JONES, J. G. 1986 (A318)
Anaerobic aquatic environments. In *Anaerobic bacteria in habitats other than man.* (ed. E. M. Barnes & G. C. Mead), 101–13, Oxford. Blackwell Scientific Publications. *(Society for Applied Bacteriology Symposium Series* No. 13).

KIPLING, C. 1984 (A319)
Charr fisheries in Windermere, England, during the past four hundred years: Organisation and management. In *Biology of the Arctic charr: Proceedings of the International Symposium on Arctic charr.* (ed. L. Johnson & B. Burns), 533–6, Univ. Manitoba Press.

MITCHELL, G. J., JONES, J. G. & COLE, J.A. 1986 (A320)
Distribution and regulation of nitrate and nitrite reduction by *Desulfovibrio* and *Desulfotomaculum* species. *Arch. Microbiol.* 144, 35–40.

PINDER, L. C. V. 1986 (A321)
Biology of freshwater Chironomidae. *A. Rev. Ent.* 31, 1–23.

ROBINSON, N., CRANWELL, P. A., FINLAY, B. J. & EGLINTON, G. 1984 (A322)
Lipids of aquatic organisms as potential contributors to lacustrine sediments. *Org. Geochem.* 6, 143–52.

SZUMIEC, M. A. 1985 (A323)
Formation of thermal stratification in a small temperate lake. *Freshwat. Biol.* 15, 581–6.

WRIGHT, J. F. 1985 (A324)
Predicting biological change. *Wat. Bull.* No. 188, 8–9.

REVIEW ARTICLES

PHYSIOLOGICAL ECOLOGY OF THE CILIATED PROTOZOON *LOXODES*

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Introduction

Protozoa tend to thrive in the same places as other microorganisms. Whether in sewage plants, the stomachs of ruminants, *Sphagnum* bogs or the faecal pellets of zooplankton, large numbers of bacteria and other microorganisms invariably provide favourable conditions for the grazing activities and growth of Protozoa. Microorganisms also flourish in the soft sediments of productive lakes and Protozoa too find a variety of niches there. Some live in and graze on the superficial algal mat, some occupy the interstices and some seek out the region of low oxygen tension and reducing conditions that invariably exists a few millimetres below the sediment surface. Many Protozoa, especially the smallest forms, are quite difficult to identify and this has often been a hindrance in ecological investigations, but the larger Protozoa, in particular those with prominent features like the ciliate *Loxodes* (Fig. 1), are easily identified. *Loxodes* was first recorded in the late 18th century when it was assigned to the genus '*Kolpoda*' by Müller. Its current name was provided by Ehrenberg who erected a special genus for his 'Lippenthierchen' (small-lipped animals). The two common species are the relatively large *L. magnus* (0·3-0·6 mm) and the much smaller *L. striatus* (0·1-0·25 mm) (Figs. 2 and 3) but there are probably several others. They feed principally on algae, but they will also ingest rotifers, testate amoebae, colonial photosynthetic bacteria and most other particles larger than about 5 μm with some organic content (Figs. 4 and 5).

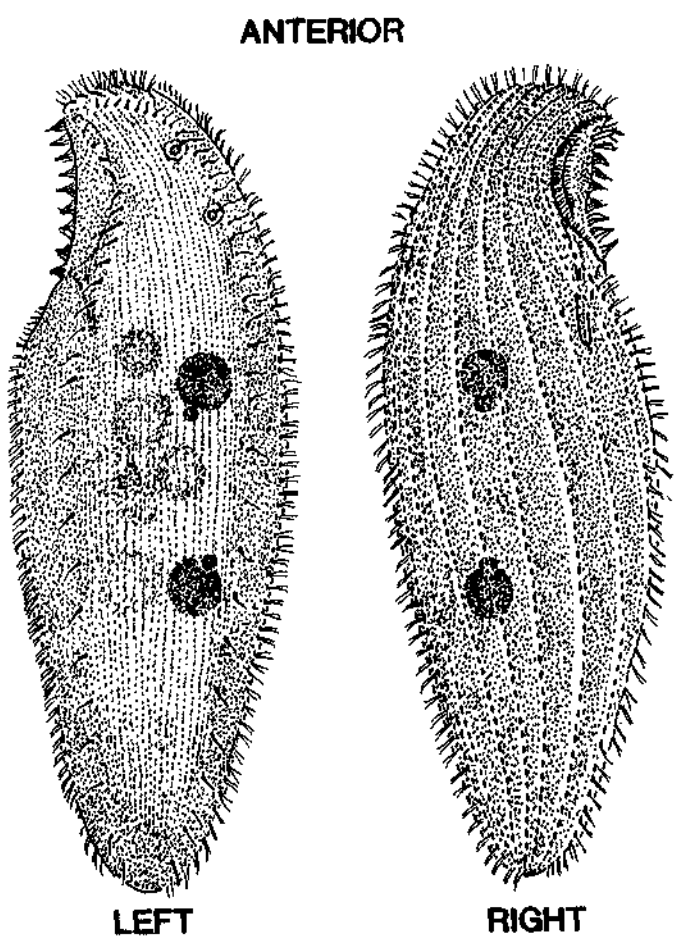

FIG. 1. *Loxodes striatus*. Most cells are about 0·2 mm long. The mouth is the large indentation at the anterior end. Two Müller bodies are shown at the anterior end (left), associated with the dorsal kinety (row of cilia). The large, dark circular structures in the cytoplasm are the macronuclei with associated micronuclei.

There are two principal reasons for our pre-occupation with *Loxodes*: the first is based on its extreme abundance in some lakes. Protozoa are voracious consumers of microorganisms and they probably have an important, even controlling influence on the size and activity of microbial populations. As a consequence they must also play an important

role in such processes as the release and cycling of nutrients and the flow of energy through ecosystems. In some lakes *Loxodes* may dominate not only the protozoan community (Finlay 1982) but the whole community of microfauna (Finlay 1985). It is also a large Protozoon so its population biomass can be considerable, and when we consider that *Loxodes* may double its number every few days (Bark & Watts 1984; Finlay & Berninger 1984), its potential importance in the economy of lakes becomes clearer. This point can be illustrated with a specific example. On 7 July 1982 Priest Pot contained 2×10^{12} *Loxodes*. Each cell has a wet weight of about 0·2 μg so the total weight would be about 400 kg. This is about four times the biomass of fish in the pond.

The other reason is that its biology is particularly interesting and at times unique. It is the only freshwater member of the family Loxodidae but in common with its marine relatives and unlike other freshwater Protozoa it has no contractile vacuole nor any other obvious method of osmoregulation. Its Golgi apparatus is unusually well developed for a ciliate but its nuclear apparatus is primitive and the macronuclei never divide. Its cell surface is covered with membrane-bound granules containing a yellow-brown pigment and much of the cell interior is composed of vacuoles of various types and sizes. Some vacuoles contain small mineral inclusions and are located in specific positions along the dorsal rim of the cell. These were discovered by Johannes Müller in 1865 and they subsequently became known as 'Müller bodies'. They have been claimed to perform various functions but we now believe they enable *Loxodes* to perform the only known taxis in a ciliated Protozoon (Fenchel & Finlay 1984; 1986a).

Our investigation of *Loxodes* began with observations of where it lived and how it was distributed in lakes. We estimated its abundance and we resolved the factors that most probably controlled its spatial distribution. We then extended our investigation into some relevant aspects of its biology, especially its physiology. We have consistently tried to relate our understanding of the physiology of the organism to our observations and understanding of its ecology. The review that follows is an account of our progress so far.

Spatial distribution

Loxodes was discovered to be an important member of the ciliate community living in the sediment of a small lake in Scotland (Airthrey Loch, Stirlingshire; Finlay, Bannister & Stewart 1979). The lake was biologically productive and quite shallow (mean depth ~1·5 m) and frequent wind-induced mixing of the water column ensured a permanent supply of oxygen at the sediment surface. This meant that the

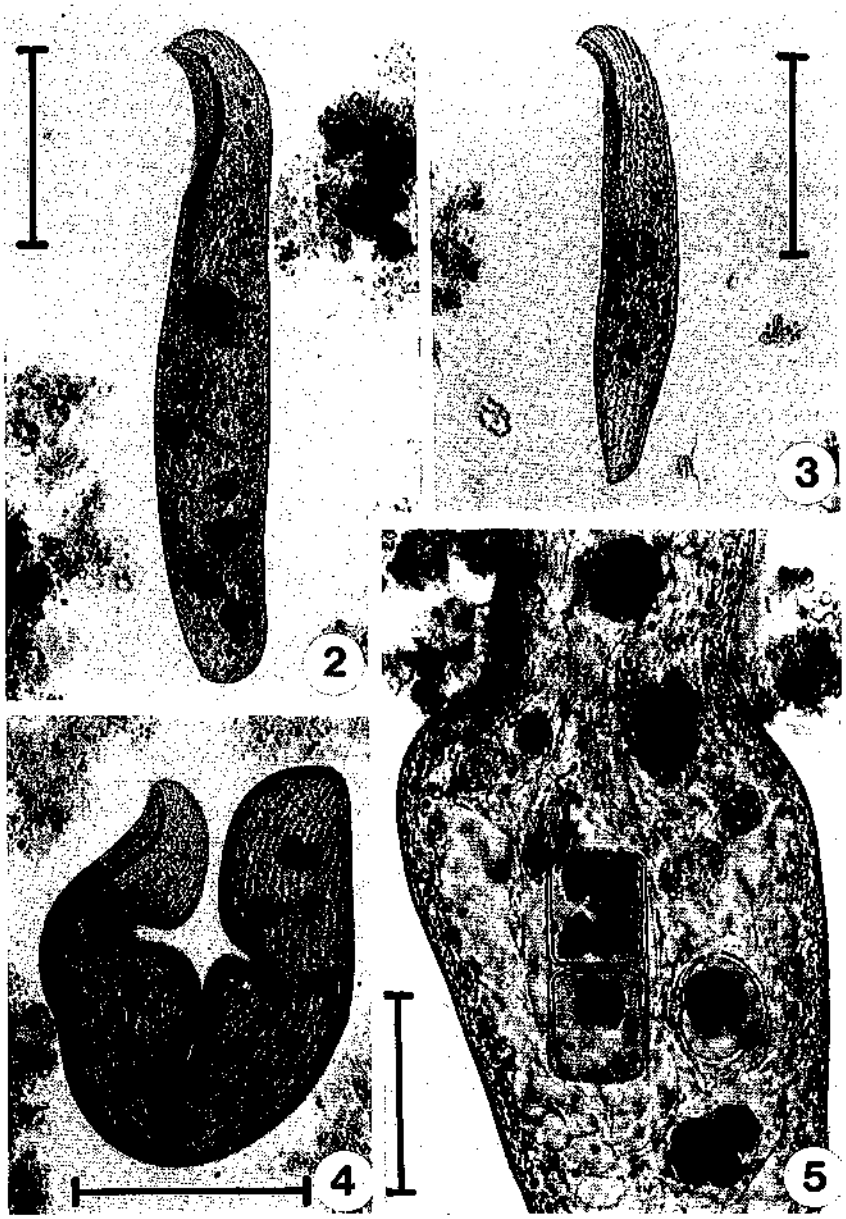

FIG. 2. *Loxodes magnus* Stokes. Scale bar 100 μm.
FIG. 3. *Loxodes striatus* Penard. Scale bar 100 μm.
FIG. 4. Typical contortion of *L. magnus* in sediment. Scale bar 100 μm.
FIG. 5. A section of *L. magnus* showing ingested diatoms and dinoflagellates. Scale bar 100 μm. Figs. 2–5 by H. Canter-Lund.

benthic organisms that might otherwise be forced out of the sediment by anaerobic conditions could remain in the sediment during the summer months. There they could feed on sedimenting algae and, at shallower sites, on the algal mat flora developing at the sediment surface. Benthic oxygen consumption was also stimulated during the summer and this was recorded as a gradual displacement towards the sediment surface of the *oxic-anoxic boundary and the redox potential discontinuity. Many ciliates, including *Loxodes*, followed this upwards migration until they met oxygen close to the sediment surface. Numbers of *Loxodes* were recorded for two years and a quite simple cyclical pattern emerged; their number increased to a maximum (2500 per ml of sediment) during the summer and decreased to a minimum (<100 ml^{-1}) during the winter (Finlay, 1978, 1980).

Airthrey Loch is not typical of productive lakes, especially the deeper ones where summer stratification becomes established for long periods and the hypolimnion becomes progressively deoxygenated. How would *Loxodes* fare if it became isolated from a supply of oxygen? Would the population die, would the cells encyst and await better conditions, or would they migrate?

The answer was found during a study of Esthwaite Water in the English Lake District. Goulder (1974) had shown that numbers of *Loxodes* in the sediments of Esthwaite decreased almost to zero during the summer months. He had also recorded planktonic *Loxodes* in Priest Pot, a small pond lying adjacent to Esthwaite (Goulder 1972) so it seemed likely that the planktonic population might simply be the displaced benthic population, forced into the water column by the arrival of anoxia at the sediment-water interface. This was later confirmed (Finlay 1981, 1982); *Loxodes* escaped into the water column when the water overlying the sediment became anoxic. The profile of population abundance was especially interesting: it showed a peak in the metalimnion which appeared to move in phase with the drift of the oxycline, eventually returning to the sediment in the autumn when the collapse of stratification made oxygen available again at the sediment surface (Fig. 6). *Loxodes* living close to the oxic-anoxic boundary presumably had access to sufficient oxygen so their migrations were considered to be the necessary behaviour of an obligate aerobe that required oxygen for

* The term 'oxic' is used throughout, to mean water in which oxygen can be detected, i.e. ≥ 1 μmol l^{-1}.

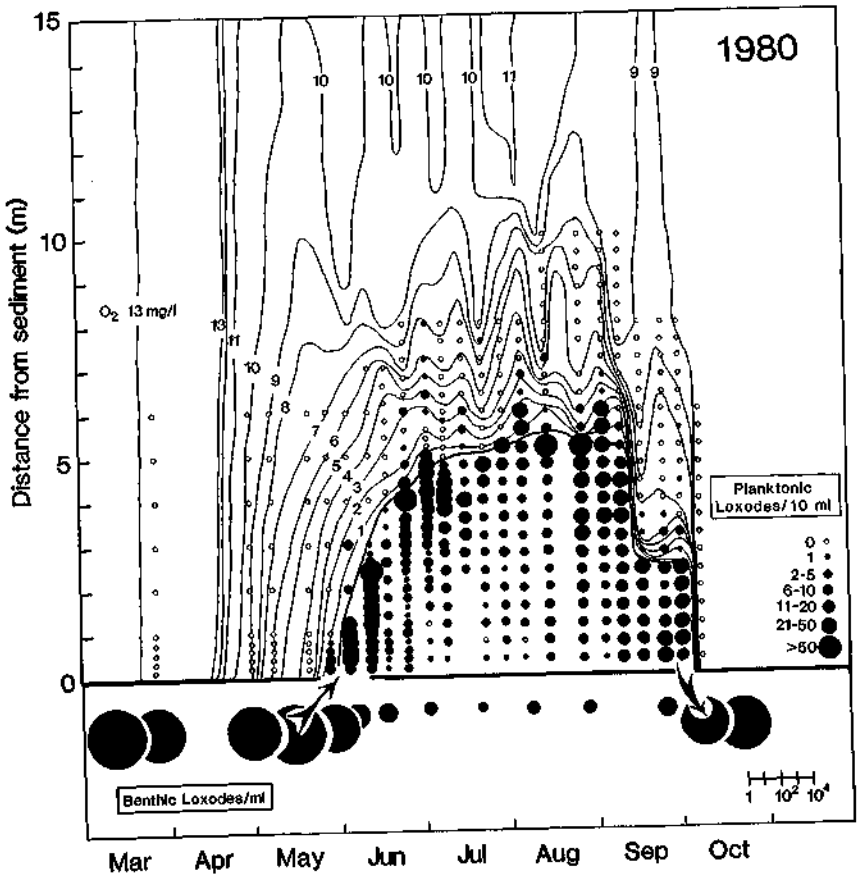

FIG. 6. Abundance and distribution of *Loxodes (L. magnus+L. striatus)* in Esthwaite Water in 1980. (Adapted from Finlay 1981, 1982.)

respiration. It became obvious however that this simple explanation was incomplete. If *Loxodes* migrated into the water column to find oxygen, why did it avoid the epilimnion, where there was plenty of oxygen, and why were these 'aerobic' cells also distributed throughout the anaerobic, sulphide-rich hypolimnion?

Nitrate respiration

We required more precise information about the behaviour of *Loxodes* in the field and we transferred our attention to the neighbouring Priest Pot. This small (1 hectare) pond was once part of Esthwaite but it is now physically separated save for a narrow connecting ditch. It receives substantial levels of plant nutrients from the surrounding agricultural land and it supports dense crops of small algae and photosynthetic bacteria (Finlay & Berninger 1984; Davison & Finlay 1986). The high algal productivity supports a complex microbial community which includes at least 20 ciliate species, some of which are extremely abundant. During the summer, *Loxodes* is present in the water column at numbers up to about $0{\cdot}8\times10^6$ per litre which is 100 times their maximum abundance in Esthwaite.

Working on Priest Pot had several advantages. The pond is sheltered, thermal stratification of the water column is quite stable and sampling at precise depths was relatively easy. More importantly, large numbers of *Loxodes* could be obtained in small samples of water. Water was collected from discrete depths using a pump sampler fitted with a sediment detector. Oxygen was recorded polarographically in the field and immediately after returning water samples to the laboratory (Finlay 1985; Davison & Finlay 1986).

The vertical distribution of *Loxodes* in Priest Pot was quite similar to that in Esthwaite; peak abundance was close to the oxic-anoxic boundary, they avoided the epilimnion and numbers decreased exponentially with depth in the anoxic hypolimnion. Examining the position of the *Loxodes* peak more closely we could see that it was always positioned in water containing little or no detectable (1 μmol l^{-1}) oxygen. More surprising still was the observation that peak abundance also occurred in water with an elevated nitrite (NO_2^-) concentration. Localized accumulations of nitrite are not uncommon in either lakes or the open ocean. In the latter, where they are well-documented, they are invariably associated with mid-water oxygen minima and they are ascribed to the activities of bacteria. In the absence of oxygen, these bacteria use nitrate (NO_3^-) as a substrate, reducing it to NO_2^- by respiration, a respiratory process similar to that by which they would reduce oxygen (O_2) to water (H_2O). The nitrite so produced then accumulates in anoxic water, often as a distinct peak. If *Loxodes* was aerobic, why did the peak abundance occur in anaerobic water which also supported a nitrite peak? If as we suspected, the nitrite was produced by dissimilatory reduction of nitrate, how did *Loxodes* manage to respire O_2 in water where the accompanying bacteria were forced to respire nitrate? These questions seemed intractable until we considered the possibility that *Loxodes* itself might be capable of reducing nitrate.

Loxodes would require a key enzyme, nitrate reductase, so the activity of this enzyme was determined at different depths while simultaneously

recording the abundance of *Loxodes*. Water was passed through a 30 μm sieve which retained almost all *Loxodes* but allowed most unattached bacteria to pass. The sieve retentate was then assayed for nitrate reductase. A clear correlation existed between *Loxodes* abundance and the

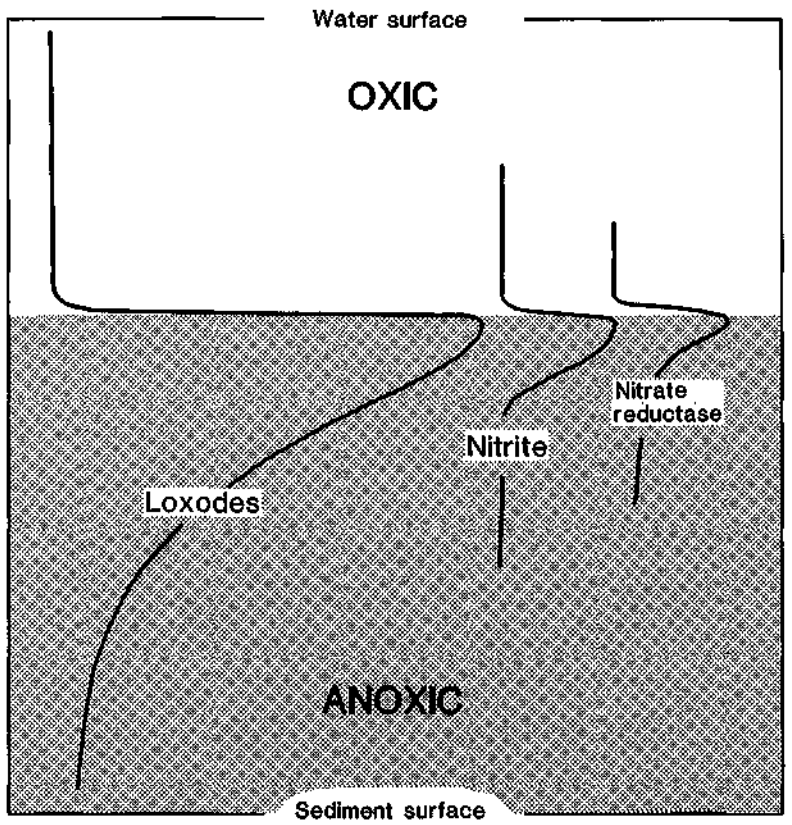

FIG. 7. Vertical profiles of *Loxodes* abundance and nitrite in the water and nitrate reductase activity in 30 μm sieve retentate from Priest Pot. (Adapted from Finlay, Span & Harman 1983.)

enzyme activity (Fig. 7): furthermore they both coincided with the nitrite peak in water containing little or no oxygen (Finlay, Span & Harman 1983).

The most likely explanation for these enzyme activities is that they were located in contaminating bacteria but there is no evidence to support this. The number of nitrate-reducing bacteria counted in the

water would account for less than 1% of the measured activity (Finlay, Span & Harman 1983; Finlay 1985) and microscopic examination of the surface of *Loxodes* revealed that it was largely free of attached bacteria. Transmission electron micrographs were also scanned for signs of prokaryote endosymbionts but none were found. Neither was there any evidence that ingested material was responsible for the activity.

If the enzyme was indeed located in *Loxodes* then we should detect it in isolated cells and the activity should be proportional to the amount of material in the assay. Both species of *Loxodes* were isolated from Priest Pot and cultured in the laboratory: each was then assayed for the enzyme. The results provided strong evidence for the presence of nitrate reductase in *Loxodes*. There was a linear relationship between nitrate reduction and the number of *Loxodes* cells in the assay and the relationship could be extrapolated to the origin (Finlay, Span & Harman 1983). The activity per cell was also roughly proportional to cell size in the two species.

Where might this enzyme activity be located in *Loxodes*? The dissimilatory nitrate reductase in bacteria is membrane-bound so the most probable site in *Loxodes* would be the membrane surfaces responsible for energy production – the inner mitochondrial membrane. We have no conclusive evidence that the enzyme is located there, merely circumstantial evidence based on quantitative analysis of *Loxodes* mitochondria. When *L. magnus* had access to low levels of oxygen, each cell had on average 46,000 mitochondria with typical tubular cristae. However when cells were collected from anaerobic water they were found to contain on average 78,000 mitochondria. The latter were apparently normal, with typical infolding of the inner membrane, so the increase in the number of mitochondria in anaerobic cells was accompanied by an increase in the surface area of the inner, energy-yielding membrane. This makes some sense, for nitrate respiration employs a shorter electron transport chain and it is less efficient than aerobic respiration in the amount of energy it provides. The evidence available for bacteria indicates that a reduction in molar growth yield of about 50% is associated with the switch from oxygen respiration to nitrate respiration so the increased energy-yielding surface area could be viewed as a compensation for the reduced energy yield accompanying the switch.

Supporting evidence for this was obtained by directly measuring the activity of the electron transport system (ETS). This can be done by exposing the ETS to a formazan dye whose subsequent rate of reduction depends on the rate of flow of reducing equivalents down the ETS (Finlay, Span & Ochsenbein-Gattlen 1982). A doubling of the specific-ETS activity accompanied the transition from oxic to anoxic water. This is quite consistent with our contention that the same transition is associ-

ated with a switch from oxygen to nitrate respiration and that the principal energy yielding processes remain within the mitochondria.

Our theory required the demonstration that *Loxodes* had access to nitrate and that the nitrite peak could be ascribed to dissimilatory nitrate reduction. The sources and sinks of metalimnetic nitrate have recently been described and some conclusions have been drawn (Finlay 1985). The nitrite peak coinciding with *Loxodes* was usually confined to anoxic water and as such it probably had its origin in dissimilatory reduction of nitrate. It was derived from nitrate which had two principal sources – runoff from the surrounding agricultural land and diffusion across the oxycline after production by aerobic, nitrifying bacteria. The nitrifiers depended on entrainment and upwards diffusion of nitrite and ammonia from the underlying anoxic hypolimnion.

Geotaxis

The profile of *Loxodes* abundance in the water column was remarkably consistent: cells avoided water containing more than a few per cent dissolved oxygen, a sharp peak in abundance lay close to the oxic-anoxic boundary and numbers decreased exponentially through the anoxic hypolimnion. *Loxodes* was apparently capable of perceiving the O_2 tension and of modifying its swimming behaviour accordingly, but how did it do this and how did it maintain its distinctive vertical profile?

We could verify that the vertical profile was a product of *Loxodes'* own behaviour by reproducing it as a scaled down version in test tube cultures that were free of other Protozoa and other potential competitors or predators. Using the same cultures we also observed patterns of behaviour that began to explain the vertical profile, for cells behaved in specific ways which depended on the level of O_2 in the tubes. In the absence of oxygen they tended to swim vertically upwards and in the presence of high levels of O_2 they swam vertically downwards. When cells entered a zone of low O_2 tension (e.g. less than about 5% of the saturation value, equivalent to about 0·05 mg l^{-1}), vertical swimming virtually disappeared and kinetic responses took over (i.e. swimming velocity decreased and the frequency of random re-orientation increased; Fenchel & Finlay 1984). As a consequence, cells tended to aggregate at a low O_2 tension. Kinetic responses to environmental stimuli are known for many microorganisms including Protozoa but orientation and swimming with respect to the force of gravity is unusual, if not unique in Protozoa. This was obviously a fundamental characteristic of the behaviour of *Loxodes* so we decided to investigate it further. Unequivocal evidence for gravity perception could be obtained quite easily: when test tube cultures like those in Fig. 8 were completely filled, then sealed with a gas-permeable membrane and turned upside

down, *Loxodes* was apparently 'fooled' into swimming in the wrong direction. Cells swimming 'up' before inversion of the tube maintained the same orientation with respect to gravity after inversion with the result that they swam towards the closed end of the test tube which now lay above them, and so into water that was devoid of oxygen. Conversely, cells that were located in a high O_2 tension before inversion and which were poised for 'downwards' swimming, did indeed swim downwards after inversion, to collect on the dialysis membrane and end up in water with an even higher O_2 tension. However in its natural habitat, movement by *Loxodes* against the pull of gravity will always be associated with a gradual or eventual increase in the ambient O_2 tension. Since it is apparently of some value to *Loxodes* to seek out a specific O_2 tension, it uses its ability to perceive the permanent and unambiguous cue of gravity to swim vertically and so quickly reach its preferred environment.

A prime requirement for gravity perception is the possession of a mechanoreceptor. In many other animals the various structures which fulfil this purpose are known as statocysts and they tend to share certain similarities. Each one consists of a mineral concretion with a relatively high specific gravity which is capable of limited movement in a vacuole or chamber containing sensory cells: these cells are stimulated by gravity-induced displacement of the mineral body. Any such structure in a Protozoon must of course be quite simple and enclosed within the single cell of the organism. Penard (1917) was the first to suggest that the so-called Müller vesicles, a series of mineral bodies enclosed in vacuoles and located along the dorsal rim of *Loxodes*, might function as statocysts. Since we now knew that *Loxodes* could perceive gravity and that it must therefore have a mechanoreceptor, we pursued Penard's suggestion and examined the structure of the Müller vesicles.

By simply considering the physical properties of these organelles it was clear that they could function as statocysts. They were large enough and, with barium sulphate as the principal component of the mineral body, they were sufficiently dense to quickly respond to the influence of gravity. Furthermore, gravity alone would pull the mineral body to one side of the vacuole in about one fifth of a second, thus nullifying the background 'noise' produced by Brownian motion.

The mineral body is roughly spherical and about 3 μm in diameter. It is located in a larger (7 μm) vacuole and apparently connected to the wall of the latter by a stalk. This much can easily be seen with the light microscope (Figs. 9, 10) but it was clear that a fuller understanding of the functional morphology of the organelle would depend on an analysis using the electron microscope.

Serial sections were obtained of both fully-developed and immature Müller vesicles. The images on individual micrographs were then

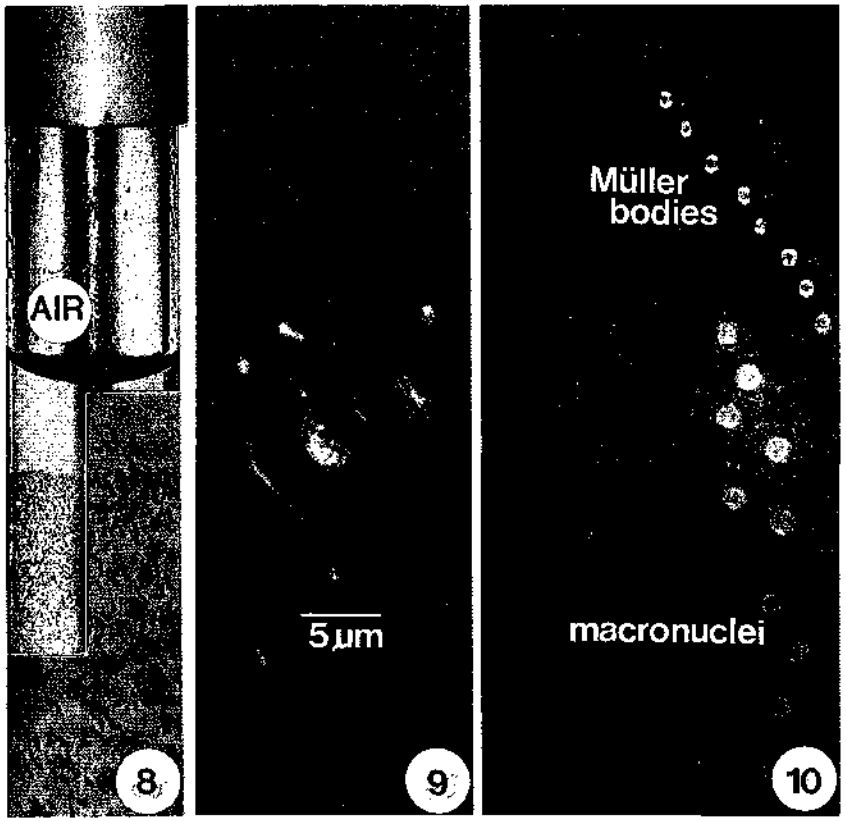

FIG. 8. Vertical distribution of *Loxodes striatus* in a test tube culture (two magnifications).
FIG. 9. A Müller body in *L. striatus*, attached by a stalk to the wall of its fluid-filled vesicle.
FIG. 10. Numerous Müller bodies along the dorsal rim of *L. magnus*.

transferred to perspex plates piled on top of each other to produce a 3-dimensional scale model of the vesicle and its associated structures. This model revealed an intimate association between the Müller vesicle and the cilia of the kinety (row of cilia) on the left side of the cell (Fig. 11). The 'stalk' supporting the mineral body consisted of a fairly rigid sheet of cytoplasm containing nine microtubules. These microtubules were anchored in a non-ciliated kinetosome (ciliary basal body) lying adjacent to another, ciliated kinetosome. How might this structure provide *Loxodes* with information about the force of gravity?

An efficient gravity receptor will provide information about what is

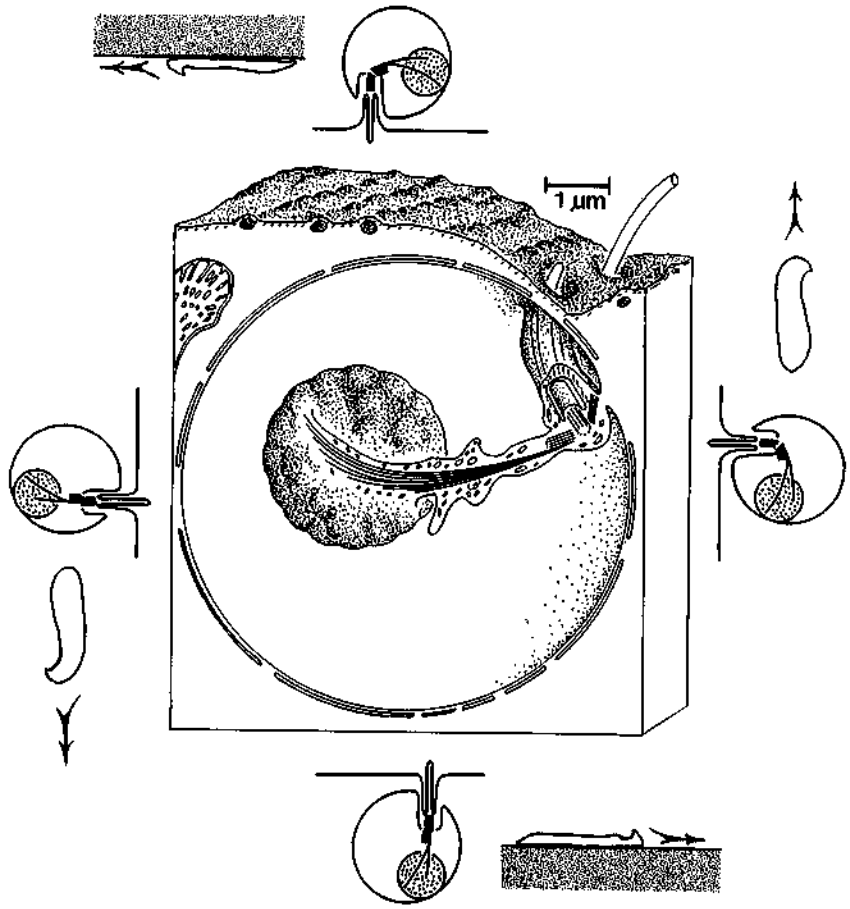

FIG. 11. A Müller vesicle and the two possible orientations of the Müller body for the four principal ways in which *Loxodes* orients itself. Drawn from a 3-dimensional reconstruction based on serial sections. (Adapted from Fenchel & Finlay 1986a.)

'up' and what is 'down' and it is most likely to do this if the receptor has only two possible orientations. But the receptor must continue to function when *Loxodes* glides horizontally across the sediment surface or on the underside of surfaces suspended in the water so there are four principal ways in which *Loxodes* can orient itself with respect to gravity. The relationship between the orientations of both *Loxodes* and

its Müller bodies was resolved after some patient observation of living cells (Fig. 11).

Only one side of the cell is fully ciliated (Fig. 1) and if *Loxodes* maintains contact with a solid surface it glides over the surface on its ciliated side. Thus it adopts a specific orientation even when moving horizontally. Furthermore, we could see that although the cells had four principal orientations, the Müller body took only one of two positions. When cells swam vertically downwards or if they glided with the ciliated side downwards (e.g. over the sediment surface), the Müller body fell to the anterior right side of the vesicle. Conversely, when the ciliate swam vertically upwards or if it glided across the underside of a horizontal surface the body fell to the posterior left side of the vesicle. The total movement of the Müller body between these two positions was 3-4 μm and, because of the rigidity of the supporting stalk the latter also moved, changing the angle between the stalk and the ciliary tube by about 90°.

Most if not all gravity receptors in other animals also involve some attachment or association with cilia and ciliary basal bodies and there is now general acceptance that the transduction of the mechanical sensory input occurs at or close to the basal bodies. In *Loxodes* too we can see how this might come about. Movement of the Müller body from one side of the vesicle to the other will cause some stretching of the membranes between the two basal bodies. This mechanical stress will change the ion permeability and hence change the membrane potential – change that will be propagated across the cell surface to modify the behaviour and beating frequency of the cilia. Any electrical signal travelling over the cell membrane will eventually be dampened so large cells will require more Müller vesicles. Our subsequent analysis of the occurrence of Müller vesicles in cells of different sizes did indeed show that the number was proportional to cell surface area (Fenchel & Finlay 1986a).

A remarkable consequence of *Loxodes'* accumulation of barium is that it accounts for most of the particulate fraction of this element in the lake. The first indication of this was the close coincidence in the vertical profiles of *Loxodes* abundance and particulate barium in Priest Pot (Fig. 12) and it was substantiated by our calculation that the contribution from *Loxodes* would be at least 60%. Furthermore, measurements of particulate barium in Esthwaite and Priest Pot could be shown to be proportional to *Loxodes* abundance over three orders of magnitude (Finlay, Hetherington & Davison, 1983). *Loxodes* presumably plays a significant but as yet poorly understood role in the biogeochemical cycle of barium in productive lakes.

Kinetic responses

Geotaxis allows *Loxodes* to reach its optimum environment at a rate

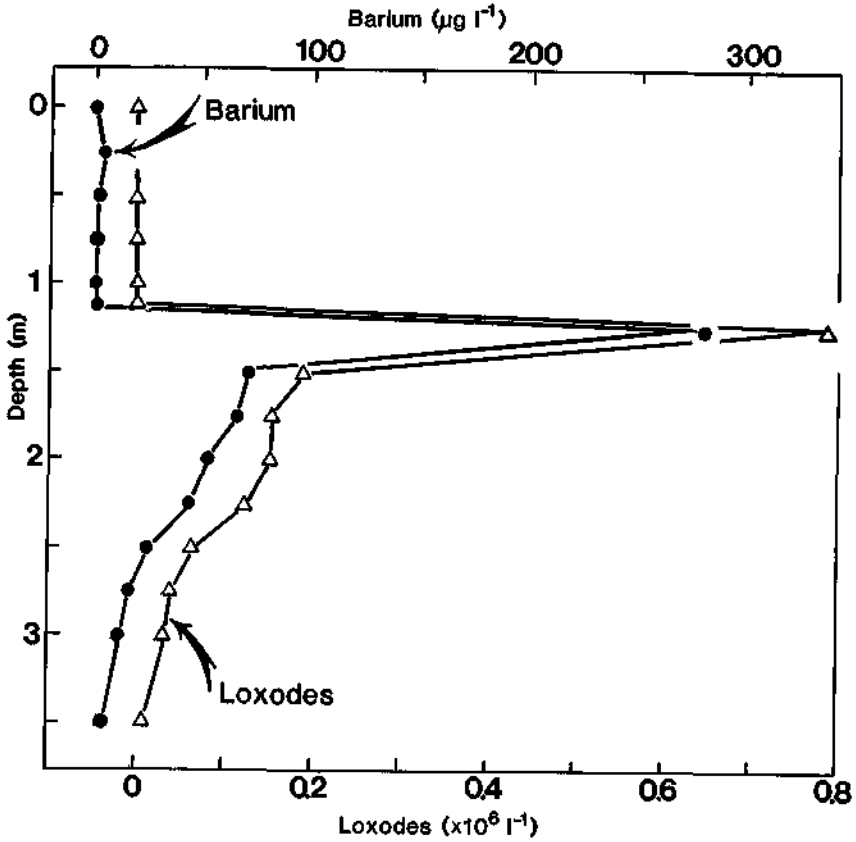

FIG. 12. Profiles of particulate barium and *Loxodes* abundance in Priest Pot, 26 July 1982. (Adapted from Finlay, Hetherington & Davison 1983.)

equivalent to about 70% of its maximum swimming velocity but it can also orientate itself without exploiting this ability. This is best demonstrated by manipulating cultures in such a way that gravity ceases to have any value for orientation, by establishing O_2 gradients in a horizontal plane. Figure 13 shows such a system, created in a Sedgewick-Rafter cell. Oxygen diffuses from the atmosphere and is consumed by bacteria and protozoa in the medium enclosed by the chamber. By taking long exposure photographs of the behaviour of a population of *Loxodes* in such a gradient it is possible to record their kinetic responses to oxygen tension (Fig. 14). Cells close to the oxic-anoxic boundary live

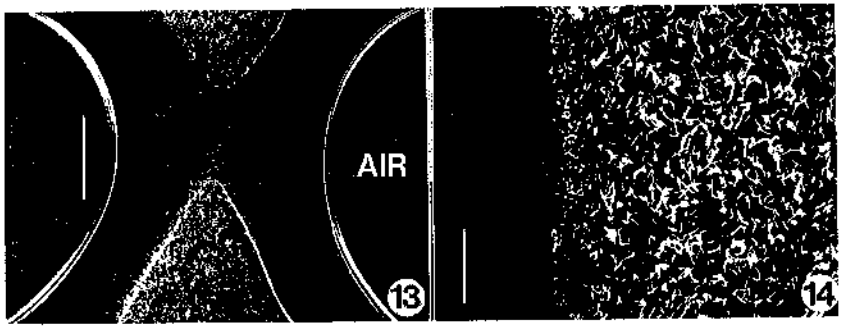

FIG. 13. A population of *L. striatus*, divided into two parts in a horizontal double oxygen gradient (O_2 diffusing from left and right) created in a Sedgewick-Rafter cell. Bands of bacteria can be seen close to the oxic-anoxic boundaries. Two second exposure. Scale bar 5 mm.

FIG. 14. A five second exposure of a population of *L. striatus* showing mainly stationary cells close to the oxic-anoxic boundary and swimming cells in the anoxic zone to the right. Scale bar 2 mm. (Figs. 13 and 14 adapted from Finlay, Fenchel & Gardener 1986.)

in their optimum environment and they respond with a significant reduction of their random motility; many cells appear almost motionless in a photograph with an exposure of several seconds (Fig. 14). But the other cells in the population live in a suboptimal environment and their random motility increases: swimming velocity and pathlength are both much higher. The resulting population profile is quite similar to that in the lake and in test tube cultures although it is produced without the benefit of gravity: it therefore takes much longer to reach equilibrium. It is very likely that the kinetic responses observed in Fig. 14 are the same behavioural responses which trap *Loxodes* in their optimum environment in the vertical O_2 gradient of a lake.

Oxygen toxicity and photosensitivity

Why should the optimum environment for *Loxodes* be depleted in oxygen and why do cells avoid water containing O_2 levels higher than about 5% of saturation? Our first attempt to answer this question was based on anticipated similarities between *Loxodes* and other microaerophilic and anaerobic organisms, especially bacteria. Oxygen is toxic to many anaerobic bacteria, probably because they lack one or more of the enzymes that confer some protection against oxygen radicals, especially the superoxide radical anion (O_2^-). The enzyme superoxide dismutase (SOD) catalyses the removal of superoxide so if *Loxodes* should fail to synthesise this or other protective enzymes we would, we

suspected, have the basis of a biochemical explanation underlying the behavioural avoidance of oxygen. The intracellular production of oxygen radicals would presumably be much greater at high partial pressures of oxygen.

We assayed *Loxodes striatus* for two enzymes, superoxide dismutase and catalase; the latter catalyses the removal of hydrogen peroxide (H_2O_2). For comparison and as a control of the methods, we used the aerobic ciliate *Tetrahymena* which was known to contain both enzymes. The results obtained were of little help in explaining the absence of *Loxodes* from oxygenated waters for both enzymes were found to be present, albeit at much lower levels (25% and 5% for SOD and catalase respectively) than in *Tetrahymena*. These activities are low but they are probably consistent with the microaerophilic habit of *Loxodes*. Unfortunately, little more can be inferred. If *Loxodes* is capable of synthesising the enzymes it might simply manufacture greater quantities to deal with the higher substrate levels as the need arose. We apparently cannot claim the absence of protective enzymes as a reason for *Loxodes'* avoidance of oxygenated waters. We also considered the possibility that oxygen avoidance might not result from a direct interaction with the dissolved oxygen tension but that some oxygen-related factor (e.g. sulphide, Fe^{2+}/Fe^{3+}, CO_2 and pH) had a controlling influence. Two types of evidence indicated that these other factors were of little importance. Firstly, the vertical profiles of CO_2, pH, sulphide and ferrous (Fe^{2+}) iron in Priest Pot were not closely related to *Loxodes* abundance. Secondly, by manipulating the gas phase surrounding cultures in Sedgewick-Rafter cells we could show that *Loxodes* followed a retreating source of O_2 and was repelled by a high oxygen tension. This behaviour was sustained when cells simultaneously swam up or down gradients of both CO_2 and pH.

There was also no evidence that *Loxodes* was repelled by high levels of potentially toxic radicals produced in the surrounding water. It is known that superoxide and hydrogen peroxide (H_2O_2) for example can be produced in some natural waters, especially if they are illuminated and they contain elevated levels of humic substances. We could detect both superoxide and hydrogen peroxide in the surface water of Priest Pot but there was virtually no correlation between concentration and *Loxodes* abundance (Finlay, Fenchel & Gardener 1986). Neither was there any obvious way by which these or any other potentially toxic radicals (e.g. OH, $^1O_2$) could be generated in the dark, even allowing for probable interactions with transition metal ions.

Thus we returned to our original assumption that the dissolved oxygen tension and probably that alone repelled *Loxodes* from well-oxygenated waters. But how did oxygen repel *Loxodes*? Two quite separate observations helped to provide the answer. We had noticed that under certain

conditions *Loxodes* appeared to respond to light: cells surrounded by anoxic water remained insensitive but cells in contact with oxygen were sensitive to light. By shining a beam of light from a fibre-optic probe onto cells gathered close to the oxic-anoxic boundary in a spectrophotometer cell we could see that they responded in three specific ways. The initial response was transient and involved an increase in tumbling frequency. Thereafter, tumbling subsided but swimming velocity and pathlength increased (kinetic response) and thirdly, positive geotaxis was initiated (Figs. 16, 17). Thus, illumination stimulated cells to quickly move away from any contact they had with oxygen. They remained in anoxic water until the light was switched off, whereupon they returned to their optimum low O_2 tension. If cells were exposed to light and oxygen but prevented from swimming into anoxic water they soon began to look abnormal: numerous vacuoles developed beneath the cell membrane and the cells exploded. Similar effects eventually developed in cells kept in the dark if they were continuously exposed to a high O_2 tension.

Insofar as light apparently exacerbated the deleterious effects of high oxygen levels the phenomenon was similar to the initiation of positive geotaxis in cells exposed to trace levels of O_2, for in the latter case also, cells reacted to light as if the oxygen tension had been further increased. This indicated that light and oxygen interacted in the physiological effects they produced. The interaction could be important, for *Loxodes* living in the wild will occasionally be exposed to various combinations of light and oxygen. It became apparent that a complete understanding of the relevance of O_2 responses would have to take account of probable interactions with the light level.

We have previously shown (Fenchel & Finlay 1986b) that the *motility of a population of *Loxodes* is proportional to swimming velocity so we can use the easily measured swimming speed as an index of motility. Motility was minimal when cells were in the dark at an O_2 tension of about 5%, it increased in anoxic water and it was highest when the O_2 tension was greater than 50% (about 5 mg l^{-1}). When cells were also exposed to a sufficiently high light level, swimming speed increased at all oxygen tensions except for anoxia, where swimming remained unaffected. These results, obtained using populations of *Loxodes* oriented horizontally in flattened capillary slides, are summarised in Fig. 18. We can see that the minimum swimming velocity (proportional to motility) occurs at a progressively lower O_2 tension as the light level is increased, and at all irradiances above about 5 Wm^{-2} it takes a

* The motility of *Loxodes* and other protozoa can be described as a random walk consisting of more-or-less straight runs and random changes in direction (tumbling). The motility of a population can therefore be quantified by a diffusion coefficient; increasing the swimming speed and decreasing the frequency of tumbling, increases the motility (see Fenchel & Finlay 1986b).

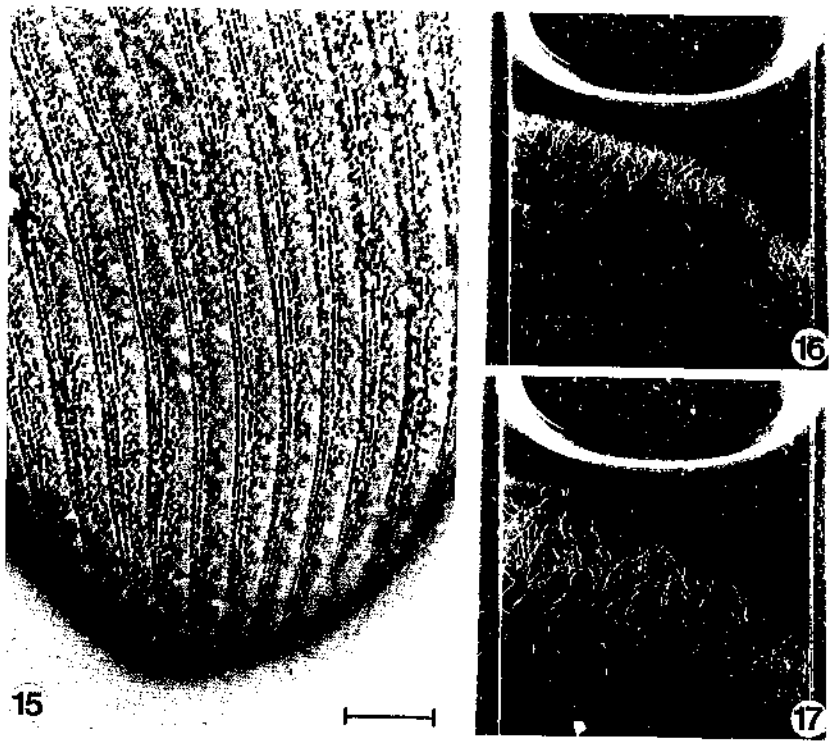

FIG. 15. Rows of pigment granules in *L. magnus*. Scale bar 10 μm. (Photo H. Canter-Lund.)

FIG. 16. A population of *L. striatus* stratified close to the oxic-anoxic boundary in a spectrophotometer cell (2 mm deep, 10 mm wide) immediately after illuminating with white light (128 Wm^{-2}). 20 s exposure.

FIG. 17. As in Fig. 16 but 30–50 s after switching on light. Pathlength has increased markedly and most cells have swum into the anoxic zone. Note the band of bacteria left at the oxic-anoxic boundary.

minimum in anoxic water. Since cells will tend to aggregate wherever their motility is lowest, cells exposed to more than 5 Wm^{-2} will aggregate in anoxic water.

A similar 'critical' light level was determined using different methods and populations of cells stratified vertically in spectrophotometer cells, a system that mimics the distribution of *Loxodes* in an O_2 gradient in the wild. *Loxodes* accumulated at a low O_2 tension in the dark. When the light was switched on, the cells swam down and so out of contact with oxygen. The rate of escape was dependent on the intensity of the

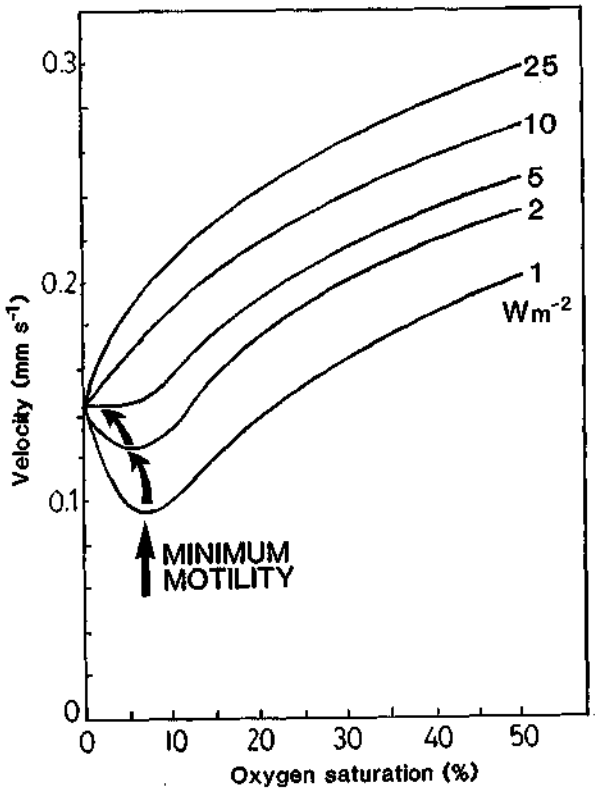

FIG. 18. Swimming velocity of *L. striatus* at different combinations of light and oxygen. Motility is a function of swimming velocity, cells aggregate where motility is lowest so *Loxodes* seeks out a progressively lower oxygen tension as the light is increased. 50% saturation is equivalent to about 5 mg l^{-1}. (Adapted from Fenchel & Finlay 1986b.)

step-up in light level and results obtained from a large number of cultures showed that escape could be detected at irradiances slightly less than 10 Wm^{-2}, broadly in agreement with the response of cells in horizontal capillary tubes.

In an effort to relate these findings to the ecology of *Loxodes* in the field, we have examined the vertical profiles of *Loxodes* abundance, O_2 and the light regime in Priest Pot. During the summer, light intensities in excess of the critical irradiance of 5-10 Wm^{-2} often penetrate to depths where the O_2 tension is about 5 per cent. These depths are

usually within a few centimetres of the upper limit to the *Loxodes* distribution so it is probable that on some occasions light and oxygen interact to exclude *Loxodes* from oxygenated water.

Pigments

The pigment mediating the photosensitivity of *Loxodes* is probably located in the vast number of granules embedded in the cell membrane (Fig. 15). These granules are usually referred to as pigment granules and they are morphologically similar to the pigment-bearing granules in other, brightly coloured ciliates such as *Blepharisma*. The principal pigment in *Blepharisma* is known to be the potent photodynamic agent hypericin but we know that the pigment in *Loxodes* is something quite different. Absorption spectra of solvent extracts indicate that it absorbs principally at the blue end of the visible spectrum (Finlay & Fenchel 1986). The action spectrum for escape from oxygenated water also indicates a blue light receptor. Interestingly the action spectrum shows at least two peaks, at about 360 and 435 nm; this is consistent with the pigment being a flavin. Many of the blue light responses in a wide variety of organisms involve photoreception by flavins but it is usually unclear how they work. We cannot claim to have a clear impression of the function of the pigment in *Loxodes* but we have made some observations which we think point us in the right direction.

The most significant clue was obtained when Steve Gardener noticed that the yellow-brown pigment remaining on top of polyacrylamide gels after electrophoresis of *Loxodes* homogenates would, in the presence of light and a tetrazolium dye, turn dark blue. This indicated that the pigment had produced something which had reduced the dye. We suspected that the active agent was superoxide and this was confirmed in a subsequent experiment. The capacity to generate superoxide in the presence of light is also a characteristic of flavins so the experiment provided further evidence for the existence of such a compound in the pigment granules of *Loxodes*.

The other relevant observations were made while searching for inhibitors of the oxygen response. The most obvious and consistent effect was obtained with micromolar concentrations of cyanide which apparently blocked *Loxodes'* ability to perceive the oxygen tension. Random motility also increased and cells generally behaved as they would do in anoxic water (Fenchel & Finlay 1986b). Of particular interest was the observation that the response was independent of the light level: in the presence of cyanide, swimming behaviour remained the same in the dark, in bright light and at all O_2 tensions. The demonstration that *Loxodes* becomes insensitive to light when it also becomes insensitive to O_2 is important, for it provides strong evidence that the 'receptors'

for light and for oxygen are probably identical. This would also be consistent with our earlier observation that behavioural responses to an increase in light level are identical to responses to increased O_2 tension in the dark – in other words the 'active agent' produced in the presence of light may be identical to that produced in the dark at high oxygen tensions.

When we come to consider the nature of this 'active agent' and its probable receptor we must admit we have little firm evidence. Our experiments with the *Loxodes* pigment would indicate that superoxide may well be the agent and the observation that cyanide is effective at micromolar concentrations indicates that a cytochrome oxidase is the receptor but how might these function in the perception of light and oxygen by *Loxodes* and how might they interact with gravity perception?

Let us assume that the common currency for the interaction of light and oxygen is a source of reducing power, for example superoxide which is produced in excess in the dark at a high O_2 tension and in similar quantities in the light at a low O_2 tension. It is known that superoxide will reduce some components of the electron transport system, especially cytochrome *c* and this reducing power will presumably be transferred to the cytochrome oxidase. Work on various bacteria indicates that the activity of the ETS and the redox state of the cytochrome oxidase can influence the cell membrane potential on which we know the frequency and rhythm of ciliary activity in protozoa is based. Thus the intracellular production of oxygen radicals like superoxide could, we suspect, modulate swimming behaviour.

We must of course also incorporate the role of gravity perception in this scheme. It is likely that the movement of the Müller body creates an electrical stimulus close to the basal bodies in the wall of the vesicle. This stimulus will be propagated over the cell membrane and it will doubtless influence the ciliary beat and hence the swimming behaviour. We can imagine then, two quite separate but interacting processes which modify the membrane potential – an input dependent on the redox state of the cytochrome oxidase and another from the orientation of the Müller body.

When *Loxodes* enters a sub-optimal O_2 tension it displays such transient responses as repeated tumbling and changes in swimming direction. This behaviour will be accompanied by displacements of the Müller body. When entering a high O_2 tension the oxygen 'receptor' will undergo an excessive reduction and the cell will tumble until the Müller body is oriented anterior right within the vesicle. This orientation will create an electrical stimulus which interacts with the membrane potential: the most important result will be that the cell continues to swim vertically downwards (positive geotaxis). The cell may successfully deploy kinetic responses to trap itself in the zone of optimum low O_2 tension or it may continue swimming down into the anoxic zone. In the latter case the flow of reducing equivalents down the ETS will cease, the redox state of the cytochrome oxidase will change and the cell will tumble until it is heading vertically up. The Müller body will then be oriented posterior left creating a new electrical stimulus which interacts with the cell membrane potential and keeps the cell swimming vertically. When the cell reaches its optimum environment the redox state of the cytochrome oxidase is presumably 'ideal' and a new membrane potential is established, the oxygen tension is sufficient for respiration, the flux of superoxide can be disposed of by SOD, the Müller body fails to interact significantly with the new membrane potential, and the probability of geotaxis becomes minimal.

Many organisms aggregate at some O_2 tension less than atmospheric saturation but the function of this behaviour is not usually understood. In *Loxodes* it probably represents a compromise between avoidance of high O_2 tensions and a periodic requirement for aerobic metabolism but the behaviour has another obvious benefit, for it will bring cells into a zone where competition and predation by other animals are probably unimportant. Most of the zooplankton for example avoid the anoxic zone and the zone of low oxygen tension inhabited by *Loxodes*.

Conclusions

Loxodes faces special problems in living close to the oxic-anoxic boundary. In tightly-stratified ponds like Priest Pot its optimum environment may be quite narrow and it can be displaced by the slightest turbulence. *Loxodes* cannot sense an O_2 gradient directly but its ability to perceive gravity allows it to make relatively long vertical migrations. It is also sensitive to light and oxygen and it uses these environmental cues to modulate the parameters of its random motility: in the dark, it aggregates at a low O_2 tension and in bright light it aggregates in anoxic water. The oxic-anoxic boundary is also a zone where O_2 may be a scarce and transient resource, but *Loxodes* can switch to nitrate respiration and exploit the pool of nitrate that often exists close to the base of the oxycline.

Acknowledgement

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References

Bark, A. W. & Watts, J. M. (1984). A comparison of the growth characteristics and spatial distribution of hypolimnetic ciliates in a small lake and an artificial lake ecosystem. *J. gen. Microbiol.* 130, 3113–22.

Davison, W. & Finlay, B. J. (1986). Ferrous iron and phototrophy as alternative sinks for sulphide in the anoxic hypolimnia of two adjacent lakes. *J. Ecol.* (in press).
Fenchel, T. & Finlay, B. J. (1984). Geotaxis in the ciliated Protozoon *Loxodes*. *J. exp. Biol.* 110, 17–33.
Fenchel, T. & Finlay, B. J. (1986a). The structure and function of Müller vesicles in loxodid ciliates. *J. Protozool.*, 33, 69–76.
Fenchel, T. & Finlay, B. J. (1986b). Photobehaviour of the ciliated Protozoon *Loxodes*: taxic, transient and kinetic responses in the presence and absence of oxygen. *J. Protozool.* (in press).
Finlay, B. J. (1978). Community production and respiration by ciliated Protozoa in the benthos of a small eutrophic loch. *Freshwat. Biol.* 8, 327–41.
Finlay, B. J. (1980). Temporal and vertical distribution of ciliophoran communities in the benthos of a small eutrophic loch with particular reference to the redox profile. *Freshwat. Biol.* 10, 15–34.
Finlay, B. J. (1981). Oxygen availability and seasonal migrations of ciliated Protozoa in a freshwater lake. *J. gen. Microbiol.* 123, 173–8.
Finlay, B. J. (1982). Effects of seasonal anoxia on the community of benthic ciliated Protozoa in a productive lake. *Arch. Protistenk.* 125, 215–22.
Finlay, B. J. (1985). Nitrate respiration by Protozoa (*Loxodes* spp.) in the hypolimnetic nitrite maximum of a productive freshwater pond. *Freshwat. Biol.* 15, 333–46.
Finlay, B., Bannister, P. & Stewart, J. (1979). Temporal variation in benthic ciliates and the application of association analysis. *Freshwat. Biol.* 9, 45–53.
Finlay, B. J. & Berninger, U.-G. (1984). Coexistence of congeneric ciliates (Karyorelictida: *Loxodes*) in relation to food resources in two freshwater lakes. *J. Anim. Ecol.* 53, 929–43.
Finlay, B. J. & Fenchel, T. (1986). Photosensitivity in the ciliated Protozoon *Loxodes*: pigment granules, absorbance and action spectra, evidence for a flavin photoreceptor and ecological significance. *J. Protozool.* (in press).
Finlay, B. J., Fenchel, T. & Gardener, S. (1986). Oxygen perception and oxygen toxicity in the freshwater ciliated Protozoon *Loxodes*. *J. Protozool.* (in press).
Finlay, B. J., Hetherington, N. B. & Davison, W. (1983). Active biological participation in lacustrine barium chemistry. *Geochim. Cosmochim. Acta.* 47, 1325–9.
Finlay, B. J., Span, A. S. W. & Harman, J. M. P. (1983). Nitrate respiration in primitive eukaryotes. *Nature*, 303, 333–6.
Finlay, B. J., Span, A. & Ochsenbein-Gattlen, C. (1983). Influence of physiological state on indices of respiration rate in Protozoa. *Comp. Biochem. Physiol.* 74A, 211–19.
Goulder, R. (1972). The vertical distribution of some ciliated Protozoa in the plankton of a eutrophic pond during summer stratification. *Freshwat. Biol.* 2, 162–76.
Goulder, R. (1974). The seasonal and spatial distribution of some benthic ciliated Protozoa in Esthwaite Water. *Freshwat. Biol.* 4, 127–47.
Penard, E. (1917). Le genre *Loxodes*. *Revue Suisse Zool.* 25, 453–89.

AFRICA: THE FBA CONNECTION

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1. Introduction

Since its inception the F.B.A. has had an involvement with African lakes and rivers, which has been maintained throughout its history. Members of its staff have at various times worked on these waters and some of them – including the present Director, R. T. Clarke – spent several of their formative years in Africa. This pattern was set by the first officer-in-charge, R. S. A. Beauchamp, who was later to spend many years on African lakes, and by the first director, E. B. Worthington, who, when appointed, already had considerable experience of these lakes and was familiar with many of their fishes and fisheries. This connection was subsequently maintained by traffic in both directions, some members of the FBA staff later taking appointments in, or making visits to, Africa, others joining the association after service in that continent.

The attractions of African lakes and rivers (Fig. 1) are easy to appreciate. The rivers include some of the world's greatest, including the 6670 km (4145 miles) long Nile, that flows through landscapes as different as swamps and deserts, before discharging into the Mediterranean. Including some of the oldest, largest and deepest lakes of the world, African lakes display an enormous diversity in mode of origin and physiography, present distinctive physical and chemical environments, and often have extremely rich faunas and floras. Lake Victoria, with an area of c. 69 000 km^2, is smaller only than Lake Superior, while Tanganyika and Malawi are the world's 7th and 9th largest lakes. Lake Victoria is relatively shallow (maximum depth c. 93 m) but Tanganyika and Malawi, that lie in arms of the Great Rift, are among the deepest of lakes. Tanganyika, with a depth of 1470 m, is second only to Baikal; Malawi, at 704 m, ranks fourth in order of depth. These facts alone, taken in conjunction with their tropical settings, explain some of the peculiarities and diversity of these lakes, such as the presence of enormous oxygenless lower zones