Note

Dissolved H$_2$S around Chromatium sp. Blooming in Lake Kaiike Protects against Feeding Pressure by a Hypotrich Ciliate

Michiro MATSUYAMA and Sang-Wook MOON

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 x 10$^4$ cells l$^{-1}$ at 5 m depth and 1 x 10$^6$ cells ml$^{-1}$ at 5.5 m depth, respectively where H$_2$S 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 H$_2$S, feeding activity of the ciliate on bacterial cells was completely suppressed. Dissolved H$_2$S around the habitat of Chromatium sp. was considered to protect against feeding pressure by Euplotes sp.

Key words: Dissolved H$_2$S, 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 H$_2$S 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 x 3-6 $\mu$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 H$_2$S 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 3-1 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 × 10⁶ 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 × 10⁴ cells and 2.3 × 10⁴ 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 × 10⁵ cells ml⁻¹ to 1 × 10⁷ cells ml⁻¹. The flasks were tightly closed with silicon rubber stoppers and incubated in
Dissolved H₂S Protects Chromatium sp. from Ciliate Feeding

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 μmole m⁻² 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 cm²) 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 7-10×10⁶ cells ml⁻¹. A determination of dissolved O₂ at the end of incubation by the Winkler method (13-15 mg O₂ 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: \(5 \times 10^5\), b: \(1 \times 10^6\), c: \(2 \times 10^6\), d: \(3 \times 10^6\), e: \(5 \times 10^6\), f: \(7 \times 10^6\), g: \(1 \times 10^7\) 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 \(7 \times 10^6\) cells ml\(^{-1}\) indicates that a single ciliate cell needs about 5000 bacterial cells for cell division.

The specific growth rate of ciliates at the bacterial level of \(1 \times 10^6\) 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 proportional to the bacterial cells added (X) when the bacterial addition was below the level of \(7 \times 10^6\) 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).
Dissolved H₂S Protects Chromatium sp. from Ciliate Feeding

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 × 10⁶ 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.

Without the addition of H₂S (Fig. 3-A), the ciliates grew at a rapid rate to the flasks to give a final concentration of 5.2 × 10⁶ 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 cells ml$^{-1}$, while the bacterial abundance dropped below $10^4$ cells ml$^{-1}$.

An addition of H$_2$S 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$_2$S 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$_2$S-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$_2$S 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$_2$S 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$_2$S 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 the significant amount of H$_2$S, which probably exceeded the tolerable level. In Lake Kaiike, *Euplotes* sp. seems to penetrate into the H$_2$S 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 ($1 \times 10^4$ cells ml$^{-1}$ 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).
Dissolved H₂S Protects *Chromatium* sp. from Ciliate Feeding

Fig. 4. Growth of *Euplotes* sp. at different rate of addition of *Chromatium* sp. (a: 0 cells, b: 1×10⁴ cells, c: 2×10⁴ cells, d: 7×10⁴ cells, e: 1×10⁵ cells ml⁻¹ 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 ml⁻¹ 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 cm² lake surface down to 6 m depth (2,300 cells cm⁻² on 24 October 1997) was suggested to feed on 1.5×10⁵ cells of *Chromatium* sp. daily to sustain the biomass. However, the actual biomass of the bacterium in the same water column amounted to 6×10⁷ 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 *Macrononas* sp. in their bodies.

of *Spirotrichia* (B) collected from Lake Kaiike. They contained many *Chromatium* sp. and *Macrononas* 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**


Dissolved H$_2$S Protects *Chromatium* sp. from Ciliate Feeding


Michiro MATSUYAMA : Faculty of Fisheries, Nagasaki University, 1-14, Bunkyo-machi, Nagasaki 852-8521 (松山通郎：〒852-8521 長崎市文教町1-14，長崎大学水産学部)

Sang-Wook MOON : Faculty of Fisheries, Nagasaki University, 1-14, Bunkyo-machi, Nagasaki 852-8521 (文　尚郁：〒852-8521 長崎市文教町1-14，長崎大学水産学部)

(Received : 6 February 1998 ; Accepted : 6 October 1998)
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 H₂S

松山通郎・文 尚郁

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
飼として捕食する。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 H₂S が出現はじめる5.5 mで、それぞれ 1.2×10⁴細胞 l⁻¹、1×10⁶細胞
ml⁻¹に達した。好気的環境で、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。H₂S 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 H₂S は Euplotes sp.による捕食圧への防御と
なると考えられた。