Foraging behaviour and dispersal capacity of a marine, benthic ciliate in flume flow

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ABSTRACT

The ability of a marine benthic ciliate *Euplotes* sp. (Hypotrichida), to find patches of food, exploit them and leave for new patches, were studied in both still water and flume flow. The ciliates efficiently accumulated in patches of food independent of flow. *Euplotes* sp. walked on surfaces with bundles of cilia, cirri, and when swimming they moved in a helix. These movements were observed in both still water and in the lower region of the boundary layer in laboratory flume flow. To explore the behaviour used by *Euplotes* sp. to find patches of food, the sinuosity, walking speed and response to food gradients, were compared between food and control patches in still water. The speed and sinuosity did not differ between food patches and the control, but the ciliates changed direction when approaching a patch boundary, which prevented the ciliates to leave the food patch. This indicates that a transient response was induced by the strong food gradient. When *Euplotes* sp. was subjected to food depletion the proportion of swimmers increased. In flume flow, the ciliates seemed to actively settle on and leave the sediment. *Euplotes* sp. was further observed to slowly drift immediately above the sediment surface and occasionally make contact with the sediment to resume walking. The analysis of the swimming activity and the observations of *Euplotes* sp. in flowing water, indicate that this ciliate can use the flow to find patchily distributed resources. This together with the ability to stay in patches of food can in part explain how microfauna can find and exploit patchy environments.

INTRODUCTION

Protozoa are mostly single-celled eukaryotes with heterotrophic nutrition. It is a large and polyphyletic group of organisms showing a fascinating diversity of sizes, shapes and life cycles. Protozoa are found in a wide range of habitats including soil, limnic and marine environments, and many are found as commensals and parasites (Fenchel 1987). Ciliates are a dominating group of protozoa in many marine, benthic environments. Many benthic ciliates exploit ephemeral resources and the life history is often characterised by periods of rapid growth leading to depletion of resources and subsequent starvation (Fenchel 1989). This 'feast and famine' life style results in large spatial and temporal fluctuations of many ciliate populations. Marine ciliates are thought to play a quantitatively important role in the transformation of energy and substances in the biosphere (Fenchel 1987). To improve models of carbon cycling in the sea it will be essential to learn more about how ciliates respond to heterogeneous resources and how this affects population dynamics. The importance of including spatial and temporal variation in order to understand the mechanisms controlling population dynamics has recently been stressed (Thrush 1991, Levin 1992, Morrisey et al. 1992a, b). An interesting question is if ciliates have the behavioural ability to find patches of food, exploit them, and then leave in search for new patches (Fenchel 1989, Levin 1992). Ciliates and other protozoa are typically viewed as simple organisms incapable of most behavioural responses. However, it is known that ciliates possess a range of sensory systems and can respond to light (Song et al. 1980), chemicals (Van Houten et al. 1975), mechanical stimuli (Naitoh & Eckert 1974), and gravity (Fenchel & Finlay 1984, 1986a). Only a few studies treat the function of these sensory systems and their ecological consequences (Kuhlmann & Heckmann 1985, Ricci et al. 1989, 1991, 1992). Fenchel and Jonsson (1988) showed that the benthic ciliate, *Strombidium sulcatum*, can find and remain in patches of food in still water. There are also some still-water studies showing that the migration of some ciliates in the water column can be regulated by the oxygen level (Fenchel & Finlay 1984, 1986a, b, Fenchel 1987).

To my knowledge there are no studies on ciliates in flowing water. In part, this may be explained by technical difficulties, since ciliates are small. Yet, many benthic ciliates have evolved in natural settings where calm water is rare, and hydrodynamic processes could be an important factor regulating their survival and reproduction.

In this work I studied the behaviour and movement patterns of a benthic ciliate *Euplotes* sp. (Hypotrichida), in both still water and in laboratory flume flow. *Euplotes* sp. was selected because it is an abundant epibenthic ciliate which is expected to be particularly exposed to water flow in its natural environment. The key question raised in my study is if benthic ciliates can actively find patchily distributed resources in flowing water. I first test the hypothesis that *Euplotes* sp. can respond to patches of food and what behavioural patterns may be used. It is further tested if behavioural patterns change as food is depleted. In flume flow the ability to settle and stay on the sediment surface is investigated and the efficiency of patch behaviour in water flow is tested. Finally, I discuss the adaptive significance of the interaction between flow and the behavioural pattern shown by *Euplotes* sp.

MATERIALS AND METHODS

THE STUDY ORGANISM

Sediment samples were collected on the west coast of Sweden, close to the Tjärnö Marine Biological Laboratory. An abundant and medium sized (50µm) species of the hypotrich genus *Euplotes* was isolated in small culture dishes (Nunc multidisc). The ciliates were fed every two days with the microalgae *Isocrysis galban*a and *Rhodomonas baltica.* Filtered (Millipore CWSS01TP3, ca 0.2 µm), autoclaved (20 min, 120 ºC) surface sea water (30 ‰ S) was used as culture medium and was changed two days a week. As the cultures started to grow exponentially, individuals of *Euplotes* sp.were moved to larger culture flasks (50 ml, Nunc), where the handling was similar as for the small culture dishes. The cultures were then kept in plastic trays (40 x 20 x 10 cm) with the same treatment. All cultures were maintained in room temperature (20 ºC).

PATCH BEHAVIOUR IN STILL WATER

A small scale experiment was conducted in still water to see if *Euplotes* sp. was attracted to patches of food and to test for discrimination of prey species. Three prey species: the microalgae *Rhodomonas baltica, Isocrysis galbana* and *Chaetoceros calcitrans*, were compared with a control patch without food. Each treatment was replicated three times (a total of twelve patches).

Patches were created by filtering 40 ml of algal suspension ($6.4 \cdot 10^5$ cells ml^{-1}) onto 47 mm GF/C glass fibre filters; for the control patches 40 ml filtered (Millipore CWSS01TP3, ca 0.2 µm) and autoclaved (120 ºC, 20 min) sea water was used. Identical patches were achieved by filtering the suspensions through a mask on top of the filter, making a 35 mm patch in the middle of the GF/C filter. The filter papers with patches were cut to fit 3.5 cm dishes where they were placed and to which culture medium and ciliates (50 in each dish) were added. Motility pattern of *Euplotes* sp. and accumulation in the patches were video recorded from above (Oscar video camera, Panasonic NV-FS90EB recorder) attached to a dissecting microscope (Wild M5A, 6 to 50 X). The number of ciliates in each patch was counted from the video tapes, every two minutes for one hour and then every five minutes for another hour. The number of ciliates in each patch was plotted against time to obtain the accumulation rate (k) and the maximum number of ciliates (max) in each patch. Tests for homogeneity of variances were conducted for both k and max. If the variances were homogenous, k and max were used separately as dependent variables in a one factor analysis of variance (ANOVA, Winer et al. 1991). The three food treatments (*R. baltica, I. galbana, C. calcitrans*) together with the control were considered as a fixed factor. A contrast was used for the a priori planned comparisons between food and control. For the multiple comparisons among food treatments I used a Student-Newman-Keuls test. All statistical analyses were performed with the Super ANOVA software (Abacus Inc.).

ANALYSIS OF MOTILITY PATTERNS IN STILL WATER

Motility on surfaces as a function of food concentration

Motility patterns of *Euplotes* sp. in still water were studied in two series of experiments. In the first series, I looked for differences in behaviour between surfaces with homogeneous food distribution and surfaces with no food. Food surfaces were prepared by filtering 100 ml suspension of *Isocrysis galbana* ($3.2 \cdot 10^6$ cells ml^{-1}) onto 47 mm GF/C glass fibre filters, and 100 ml filtered and autoclaved sea water was used to create control surfaces. The filters were cut to fit 3.5 cm dishes where they were placed and to which culture medium and ciliates were added. Three individual ciliates on three replicate filters were followed from above through a video camera (Oscar video camera, Panasonic NV-FS90EB recorder) attached to a dissecting microscope (Wild M5A, 6 to

50x). Motions of individual ciliates were analysed by plotting ciliates frame by frame (time resolution: 5 s) on a plastic film covering the monitor screen. The fractal dimension of each plotted path of motion was calculated by using the dividers method (Coughlin et al. 1991, Erlandsson & Kostylev 1995). The basis for this calculation is a comparison of the length of a path measured with a given divider size over a range of dividers. Fractal dimension (D_f) is based on log/log regression of the obtained lenght of the path versus the divider size (fig. 1), and D_f is estimated as:

$$D_f = 1-a \qquad (1)$$

where a is the slope of the power function of path lenght versus divider size (Frontier 1987).

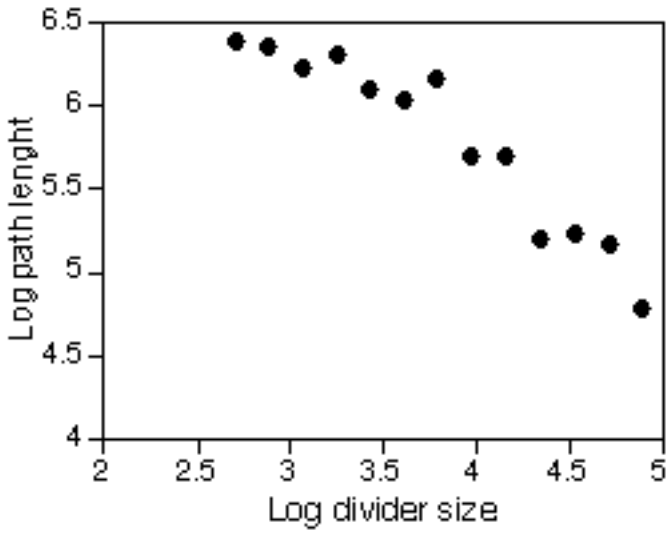

Fig. 1. Log/log plot of path lenght versus divider size, used in the calculation of fractal dimension (D_f) of the walking path of an *Euplotes* sp. D_f = 1-slope.

Plotted paths were transferred to a cartesian coordinate system (170 x 250 mm), and divider lengths were selected as to be represented by all paths. Dividers used were 15, 18, 21.6, 25.9, 31.1, 37.3, 44.8, 53.7, 64.5, 77.4, 92.9, 111.5 and 133.7 mm. Analyses of plotted paths were made in a computer program written by Kostylev (Erlandsson & Kostylev 1995). The results were analysed with a one-factor ANOVA comparing D_f of paths in food with no food. I also compared avarage walking speed for each path in food with no food (one factor ANOVA).

In the second experiment I tested the hypothesis that a change of behaviour was induced by a food gradient. Small patches of *Isochrysis galbana* were prepared in the middle of 47 mm GF/C glass fibre filters, by filtering the algal suspension through a mask on top of the filter, making a 35 mm patch on the GF/C filter. The filters with patches were cut to fit 3.5 mm culture dishes, where they were placed together with culture medium and ciliates. Two individual ciliates on three replicate patches (6 ciliates all together) were video recorded with the same equippment as described above. Motions of individual ciliates were analysed by plotting ciliates frame by frame (time resolution: 5 s) on a plastic film covering the monitor screen. The paths were analysed by comparing the number of times the ciliates changed direction when encountering the patch boundary, with the number of times the ciliates walked out of the patches without a response (G-test, Winer et al. 1991). I also measured the walking speed of the ciliates as a function of the time spent in a patch, by measuring the average speed from an interval of thirty seconds, immediately after entering the patch, after five minutes in the patch and after ten minutes in the patch. The change of speed was tested with a two factors ANOVA where individual paths of ciliates and time were considered as fixed factors, and avarage speed as the dependent variable.

Swimming activity as a function of food depletion

To test if *Euplotes* sp. displays any dispersal behaviour as a response to food depletion, I performed an experiment where the proportion of swimmers was monitored during food depletion.

Dishes (diameter 3.5 cm, volume 12 ml) were prepared with three different concentrations of food (0.72, 1.4, $2.2 \cdot 10^6$ ml^{-1}), replicated sex times. Fifty ciliates were added to each dish and followed for nine days. During that time, the dishes were examined twice a day. Each time the number of ciliates on the bottom of the dish, at the air-water interface and the number of swimming ciliates was counted. Only ciliates in one field of view (Wild M5A, 25 x), located in the middle of each dish, was counted by slowly changing the focus from the bottom to the surface.

The swimming activity was quantified as the number of ciliates swimming compared to the total number of ciliates in each field of wiew. The swimming activity was plotted against time to test if the swimming activity of *Euplotes* sp. changed as food was depleted. Tests for differences among initial food concentrations and for an effect of time were performed with a repeated measures analysis of variance (Winer et al. 1991).

MOTILITY IN FLUME FLOW

The behaviour of *Euplotes* sp. in flowing water was observed in a laboratory flume tank. The aim was to see if *Euplotes* sp. could settle and move on the sediment surface without being resuspended, and if special behavioural responses to flow could be detected. The flume consists of a Plexiglas tank 6.5 x 0.5 x 0.3 m with a PVC-pipe (diameter: 0.35 m) recirculating the water. Water flow is driven by a variable speed motor (0.75 kW) with a propeller on an axis mounted in the vertical exit section of the flume. Sea water was added to the flume to a depth of 12 cm, and the boundary layer was fully developed at the working section located 5 m from the entrance which was fitted with two collimating honey-comb panels.

Autoclaved (20 min, 120 ºC) sediment was added to the working section and free-stream velocity, 12 cm above the bottom, was set at 6.8 cm s^{-1}. This flow is below the critical erosion velocity for this sediment and represents a flow speed typical for the environment where *Euplotes* sp. is found. *Euplotes* sp. was added to the flume and the behaviour of the ciliates was observed and video-recorded (Oscar video-camera, Panasonic NV-FS90EB recorder) from one side of the tank through a dissecting microscope (Wild M5A, 6 to 50 x).

PATCH BEHAVIOUR IN FLUME FLOW

The small scale experiment of patch behaviour in still water was repeated in the flume tank, with the addition of flow as a factor. Thus *Euplotes* sp. was tested at two flow levels (no flow, 6.8 cm s^{-1}), replicated twice. In both treatments of flow the ciliates were given a choice of eight patches, four with and four without food. The flume tank, sediment and water depth were the same as in the study of behaviour in flume flow described above.

Patches were created by filtering 100 ml suspension of *Isocrysis galbana* ($3.2 \cdot 10^6$ cells ml^{-1}) as food and 100 ml filtered (Millipore CWSS01TP3, ca 0.2 µm), autoclaved (120 ºC, 20 min) sea water as a control, onto 47 mm GF/C glass fibre filters. The filters were then fixed to culture dishes (diameter 3.5 cm) which were weighted with 1 g of lead, placed inside the dishes to prevent them from floating. The filters with the patches were adjusted to be flush with the sediment surface and arranged in a regular pattern (Fig. 2).

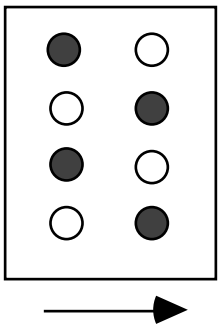

Fig. 2. Patches regularly arranged on the sediment surface in the flume. The rectangle represents the working section in the flume, and circles indicate the patches, ● food, ○ without food. The arrow show the direction of the flow.

For each experiment, the patches were placed in the flume and the flow speed was adjusted appropriately. Then 2 l of *Euplotes* sp. culture were added to the flume tank, 1 l at each side of the working section as close as possible without disturbing the sediment surface. Each experiment was run for 4 hours after which patches were recovered from the sediment with a specially made suction device. This tool collects some of the water above the dish, and prevented ciliates from being washed off the patches. The number of ciliates in each patch was counted by labelling their DNA with a fluorochrome (proflavin 16 mg per 50 ml sample, for 2 min, ciliates fixed with 6 % glutaraldehyd 2 ml per 50 ml sample). Labelled ciliates were filtered onto membrane filters (MFS, diameter 25 mm, pore size 5 µm) mounted on slides in a drop of oil and examined under an epifluorescence microscope (Leitz Dialux, filterblock I2, 250 x). With this method *Euplotes* sp. was easy to recognize and distinguished from other organisms found in the patches.

For statistical analysis a three factor ANOVA was used. The fixed effects were flow/no flow and food/no food, and the two separate flume runs were considered a random factor nested within the flow/no flow factor. Dependent variable was the number of ciliates in each patch.

RESULTS

PATCH BEHAVIOUR IN STILL WATER

When *Euplotes* sp. was offered patches of food in still water they immediately accumulated in all three food treatments (*Rhodomonas baltica*, *Isocrysis galbana*, *Chaetoceros calcitrans*), (Fig. 3). *Euplotes* sp. seemed to prefer *I. galbana* and *R. baltica* compared to *C. calcitrans*, though this was not statistically significant.

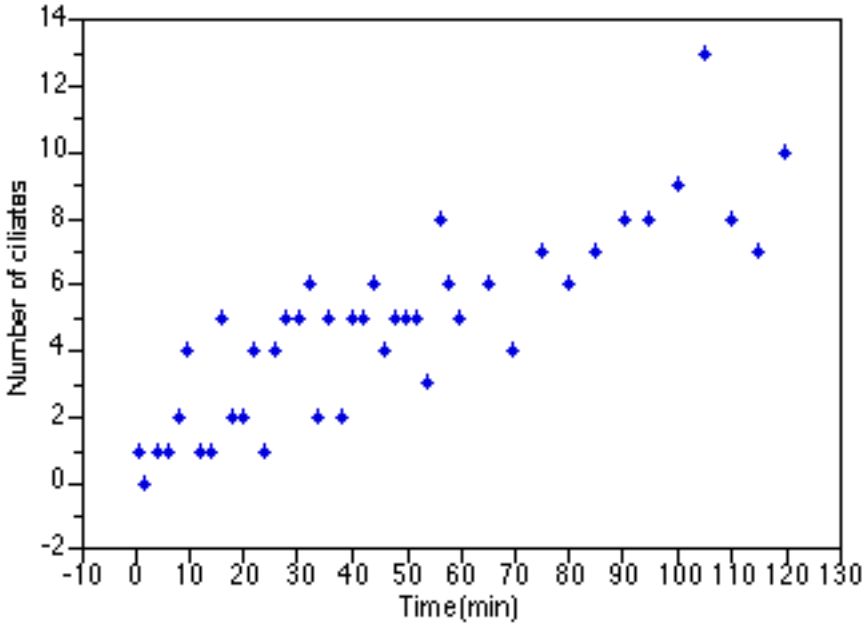

Fig. 3. Accumulation of *Euplotes* sp. in a patch of food (*Isocrysis galbana*).

A one-factor ANOVA using maximum number of ciliates in a patch ($x^{0.25}$ transformed) as the dependent variable, separated food patches from control patches ($F_{3,8}$=10.119, P=0.0043). Multi-comparision (SNK) among the food treatments and control showed that number of ciliates was higher in all food treatments compared to the control (*I. galbana* versus control: $F_{1,4}$= 24.11, P=0.0012, *R. baltica* versus control: $F_{1,4}$=21.3, P=0.0017, *C. calcitrans* versus control: $F_{1,4}$=11.0, P=0.011), (Fig. 4a). When comparing the accumulation rate in a patch, no difference was found between food treatments and the control ($F_{1,8}$=1.01, P=0.44), (Fig. 4b).

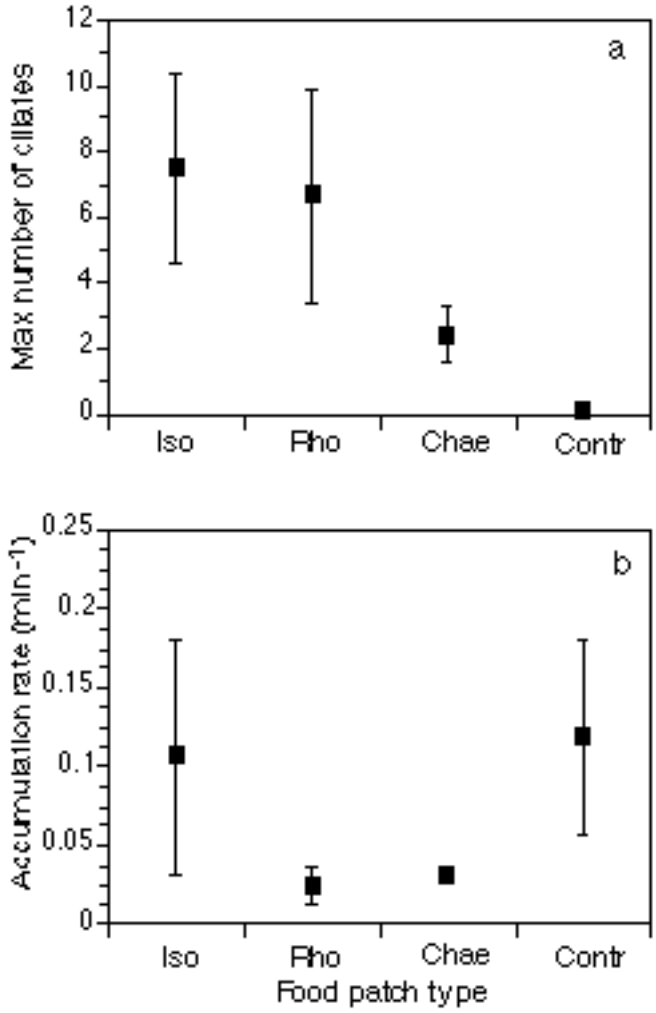

Fig. 4. Accumulation in food patches by *Euplotes* sp., Iso= *Isochrysis galbana*, Rho= *Rhodomonas baltica*, Chae= *Chaetoseros calcitrans*, Contr= Control. (a) Maximum number of *Euplotes* sp. in a patch (mean value ± SE of three replicates). (b) Accumulation rate of *Euplotes* sp. in a patch (mean value ± SE of three replicates).

ANALYSIS OF MOYILITY PATTERNS IN STILL WATER

Motility on surfaces as a function of food concentration

The study of the paths of *Euplotes* sp. in food and in no food, could not reveal a difference in behaviour; both the walking speed and the sinuosity of the paths were highly variable. Figure 5 shows an example of a path in food and a path in no food. Analyses (one-factor ANOVA) of fractal dimension (D_f) of the paths could not separate the food/no food treatment ($F_{1,16}$=3.09, P=0.098), (Fig 6a). The walking speed in food compared to control patches did not differ (one factor ANOVA, $F_{1,16}$=0.758, P=0.397), (Fig 6b).

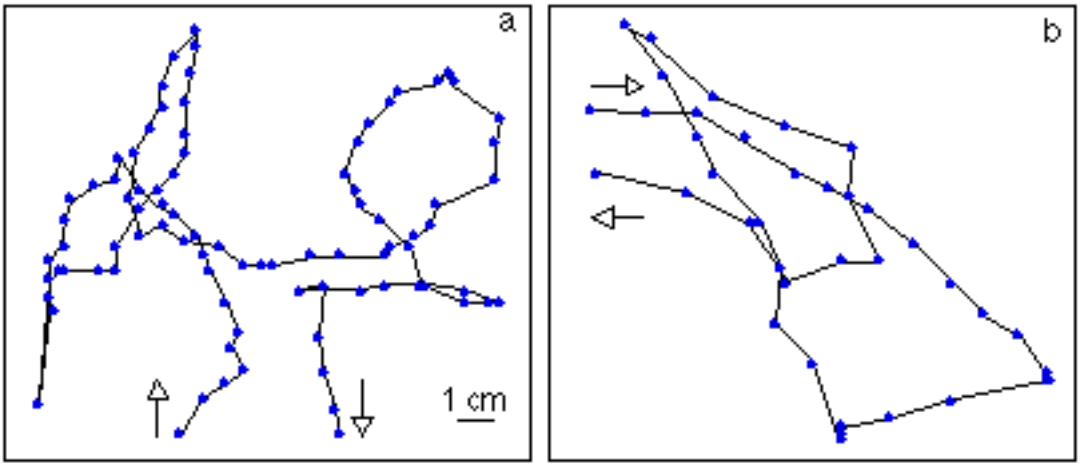

Fig. 5. Example of walking trajectories of *Euplotes* sp. in (a) no food (b) food, (time resolution 5 s). The arrows indicate the direction of the movement.

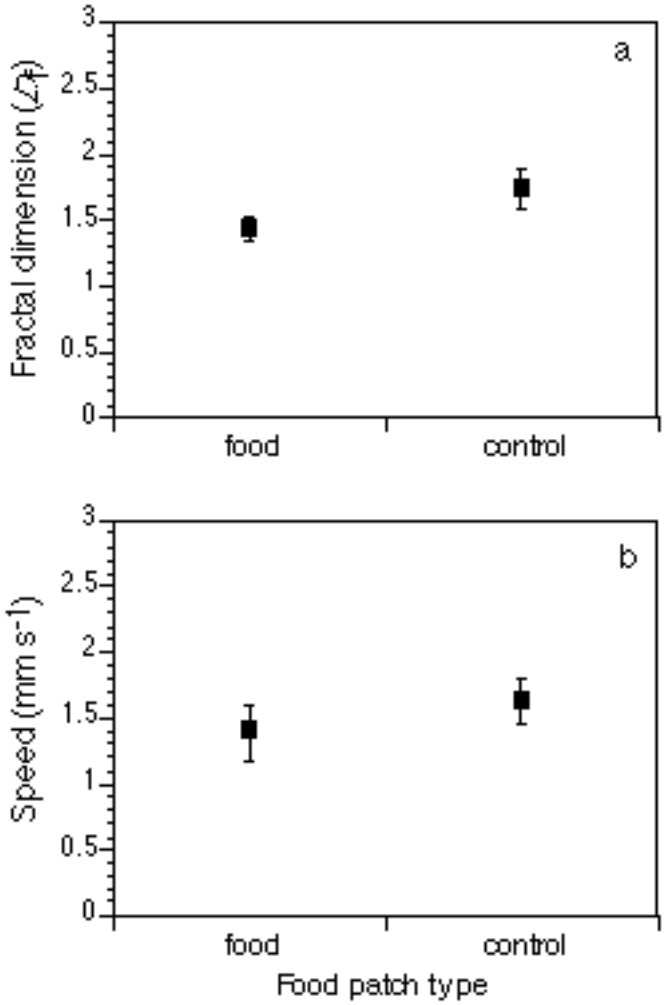

Fig. 6. Comparison of (a) fractal dimension (D_f) of the walking paths of *Euplotes* sp. in patches of food and control (mean value ± SE of nine replicates). (b) walking speed of *Euplotes* sp. in patches of food and control (mean value ± SE of nine replicates).

When *Euplotes* sp. was observed at a boundary of a food patch, they reacted in two ways. When first approached the patch, the ciliates often stopped or slowed down at the patch boundary before walking into the patch. When the ciliates approached the patch boundary from within the patch, they changed direction by quickly reversing ciliary motion. In this way the ciliates remained inside the patch of food (Fig. 7). This reversal behaviour was induced in 61 of totally 67 studied occations when the ciliates encountered a patch boundary, (G-test, $X^2_1 = 26$, $P < 0.001$).

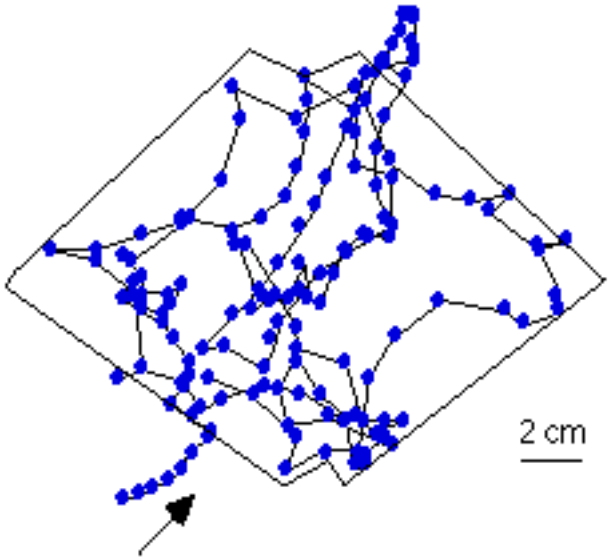

Fig. 7. Exampe of a plotted path of a *Euplotes* sp. in a patch of food, (time resolution 5 s). The arrow indicates the direction of the movement.

When the ciliates walked inside the patches, the walking speed and the sinuosity of the path were highly variable. The walking speed seemed to increase with time, though there was too much variability between different ciliates to discover a change in speed ($F_{2,10}=1.85$, $P=0.207$, Fig. 8).

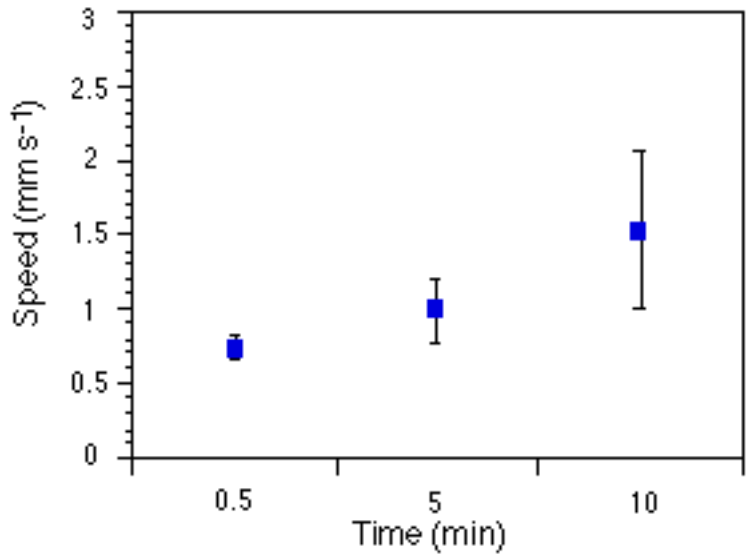

Fig. 8. Walking speed of *Euplotes* sp. with time inside a patch of food (mean value ± SE of six replicates).

Swimming activity as a function of food depletion

Euplotes sp. initially grew exponentially with most individuals walking on surfaces in the dish (Fig. 9).

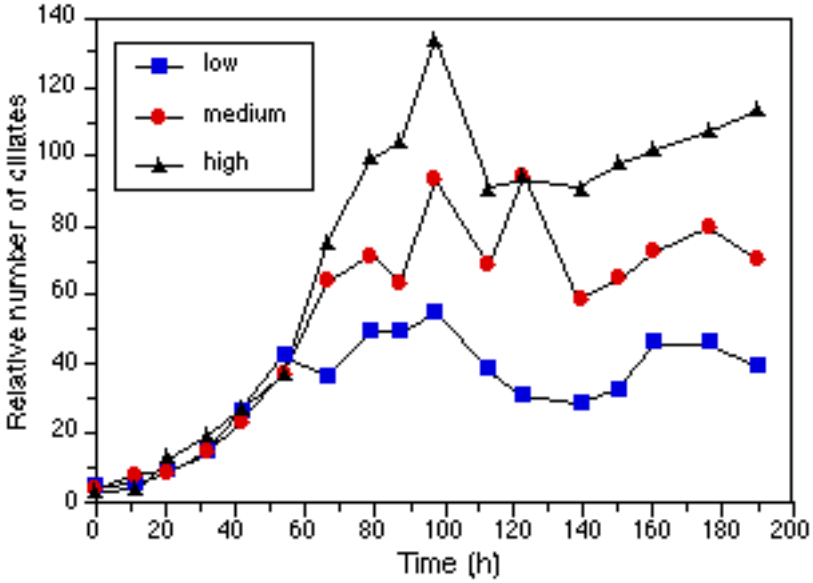

Fig. 9. Relative number of *Euplotes* sp. in dishes of food with time. Three different concentrations of food were used, low = $0.72 \cdot 10^6$ ml l^{-1}, medium = $1.4 \cdot 10^6$ ml l^{-1}, high = $2.2 \cdot 10^6$ ml l^{-1}, with six replicates for each concentration.

As food became increasingly depleted a shift in behaviour was observed and the proportion of swimming ciliates increased (Fig. 10). A repeated measures ANOVA shows a significant effect of time ($F_{16,240}$=23.2, P<0.001). At the end of the experiment, at very low food concentrations, the swimming activity decreased again and the ciliates were generally less active. This trend was true for all three initial food concentrations examined (Fig. 10). There were, however, differences in swimming activity among the different concentrations, where the lowest concentration showed the lowest proportion of swimming ciliates ($F_{32,240}$=2.19, P=0.029).

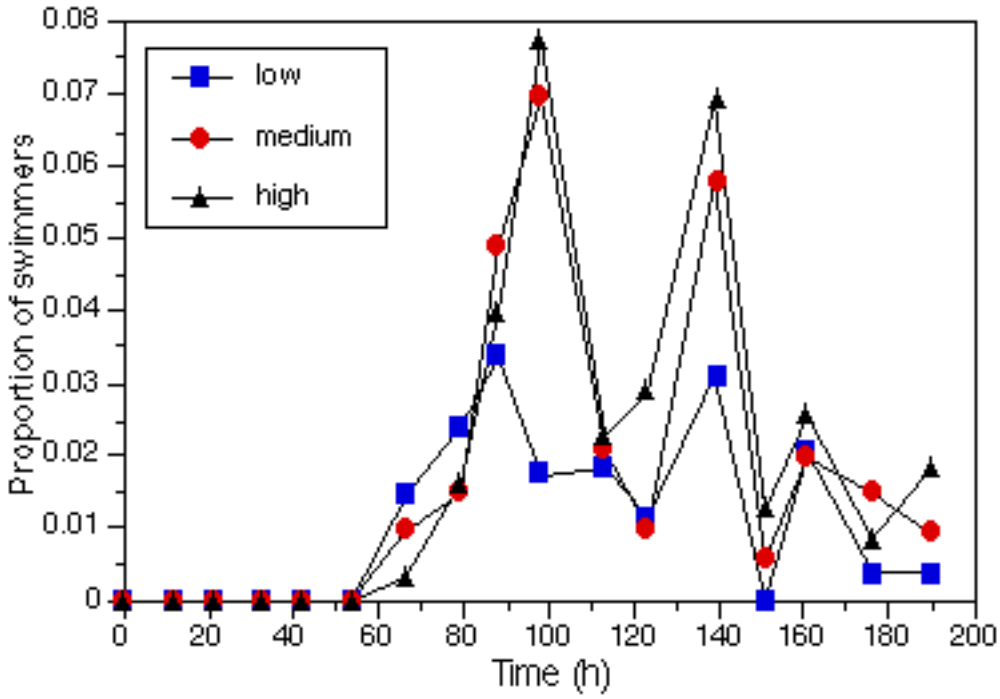

Fig. 10. Proportion swimming *Euplotes* sp. with time for three initial food concentrations (low = 0.72· 10^6 ml l^{-1}, medium = 1.4 · 10^6 ml l^{-1}, high = 2.2· 10^6 ml l^{-1}), (mean values from six replicates for each concentration).

When *Euplotes* sp. was observed in flume flow I could see many similarities with movements in still water but also several new behaviours. The walking movements were similar in flow as compared to still water; *Euplotes* sp. was walking with the cirri on the sediment seemingly undisturbed by the water flow. The flow speed used (6.8 cm s^{-1}) was far too strong for the ciliates to swim against, but they seemed to have some vertical control when drifting with the flow. *Euplotes* sp. was observed to swim in a helix against the direction of the current, with their anterior end tilted down towards the sediment. This orientation and the swimming activity brought ciliates closer to the sediment. When the ciliates approached the more slowly flowing water near the sediment surface, they swam down in an arc towards the sediment (Fig. 11a). In this way *Euplotes* sp. seemed to actively settle on the sediment surface. *Euplotes* sp. was also observed to actively swim up from the sediment surface (Fig. 11b). Ciliates walking on the sediment surface were leaving the sediment by swimming upwards in a strong helix, until they were carried away with the flow. An intriguing slow drift behaviour was observed where *Euplotes* sp. only swam up just above the sediment surface and drifted close to the sediment, occasionally stopping to walk (Fig. 11c).

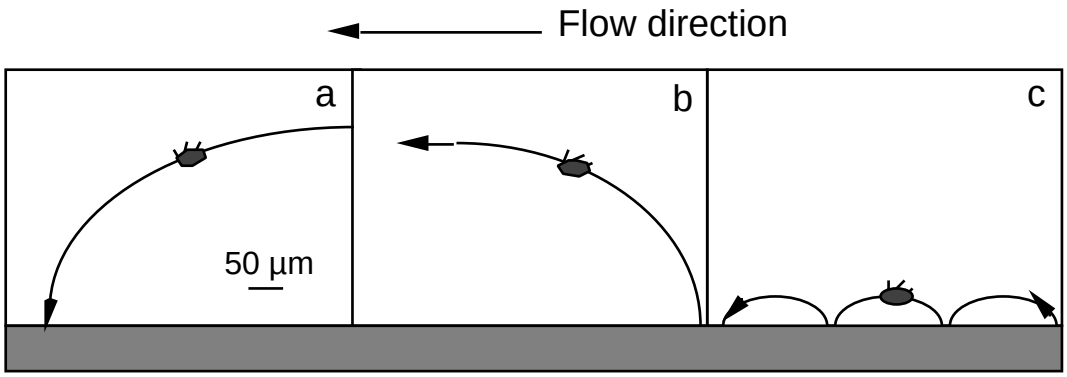

Fig. 11. Schematic drawing of the behaviour of *Euplotes* sp. in flume flow (free-stream velosity: 6.8 cm s^{-1}). (a) Settlement on the sediment surface. (b) A ciliate actively swims up from the sediment. (c) 'Slow drift' behaviour where the ciliate drifted close to the sediment surface, occasionally stopping to walk.

When *Euplotes* sp. was given a choice of patches of food and patches without food, ciliates accumulated in the patches of food, both in still water and in flume flow ($F_{1,24}$=29.3, P<0.0001, Fig. 12). Note though, that the number of ciliates differed between the treatments ($F_{1,24}$=10.2, P=0.004). There were more ciliates in all patches in still water, possibly because a greater number of ciliates were initially exposed to the working section in still water. Using the logarithm of the number of ciliates, shows that the relative efficiency in finding patches were similar in flow and in still water as revealed by a non-significant interaction between patch behaviour and flow speed ($F_{1,24}$=0.401, P=0.533).

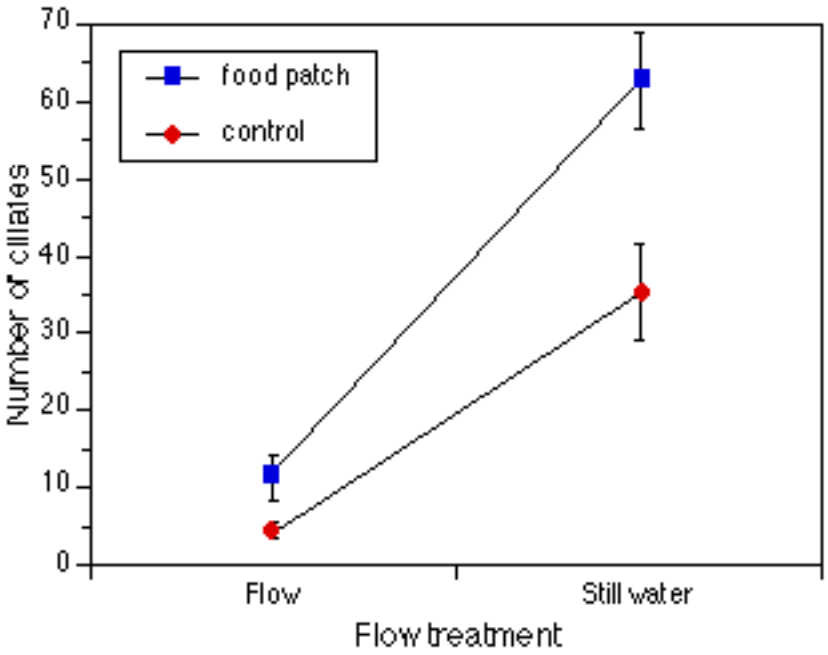

Fig. 12. Comparison of the number of *Euplotes* sp. reaching patches of food and control (cell means ± SE of four patches) in two flow treatments.

DISCUSSION

In a series of experiments I have explored the behaviour of *Euplotes* sp. It is shown that this epibenthic ciliate can find and stay in patchily distributed resources, in both still water and in flume flow. The analysis of the swimming activity and the analysis of *Euplotes* sp. in flowing water, indicate that this ciliate can use the flow to find new resources.

The physiology and movements of ciliates have previously been studied in still water (Blake & Sleigh 1974, Van Houten et al. 1981). Hypotrich ciliates, like *Euplotes* sp., are known to walk on surfaces with bundles of cilia, cirri, and when swimming they move in a helix (Ricci et al. 1989, 1991, 1992). These movements were observed in both still water and laboratory flume flow. *Euplotes* sp. swims at ca. 1 mm s^{-1} and was passively advected in the flume at the flow speed used (6.8 cm s^{-1}). Because of the high flow speed it was impossible to observe any ciliate behaviour in the bulk flow with the recording technique used. However, in the lower region of the boundary layer, where flow was slower, apparent rheotaxis was observed. The counter-current swimming towards the sediment could be an active response to bottom shear or it may be a passive reorientation caused by interactions between flow and the ciliate morphology. The way the ciliates left the sediment suggests that an active behaviour is involved. Two different upward motions were observed. One where *Euplotes* sp. left the sediment surface in steep ascent until the ciliates reached the faster flow, where they were carried away by the current. A second behaviour was observed where the ciliates slowly drifted close to the sediment surface, occasionally making contact with the sediment to resume walking. Again, it can not be concluded if this is an active response to shear rate, or a passive consequence of boundary-layer flow and ciliate morphology.

In a few studies, ciliates have previously been shown to possess patch behaviour in still water (Fenchel & Jonsson 1988, Verity 1988). My observations on the movement of *Euplotes* sp. show that the ciliates can increase their residence time in patches of food in both still waters and in flume flow. The flume experiments tested the patch response of *Euplotes* sp. that were initially suspended. This procedure probably exposes more ciliates to the working section in the still water treatment, explaining the higher settling intensity in the patches. It would be interesting to design an experiment where the ciliates were first added and then left to settle some distance upstream of the working section, before the start of the flume flow. The ability and time to reach the patches in different flow speeds could then be investigated.

The small-scale experiments on patch behaviour in still water, were used to analyse what behavioural mechanisms could lead to patch accumulation. Within minutes *Euplotes* sp. reaches equilibrium density in the food patches. The non-significant accumulation rate (k) between food patches and control suggests that the ciliates do not detect the patch from a distance but encounter them randomly. Once inside the patch, several mechanisms may be responsible to increase patch residence time. I tested two hypotheses about how *Euplotes* sp. may be prevented from leaving the patch. Firstly, a kinetic response could be used through a reduction of walking speed within the patch (orthokinesis). Analysis of the walking paths outside and inside patches did not show any clear evidence of an orthokinetic response. Although not significant, the ciliates seemed to increase, rather than decrease, the walking speed inside the patch. Secondly, a transient response (Fenchel & Jonsson 1988) to the strong gradient at the patch boundary may induce a direction change (tumble) preventing the ciliates from leaving the patch. There was a clear effect of the patch boundary on the probability of tumbling and I conclude that this was the main mechanism responsible for the accumulation of *Euplotes* sp. in the food patches. Future experiments may show if a similar transient response at patch boundaries can be observed in flowing water. The stimulus eliciting the transient response of *Euplotes* sp. and leading to patch accumulation is probably a dissolved chemical substance. Fenchel & Jonsson (1988) managed to induce patch behaviour in an epibenthic ciliate with dissolved amino acids. However, in the present study it is possible that *Euplotes* sp. could detect differences in surface texture between filter areas covered with and without algae. Texture induced responses have been described for other hypotrich ciliates (Ricci et al. 1989). The weaker response to the microalga *Chaetoceros calcitrans* compared to the other food species could probably be used to argue for a difference in perception of either chemical or surface properties.

To explore how *Euplotes* sp. may disperse and move to new unexploited resources I studied how swimming activity changed during food depletion. A clear increase in the propensity to leave the substratum and swim was observed as food was depleted. This behaviour has been described for both ciliates (Fenchel & Jonsson 1988, Fenchel 1990) and for heterotrophic flagellates (Fenchel 1982). It may be speculated that this might be a way for the ciliates to faster colonise new patches of food. It would especially be a great advantage in flowing water where ciliates could swim up into the flow and be quickly transported to new areas.

The onset of starvation as food was depleted is the most likely explanation for the induction of the swimming behaviour. The pattern was obvious for all three initial food concentrations although the low concentration treatment showed a lower proportion of swimmers. Differences in initial food concentration will translate into different population densities in the dishes, and because growth is exponential the depletion of food occurs at about the same time, irrespective of initial concentration. Exponential growth of ciliates as a function of food concentration (c) may be modelled as:

$$\mu=\frac{c\mu_{max}}{c+K} \tag{2}$$

where μ is growth rate, μ_{max} is the maximum growth rate and K the half-saturation constant (Jonsson 1986). Food uptake (u) as a function of food concentration (functional response) may be similarly modelled with a maximum uptake rate (u_{max}):

$$u=\frac{cu_{max}}{c+K} \tag{3}$$

The number (N) of ciliates grows with time (t) as:

$$N=N_0e^{\mu t} \tag{4}$$

where N_0 is initial number of ciliates. During growth of ciliates the food concentration will decrease with the rate:

$$\frac{dc}{dt}=-Nu \tag{5}$$

This process of food uptake, growth rate and food depletion, can be simulated to find the time at food exhaustion and the final number of ciliates as a function of initial food concentration. Assuming homogeneous conditions in the dishes and that μ_{max} is 0.057, u_{max} is 200 *Isochrysis galbana* h^{-1}, K is 10000 cells ml^{-1} (Jonsson 1986), the three initial concentrations (0.72, 1.4 and $2.2\cdot10^6$ cells ml^{-1}), should theoretically yield 1200, 2400 and 3500 *Euplotes* sp. per dish. Total food depletion should occur after 58, 68 and 75 h respectively. Apparently, the time at food exhaustion does not differ much among treatments, and these times coincide well with the first occurrence of swimmers (Fig. 10).

The lower proportion of swimmers in the treatment with low initial food concentration, is likely an artefact caused by the low frequency of swimmers in the samples which will underestimate the true proportion. However, an alternative explanation is that crowding affects the induction of swimming behaviour. Interestingly, the swimming activity as well as the walking activity decreased after ca 4 days of starvation. This may be interpreted as a metabolic shift-down response to long-term starvation. It may be argued that the swimming response is used to quickly exploit spatial heterogeneity of resources, while a reduction in activity and general metabolism is a response to survive temporal resource heterogeneity.

With my experiments I have been able to show that *Euplotes* sp. can disperse and settle in flowing water and that they can find patches of food in both still water and in flume flow. The general impression is that *Euplotes* sp. can walk and swim in a small layer close to the sediment, unimpeded by the overlying flow. *Euplotes* sp. seems to settle and leave the sediment by active behaviour. These responses, however, need to be studied in more detail and at several flow speeds to elucidate if these behaviours are active responses to flow. It may be speculated that the alternation between walking, swimming up in the bulk flow, and settlement is an adaptive behaviour to efficiently exploit patchy environments. Some members of the meiofauna have been studied in different hydrodynamic regimes and correlated to a suit of factors. Palmer studied in a series of experiments, meiofauna in the field and in flume flow (Palmer 1986, 1988, 1988). She could show that hydrodynamic processes together with predation and behaviour were important factors when trying to explain the patchiness found in the field for benthic harpacticoid copepods. The slow drift just above the sediment surface could be a way for the ciliates to actively, and more quickly than walking explore the sediment surface. In this way *Euplotes* sp. may select patches based on the perception of diffusive chemical gradients that may be persistent in the viscous sublayer. A similar behaviour was observed for bivalve larvae (Jonsson et al. 1991). These behaviours can in part explain how microfauna can find and exploit patchy environments.

ACKNOWLEDGEMENTS

My thanks go to all the wonderful people working at Tjärnö Marine Biological Laboratory. I specially like to thank Mats for helping with the statistics, Kent B. for the algae and inspiring discussions, Kent H. for helping me with the electrical work, Kalle for making all my tools, Vladimir for giving me a new dimension of fractals and Kerstin for giving me a lot of good advice. I also like to thank those people who have been like a family during this time; Hans with his never ending word-games, Ulrika who answered my phone, Frank who kept my spirit up with 'palt', the worm-people who kept my pulse up and all the 'artist people' (including the 'killer cat'). Most of all, I thank my supervisor Per, for trusting me enough to work on my own, just leading me back to the right path when I got lost, and for always showing enthusiasm for my work.

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