

GEOTAXIS IN THE CILIATED PROTOZOON *LOXODES*

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Accepted 14 November 1983

SUMMARY

Geotaxis is demonstrated in the ciliated protozoon *Loxodes*. This behaviour is mediated by a mechanoreceptor which is probably the Müller body, an organelle characteristic of loxodid ciliates. The geotactic response is sensitive to dissolved oxygen tension: in anoxia or at very low O₂ tensions the ciliates tend to swim up and at higher O₂ tensions they tend to swim down. This behaviour, in conjunction with a kinetic response allows the ciliates to orientate themselves in vertical O₂ gradients and to congregate in their optimum environment. In two appendices, models of the behaviour predicting vertical distribution patterns and considerations of the minimum size of a functional statocyst are offered.

INTRODUCTION

Many protozoa display a pronounced positive or negative geotaxis. The mechanisms responsible for this have been the subject of a prolonged dispute (for references see Roberts, 1970). In the species studied so far, however, there is no evidence of mechanoreceptors. Rather, the geotactic behaviour can be explained as the result of the interactions between sinking velocity, swimming velocity, rate of random reorientation and the net result of gravitational and hydrodynamical forces. These tend passively to orientate the anterior end of the cells upwards. Through modulations of the swimming velocity and the rate of random reorientation, the cells can change their probability of moving upwards and hence their vertical distribution in the water column. These mechanisms do not imply any perception of gravity (Roberts, 1970, 1981; Winet & Jahn, 1974). In this paper we demonstrate a geotactic response in the ciliate *Loxodes* which must depend on mechanoreceptors. This response to gravity depends on oxygen tension. In conjunction with a kinetic response this leads to the congregation of these microaerophilic organisms within a restricted layer in a vertical oxygen gradient.

The genera of the ciliate family Loxodidae, the limnic *Loxodes* and the marine *Remanella*, are mainly found in sediments. Species of *Remanella* are known to occur

in great numbers in sandy sediments, but vertically they are restricted to a more or less narrow zone beneath the superficial oxidized layer and above the anoxic, sulphide-containing sediment (Fenchel, 1969). In the case of *Loxodes*, similar vertical distribution patterns are best known from lakes and ponds which periodically develop an anoxic hypolimnion. During such episodes, the ciliates leave the sediment and occur in the water column around or immediately beneath the oxycline often several metres above the sediment. As in the case of *Remanella*, some individuals may be found deeper in the totally anoxic zone, but they totally avoid higher oxygen tensions (Bark, 1981; Finlay, 1981, 1982; Goulder, 1980). The ecological significance of this is probably that loxodids utilize food resources in a habitat inaccessible to competitors with demands for higher O_2 tensions. The recent finding that *Loxodes* can respire NO_3^- (Finlay, Span & Harman, 1983b) also explains their vertical distribution. Finally, loxodids may be sensitive to higher O_2 tensions, but this has not been studied. It remains to be shown how these organisms find and maintain their position in the chemical gradient and to which chemical species they react.

Loxodes and *Remanella* possess variable numbers of a peculiar organelle, the Müller body. These organelles are situated along the dorsal rim of the left side. Each body consists of a vacuole close to the cell surface and it contains a granular mineral concretion (Fig. 1). It has recently been found that the Müller body of *Loxodes* contains Ba and that of *Remanella* contains Sr, both metals presumably being in the form of the sulphate salt, (Finlay, Hetherington & Davis, 1983a; Rieder, Ott, Pfundstein & Schoch, 1982). Depending on the species, the ciliates possess one or more Müller bodies. Recent studies (Bedini, Lanfranchi & Nobili, 1973; Foissner & Rieder, 1983; Rieder, 1971) have demonstrated a rather complex structure within the organelles. They are associated with a ciliary row. In conjunction with each Müller body a pair of basal granules is situated a few μm beneath the other basal granules of the kinety in an opening leading to the body. One basal granule carries a normal cilium which protrudes through the opening to the surface of the cell; the other basal granule attaches to the Müller body *via* a stalk which is presumably ciliary in nature. Long before the fine structure of the organelles was known, it was suggested that they were mechanoreceptors (Penard, 1917). Since then, this idea has frequently been repeated in the literature, but never supported by experimental evidence.

MATERIALS AND METHODS

Most experiments were carried out on *Loxodes striatus* (Engelmann) (length, approx. 200 μm) with additional observations on the somewhat larger *L. magnus* Stokes. Both species were isolated from lakes in the English Lake District and grown in a soil extract medium. The containers were either tissue culture flasks entirely filled with medium or test tubes with a 1- to 2-cm layer of autoclaved soil. The cultures did not contain other protozoa, but a bacterial flora was present and necessary in order to maintain low O_2 levels. The ciliates were fed with an axenic culture of the flagellate, *Euglena gracilis*. Cultures were kept in the dark at either 10, 15 or 20 °C.

For observations and experiments we used test tubes, spectrophotometer cells or capillary glass tubes, and a horizontally mounted dissection microscope. The latter could be elevated or lowered and was fitted with an ocular grid so that cells could

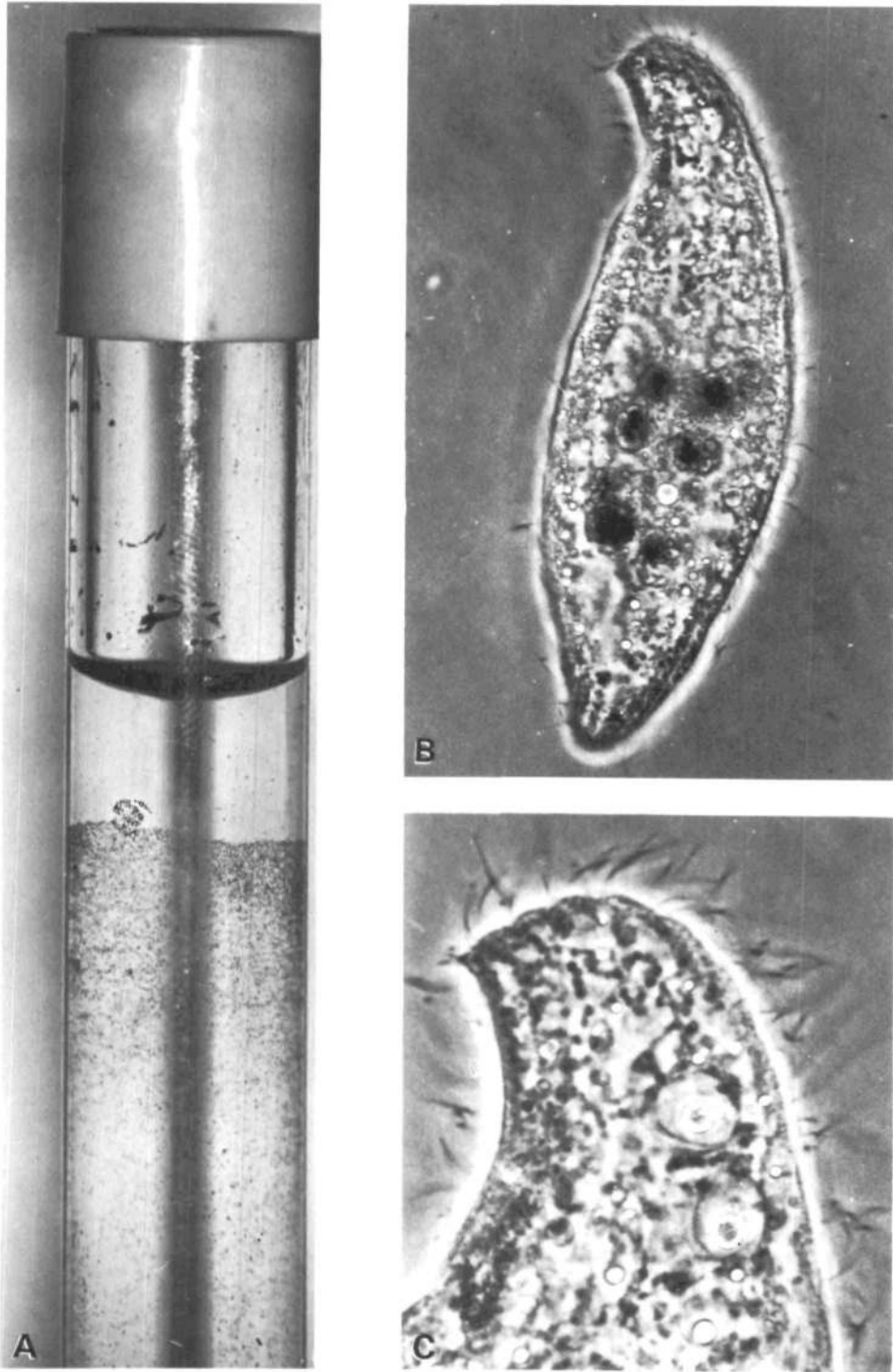


Fig. 1. (A) Distribution of *Loxodes striatus* cells in a test tube culture. There is a sharp oxygen gradient and cells avoid the upper, oxygenated water. (B) A single cell of *L. striatus*. The anterior end is at the top and the cell is about 200 μm in length. (C) Two Müller bodies located antero-dorsally in a cell of *L. striatus*. Each spherical mineral concretion has a diameter of about 3 μm in a vesicle of about 7.5 μm .

Counted at different depths in the containers. At each level the number of cells in a volume extending through the tube and delimited by a rectangle measuring either $1.2 \times 1.5 \text{ mm}^2$ or $1 \times 2 \text{ mm}^2$ was counted. The density of cells at a given level is expressed as ciliates mm^{-2} or as a percentage of the total number counted. Water (sterile soil extract medium) with different levels of dissolved O_2 was prepared by mixing N_2 -bubbled water with air-saturated water. The behaviour of the ciliates in O_2 -gradients was studied either in the above mentioned test tube cultures or in photometer cells. In the latter case, the cells were initially filled with totally O_2 -free water and ciliates and left uncovered at a constant temperature for 1–2 days so that a suitable diffusion gradient of O_2 formed. In laboratory systems, oxygen gradients were measured with a micro oxygen electrode (Baumgärtl & Lübbers, 1973). Most experiments and quoted rates apply to 10°C . Some additional experiments took place at 20°C .

Since *Loxodes*, when exposed to a sudden increase in light intensity, shows an avoidance reaction and since Goulder (1980) claimed that it does not survive long in the light, we initially carried out observations in dim red light. We later found that *Loxodes* adapts to and survives normal daylight so we discontinued the use of very dim light.

The vertical distribution of *L. striatus* in the field was studied during the summer of 1982 in Priest Pot, a shallow 1-ha water body in the English Lake District. Samples at various depths were collected with a peristaltic pump and their O_2 contents measured polarographically.

RESULTS

Vertical distribution in O_2 gradients

Fig. 2A shows two examples of the vertical distribution in Priest Pot during summer stratification and Fig. 2B shows similar distributions in two test tube cultures. The main difference between the two sets of data is the vertical scale; in the former case it is about 4 m and in the latter case about 8 mm. In both cases the organisms show a pronounced maximum abundance at O_2 tensions somewhere within the range 1–10% atmospheric O_2 saturation (atm O_2). Upwards they are sharply delimited and downwards the numbers decline more gradually. The vertical distribution correlates equally well with electrode potentials (E_h); a ciliate maximum is found in the range +100 to +250 mV (relative to an H_2 -electrode) and they are absent at potentials exceeding +300 mV. Natural waters saturated with atmospheric air show an E_h of +400 to +450 mV (Fenchel, 1969).

Behaviour at different O_2 tensions

When ciliates are placed in water in sealed photometer cells they display different orientations of their bodies according to the O_2 tension. In experiments we included a tiny magnet and stirred the water at time zero. For each following minute the percentage of cells orientated 0° , 45° , 135° and 180° relative to the vertical was determined. About 60 cells could be scored per minute. Four examples are shown in Fig. 3. Within the range 0–2.5% atm O_2 and after about 1 min, some 60% of the cells

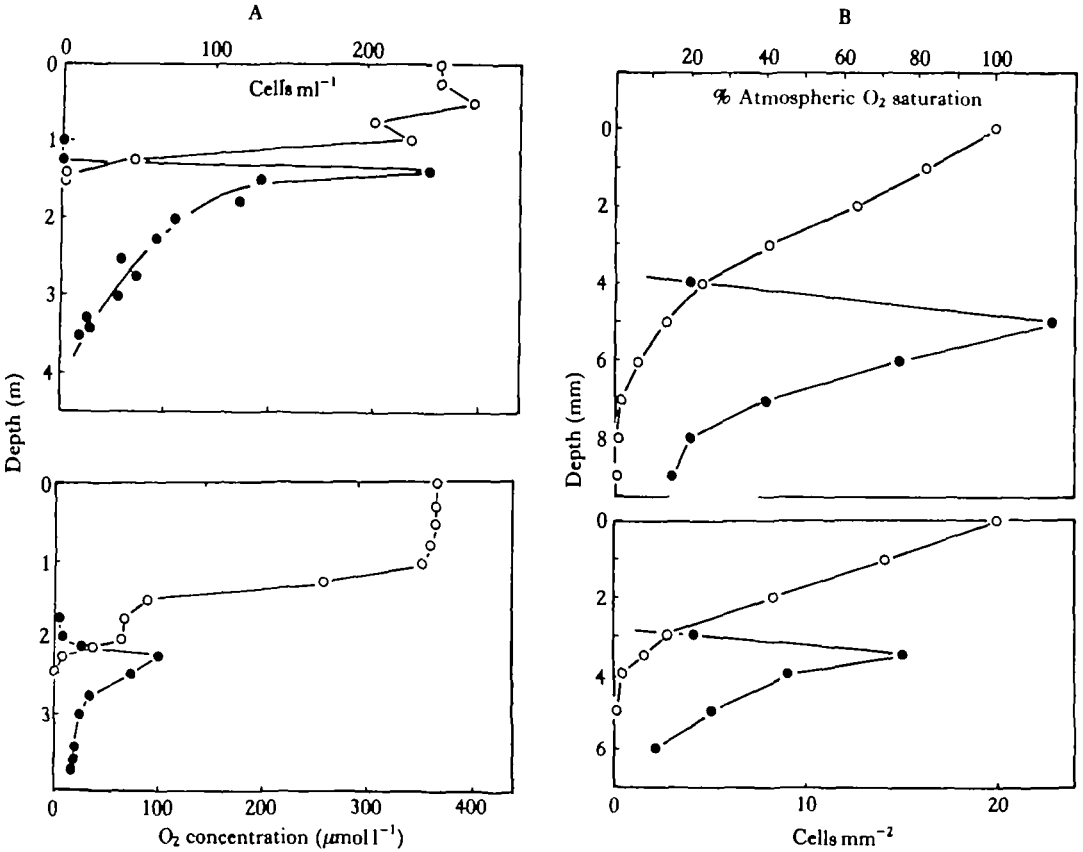


Fig. 2. (A) The vertical distribution of *Loxodes striatus* (filled circles) and of O₂ (open circles) in Priest Pot on July 21 (top) and September 15, 1982. (B) The vertical distribution of *L. striatus* in two test tube cultures.

swim straight upwards and about 20 % swim obliquely upwards. At high O₂ tensions the converse is true; the great majority of cells swim downwards. Intermediate O₂ tensions yield intermediate results and the higher the O₂ tension the larger is the fraction of downward-swimming individuals. Observations in the photometer cells also allowed the measurement of swimming velocities. In completely anoxic water as well as in water with 100 % atm O₂, the horizontal, upward and downward swimming velocities are, respectively, 0.14 mm s⁻¹, 0.1 mm s⁻¹ and 0.18 mm s⁻¹. Hence the settling velocity (which was also measured independently on OsO₄-fixed cells) is about 0.04 mm s⁻¹. Within the range of 5–10 % atm O₂ the horizontal swimming velocity is reduced to about 0.07 mm s⁻¹. Altogether, the swimming velocities of about 100 cells were measured.

Within the range of 5–10 % atm O₂, the cells tend to attach to the glass walls of the containers and glide along them rather than to swim freely in the water. Finally, the frequency with which the cells change swimming direction was measured. In anoxic water and in air-saturated water the ciliates change their swimming direction about every 45 s. In the range of about 5–10 % atm O₂ this time interval decreases to 10–13 s. These data are based on 42 cells making altogether 125 turns. From the data

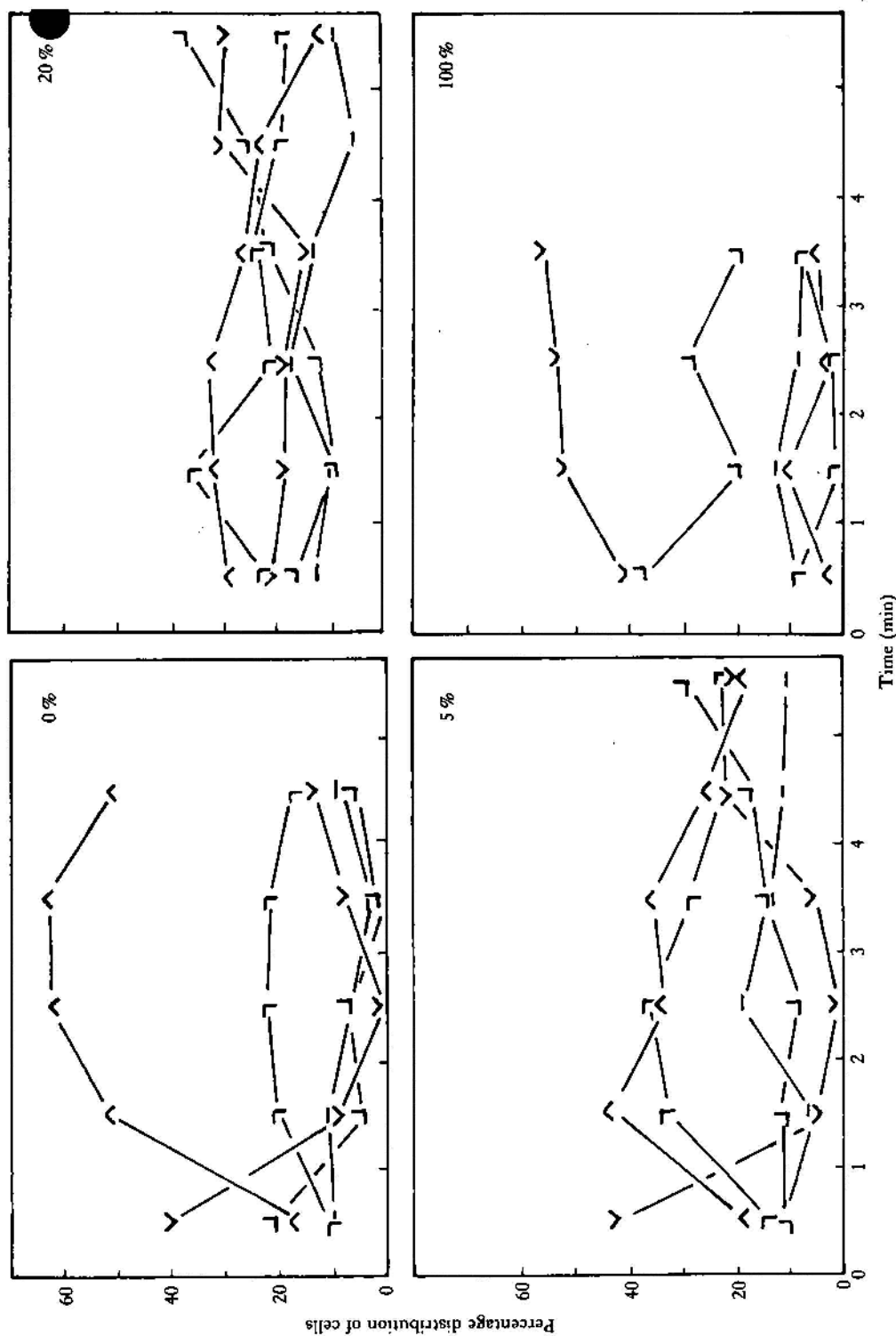
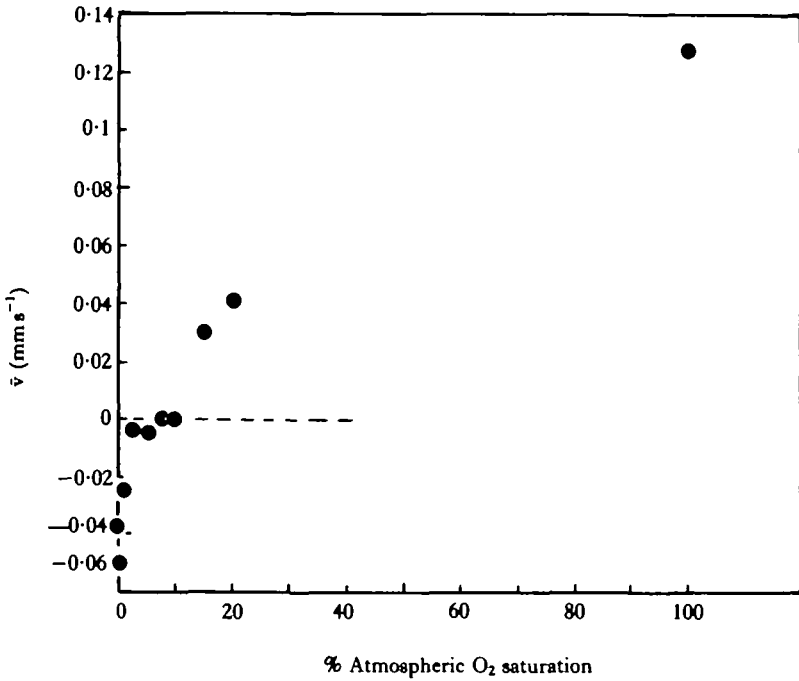


Fig. 3. Percentage distribution of cells turning their anterior ends 0° , 45° , 90° , 135° and 180° relative to the vertical (as indicated by the direction of the arrowheads) for each minute following stirring and in media with different O₂ contents. About 60 cells were scored each minute.

A



B

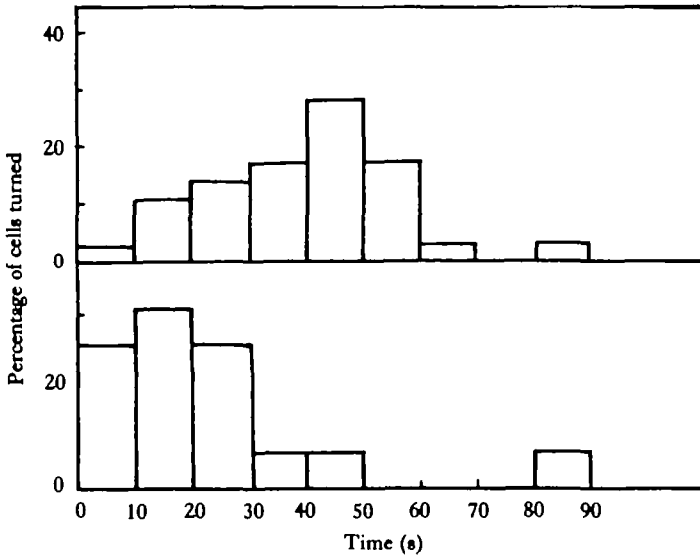


Fig. 4. (A) Mean vertical velocity (\bar{v}) of *Loxodes striatus* as a function of O₂ tension. Calculated as described in Appendix A, equation (1), and based on the orientation of the cells in the periods 2–3 and 4–5 min following stirring. Negative values indicate net upward drift. Each data point is based on about 120 cells. (B) Time taken to turn 180° after being turned upside down. Top, turning the anterior end up; bottom, turning the anterior end down.

Obtained in these experiments three parameters can be calculated using equations (1), (2) and (3) in Appendix A. Among these is the mean net vertical velocity or vertical drift, \bar{v} , shown in Fig. 4A as a function of O_2 tension. Here negative values indicate net upward swimming. It can be seen that the cells tend to swim upwards at O_2 tensions below 5–10% atm O_2 and otherwise they swim downwards. The other parameters calculated from the measurements are the mean vertical path length, λ , and the random motility or diffusion coefficient, D . The latter is in the range $0.1\text{--}0.2\text{ mm}^2\text{ s}^{-1}$ under fully anoxic and under fully aerobic conditions. It is reduced to about $0.025\text{ mm}^2\text{ s}^{-1}$ within the range 5–10% atm.

In order to study the turning of individual cells, they were enclosed in capillary glass tubes with either air-saturated or O_2 -free water. The tubes were mounted with tape onto a rotating, vertically mounted microscope stage so that the ciliates could be rapidly turned upside down during observation. When cells swimming upwards under anoxic conditions or cells swimming downwards under aerobic conditions are

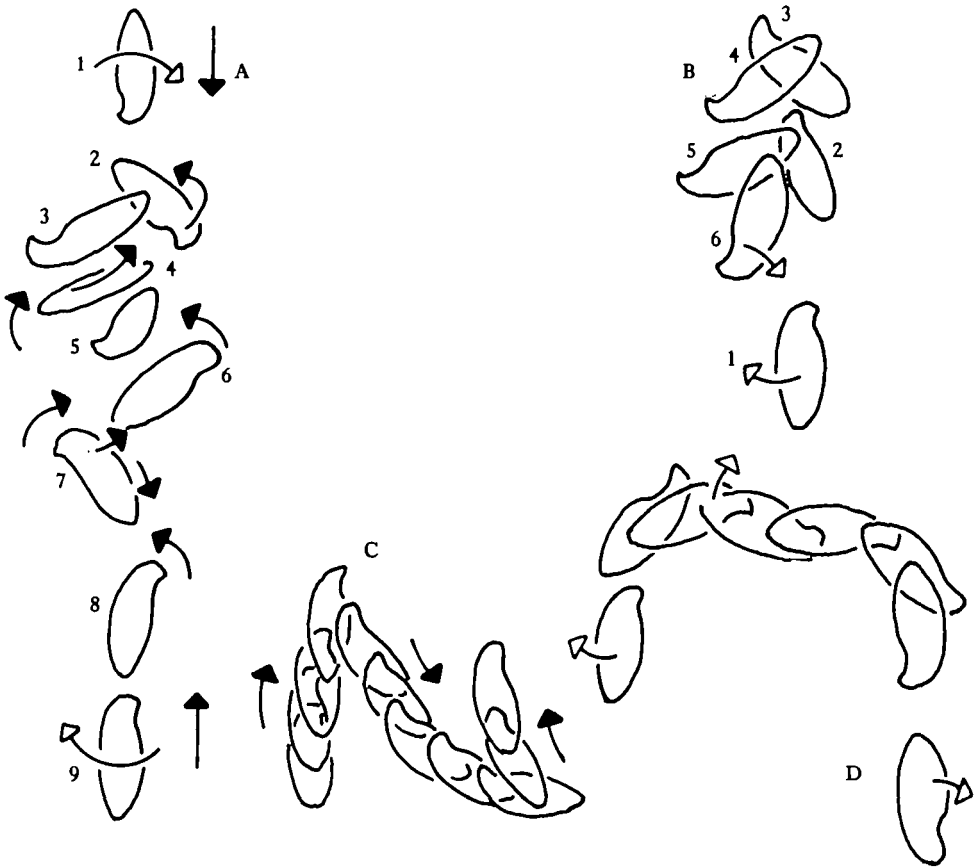


Fig. 5. Behaviour of turning. (A) An initially upward-swimming cell has been turned head down. (B) An initially downward-swimming cell has been turned head up. (C) A cell gliding on the glass wall in an O_2 gradient. (D) A cell swimming through an O_2 gradient. Time intervals between drawings: (A) and (B), 5 s; (C) and (D), 1 s. Length of ciliate is about 0.2 mm. Open arrows indicate rotation around cell axis; filled arrows indicate other movements.

turned through 180° , they practically always react within a few seconds and turn themselves back to their original direction of swimming. Fig. 4B, which is based on some 30 cells in each case, shows that to turn the anterior end downwards usually takes less than 30 s while the opposite process takes about twice as long. An individual cell which is turned upside down several times will repeatedly turn back to its preferred orientation.

Fig. 5A, B depicts the movements of the cells after being turned upside down. Normally, swimming *Loxodes* cells rotate clockwise around the axis of the swimming direction when swimming towards the observer (open arrows in Fig. 5). When a previously upward-swimming cell is turned upside down (A) it reacts nearly immediately with several ciliary reversals. This brings the body to form an increasing

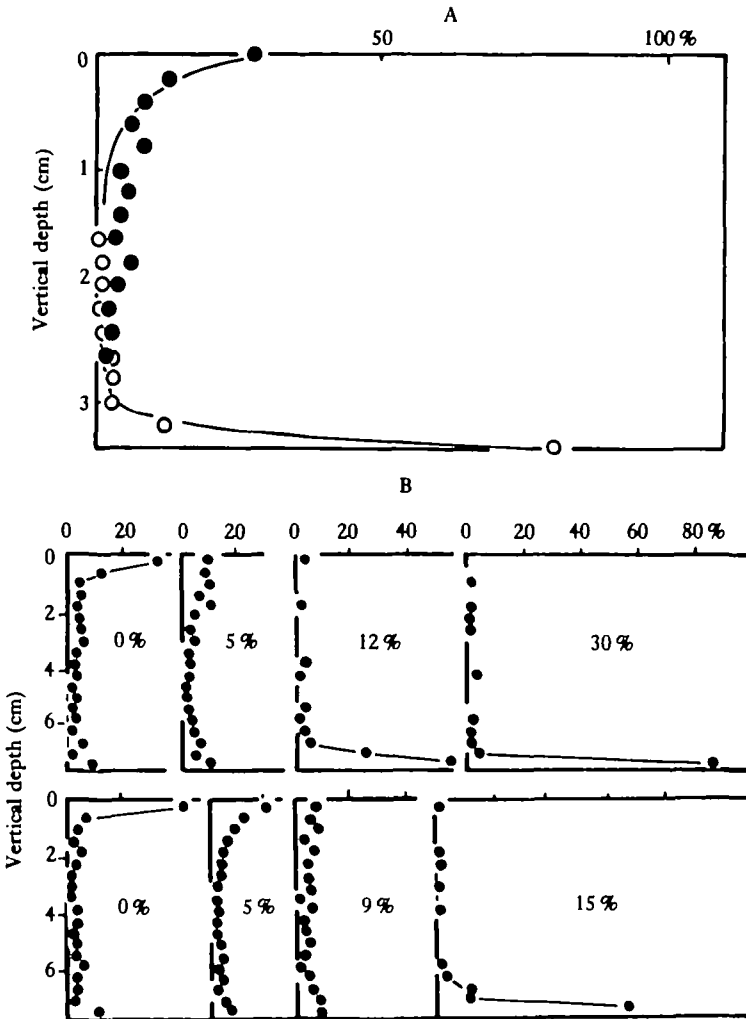


Fig. 6. (A) Vertical distribution in an anoxic medium (filled circles) and in 100% atm O_2 (open circles). About 200 cells were counted in either case. Also shown are the curves $u(x) = \text{constant} \times e^{x^2/D}$ for the two cases. (B) The vertical distribution in a water column equilibrated with air with different contents of O_2 . About 110 cells were counted in each case. The standard deviations for the maximum points in the anoxic experiments and those for O_2 pressure exceeding 12% are within the range of 8–12%. For all other data points, s.d. is between 20 and 40%.

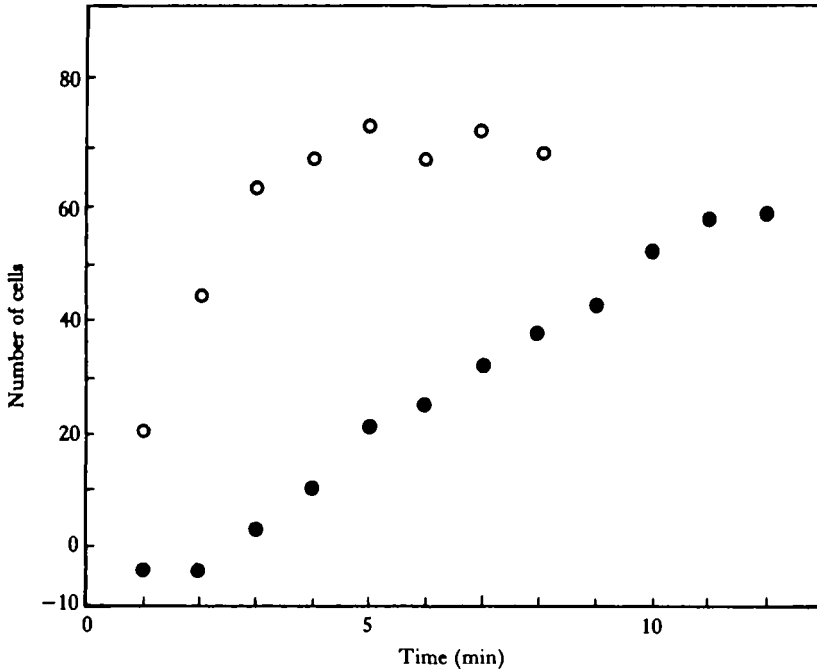


Fig. 7. Net migration of cells across a horizontal line 2 mm from the surface (anoxic case, filled circles) and across a horizontal line 2 mm above the bottom (100% atm O₂, open circles) as a function of time following stirring.

angle to the vertical. Eventually this brings the anterior end to point upwards and the ciliate resumes normal swimming. During this process the ciliate will have moved downwards about 1 mm in accordance with the settling velocity. Turning the anterior end downwards (B) seems also to involve a series of ciliary reversals. In both cases, the normal rotation of swimming cells stops during the turning process.

Stationary vertical distribution

When cells are left in a container with a homogenous O₂ tension, they will show a characteristic vertical distribution pattern. The time taken to reach the stationary distribution from a homogenous distribution depends on the height of the vessel. The form of this distribution is dependent on the O₂ tension. Fig. 6A shows the vertical distributions in a 3.4-cm photometer cell containing anoxic water and subsequently air-saturated water.

As could be expected from Fig. 4A, the cells tend to aggregate at the bottom under aerobic conditions and to aggregate at the top under anoxia. In Appendix A it is shown that the number of cells per mm at depth x (positive in the downward direction) can be expected to conform with the equation $u(x) = \text{constant} \times e^{xv/D}$. Fig. 6A shows these predictions based on independently measured values of \bar{v} (anoxic, -0.05 mm s^{-1} ; aerobic, 0.12 mm s^{-1}) and D ($0.15 \text{ mm}^2 \text{ s}^{-1}$).

At intermediate O₂ tensions, the above equation in conjunction with the data in Fig. 4A predict less steep gradients. There is also some O₂ tension at which the distribution is even. This is confirmed by the experiment shown in Fig. 6B. A cell

population (about 1000 cells) was added to a 7.5-cm water column in a test tube. The tube was capped with an airtight seal leaving 10 ml head space above the water. Oxygen was stripped from the water by inserting a hypodermic needle through the seal and bubbling the water with N_2 for 10 min. Thirty minutes after the needle had been removed, the anoxic steady state distribution was recorded. Thereafter a known amount of air was injected into the head space and the tube was shaken gently to equilibrate the water with the new air composition. After 30 min the distribution was recorded again. This procedure was repeated with increasing O_2 tensions. In the experiment shown, the tube was rendered anoxic again after a period with 30 % atm O_2 and the experiment repeated (lower row). At 30 % atm O_2 the vertical distribution is no different from that recorded for 100 % atm O_2 (Fig. 6A) and it can be seen that within the range 5–10 % atm O_2 , \bar{v} must take a value close to zero.

The rate at which the steady state distribution is approached was studied in an experiment, the results of which are shown in Fig. 7. Ciliates in a 3.4-cm deep cell were distributed evenly by stirring. The number of individuals passing a horizontal line 2 mm below the surface (anoxic case) or 2 mm above the bottom (aerobic case) was then recorded every minute. Since cells passing the lines from above and from below were recorded separately, the net migration could be calculated. When this eventually became zero (equally many cells passing either way per unit time) steady state was achieved. The depth of the container divided by the time indicated by extrapolation of the initial linear part of the curve to the equilibrium level is the mean vertical drift. It is about 0.13 and -0.06 mm s^{-1} under aerobic and anaerobic conditions respectively and in accordance with the values in Fig. 4A.

Behaviour in O_2 gradients

At the level in an O_2 gradient where *Loxodes* cells congregate (5–10 % atm O_2) they behave as they do in containers with a homogenous O_2 tension within that range. In contrast to the cells found deeper in the container or the very rare ones found higher up, they tend to position their ciliated right side next to the glass surface and to glide rather than swim. The swimming velocity is relatively low (around 0.07 mm s^{-1}) and the frequency of changing swimming direction is increased so that random motility as well as drift is much reduced. Most cells are orientated upwards or obliquely upwards, whether swimming or gliding. Consequently, they may slowly drift upwards to reach a higher O_2 tension than the optimum one. For a cell gliding on the wall the events are shown in Fig. 5C. At some point, a few hundred μm above the upper limit of the bulk of the ciliates, the individual displays a ciliary reversal, slides obliquely downwards, comes to a stop some $500 \mu\text{m}$ lower down, whereafter it may slowly glide upwards again. Cells which swim upwards through the gradient may display the ciliary reversal behaviour shown in Fig. 5B. Sometimes, however, the cells seem to turn gradually without ciliary reversal and without stopping the characteristic rotation of normally swimming cells (Fig. 5D).

In Appendix A the model for the steady state distribution is extended to cover the case of an O_2 gradient. In Fig. 8 this model is applied to the O_2 gradient in a test tube culture and compared to the actual distribution of the cells. The numerical calculation is based on values of vertical drift for different O_2 tensions determined experimentally. Two cases are considered. In one a constant random motility ($0.15 \text{ mm}^2 \text{ s}^{-1}$) is

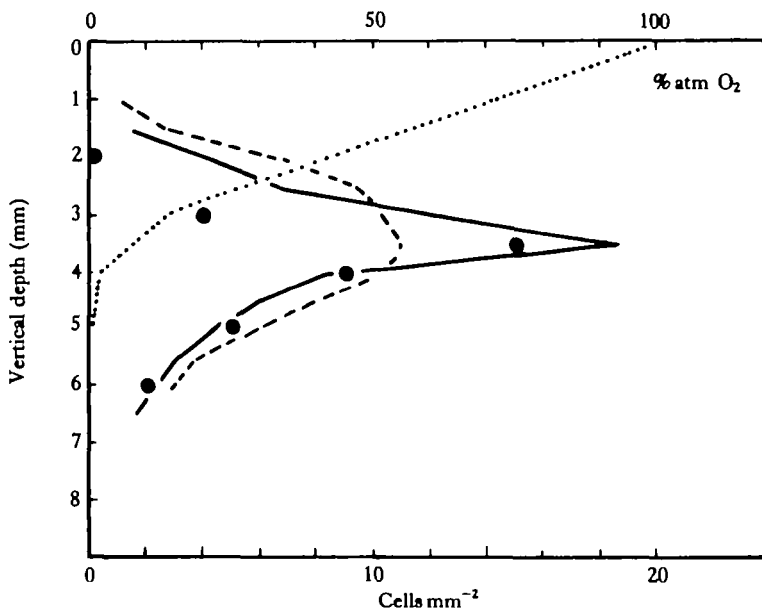


Fig. 8. The vertical distribution of *Loxodes striatus* (filled circles) and of O_2 (dotted line) in the upper 7 mm of a test tube culture. Also shown are predictions of the gradient model in Appendix A, based on values of \bar{v} from Fig. 4A and assuming either $D = 0.15 \text{ mm}^2 \text{ s}^{-1}$ at all O_2 tensions (interrupted line) or that D decreases to $0.025 \text{ mm}^2 \text{ s}^{-1}$ within the range of 2–12 % atm (solid line).

assumed; in the other one D is (in accordance with experimental evidence) assumed to decrease to $0.025 \text{ mm}^2 \text{ s}^{-1}$ within the region 2–12 % atm O_2 . It can be seen that this leads to a more pronounced peak and predicts the actual distribution quite closely. However, we do not know the exact threshold values at which D decreases nor whether this happens gradually or abruptly with changing O_2 tension.

Distribution in inverted O_2 gradients

As can be seen intuitively and as predicted in Appendix A, the distribution of cells in an inverted O_2 gradient (O_2 increases downwards) should be bimodal with a minimum at the level of optimum O_2 tension and with maxima at the upper and lower boundaries of the water column. Such O_2 gradients hardly exist in nature, but they may be produced in the laboratory. We placed a 2 cm thick sediment consisting of a mixture of autoclaved soil and a 2 % agar solution in a test tube. After the sediment had solidified we filled the tube to the rim with culture medium inoculated with ciliates and food organisms and covered the tube with dialysis membrane taking care to avoid air bubbles. The membrane was kept moist to avoid evaporation. After a few days, an O_2 gradient had developed and the ciliates were distributed accordingly with a pronounced maximum 4 mm beneath the membrane (Fig. 9). The tube was then turned upside down without disturbing the gradients and the distribution was recorded again after 5 h. A maximum number of cells was now found at the top, just beneath the sediment surface. Here the cells were trying to swim upwards with their anterior ends touching the agar surface. A rubber band holding the membrane prevented observation close to the dialysis membrane, but since in the upright position a total of 1006 cells was counted and in the inverted case only 828, the missing cells (about

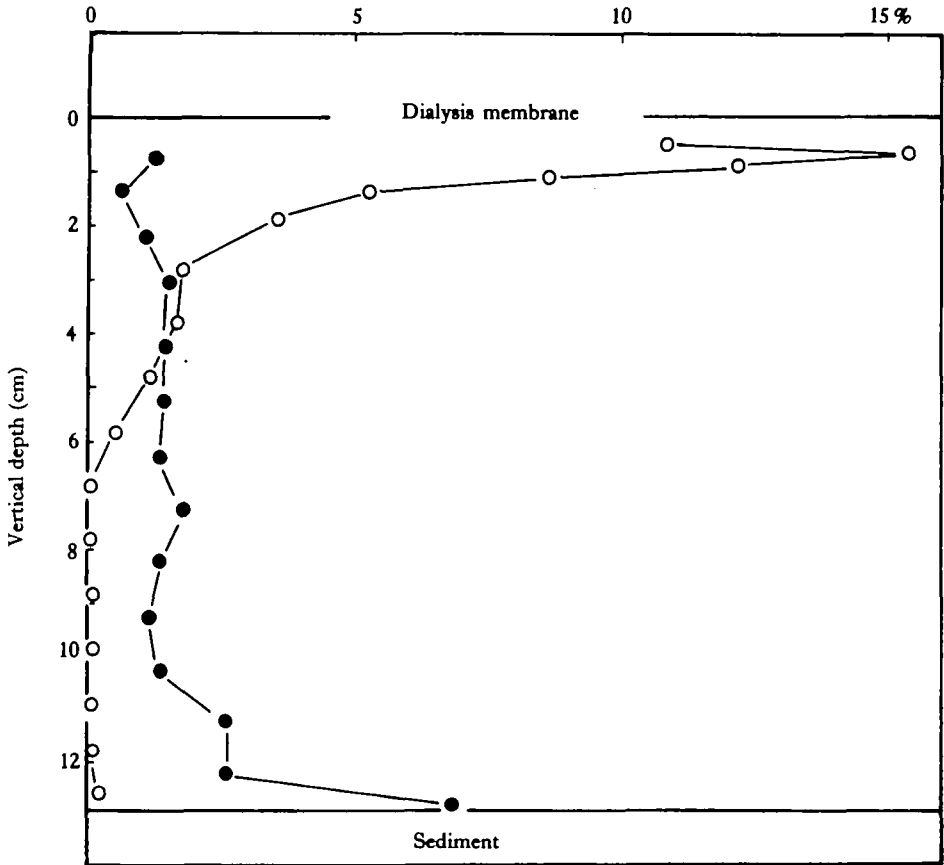


Fig. 9. Open circles: distribution of *Loxodes striatus* in a test tube culture. Filled circles: distribution of the cells 5 h after turning the culture upside down (sediment is then at the top). Cell density was counted every 2 mm and the data averaged for every cm. s.d. for each data point is between 10 and 15%.

180) must have been situated on the dialysis membrane, thereby producing a maximum in this region. In the upright position about 70% of the ciliates were situated beneath the maximum peak but virtually none were found beneath 6 cm. In the inverted tube the corresponding number of cells was found between the sediment and about 9 cm, so the bulk of these cells must have migrated upwards after the tube was turned upside down.

DISCUSSION

Our results show that *Loxodes* must possess a mechanoreceptor which tells it what is up and what is down. This mechanism in conjunction with a kinetic response, viz, a decreased random motility within the optimum zone, explains the vertical distribution pattern and the ability of the organisms to respond to changes in the chemical gradients. Many, if not all motile micro-organisms are capable of responding to chemical gradients of one sort or another. In the great majority of cases the only

Means of orientation is by kinetic responses (changes in swimming velocity and/or frequency of random turns, possibly in conjunction with some refinements such as 'memory'; for a review and discussion, see Lapidus & Levandowsky, 1981). Orientation in oxygen and redox gradients is potentially easier since in nature (water bodies, sediments) reducing and anoxic conditions prevail downwards and oxidizing conditions prevail upwards. Therefore, gravity in conjunction with the surrounding environment offers a clue concerning where to swim.

The model developed in Appendix A links the studied behaviour with the observed vertical distribution patterns. *Loxodes* cells respond rapidly to changes in O_2 gradients and are able to fill out the entire optimum environment irrespective of the steepness of the gradient and once the cells have found their preferred habitat, few of them venture out towards more adverse conditions. When the small size of the cells is considered, it is improbable that they can detect the orientation of a chemical gradient directly. A kinetic response alone (changes in swimming velocity and/or rate of random reorientation dependent on the external environment) could in principle account for the observed steady state distributions found (except for that of inverted O_2 gradients), but it would take an impracticably long time to achieve this distribution over longer distances such as in the water columns of lakes. Certain other mechanisms could possibly explain the ability of the ciliates to orientate themselves in gradients. For cells swimming through such a gradient a 'memory' could inform them about the orientation and, in conjunction with a time lag in response, it could be ensured that the entire habitat niche is filled. The significance of the model in Appendix A is to show that it is not necessary to infer such mechanisms in order to explain the observed distribution patterns or the short time needed for the cells to adjust to changes in the gradients. The model adequately predicts the steady state distribution solely on the basis of the behavioural properties studied: a geotactic response and a kinetic one both of which are modified by O_2 pressure. It further shows that the random element of motility is an adaptive aspect of the behaviour. Finally the model correctly predicts that if the O_2 gradients are reversed relative to gravity, the orientation ability breaks down. In a normal O_2 gradient the geotactic response enables the cells to approach the optimum environment with 60–70 % of their maximum swimming velocity. Once they have arrived there, a kinetic response traps the cells there. The efficiency of these achievements is independent of the steepness of the O_2 gradient.

For loxodids living in sediments with steep chemical gradients and in the absence of turbulent diffusion, our test tube cultures constitute a perfect experimental model. In the case of the distribution in the free water column a complete description must include turbulence. Since the drift is only of the order of 30 cm h^{-1} even slight convection in the water will constitute a considerable additive effect relative to the random motility of the organisms themselves.

Our experiments show that at least one of the parameters to which *Loxodes* responds is O_2 . It is possible that other chemical species (absence or presence of HS^- , Fe^{2+}/Fe^{3+} , etc.) may also influence the geotactic behaviour, but this was not investigated.

'Statocysts', that is organs which – mainly due to their morphology – are assumed to be mechanoreceptors, are widely distributed among smaller metazoans (turbellarians, coelenterates). But to our knowledge, *Loxodes* is the first example of a

unicellular organism which has been demonstrated to sense gravity. A somewhat parallel and ecologically related example is that of magnetic bacteria. These peculiar prokaryotes follow magnetic field lines for much the same reason that *Loxodes* swims downwards, that is to reach microaerophilic or anoxic conditions in the sediment (Blakemore, Frankel & Kalmijn, 1980).

Our results do not provide evidence of the mechanism by which the Müller body functions but they do make the hypothesis that they are gravity sensors more convincing. In Appendix B we show that the Müller body is sufficiently large and heavy to overcome the effect of Brownian movements so that at least from the viewpoint of mechanical physics the organelles could function as gravity sensors. While Müller bodies are so far only known from loxodids there are a large number of other ciliate species which respond to oxygen or redox gradients in natural environments (Fenchel, 1969; Finlay, 1982). Whether these forms also use gravity as a clue remains to be shown.

APPENDIX A: MODELS OF VERTICAL DISTRIBUTION

The model used here as a diffusion approximation to describe *Loxodes* behaviour is identical to the 'Kolmogorov forward equation' (see Lapidus & Levandowsky, 1981; Okubo, 1980). Let x represent the depth ($x=0$ at the surface, $x=L$ at the bottom, see Fig. 10), $u(x)$ the number of cells at depth x and $j(x)$ the net flux through level x (positive downwards). Let v_h be the horizontal swimming velocity, v_s the settling velocity and, θ the angle of the axis of the ciliate relative to the vertical (Fig. 10). The mean vertical swimming velocity (drift) in a population of n individuals is then given by,

$$\bar{v} = 1/n \sum^n (-\cos\theta v_h + v_s). \quad (1)$$

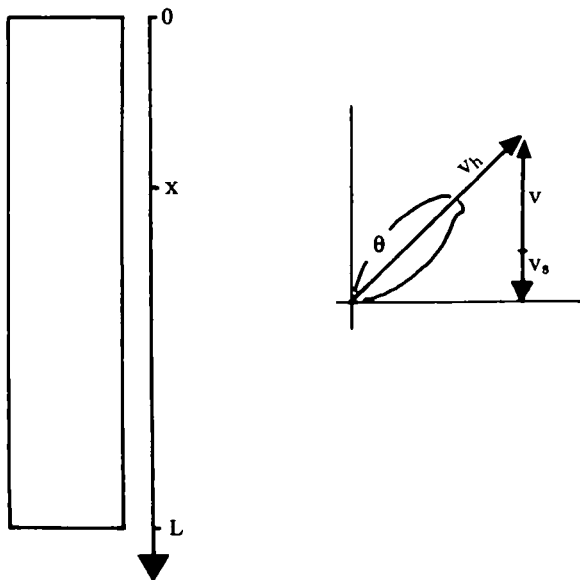


Fig. 10. Left: coordinate system for the biased diffusion model. Right: calculation of the vertical velocity component.

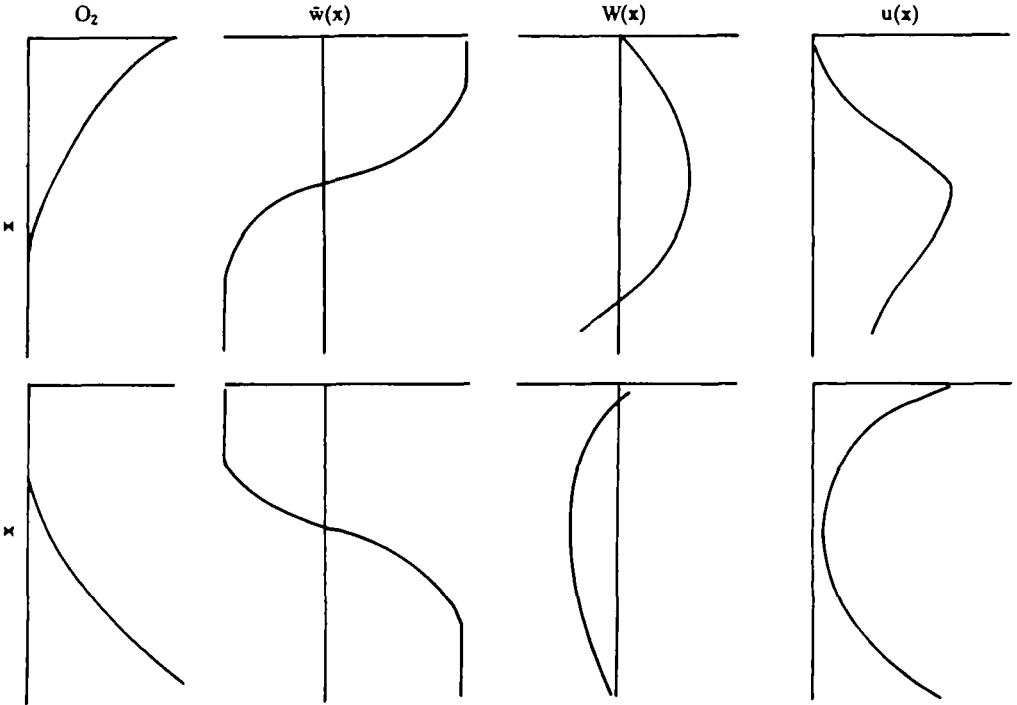


Fig. 11. Qualitative predictions of the vertical distribution pattern in the case of a normal (above) and of an inverted (below) O_2 gradient.

If the mean numerical vertical velocity component is given as,

$$v_v = 1/n \sum^n |(-\cos\theta v_h + v_s)|, \quad (2)$$

then the mean vertical path length is $\lambda = v_v \tau$, where τ is the time interval between random changes in swimming direction. The vertical random motility or diffusion coefficient is then approximated as:

$$D = \lambda^2 / 2\tau. \quad (3)$$

The equation of continuity is $\partial u / \partial t = -\partial j / \partial x$, and that of transport is,

$$j = u\bar{v} - D\partial u / \partial x, \quad j(0) = j(L) = 0.$$

If we assume that \bar{v} and D are invariant with u and D is invariant with O_2 tension, the following two cases can be considered.

(i) *Constant O_2 with depth.* At steady state, $j = 0$ and $du/dx = \bar{v}u/D$, or $u(x) = \text{constant} \times e^{x\bar{v}/D}$. Since \bar{v} is negative at low O_2 tensions and positive at high O_2 tensions, the cells will concentrate at the top in the former case and at the bottom in the latter.

(ii) *An O_2 -gradient is present.* In this case, \bar{v} is a function of O_2 and O_2 is again a function of depth, x , and we have $\bar{v}[O_2(x)] = \bar{w}(x)$. Then, $du/dx = w(x)u/D$, with the solution,

$$u(x) = \text{constant} \times \exp \left[\int_0^x \bar{w}(y)/D dy \right] = \text{constant} \times e^{W(x)}.$$

With the established dependence of \bar{v} on O_2 tension, the equation predicts a maximum for $\bar{v} = 0$ if O_2 decreases with depth and a minimum for $\bar{v} = 0$ if O_2 increases with depth and that the gradients of the ciliates will be steeper as the O_2 gradients become steeper (Fig. 11).

This assumes a constant value of D . In fact, D decreases in the region where \bar{v} takes the smallest numerical values, an effect which will tend to trap the cells (see also Fig. 8). Thus the maximum will tend to be more pronounced in the case of a normal O_2 gradient and the minimum will tend to be less pronounced in an inverted one (cf. Fig. 9).

APPENDIX B: THE MÜLLER BODY AS A STATOCYST

Each Müller body consists of a spherical mineral granule (radius r) attached to a cilium which is directed perpendicularly to the long axis of the cell. The organelle is situated in a vacuole. The free distance from the body to the bottom of the vacuole is L and it is assumed that the body can move freely from the top to the bottom of the vacuole. It is further assumed that the ciliate can somehow sense the bending direction of the supporting cilium.

The Müller body is subject to movement due to gravity and to Brownian motion. The settling velocity is given by $v_s = 2/9r^2 (\rho_m - \rho_w)g/\eta$, where ρ_m and ρ_w represent the specific weight of the Müller body and of water respectively, η is water viscosity (assumed to be $10^{-3} \text{ Pa s}^{-1}$ in the following) and g is the acceleration due to gravity. The Brownian motion is characterized by a diffusion coefficient, $D = kT/6\pi\eta r$, where T is absolute temperature and k is Boltzmann's constant. The time taken to move the sphere some vertical distance, s , by diffusion is $T_D = s^2/D$ while gravity will move the sphere the same distance in $T_g = s/v_s$ time units. Clearly, for a statocyst to work, $v_s \gg D/L$.

Individual cells of *L. striatus* invariably contain one to four Müller bodies ($\bar{x} = 2.27$, Finlay *et al.* 1983a). They are spherical with diameters between 2 and $4 \mu\text{m}$ (mean approximately $3 \mu\text{m}$ in the cells used in this study). The vacuole has an average diameter of $7.5 \mu\text{m}$, so L is about $2.3 \mu\text{m}$. The specific weight of BaSO_4 is 4.5 so the settling velocity works out to be $1.71 \times 10^{-3} \text{ cm s}^{-1}$ while D is $1.39 \times 10^{-9} \text{ cm}^2 \text{ s}^{-1}$. Therefore, $D/L = 6.0 \times 10^{-6} \text{ cm s}^{-1} \ll v_s$. Gravity should pull the Müller body to the bottom of the vacuole in about 0.13 s. During this time period the mean displacement of the body due to Brownian movement would only be about $0.05 \mu\text{m}$ and the probability that it would be found in the upper half of the vacuole is extremely small. Therefore, at least theoretically, the Müller body could function as a gravity detector. If the composition of the body was of some lighter material such as CaSO_4 rather than BaSO_4 or SrSO_4 , the organelle would need roughly twice the time to yield unambiguous information about the orientation of the cell.

We are grateful to Professor Ebbe Thue Poulsen for discussions and help concerning the contents of Appendix A. Our thanks are also extended to Dr Niels Peter Revsbech for help with the use of oxygen microelectrodes and to Trevor Furnass and Annie Sølling for assistance with photography.

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