector swam about 20% more slowly than those swimming in any other direction (Fig. 2b). When the organisms were exposed to supplementary white light from above while swimming in the vertical cuvette, the degree of orientation increased even at moderate fluence rates. At 250 lx the r-value was 0.34, and 70% of all cells swam in the upward direction deviating less than ±90° from the vertical axis (Fig. 3a). At 1 klx more than 72% of all cells oriented in the upper two quadrants, and the r-value amounted to 0.39 (Fig. 3b). Further increase of the white light irradiance increased the directedness as the phototactic orientation increased (Fig. 3c). This trend continued until about 10 klx. Above this value up to about 70 klx, the cells showed a moderate orientation in the upward direction with r-values around 0.39, and about 76% of the cells moved in the top two quadrants (Fig. 3d). Higher fluence rates drastically decreased the degree of orientation and the percentage of cells in the top two quadrants (Fig. 3e). This effect is due to an increasing number of cells swimming perpendicular to the impinging light beam.

Compared with the 1 *g* control, the gravitactic orientation was significantly enhanced by accelerating the cells to 2 *g* (Fig. 4a). The velocity difference between those cells swimming against the acceleration vector and those swimming in the direction of the vector was even more pronounced than that at 1 *g* (Fig. 4b). The optimum of gravitactic orientation was reached at 3 *g* (Fig. 4c). Increasing the acceleration further strongly impaired the degree of orientation. At 3.6 *g* the orientation was even lower than at the 1 *g* control (Fig. 4d), and the velocity histogram indicates that cells moving against the acceleration vector were much slower and in fact many cells were literally centrifuged by the acceleration force (Fig. 4e). Plotting the degree of gravitactic orientation (r-value) in dependence of the acceleration force indicates an increase in the directedness of movement up to about 3 *g* followed by a drastic decline in orientation at higher forces (Fig. 5). Likewise, the percentage of cells moving in the two quadrants pointing upward

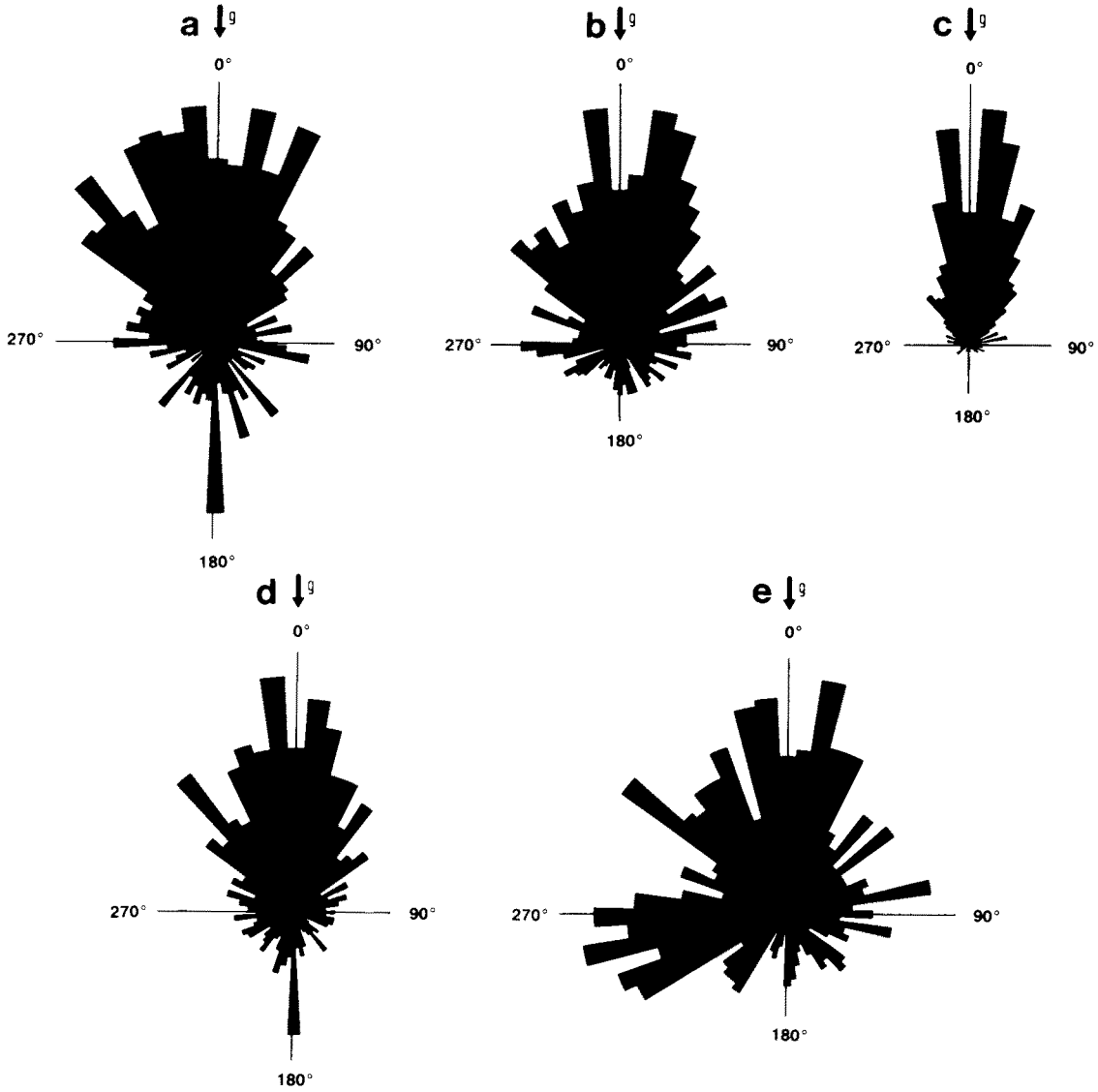

Fig. 3. Circular histograms of the movement vectors of *Peridinium gatunense* in a vertical cuvette under the simultaneous irradiation from above at fluence rates of (a) 250 lx, (b) 1 klx, (c) 5 klx, (d) 60 klx, and (e) 100 klx; 1000 tracks have been analyzed for each histogram.

increased up to 3 *g* and then declined; at 3.8 *g* almost the same percentage of cells swam upward and downward (Fig. 6). The dependence of the average linear velocity on the increasing acceleration force indicates a steady decline (Fig. 7). A linear regression curve through the data points cuts the abscissa at about 5 *g*.

Discussion

Peridinium, like the unicellular flagellate *Euglena* [30], shows an exclusive negative gravitaxis, which takes the organisms to the surface of the water column. Thus, the negative gravitaxis supports the positive phototaxis and helps the cells to accumulate in a band of suitable light conditions for growth and survival. The precision of orientation reaches an optimum at about 10 klx. At higher fluence rates approaching those measured at the surface of the natural habitat of *Peridinium gatunense*, the degree of orientation drastically declines, which stops the upward movement.

This behavior is certainly ecologically meaningful for photosynthetic microorganisms since the cells

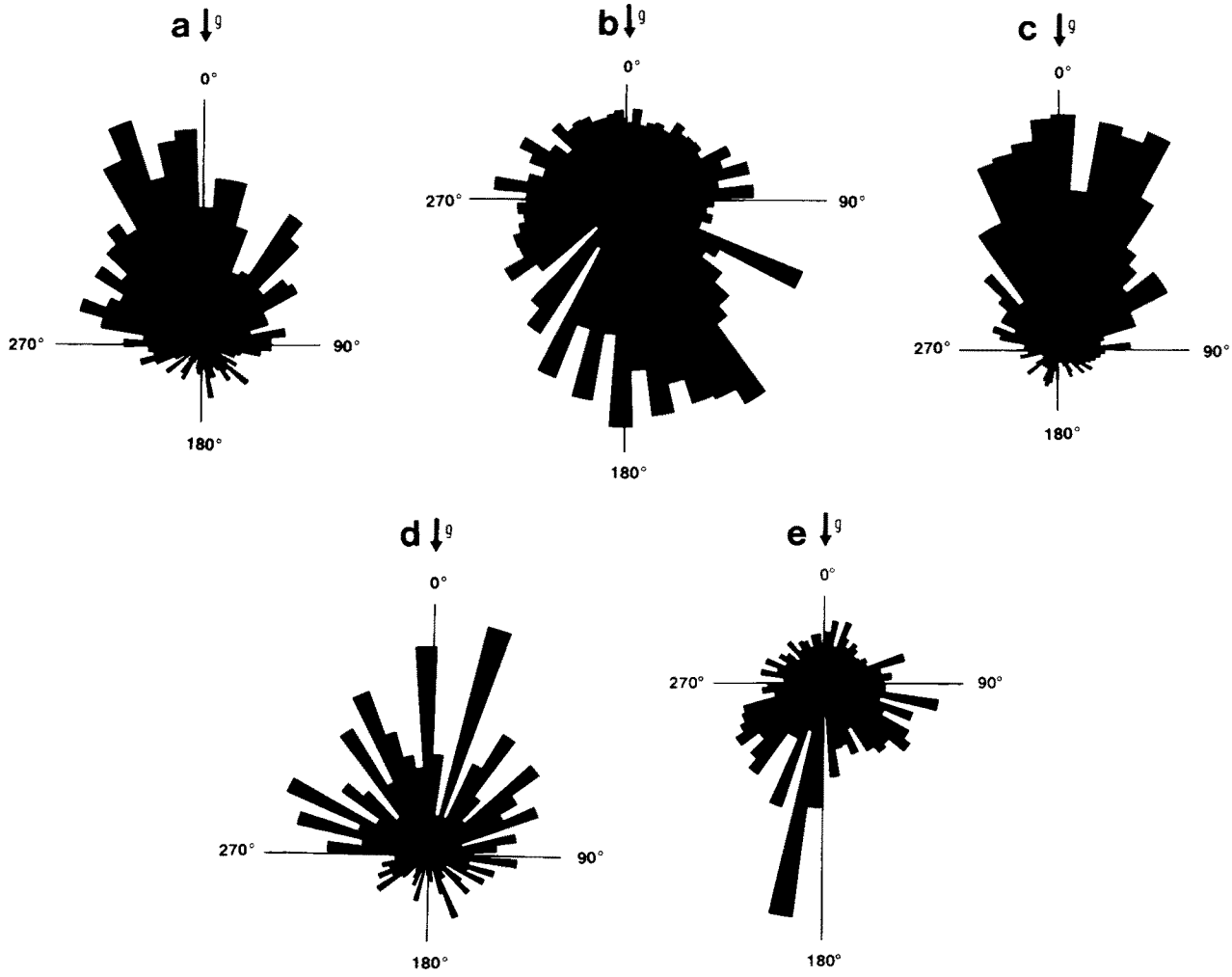

Fig. 4. Circular histograms of the movement vectors at 2 *g* (a), velocity distribution at 2 *g* (b), movement vectors at 3 *g* (c) and 3.6 *g* (d), and velocity distribution at 3.6 *g* of *Peridinium gatunense* in the NIZEMI; 1000 tracks have been analyzed for each histogram.

are easily killed by the solar radiation close to the surface and the cellular chromoproteins are photobleached by the too bright light intensity [17, 41, 42, 56, 59, 60]. In addition, the UV-B component in solar radiation has been found to impair both photoorientation and motility in *Peridinium* [43, 44] as well as in *Euglena* [26, 28, 33, 34] and other photosynthetic and nonphotosynthetic microorganisms [25, 29, 35–37, 40].

In the NIZEMI the cells are capable of swimming against acceleration forces up to 3.8 *g*. The degree of orientation even increases up to an optimum of 3 *g*, which is interesting since under natural conditions the cells have never been subjected to such acceleration forces during their evolution on earth. This result, however, does not clarify the question whether gravitactic orientation in flagel-

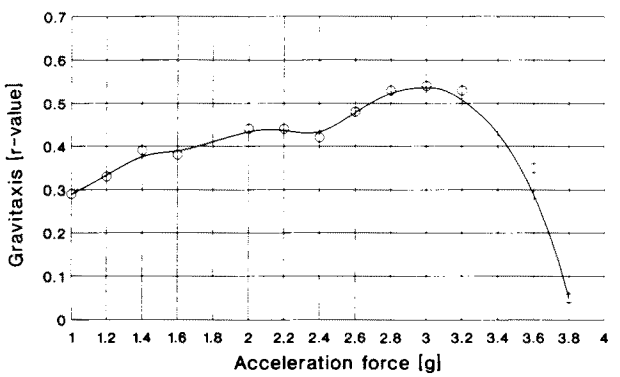

Fig. 5. Dependence of the degree of orientation (r-value) of *Peridinium gatunense* in dependence of the acceleration force in the NIZEMI.

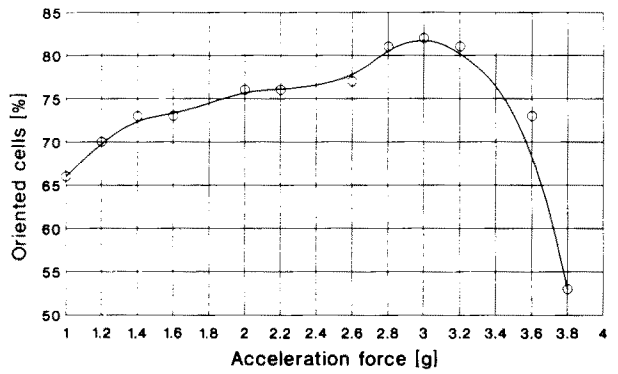

Fig. 6. Quantification of the orientation of *Peridinium gatunense* in the NIZEMI, plotting the percentage of those cells moving in the top two quadrants in dependence of the acceleration force.

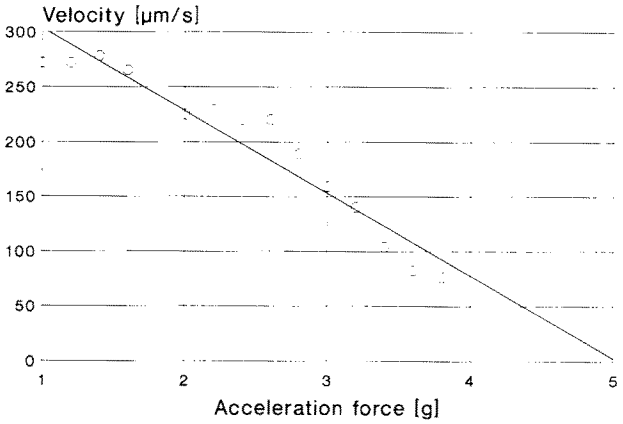

Fig. 7. Linear velocity of *Peridinium gatunense* moving against the acceleration vector in dependence of the acceleration force.

lates is owing to an active physiological process involving a specific (unknown) receptor or owing to a passive physical process such as an asymmetric mass distribution within the cell, since both mechanisms would be enhanced by higher acceleration forces.

The dependence of the cells' velocity on the acceleration force can be utilized for an estimation of the energy requirements for locomotion. The cells encounter a frictional component during forward propulsion. Furthermore, the cells have to provide additional energy to swim against the acceleration vector. The latter component depends on the acceleration force. An extrapolation of the velocity curve to zero suggests that the total energy requirement of movement equals that necessary to overcome an acceleration force of 5 *g*.

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