Note

Dissolved H₂S around *Chromatium* sp. Blooming in Lake Kaiike Protects against Feeding Pressure by a Hypotrich Ciliate

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ABSTRACT

Euplotes sp., a hypotrich ciliate isolated from Lake Kaiike, feeds on *Chromatium* sp. (phototrophic bacterium) as an effective food source. On 24 October 1997, this predator and its prey reached a maximum abundance of 1.2×10^4 cells l⁻¹ at 5 m depth and 1×10^6 cells ml⁻¹ at 5.5 m depth, respectively where H₂S began to appear. In aerobic conditions, the ciliates grew rapidly by feeding on about 5,000 bacterial cells for the cell division. A minimum level of bacterial cells for the ciliate population to sustain the biomass was estimated to be about 60 cells day⁻¹ per single ciliate cell. In the presence of H₂S, feeding activity of the ciliate on bacterial cells was completely suppressed. Dissolved H₂S around the habitat of *Chromatium* sp. was considered to protect against feeding pressure by *Euplotes* sp.

Key words : Dissolved H₂S, *Chromatium* sp., *Euplotes* feeding, Lake Kaiike

The Kaiike Strain of *Euplotes* sp. was found to feed on *Chromatium* sp., a phototrophic bacterium blooming at an upper boundary of the H₂S layer, as an effective food source (MATSUYAMA and MOON, 1997). Without any environmental control, the ciliates were thought to consume the *Chromatium* population within a week (MATSUYAMA and MOON, 1997). The size of the bacterium, $3-4 \times 3-6 \mu\text{m}$, seems ideally suited to the filter-feeding of *Euplotes* species (FENCHEL, 1987).

However, *Chromatium* sp. as well as a coexisting bacterium, *Macromonas* sp., were able to maintain dense populations at the upper boundary of the H₂S layer throughout the years, although they appeared to grow very slowly in the lake (MATSUYAMA, 1988, 1993).

Although the presence of ciliates in many anoxic waters has been described (FENCHEL and FINLAY, 1995), very little work has been done to elucidate the ecological role of these ciliates in such waters. MASSANA and PEDRÓS-ALIÓ (1994) studied the dynamics of two populations of anaerobic ciliates of *Plagiopyla* sp. and *Metopus* sp., and of their potential prey of heterotrophic and phototrophic bacteria in Lake Ciso. They showed that the impact of predation by the *Plagiopyla* population on bacterioplankton was insignifi-

cant, being less than 0.1% of the bacterial biomass consumed per day.

This study aims to elucidate the feeding pressure from *Euplotes* sp. on the *Chromatium* population in Lake Kaiike.

Lake Kaiike (surface : 0.15 km², max. depth : 11.6 m) is stratified ; the seawater layer below 2–3 m depth is consistently covered by less saline water, and the water below 4–5 m contains a significant amount of H₂S (~36 mg S l⁻¹) throughout the year.

Field observations were done at the deepest point of the lake on 24 October 1997. Water samples from different depth were taken with a 3-l VAN DORN bottle. Biological samples were immediately fixed by adding a few drops of neutralized formalin with a pipette (final concentration : 0.3%) and poured into 100-ml screw-capped bottles for transfer to the laboratory under cool and dark conditions. In the laboratory, the samples were centrifuged (20 min, 1,000 × g). One ml of centrifuged sample was transferred into a counting cell which consisted of a rectangular rim closely fixed to a slide glass with an inside dimension of 50 × 20 mm and 1 mm deep. A coverglass was gently placed on the rim (If laboratory-cultured cells of *Euplotes* sp. were killed with formalin and a coverglass was placed directly on them, the cells began to swell and finally ruptured (MATSUYAMA and MOON, 1997)). However, such deformed cells were hardly encountered in the wild strains. Ciliates in the counting cells were enumerated using a low power stereoscopic microscope (Nikon SMZ-10). The numbers of *Chromatium* sp. and *Macromonas* sp. were determined using a THOMA hemocytometer. Ciliate and bacteria counts were performed within 48 hr after collection. Apparatus and methods for the measurements of environmental factors were the same as those described in MATSUYAMA and SHIRO-UZU (1978) and MATSUYAMA (1995).

Figure 1 shows the vertical profiles of two dominating bacteria and ciliates as well as some environmental factors. Members of Copepoda and Spirotrichia were also found, but they were less than 1,000 ind. l⁻¹ at any depth.

Chromatium sp. reached a maximum of 1×10^6 cells ml⁻¹ at 5.5 m where H₂S began to appear (1.5 mg S l⁻¹). *Euplotes* sp. and *Tracheloraphis* sp. reached the maximum abundances of 1.2×10^4 cells and 2.3×10^4 cells l⁻¹ at 5 m, respectively. The numbers of both species decreased below this depth.

To elucidate the potential feeding activity of *Euplotes* sp. on *Chromatium* sp., the ciliate was cultured by the addition of elevated amounts of the bacterial cells. *Euplotes* sp., which had been isolated from Lake Kaiike and maintained in a glass-fiber (Whatman GF/C) filtered seawater by adding living cells of *Chromatium* sp. as a sole food source, was used for the experiment. To consumption of bacterial cells from the culture, the ciliates were starved for a month, then uniformly distributed in a series of polystyrene flasks (Corning 25 cm² Tissue Culture Flask, 75 ml-capacity), and filled with glass-fiber filtered seawater. Living cells of *Chromatium* sp., which had been grown in the inorganic medium of PFENNIG (1965), were added to the flasks at different abundances from 5×10^5 cells ml⁻¹ to 1×10^7 cells ml⁻¹. The flasks were tightly closed with silicon rubber stoppers and incubated in

Fig. 1. Vertical profiles of *Chromatium* sp. (A), *Macromonas* sp. (B), *Euplotes* sp. (C), *Tracheloraphis* sp. (D), together with photosynthetically available radiation (PAR) in Lake Kaiike (24 October 1997). Vertical profiles of some environmental factors are shown in the left column. Profiles between 2–6 m were based on 0.5-m interval samplings. Otherwise on 1-m intervals. PAR at 5.5 m was $2.4 \mu\text{mole m}^{-2} \text{sec}^{-1}$ (at noon).

the dark at a constant temperature of 25°C . Duplicate flasks were used for the growth experiment at respective levels of additional bacteria. Growth of the ciliates were measured by directly counting cell numbers in the flasks without subsampling (MATSUYAMA and MOON, 1997); i.e., after the flask was gently agitated to distribute ciliates uniformly, the flask was mounted on the stage of a Nikon SMZ-10 microscope. Ciliates in the field of vision (usually 0.2 cm^2) were enumerated by slowly scanning the focal plane from the bottom to the top layer (2 cm deep). Counting was repeated 3 times for each flask.

Figure 2-A shows the growth of *Euplotes* sp. at different concentrations of *Chromatium* sp. The ciliate immediately began to grow. Their growth rate, simply obtained from the increase in their numbers during the first 3 days of incubation, increased with the level of bacterial cells added. A maximum rate of 1.5 day^{-1} was obtained at a level of $7\text{--}10 \times 10^6 \text{ cells ml}^{-1}$. A determination of dissolved O_2 at the end of incubation by the WINKLER method ($13\text{--}15 \text{ mg O}_2 \text{ l}^{-1}$) indicated that ciliate growth was continuous throughout the aerobic condition.

Similar relationships between the growth rate and abundance of bacterial prey were reported for the phagotrophic chrysomonad, *Paraphylosomonas vestita* (FENCHEL, 1987) and *Plagiopyla nasuta* and *Metopus* sp. (MASSANA *et al.*, 1994).

Maximum yields of the ciliates attained in each flask (Y) were linearly

Fig. 2. A : Growth of *Euplotes* sp. added with different amounts of *Chromatium* sp. (a : 5×10^5 , b : 1×10^6 , c : 2×10^6 , d : 3×10^6 , e : 5×10^6 , f : 7×10^6 , g : 1×10^7 cells ml⁻¹). Vertical lines represent standard deviation. B : Maximum yields of *Euplotes* sp. attained at different levels of *Chromatium* cells added. A linear relationship below the bacterial addition of 7×10^6 cells ml⁻¹ indicates that a single ciliate cell needs about 5000 bacterial cells for cell division.

proportional to the bacterial cells added (X) when the bacterial addition was below the level of 7×10^6 cells ml⁻¹ (Fig. 2-B), and the following relation was suggested :

$$\Delta Y : \Delta X = 1.89 \times 10^{-4} : 1.00$$

A single ciliate cell was considered to need about 5,000 *Chromatium* cells for cell division, and this estimation was roughly consistent with that reported in our previous study (MATSUYAMA and MOON, 1997).

The specific growth rate of ciliates at the bacterial level of 1×10^6 cells ml⁻¹ (=maximum abundance in the lake on 24 October 1997) was estimated to be around 0.6 day⁻¹, whereas the *in situ* growth rate of *Chromatium* sp. had been put at 0.17 day⁻¹ (MATSUYAMA, 1993). Thus, the *Euplotes* population at 5.5 m depth (4,000 cells l⁻¹ on 24 October 1997) was suggested to consume the entire *Chromatium* population within a week unless inhibited by some environmental factors affecting their feeding activity.

Figure 3 shows the growth of *Euplotes* sp. in a series of Corning flasks filled with glass-fiber filtered seawater. Living cells of *Chromatium* sp. were added

Fig. 3. Growth of *Euplotes* sp. at different concentrations of H₂S (A : 0 mg, B : 0.1–10 mg, C : 6–20 mg, D : 13–30 mg S l⁻¹) after addition of *Chromatium* sp. (5.2×10^6 cells ml⁻¹). H₂S was added as neutralized H₂S solution at 0, 1, 3, 5, 7 and 9 days of incubation. Vertical lines represent standard deviation. Broken lines denote the abundance of *Chromatium* sp. Initial abundance of *Euplotes* sp. was 97 cells ml⁻¹.

to the flasks to give a final concentration of 5.2×10^6 cells ml⁻¹, and different amounts of neutralized H₂S solution were added (0–30 mg S l⁻¹). The flasks were tightly closed with silicon rubber stoppers and incubated in the dark at a constant temperature of 25 °C. At 1, 3, 5, 7 and 9 days of incubation, 0.2 ml of the culture was withdrawn by syringe through the silicon rubber stoppers to measure the abundance of *Chromatium* sp. and H₂S concentration by the spectrophotometric method of CLINE (1969) using *N,N*-dimethyl-*p*-phenylenediamine sulfate and ferric chloride. After the H₂S measurement, neutralized H₂S solution was injected into each flask to bring the H₂S concentration to the initial levels (B : 10 mg, C : 20 mg, D : 30 mg S l⁻¹).

Without the addition of H₂S (Fig. 3-A), the ciliates grew at a rapid rate

of 0.4 day^{-1} for the first 5 days, and bacterial numbers rapidly decreased. At 9 days of incubation, the ciliates reached $900 \text{ cells ml}^{-1}$, while the bacterial abundance dropped below $10^4 \text{ cells ml}^{-1}$.

An addition of H_2S to a level of 10 mg S l^{-1} (Fig. 3-B) markedly inhibited both the growth of the ciliate as well as the decrease in bacterial abundance, indicating that the feeding activity on the bacterial cells was completely suppressed. These findings were markedly different from those reported for anaerobic *Plagiopyla nasuta* by MASSANA *et al.* (1994). They showed that H_2S concentrations between 0 and 32 mg S l^{-1} did not affect feeding activity, but concentrations greater than 64 mg S l^{-1} inhibited it.

However, the abundance of *Euplotes* sp. remained at the initial level until the end of incubation (11 days), and most of the ciliates were viable throughout the incubation since they immediately began to grow when the culture was put in an aerobic condition. The ciliates seemed to grow aerobically, but only survived in the H_2S -containing condition as long as it was below 10 mg S l^{-1} . This was consistent with the findings of FENCHEL and FINLAY (1995). They showed that *Euplotes* sp. survived when H_2S was less than 160 mg S l^{-1} . At a level of 160 mg S l^{-1} , its survival time (50% survival) was about 24 hr.

At elevated levels of H_2S addition (Fig. 3-C, D), both *Euplotes* sp. and *Chromatium* sp. decreased in abundance with incubation. It must be noted that even at a more elevated levels of H_2S up to 60 mg S l^{-1} (not shown here), motile but slowly crawling ciliate cells which contained bacterial cells inside their bodies were observed for several days. The ciliates seemed to ingest the bacterial cells even in the presence of a significant amount of H_2S , which probably exceeded the tolerable level. In Lake Kaiike, *Euplotes* sp. seems to penetrate into the H_2S layer to feed on the bacterial cells and then return to the overlying layer to grow.

To estimate the predatory impact of *Euplotes* sp. on the *Chromatium* population in Lake Kaiike, the ciliate was grown by adding with limited numbers of bacterial cells. Figure 4-A shows the growth of the ciliate in the Corning flasks, to which different amounts of *Chromatium* sp. were added at 0, 1, 4, 6, and 8 days of incubation. At all levels of bacterial addition, the ciliates had consumed the bacterial cells completely before the next addition.

Without the addition of bacterial cells (a), the ciliates gradually decreased in number. However, they increased even at a minimum rate of bacterial addition ($1 \times 10^4 \text{ cells ml}^{-1} \text{ day}^{-1}$) (b). Relationships between the mean specific growth rate of ciliates (simply obtained from the change in numbers during incubation) and the rate of bacterial addition (Fig. 4-B) showed that the ciliate population (80 cells ml^{-1}) needed about 5100 bacterial cells daily to sustain the biomass (a single ciliate cell needed about 60 bacterial cells per day). Comparison of this rate with that necessary for cell division (about 5000 bacterial cells per single ciliate cell, Fig. 2-B) indicated that a larger part of energy was involved in macromolecular synthesis for cell division and only a very small fraction of the energy budget was spent on work to maintain cell integrity, as already stated by FENCHEL (1987).

Fig. 4. Growth of *Euplotes* sp. at different rate of addition of *Chromatium* sp. (a: 0 cells, b: 1×10^4 cells, c: 2×10^4 cells, d: 7×10^4 cells, e: 1×10^5 cells $\text{ml}^{-1} \text{ day}^{-1}$). Bacterial cells were added at 0, 1, 4, 6 and 8 days of incubation. Vertical lines represent standard deviation. B: Specific growth rate of *Euplotes* sp. simply obtained from the change of the abundance during the incubation. Growth rate was thought to become zero when the bacterium was added at a rate of about 5,060 cells $\text{ml}^{-1} \text{ day}^{-1}$, indicating that a minimum requirement of bacterial cells was about 60 cells day^{-1} for a single ciliate cell.

If this rate of *ca.* 60 bacterial cells day^{-1} for a single ciliate cell is accepted as a minimum requirement of *Euplotes* sp. for the bacterium, the ciliate population in an upper water column of Lake Kaiike from 1 cm^2 lake surface down to 6 m depth (2,300 cells cm^{-2} on 24 October 1997) was suggested to feed on 1.5×10^5 cells of *Chromatium* sp. daily to sustain the biomass. However, the actual biomass of the bacterium in the same water column amounted to 6×10^7 cells cm^{-2} . The impact of predation by *Euplotes* sp. seemed to exert no significant effect on the *Chromatium* population as long as the feeding activity of the ciliates was limited to sustaining the population. It is apparent that dissolved H₂S in the habitat of *Chromatium* sp., which was usually less than 5 mg S l^{-1} at the bacterial peak (but always present) plays a decisive role in protecting the bacterium from the feeding pressure of *Euplotes* sp.

Figure 5 shows microphotographs of *Tracheloraphis* sp. (A) and a member

Fig. 5. Microphotographs of an anterior part of *Tracheloraphis* sp. (A) and a member of *Spirotrichia* (B) collected from Lake Kaiike. Both species included many cells of *Chromatium* sp. and *Macromonas* sp. in their bodies.

of *Spirotrichia* (B) collected from Lake Kaiike. They contained many *Chromatium* sp. and *Macromonas* sp. cells inside their bodies. Members of *Tracheloraphis* are believed to be an interstitial species (FENCHEL, 1987). This figure shows the importance of these bacteria in the planktonic food webs in Lake Kaiike.

References

- CLINE, J. D. (1969) : Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnol. Oceanogr.*, 14 : 454-458.
- FENCHEL, T. (1987) : *Ecology of Protozoa*. Science Tech Publishers, Madison/ Springer-Verlag, Berlin.
- FENCHEL, T. and B. J. FINLAY (1995) : *Ecology and Evolution in Anoxic Worlds*. Oxford University Press, Oxford.

- MASSANA, R. and C. PEDRÓS-ALIÓ (1994) : Role of anaerobic ciliates in planktonic food webs : Abundance, feeding, and impact on bacteria in the field. *Appl. Environ. Microbiol.*, 60 : 1325-1334.
- MASSANA, R. C. STUMM and C. PEDRÓS-ALIÓ (1994) : Effects on temperature, sulfide, and abundance on growth and feeding of anaerobic ciliate. *Appl. Environ. Microbiol.*, 60 : 1317-1324.
- MATSUYAMA, M. and E. SHIROUZU (1978) : Importance of photosynthetic sulfur bacteria, *Chromatium* sp. as an organic matter producer in Lake Kaiike. *Jpn. J. Limnol.*, 39 : 103-111.
- MATSUYAMA, M. (1988) : Ecology of a large-celled phototrophic bacterium densely populating the upper boundary of H₂S water of Lake Kaiike. *Bull. Jpn. Soc. Microbiol. Ecol.*, 3 : 35-46. (in Japanese)
- MATSUYAMA, M. (1993) : Frequency of dividing cells of *Chromatium* sp. blooming in Lake Kaiike as an estimate of growth rate. *Jpn. J. Limnol.*, 54 : 137-140.
- MATSUYAMA, M. (1995) : Upward movement of *Chromatium* sp. in the H₂S layer of Lake Kaiike causing a bloom at its upper boundary. *Jpn. J. Limnol.*, 56 : 205-209.
- MATSUYAMA, M. and S-W. MOON (1997) : Feeding of a hypotrichous ciliate swimming in the H₂S layer of Lake Kaiike on *Chromatium* sp.. *Jpn. J. Limnol.*, 58 : 61-67.
- PFENNIG, N. (1965) : Anreicherungskulturen für rote und grüne Schwefelbakterien. *Zbl. Bakt. 1. Abt. Orig. Suppl. 1* : 179-189, 503-504.

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貝池でブルームを形成している *Chromatium* sp. の下毛類 繊毛虫による捕食圧への防御としての周辺に溶存する H_2S

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摘 要

貝池から分離した下毛類繊毛虫 *Euplotes* sp. は *Chromatium* sp. (光合成細菌) を重要な餌として捕食する。1997 年 10 月 24 日、湖でのこれら捕食者、被食者の最高出現数は、水深 5 m および H_2S が出現しはじめる 5.5 m で、それぞれ 1.2×10^4 細胞 l^{-1} 、 1×10^6 細胞 ml^{-1} に達した。好氣的環境で、繊毛虫は 1 細胞あたり約 5,000 細胞の細菌を捕食分裂し、急速に生長した。一方、繊毛虫は個体群を維持するのに 1 細胞 1 日あたり約 60 細胞の細菌を必要とすると推定された。 H_2S が存在すると繊毛虫は細菌捕食活動を停止した。*Chromatium* sp. の生息する周辺に溶存する H_2S は *Euplotes* sp. による捕食圧への防御となると考えられた。