NOTE

Feeding of a Hypotrichous Ciliate swimming in the H₂S layer of Lake Kaiike on *Chromatium* sp.

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ABSTRACT

A freely swimming hypotrichous ciliate was found in all samples from different depths of the H₂S layer in Lake Kaiike on 24 May 1996 (<10 cells ml⁻¹). Growth of these ciliates was tested by adding living cells of *Chromatium* sp., a phototrophic bacterium blooming at an upper boundary of the H₂S layer (bacterial plate). The bacterium was found to be an effective food for the ciliates in the lake. If the ciliate growth rate, estimated as a maximum 0.6 day⁻¹, was assumed to be determined solely by bacterial cell density, a single ciliate cell would soon grow to 2500 cells (a number sufficient to consume the entire bacterial population). However, results suggest that growth of the ciliates in the lake was greatly controlled by other environmental factors such as predation by larger zooplankton or by the occurrence of a high concentration of H₂S at and around the bacterial plate. The ciliates found in the H₂S layer appeared to be those expelled from their original habitat.

Key words : Ciliates from H₂S layer, feeding on *Chromatium* sp., Lake Kaiike.

Lake Kaiike is characterized by a steady-state blooming of bacteria of the genera *Chromatium* and *Macromonas* at an upper boundary of the H₂S layer. Freely swimming ciliates were frequently found in the H₂S layer. The present study tries to understand the ciliate population dynamics by laboratory cultivation.

Water was taken with a 3 l VAN DORN sampler at different depths through the deepest water column (11 m) in Lake Kaiike on 24 May 1996. The lake was stratified. Vertical profiles of environmental factors such as salinity and temperature were within their seasonal variations (MATSUYAMA, 1988) except for H₂S, which appeared at 5 m depth where it attained a maximum concentration (20.3 mg S l⁻¹). However, it rapidly decreased to 3.8 mg S l⁻¹ at 5.5 m, below which it gradually increased downwards (15.8 mg S l⁻¹ at 10 m depth). An H₂S profile with such a maximum at the mid-depth was not exceptional (MATSUYAMA, 1978). Two bacterial species, *Chromatium* sp. and *Macromonas* sp., were most abundant at 5 m depth, amounting to 1.4×10^6 and 7.4×10^6 cells ml⁻¹, respectively (bacterial plate). Below that depth cell

densities rapidly decreased.

Freely swimming ciliates were found in all bottles, which were filled with samples from different depths of the H_2S layer and transferred to the laboratory (<10 cells ml^{-1}). However, ciliates were not found in the bottles sampled from an upper oxic layer or those from the H_2S layer previously added with formalin as a fixative. The ciliates seemed to live almost exclusively in the H_2S layer and to be very fragile.

Ciliates were picked up from the sample at 8 m with a micropipette and transferred into a polystyrene flask (Corning 25 cm^2 Tissue Culture Flask, 75 ml capacity). A5 - 10 ml living cell suspension of *Isocrysis galbana* (Haptophyceae) was added as food to give a final cell density on the order of $10^4 - 10^5$ cells ml^{-1} . The flask was then filled with glass fiber (Whatman GF/C) filtered seawater and closed with a silicon rubber stopper for incubation at 25 °C in the dark. (All ciliate growth experiments were performed using duplicate flasks at respective levels of the food supply ; the flasks were treated under the above conditions.)

The ciliates grew well by feeding on the cells of *I. galbana*, which was true of other ciliates (GOLD, 1968). They captured food particles by generating a current which swept the particles toward ciliary membranella (filter feeder). Usually, the culture became clear within a week due to consumption of foods and entered a stationary phase of growth.

Figure 1 shows microphotographs of the living ciliates. Size of the starved cells (A) was 62 μm (s.d. = 4.6) long and 30 μm (s.d. = 3.7) wide for $n=30$. They were ovoid-shaped, the dorsal side was bent, and the ventral side flattened (left). Contractile vacuoles were clearly seen. Many cirri and several bundles of cilia as well as oral membranella were seen on the ventral side (right). Elongated C-shaped macronucleus located in the middle of the body were clearly seen in the Protargol-stained cells. Ingestion of food was readily revealed by the formation of optically dense food vacuoles (B). These characteristics conform to the genus *Euplotes* (LYNN and SMALL, 1985). When food was abundant, the ciliates swam vigorously by rotating their bodies. When food was entirely consumed, most ciliates began settling to crawl on the bottom.

Varying numbers of the living cells of *I. galbana* and *Chromatium* sp., once isolated from the blooming population at an upper boundary of the H_2S layer of Lake Kaiike and maintained in the medium of PFENNIG (1965), were added to the ciliate culture. Prior to the experiment the ciliates were starved for 1 - 2 weeks. Ciliate growth in each flask was measured by directly counting the cell density without subsampling ; i.e., after the flask was gently agitated for uniform distribution of ciliates, it was mounted on the stage of a stereoscopic microscope (Nikon, SMZ-10). Ciliates in the field of vision (5 - 8 mm in diameter) were counted by slowly scanning the focal plane from the bottom to the top layer (20 mm high). Counting was repeated 3 times for each flask. Coefficient of variation was between 0.031 and 0.357 (mean : 0.187) for the measurements of cell density in 20 flasks (at the end of incubation shown in Figure 2).

Figure 2 shows the growth experiments of ciliates on two food sources. Apparently, they grew on *Chromatium* sp. more effectively than on *I. galbana* when the growth was normalized to the respective cell densities. Although *Chromatium* sp. used here were smaller ($3\text{--}4 \times 3\text{--}6\text{ }\mu\text{m}$) than *I. galbana* ($3\text{--}5 \times 6\text{--}9\text{ }\mu\text{m}$), the latter were also consumed within a week (indicating the omnivorousness of the ciliates). *Chromatium* sp. were obviously a very effective food for ciliates. Maximum ciliate density in the *Chromatium*-fed flask was directly proportional to the initial cell density of the bacterium, showing that a single ciliate cell needs ca. 4×10^3 cells of

Fig. 1. Microphotographs of living ciliate cells. A : starved cells. B : food-ingested cell. When a few drops of formal in were added to the ciliate culture, the cells immediately began to swell and finally ruptured.

Chromatium sp. during its mean generation time.

Relatively little is known about the protozoan feeding on phototrophic bacteria. MADONI *et al.* (1990) reported that both *Chlorobium phaeobacteroides* and *Thiocapsa roseopersicina* were incapable of sustaining *Coleps hirtus*, which were isolated from Kinneret among 12 species of algae and 7 species of bacteria isolated from Lake that lake. Figure 2, however, showed that ciliates depend on *Chromatium* sp. in Lake Kaiike, because a phytoplanktonic biomass was generally low in the lake (MATSUYAMA and SHIROUZU, 1978).

To feed on *Chromatium* sp., ciliates must encounter ambient H_2S .

Figure 3 shows the growth experiments of ciliates in flasks containing equal

Fig. 2. Growth of ciliates upon addition of *Isocrysis galbana* (upper) and *Chromatium* sp. (lower) at 1, 2, 5, 6, 7 and 9 days of incubation. *I. galbana* and *Chromatium* sp. were added to obtain cell densities of 0–50 $\times 10^4$ cells ml^{-1} , and 3–300 $\times 10^4$ cells ml^{-1} , respectively. Vertical lines represent standard deviations.

levels of *Chromatium* sp. (1.5×10^6 cells ml^{-1}) but with different amounts of neutralized Na_2S (0–70 mg S l^{-1}). Just before determining ciliate cell density, a 0.5 ml of O_2 -depleted seawater (by autoclaving and cooling under a N_2 atmosphere) was injected by syringe into the flasks through a silicon rubber stopper and an equal amount of culture suspension was withdrawn. H_2S concentration in the subsample was measured spectrophotometrically (CLINE, 1969).

In the absence of H_2S (A in fig. 3), the ciliates immediately began to grow fast, showing a specific growth rate of 0.6 day^{-1} for the first 5 days of incubation. The rate was comparable with *in situ* rates of the most dominant ciliates in the eutrophic Řimov Reservoir (0.3 – 0.9 day^{-1}) (MACEK *et al.*, 1996). H_2S addition, however, retarded growth. At initial H_2S additions of 48–70 mg S l^{-1} (C, D), the growth was poor until the end of the experiment,

Fig. 3. Growth of ciliates upon addition of *Chromatium* sp. (1.5×10^6 cells ml^{-1}) and different amounts of neutralized Na_2S (0 (A), 25 mg S (B), 48 mg S (C), 70 mg S l^{-1} (D)). Vertical lines represent standard deviations. Broken lines denote H_2S concentration.

although most of the ciliates retained their motility. The ciliates appeared to tolerate H_2S , at least at the concentrations encountered in Lake Kaiike ($\sim 30 \text{ mg S l}^{-1}$) (MATSUYAMA, 1988). Active growth first occurred when ambient H_2S was decreased below ca. 5 mg S l^{-1} (B), H_2S level usually encountered at the bacterial plate (MATSUYAMA, 1988). It is noteworthy that H_2S also decreased in the flask filled with autoclaved seawater, but more slowly than shown in Figure 3. The decrease in H_2S concentration in the figure may be partly due to biological processes.

From the above growth experiments with ciliates, it may be pointed out that the growth of the ciliates in Lake Kaiike was not limited by the cell density of *Chromatium* sp., because these bacteria could form a steady-state dense population over the years.

DYER *et al.* (1986) found some heterotrophic ciliates (*Metopus*, *Plagiopyla*, an unidentified hypotrich) inhabiting the dark, H_2S -rich hypolimnion of Lake Ciso. They speculated that the low population of these ciliates was primarily due to a limited transport of food (detritus) from the upper layers as well as to a low rate of decomposition by anaerobiosis. If the growth rate of the present ciliates in this study was assumed to be controlled by *Chromatium* sp., and if a single ciliate cell was assumed to need 4×10^3 cells of *Chromatium* sp. to grow at a rate of 0.6 day^{-1} (Figs 2, 3), it would attain a population of 2500 cells within 2 weeks by consuming 10^5 cells of *Chromatium* sp. This suggests that ciliate growth may be more closely controlled by other factors such as predation by larger zooplankton which were layering just above the bacterial plate (MATSUYAMA and SHIROUZU, 1978) or of a high concentration of H_2S at the bacterial plate (as on 24 May 1996). The ciliates found in the sample from the H_2S layer on that date are presumably those expelled from the original habitat. Further studies on spatio-temporal changes in the distributions of ciliates at and around the bacterial plate are necessary.

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貝池 H₂S 層を遊泳する下毛類纖毛虫の *Chromatium* sp. 摂食

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摘 要

1996 年 5 月 24 日, 貝池 H₂S 層の色々な深さから得た試水中に自由遊泳する下毛類纖毛虫が見られた (<10 細胞 ml⁻¹)。H₂S 層上端でブルーム (バクテリアプレート) を形成している光合成細菌, *Chromatium* sp., の生きた細胞を纖毛虫に与え, その成長を検討した。細菌は湖で纖毛虫の効果的な餌となっていることが判った。纖毛虫の最大比成長速度は 0.6 日⁻¹と求められたが、その速度が細菌数に依存すると仮定すると、纖毛虫 1 個体は短時間に 2500 個体になり、細菌群集を食い尽くす。湖での纖毛虫の成長はバクテリアプレートおよびその周辺に分布する大型動物プランクトンの摂食、高濃度の H₂S の出現など他の環境要因により大きく抑制されていると推察された。H₂S 層中の纖毛虫は本来の生息層から駆逐された個体であると考えられた。