

Effects of an Artificial Breakwater on the Distributions of Planktonic Microbial Communities

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Abstract – The summer distributions of planktonic microbial communities (heterotrophic and phtosynthetic bacteria, phtosynthetic and heterotrophic nanoflagellates, ciliate plankton, microphytoplankton) were compared between inner and outer areas of Lake Sihwa, divided by an artificial breakwater, located on the western coast of Korea, in September 2003. The semienclosed, inner area was characterized by hyposaline surface water (<17 psu), and by low concentrations of dissolved oxygen (avg. 0.4 mg L⁻¹) and high concentrations of inorganic nutrients (nitrogenous nutrients >36 µM, phosphate >4 µM) in the bottom layer. Higher densities of heterotrophic bacteria and nanoflagellates also occurred in the inner area than did in the outer area, while microphytoplankton (mainly diatoms) occurred abundantly in the outer area. A tiny tintinnid ciliate, Tintinnopsis nana, bloomed into more than 10⁶ cells L⁻¹ at the surface layer of the inner area, while its abundance was much lower $(10^3-10^4 \text{ cells L}^{-1})$ in the outer area of the breakwater. Ciliate abundance was highly correlated with heterotrophic bacteria (r = 0.886, p < 0.001) and heterotrophic flagellates (r = 0.962, p < 0.001), indicating that rich food availability may have led to the T. nana bloom. These results suggest that the breakwater causes the eutrophic environment in artificial lakes with limited flushing of enriched water and develops into abundant bacteria, nanoflagellates, and ciliates.

Key words – bloom, breakwater, ciliates, heterotrophic bacteria, nanoflagellates, microphytoplankton

1. Introduction

Estuaries in general are highly productive aquatic habitats. They receive large amounts of nonliving organic matter from inflowing waters, and consequently, the primary consumers

are largely saprovores and detritivores (Barnes 1974). Estuaries are recognized as two main types, open and closed. In the former, microbial production is typically low, due to a short residence time of seawater. In contrast, large inputs of nutrients and longer residence time of seawater in enclosed estuaries often stimulate microbial blooms (Laybourn-Parry 1992).

Artificial breakwaters preventing water exchange between the protected area and open waters generally lead to chronic eutrophication in the protected area. For example, Toulon Bay on the north coast of the Mediterranean Sea is divided into a heavily polluted small bay inside and a less-polluted outer bay by an artificial breakwater. The biomass and production of phytoplankton and the structure of the zooplankton community between the two bays are very different (Jamet et al. 2001; Richard and Jamet 2001; Despiau et al. 2002). In a recent report, toxic algal booms are observed inside the inner breakwater of the Southern California coastal waters (Schnetzer et al. 2006). The distribution of plankton community was compared between the inner and outer areas divided by an artificial breakwater in Guryongpo Harbor on the southeastern coast of Korea (Kim et al. 2007). The results indicate that the breakwater induces the eutrophication in the inner area and provides a suitable condition for nanoflagellate blooms to serially trigger opportunistic increase of oligotrich ciliates.

Lake Sihwa is an artificial estuarine lake created by the entrapment of Yellow Sea water with the construction of a long dyke offshore of Ansan City, Korea in 1997. The deterioration in water quality of Lake Sihwa has been

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discussed as a hot issue for the last decade in Korea (Han and Park 1999; Park et al. 2003; Suh et al. 2004). Water quality in Lake Sihwa is more seriously deteriorated in summer than in other seasons. Aquatic microorganisms in the semi-enclosed inner area were analyzed to estimate the effect of the artificial construction in previous investigations (Choi et al. 1997b; Park and Huh 1997; Shin et al. 2000a). Microphytoplankton and mesozooplankton have been understood to be the major planktonic components. Small flagellates and bacteria among small-sized microbial components were also analyzed and recorded abundantly in summer (Choi et al. 1997a; Shin et al. 2000b). It is generally recognized that the function of microbial loop is more active, especially in summer; bacterial growth is rapid and its energy transfers to nanoplankton via flagellates to ciliates, top predators in the microbial food web. However, the lack of simultaneous consideration of a set of microbial components including bacteria, nanoflagellates and ciliates made the reasonable information limited in these previous works. Especially, ciliate plankton has been excluded from the microbial components in the previous studies in Lake Sihwa.

We investigated breakwater-related modifications to the communities of natural bacteria, nanoflagellates, ciliates, and microphytoplankton in a semi-enclosed lake. We report an enormous bloom of a tintinnid species in the ciliate community, and discuss the critical factor inducing the bloom and the potential effects of an artificial breakwater on the distributions of planktonic microbial communities.

2. Materials and Methods

Study site

The study site (Fig. 1) was an artificial estuary, Lake Sihwa, located on the west coast of Korea (37°2′–37°4′N, 126°5′–126°8′E). The sampling area was divided into two areas by a 12.7 km long breakwater. The semi-enclosed inner area has limited water exchange through a sluice gate and serious eutrophication due to an excessive inflow of polluted water from surrounding cities and industrial complexes. The outer area opens toward the Yellow Sea between Korea and China, and thus is less influenced by terrestrial discharge. Water depth ranged from 6.5 to 15 m in the inner area and 6.7 to 16.3 m in the outer area. Total volume of the lake is about 378 × 10⁸ m³ and surface area is about 61 km².

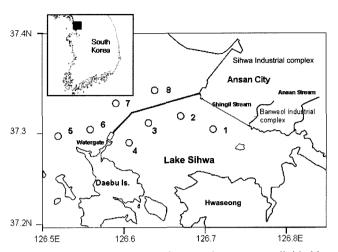


Fig. 1. Sampling stations in the inner and outer areas divided by of a breakwater in Lake Sihwa.

Sampling and analytical methods

Water samples were collected from surface and bottom layers at eight locations using Van Dorn bottles, on 8 September 2003 (Fig. 1). Microbial components and inorganic nutrients and chl-*a* concentrations were analyzed, and water temperature and salinity were recorded using a conductivity-temperature-depth (CTD) recorder. Size fractionation of chl-*a* was performed using a 20-µm mesh and filter paper of 3-µm pore size. Each size (<3 µm, 3–20 µm, >20 µm) was determined using a spectrophotometer, according to Parsons *et al.* (1984). Dissolved oxygen was determined by the Winkler method, and inorganic nutrients (NO₂-, NO₃-, NH₄-, PO₄-, SiO₄-) were analyzed with an autoanalyzer (Lachat Quickchem, Lachat Instruments, Milwaukee, WI, USA) in the inner area.

Microorganisms

Bacteria and nanoflagellates were fixed immediately in buffered formaldehyde at a final concentration of 1%, and filtered onto black Nucleopore filters (0.2 μm) stained with DAPI for bacteria (Porter and Feig 1980) and DAPI/primulin for nanoflagellates (Caron 1983). The autofluorescence of photosynthetic bacteria and nanoflagellates was distinguished under blue light excitation. Subsamples of microplankton (*e.g.* ciliates, diatoms, and dinoflagellates) were fixed immediately in 2% (v/v) Lugol's solution. The microplankton cells were enumerated under a microscope (Zeiss Axioskop 2; Carl Zeiss, Jena, Germany) using a Sedgwick–Rafter chamber, and identified at high magnification (× 400–1,000) using a wet mounted slide glass. The diversity

index of microphytoplankton (*H*) was calculated using Shannon–Weaver's (1949) formula $H = -\sum n_i N^{-1} \log_2(n_i N^{-1})$, where *n* is the cell abundance of the *t*th species and $N = n_i$.

3. Results

Hydrological conditions

The temperature range was 23.4–25.5°C inside and 22.8–25.0°C outside the breakwater, i.e., slightly higher inside (Fig. 2A). The temperature was higher at the surface layers than at the bottom layers in both areas divided by an artificial breakwater. Surface salinity was considerably lower in the inner area (1.6–16.3 psu) than in the outer area (25.1–26.3 psu). The lowest salinity occurred at the surface layer of Station (Stn.) 1 under the influence of freshwater runoff (Fig. 2B). Dissolved oxygen and inorganic nutrient concentrations in the inner area were extremely different between surface and bottom layers. Dissolved oxygen was 7.8–10.4 mg L⁻¹ at the

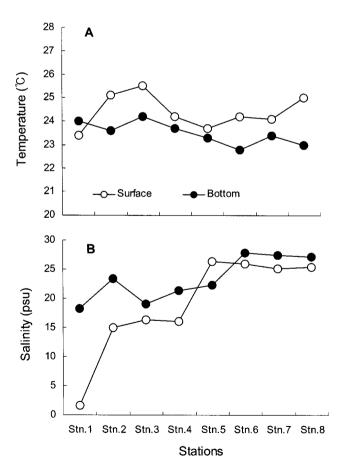


Fig. 2. Water temperature (A) and salinity (B) at the surface and bottom layers in Lake Sihwa.

surface layer, while it was very low (0.3–0.6 mg L^{-1}) at the bottom layer. The bottom layer also had extremely high concentrations of dissolved nitrogenous nutrients (>36 μ M), phosphate (>4 μ M), and silicate (>28 μ M). The highest concentration of dissolved nitrogenous nutrient was recorded at the surface layer of Stn. 1, which had the lowest salinity (Fig. 3).

Size-fractionated chlorophyll-a

A high concentration (>30 μg L⁻¹) of chl-*a* in surface layers was observed at the inner stations, Stns. 2, 3 and 4 (Fig. 4A), but this largely decreased to between 10 and 20 μg L⁻¹ at the bottom layers (Fig. 4B). The size composition of chl-*a* was different between the inner and outer areas. In the inner area, an average 68% of total chl-*a* consisted of nano fractions; the remainder (32% on average) was made up of pico and micro fractions. In the outer area, total chl-*a* concentrations were about 20% of those in the inner area, and the size composition had an opposite trend, primarily consisting of micro fractions (58% on average), whereas nano and pico fractions contributed about 37 and 5%, respectively.

Microphytoplankton

The cell abundance of microphytoplankton ranged from 1.7×10^4 to 9.8×10^5 cells L⁻¹ in the inner area and from 4.8×10^5 to 1.7×10^6 cells L⁻¹ in the outer area. Highly abundant standing crops of more than 10^6 cells L⁻¹ were recorded at the surface layers at Stns. 5 and 6, but no apparent difference was observed between the two areas in the bottom layers. A notably lower abundance occurred at the surface layer of Stn. 1, which is influenced by freshwater runoff (Fig. 5).

Diatoms dominated the micro fraction. The most abundant species in the inner area was *Cyclotella littoralis* (>57% of total abundance; Fig. 6). A small naked dinoflagellate, *Gyrodinium* sp., was also abundant, occupying 15-33% of the total abundance at Stns. 2-4. In the outer area, four diatom species, *Chaetoceros debilis, Eucampia zodiacus*, *Ch. pseudocurvisetus*, and *Ch. socialis*, were dominant. Among them, *Ch. debilis* and *E. zodiacus* were the most dominant species (50-70% of total abundance).

The diversity index of the microphytoplankton community also showed a clear difference between the two areas, being higher value (1.81 on average) at the surface and bottom layers in the outer area while exhibiting low

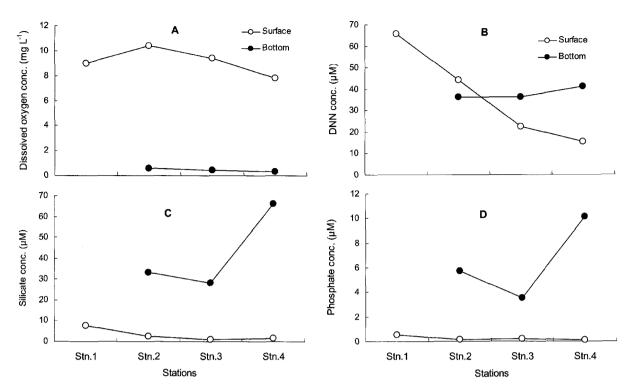


Fig. 3. Concentrations of dissolved oxygen (A), dissolved nitrogenous nutrients (B; DIN, NO₃⁺+NO₂⁺+NH₄⁺), phosphates (C; PO₄⁻), and silicate (D; SiO₄⁻) in the inner area of Lake Sihwa.

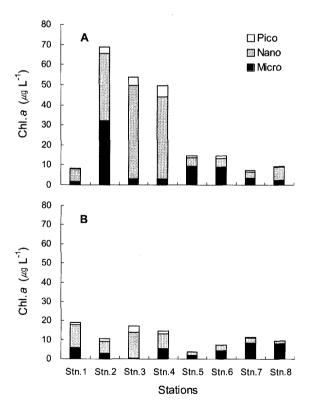


Fig. 4. Concentrations of total chlorophyll-a made up of picoplankton (>3 μ m), nanoplankton (3–20 μ m), and microplankton (>20 μ m) at the surface (A) and bottom (B) layers.

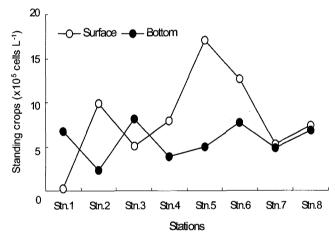


Fig. 5. Standing crops of microphytoplankton at the surface and bottom layers.

diversity (0.84 on average) in the inner area (Fig. 7).

Bacteria and nanoflagellates

The cell abundance of heterotrophic bacteria (HB) in the inner area $(2.4-14.1\times10^6\text{cells mL}^{-1})$ was 10 times higher than that of the outer area. The maximum abundance $(1.4\times10^7\text{ cells mL}^{-1})$ occurred at the surface layer of Stn. 3, and decreased markedly with depth to 50% at the bottom

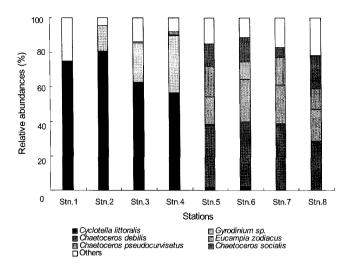


Fig. 6. Relative abundances of dominant microphytoplankton at the surface layer.

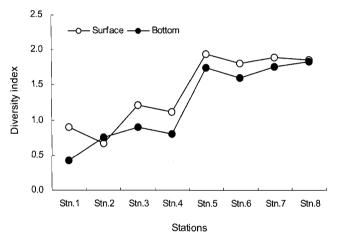


Fig. 7. Diversity index of the microphytoplankton community at the surface and bottom layers.

layer. HB abundance was uniformly low in the outer area (Fig. 8). Similarly, the cell abundances of three other microbial components, photosynthetic bacteria (PB), heterotrophic nanoflagellates (HNF), and photosynthetic nanoflagellates (PNF), were high at the surface layer but low at the bottom layer in the inner area, but uniformly low in the outer area. The peak abundance of PNF (7.0×10⁵ cells mL⁻¹) at the surface layer of Stn. 4 inside was prominent (Fig. 8A).

Ciliates

An extremely high abundance of ciliates $(1.6-2.2\times10^6)$ cells L⁻¹ occurred at the surface layers in the inner stations (Stns. 2-4). In all other samples, the abundance was 0.1-1.0% of these values (Fig. 9A). A notably low

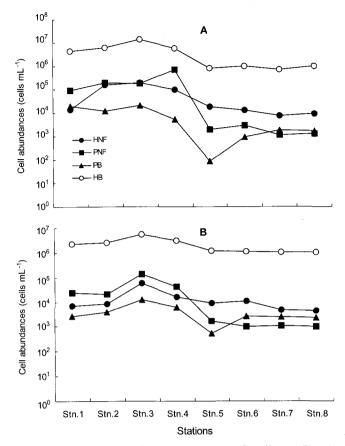


Fig. 8. Abundances of heterotrophic nanoflagellates (HNF), photosynthetic nanoflagellates (PNF), photosynthetic bacteria (PB), and heterotrophic bacteria (HB) at the surface (A) and bottom (B) layers.

abundance was observed at the surface layer of Stn. 1, indicating a similar trend between ciliate abundance and salinity (cf., Fig. 2).

Among 12 identified ciliate species, *Tintinnopsis nana*, a tiny loricate ciliate, was overwhelmingly dominant (1.8×10⁶ cells L⁻¹ on average) and generally accounted for more than 97% of the total ciliate abundance at the surface layers of Stns. 2-4 in the inner area (Fig. 9B). An oligotrich ciliate, *Strombidium* sp., and an autotrophic ciliate, *Mesodinium rubrum*, sequentially followed *T. nana* as the second and third most abundant components, comprising about 1.0% (1.8×10⁴ cells L⁻¹ on average) and 0.7% (1.2×10⁴ cells L⁻¹ on average) of the total ciliate abundance, respectively (Figs. 9B, 9C).

4. Discussion

Estuaries tend to sustain higher abundances, but less

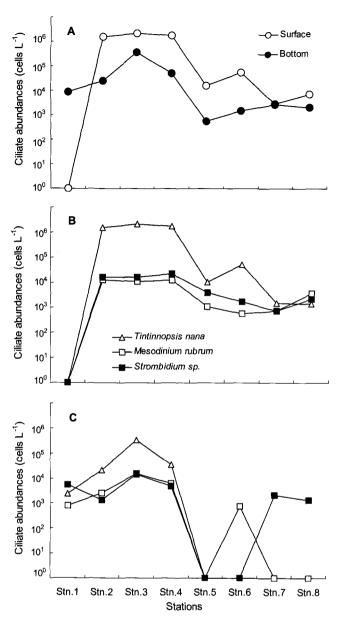


Fig. 9. Ciliate abundances at the surface and bottom layers (A) and dominant ciliate species at the surface (B) and bottom (C) layers.

diversity, of protozooplankton compared to deep and open environments (Burkill 1982) because of the relatively few species that can adapt to marked salinity gradients (Mouny *et al.* 1998; Muylaert and Sabbe 1999). In addition, large eutrophic loadings accumulate, especially in semi-enclosed estuaries as in the present study, because water exchange is hindered, resulting in algal blooms. In Lake Sihwa before construction of the breakwater, the average primary productivity was 3.98 mg C m⁻² day⁻¹ (KOWACO 1993). Year-round algal blooms broke out after a breakwater

construction and high primary production (3,972 mg C m⁻² day⁻¹ on average) occurred, and was about 1,000-fold higher value than the production before constructing breakwater (Choi *et al.* 1997b). Our results indicated a low diversity of phytoplankton in the inner area divided by the breakwater in Lake Sihwa (Fig. 7). A brackish-water diatom species, *Cyclotella littoralis*, overwhelmingly dominated in the inner area, while several species of relatively large diatoms, such as three *Chaetoceros* species and *Eucampia zodiacus*, were dominant in the outer area. This trend was also reflected in the size-fractionated chl-*a* concentration; nano fractions caused by small species in the inner area and micro fractions by larger ones in the outer area comprised the major portion of the total chl-*a* concentration.

Lake Sihwa has a large input of organic detritus, which accumulates on the bottom (Suh *et al.* 2004). As this organic matter degrades, it may lead to the anoxic conditions at the bottom layer reported in the present study, and as suggested by Choi *et al.* (1997b). The extreme difference in nutrient concentration between the surface and bottom layers showed the opposite trend to the dissolved oxygen. Phosphate (0.13–0.19 μ M) and silicate (1.1–2.4 μ M) concentrations were much lower at the surface layer than at the bottom layer, possibly reflecting nutrient uptake by the phytoplankton at the surface layer.

Salinity was not a critical factor influencing ciliate abundance in an estuary in Maine (Sanders 1987). However, the sharp drop of salinity (to 1.6 psu) at the surface layer of Stn. 1 in Lake Sihwa was likely responsible for the extremely low abundance of ciliates and other plankton (except PB and PNF). PB did not decrease sharply at Stn. 1, and PNF increased.

Ciliates usually consume bacteria and nanoflagellates (Sherr and Sherr 1987; Sherr *et al.* 1989), and Burkill (1982) reported a seasonal trophic relationship between nanophytoplankton as prey and ciliates as predators. While our study was conducted only in summer, strong relationships were found between ciliates and heterotrophic bacteria (r = 0.886, p < 0.001), and between ciliates and heterotrophic nanoflagellates (r = 0.962, p < 0.001) (Fig. 10), indicating that ciliates can be a major predator of the smaller microbial communities in the study area. The prey resources for ciliate might be sustained in the rapid growth rates, despite the potential for enhanced predation in the ciliate bloom.

Tintinnids are a crucial component of the ciliate assemblage in most coastal waters (Pierce and Turner 1992). Several

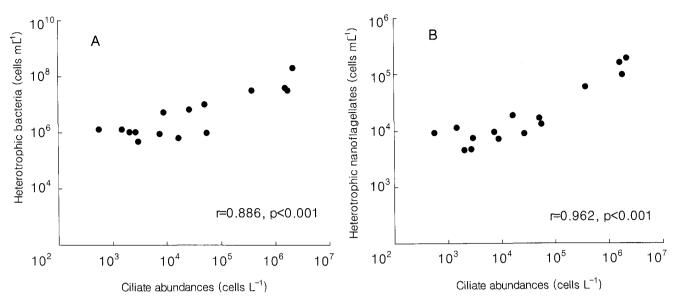


Fig. 10. Scattergrams of the abundances of heterotrophic bacteria and ciliates (A), and heterotrophic nanoflagellates and ciliates (B). Linear correlation coefficients (r) and associated probabilities are shown.

tintinnid species are capable of rapid reproduction under favorable conditions, which include optimum water temperature, rich food availability, low predation pressure, and little competition for resources from other organisms. Estuarine tintinnid communities often comprise numerous coexisting species (Rogers et al. 1981; Sanders 1987; Barría de Cao 1992; Dolan and Gallegos 2001), and a significant positive correlation exists between temperature and tintinnid diversity in temperate estuaries that peaks in summer (Urrutxurtu 2004; Barría de Cao et al. 2005). These investigations were conducted in relatively open or wide estuaries. When environmental conditions are most suited to a specific species in enclosed or semi-enclosed water, that species dominates the community. In Lake Sihwa, a semi-enclosed estuarine lake, mass occurrences of solitary species, have been found in phytoplankton and zooplankton communities (Choi et al. 1997b; Park and Huh, 1997). In this study, we found that a tintinnid species has mass occurrence.

Tintinnids respond successfully and quickly to changes in temperature and food conditions where they belong to (Laybourn-Parry 1992). A representative coastal tintinnid, *Tintinnopsis beroidea* bloomed in field samples collected from Dutch coastal waters (Admiraal and Venekamp 1986) and also occurred abundantly in a bay of southern Norway in relation to wind-induced hydrographic conditions. Mass occurrence of *T. beroidea* along the coasts was associated

with onshore winds (Dale and Dahl 1987). High abundance of a small tintinnid, T. minuta, has been observed in other coastal waters (Hargraves 1981; Verity 1987; Pierce and Turner 1994). Tintinnopsis nana in the present study is also a small species breaking out in mass occurrence like T. beroidea and T. minuta. Three species, T. nana, T. minuta and T. beroidea are morphologically similar, and have been suggested as the variants of one species (Bakker and Phaff 1976). In the present study, abundant concentrations of pico- and nano-sized microbes could be a favorite condition for the tiny T. nana. T. nana, which has a lorica diameter of 18 µm, may prefer bacteria and nanoflagellates (2-5 µm) in this study. This can be supported by the size of ingested particles corresponding up to 40-45% of the oral diameter of the lorica (Spittler 1973; Heinbokel 1978). Tintinnids typically perform one or two doublings per day (Pierce and Turner 1992), but smaller tintinnid species have shorter generation times (7.25 h), or three doublings per day (Verity 1986). This could explain the overwhelmingly high density of T. nana with more than 106 cells L-1 in the study area.

Ciliate densities largely decreased, and community composition was remarkably different in the anoxic water layer (Dolan and Coats 1991). Tintinnids avoided water less than 50% saturated with oxygen (Laybourn-Parry *et al.* 1990). As a similar result in the present study, the anoxic condition was regularly observed at the bottom of

Lake Sihwa in summer (Park *et al.* 2003). Therefore, the extremely high abundance of *T. nana* at the surface layer in the inner area may also reflect a response of escape from the anoxic conditions at the bottom layer. Together, abundant prey resources, a rapid growth rate, and aggregation at the aerobic surface layer may have been the principal factors leading to the *T. nana* bloom.

The bulk of microzooplankton in the Schlei Fjord estuary in Germany consisted of small ciliates less than 30 µm in diameter (Gast 1985). During the summer, ciliates less than 50 µm long increased considerably (Smetacek 1981), likely exploiting bacterioplankton and small flagellates as an energy source, which indicates a tight relationship among ciliates, heterotrophic flagellates, and heterotrophic bacteria. The functional role of ciliates in estuaries and other hyposaline environments must be significant. Abundant bacteriovorus ciliates can exert a considerable grazing impact on bacterioplankton. Sometimes the production of small ciliates exceeds that of heterotrophic flagellates in estuarine waters (Sherr et al. 1986; Sherr and Sherr, 1987). Consequently, the protozoan bacteriovory in Lake Sihwa, a semi-enclosed estuary, may regulate the energy flow of this ecosystem, especially during summer.

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