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Characteristics of Microbial Abundances in Hypoxic Water of Brackish Lake Shihwa

DONG H. CHOI, SANG C. NA, YONG C. PARK[1], SUNG H. HUH[2] AND BYUNG C. CHO*
Department of Oceanography and Research Institute of Oceanography, Seoul National University, Seoul 151-742, Korea
[1]Department of Oceanography, Inha University, Incheon 402-751, Korea
[2]Department of Oceanography, Pukyong National University, Pusan 608-737, Korea

A preliminary study was carried out to find characteristics of microbial trophic relations in hypoxic waters of Lake Shihwa in May and August 1996. Abundances of bacteria, viruses, and heterotrophic nanoflagellates (HNF) and HNF grazing on bacteria were measured. Dissolved O_2 (DO) saturation ranged from 13 to 34% in the bottom waters, and % of DO saturation strongly correlated with salinity. Ratios of HNF-to-bacteria abundance ($42-118\times10^{-5}$) and biomass (0.06—0.25), and ratios of virus-to-bacteria abundance (110—297) in the hypoxic water were similar to those found in the surface layer, indicating similar structures of microbial abundances and trophic functions in hypoxic and surface waters during the study period. In the hypoxic water, an energy flow from organic matter to bacteria to HNF might operate as equally as in oxic surface layer.

Studies on microbial ecology in hypoxic saline waters are rare. This is probably due to the fact that hypoxic water exists as a relatively sharp transition between oxic and anoxic layer and does not form a large water body in coastal areas (Detmer *et al.* 1993, Gast and Gocke 1988, Karl and Knauer 1991). Thus, characteristics of microbial interrelationships in hypoxic waters are not well known. Since developments of hypoxic waters in coastal areas are expected to increase (Malakoff 1998, Seki 1991), it is important to understand characteristics of microbial interactions in hypoxic water. During an investigation on recently formed hypertrophic Lake Shihwa, the saline bottom layer was found to be hypoxic over the basin of the lake (Park *et al.* 1997). Lake Shihwa provided us an opportunity to examine characteristics of microbial trophic relations in hypoxic saline water. Here, we studied abundance distributions of bacteria, heterotrophic nanoflagellates (HNF) and viruses, and HNF grazing rates on bacteria in the hypoxic zone of Lake Shihwa. We asked whether abundance structures of bacteria, HNF and viruses were similar between hypoxic and surface waters.

Lake Shihwa (Fig. 1) is monomictic and shallow with the maximum depth of 18 m near the dike. Surface waters of Lake Shihwa show hypertrophic characteristics with chlorophyll *a* concentration > 100 μg l^{-1} during the study period (Choi *et al.* 1997). In May 1996, a hypoxic zone developed at Stn 8 with thickness of 11 m and expanded to Stn 2 in August 1996 (Park *et al.* 1997). A water gate with a bottom depth of 6.5 m is in place near Stn 11. From April 1996 to July 1996, there were 4 times of discharges of surface waters and inputs of coastal waters into the lake (Park *et al.* 1997). During two investigations performed in May and August 1996, water samples were obtained with a Niskin bottle both from the surface and hypoxic waters. Measurements of dissolved oxygen (DO) were made by an oxygen probe (YSI oxygen meter). Measurements of DO and other chemical parameters were made 3 days after those of microbial parameters in May 1996 and 7 days before in August 1996. Samples for bacterial abundance counts were fixed with 0.2 μm filtered neutrally buffered formalin (final conc. of 2%). Bacterial abundance was measured by epifluorescence microscopy after staining with DAPI (Porter and Feig 1980). Samples for HNF abundance measurements were fixed with Lugol solution (final conc. of 0.5%) and immediately with borate-buffered formalin (final conc. of 3%, Sherr *et al.* 1989), and refrigerated. Primulin stained HNF collected on 0.4 μm polycarbonate black filter were counted under epifluorescence microscopy (Caron 1983). During counting, length of short and long axes of HNF were recorded for biovolume calculations. Biovolumes of bacteria and FLB were determined by

*Corresponding author: bccho@plaza.snu.ac.kr

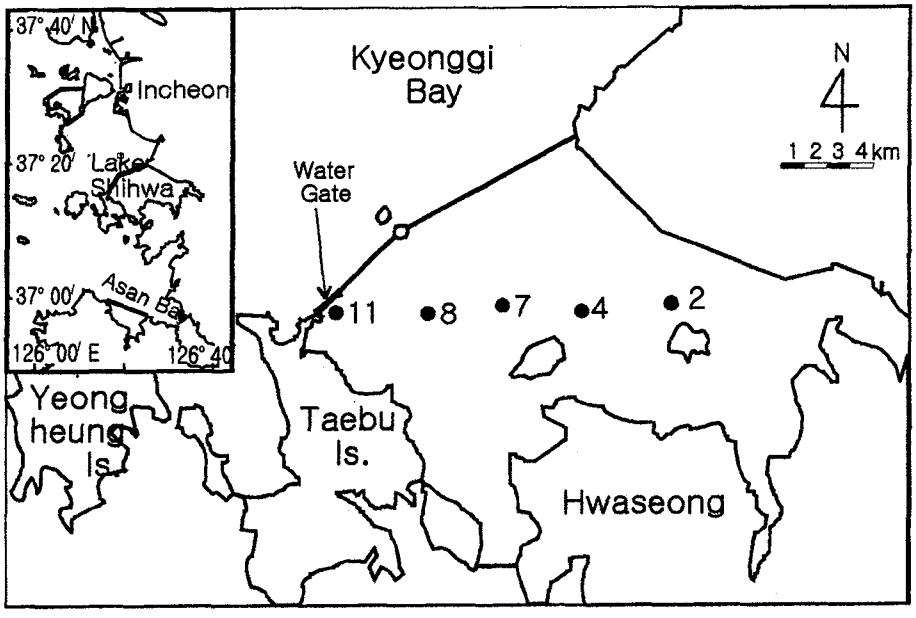

Fig. 1. A map of sampling locations in Lake Shihwa.

using microphotographs and projecting slides onto a paper screen. Projected slides of fluorescent beads of known diameter (0.4 μm and 1.0 μm, Polysciences Inc.) were used for calibration (Moran *et al.* 1991). Bacterial biomass was calculated by using the biovolume-to-biomass relationship of Simon and Azam (1989). HNF biomass was calculated by using a conversion factor of 220 fg C μm^{-3} (Børsheim and Bratbak 1987). Viral abundance was measured by epifluorescence microscopy in May, 1996 using the Yo-Pro method (Hennes and Suttle 1995). Water samples were pretreated with DNase according to Hennes and Suttle (1995). Grazing rates of HNF on bacteria were measured by the method of fluorescently labelled bacteria according to Sherr *et al.* (1987). Incubations were carried out at *in situ* temperature and in the dark for 30 min. Subsamples were collected at 0, 10, 20 and 30 min, fixed, and kept refrigerated until microscopic examinations. The added concentration of FLB was between 1 to 7% of bacterial abundances. Grazing experiments were done in duplicate. We calculated number of ingested FLB l^{-1} h^{-1} from the slope of numbers of ingested FLB l^{-1} vs. incubation time for each bottle, and average values of ingested FLB $l^{-1}h^{-1}$ were calculated from duplicate bottles. Grazing rates were calculated by multiplying the ratio of total bacterial (*i.e.* FLB+bacteria) abundances to FLB by the mean values of ingested FLB $l^{-1}h^{-1}$. No special precaution except cautiously handling hypoxic water samples was made in measurements of HNF grazing. Changes in oxygen concentrations of hypoxic samples during incubation might have occurred. Chlorophyll *a* was measured according to Parsons *et al.* (1984). Water temperature was measured by a mercury thermometer. Salinity was determined using an inducive salinometer. Parametric statistical analyses were performed using SYSTAT (version 8.0, SPSS Inc.). Bonferroni adjusted p-values were used to assess significance of *t*-test (Peres-Neto, 1999).

Oxygen concentrations were low (13−34% DO saturation or 1.3−2.6 mg O_2 l^{-1}; Fig. 2A), indicating hypoxia (< 4 mg O_2 l^{-1}, Paerl *et al.* 1998) in most of the bottom layer in Lake Shihwa. The formation of hypoxic bottom water seemed to be partly due to large flux of sinking organic matter into the bottom layer (Hong 1996) and partly due to stratification caused by large differences in salinity between surface and bottom layers (Table 1). A strong relationship was found between % DO saturation and salinity in the hypoxic, bottom water during the chemical investigation (r^2=0.69, $p<0.001$, n=26; Fig. 2A). Similarly, Paerl *et al.* (1998) observed a negative correlation between O_2 concentration in bottom waters and salinity difference between surface and bottom layers in the eutrophying Neuse River estuary. Since simultaneous measurements of oxygen concentrations were not made during the microbial investigation, we had to justify our hypoxic samples. The similar relationships of temperature vs. salinity in bottom waters in microbial and chemical investigations (Fig. 2B), and close relationship between salinity and % DO saturation suggest that changes in % DO from chemical investigations would be

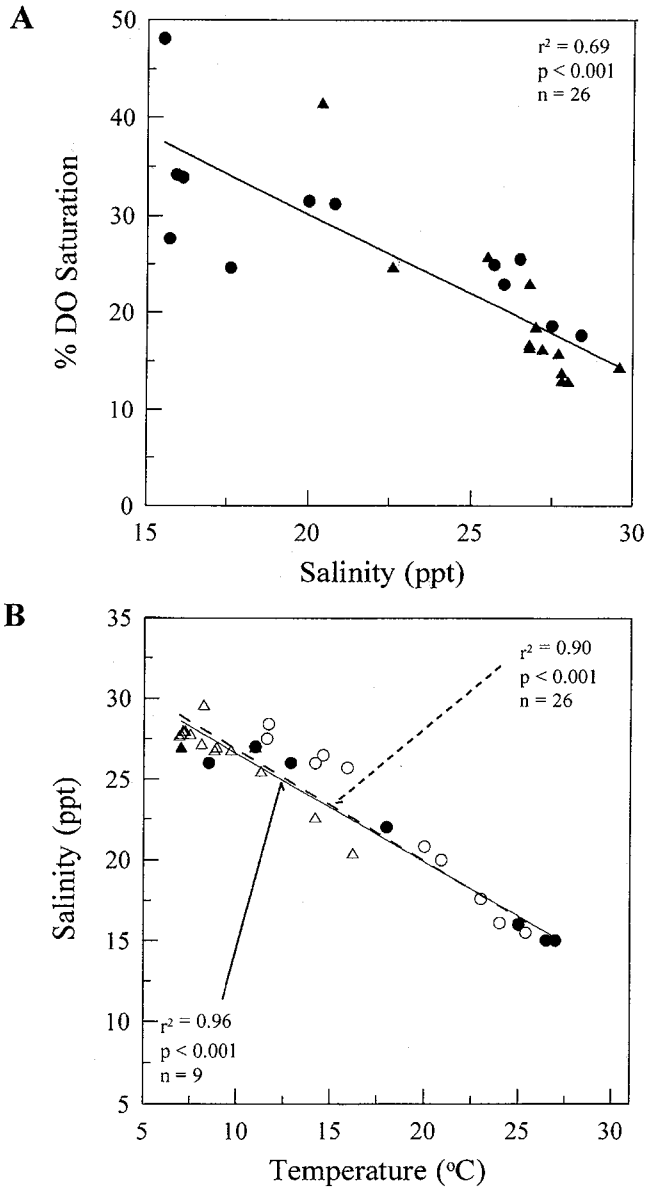

Fig. 2. (A) A plot of salinity vs percentage of dissolved oxygen saturation (% DO saturation) in hypoxic water of Lake Shihwa (r^2=0.69, p<0.001, n=26) during chemical investigations. Data were from May (▲) and August (●) 1996. (B) A plot of temperature vs salinity in hypoxic, bottom water of Lake Shihwa. Data were from microbiological (closed symbol, r^2=0.96, p<0.001, n=9) and chemical (open symbols, r^2=0.90, p<0.001, n=26) investigations in May (△, ▲) and August (○, ●) 1996. Chemical investigation was made 3 days after microbiological investigation in May 1996 and 7 days before August 1996, respectively.

expected minor in May and August 1996. Thus, we used salinity data obtained during the microbial investigation for defining hypoxic samples from the microbial investigation.

Concentration of chlorophyll *a* (chl *a*) was low in hypoxic water (4—16 μg l^{-1}) of Lake Shihwa compared to the euphotic zone (Table 1). Abundances of bacteria in the hypoxic water (1.6 to 8.4×10^9 bacteria l^{-1}) were on average 1.8-3.2 fold lower than those in surface layer (Table 1). Abundances of HNF (1.6 to 4.9×10^6 cells l^{-1}) and viruses (4.3 to 4.7×10^{11} cells l^{-1}) in the hypoxic water were on average 2.2—3.0 fold and 2.7 fold lower than those in surface layer, respectively (Table 1). The observed abundances of microbes in hypoxic water of Lake Shihwa were high compared to other coastal surface waters. Especially, virus abundance in the hypoxic zone was high compared to other marine surface waters (Maranger and Bird 1995). The co-occurrences of high abundances of bacteria, HNF and viruses suggest active and dynamic interactions between microbes in hypoxic water.

Ratios of HNF-to-bacteria abundance ranged from 42×10^{-5} to 118×10^{-5} in hypoxic and from 27×10^{-5} to 129×10^{-5} in surface waters (Table 2). Those ratios in two layers were statistically similar in May and August. Our values from the hypoxic zone are similar to those from hypoxic depth within the chemocline in the central Baltic Sea (90 to 260×10^{-5}; Detmer *et al.* 1993) and from hypoxic samples of Plussee Lake (ca. $21-37\times10^{-5}$; Weinbauer and Höfle 1998). Ratios of HNF-to-bacteria carbon biomass ranged from 0.05 to 0.58 in surface and from 0.06 to 0.25 in hypoxic layer (Table 2). Those biomass ratios in two layers were also statistically similar in May and August. There were no significant temporal changes in the abundance and biomass ratios of HNF-to-bacteria from May to August in surface and hypoxic layers (Table 2). Thus, similar abundance and biomass ratios of HNF-to-bacteria seem to be maintained in surface and hypoxic waters in May and August. The similar abundance and biomass ratios of HNF-to-bacteria in surface and hypoxic zone might indicate that bacteria-HNF interactions are similar in both oxic and hypoxic layers. This observation is interesting because there was a strong stratification between surface layer and hypoxic bottom layer during the study period (Table 1). The strong stratification of water column would prevent mixing between 2 layers. Therefore, the large-scale existence of high HNF abundance in hypoxic zone indicates that HNF might survive there. Particles falling from the surface layer (Hong 1996) would be one of the sources of HNF in hypoxic zone. Particles are known to contain hypoxic/anoxic microenvironments (Alldredge and Cohen 1987, Shanks and Reeder 1993), and might contain some HNF tolerant to hypoxic conditions.

Table 1. Ranges of temperature, salinity, chlorophyll *a* (Chl *a*), bacterial abundance (BA), viral abundance (VA), VA-to-BA ratios, heterotrophic nanoflagellate (HNF) abundance (HNFA), HNF biomass (HNFC), and HNF ingestion rate (IR) measured in the surface and hypoxic zones in Lake Shihwa. Values are mean ±1SD (range; n)

Date		Temperature (°C)	Salinity (ppt)	Chl *a* (μg l^{-1})	BA (×10^9 l^{-1})	VA (×10^{11} l^{-1})	VA/BA	HNFA (×10^6 l^{-1})	HNFC (μg C l^{-1})	IR (×10^2 HNF^{-1} h^{-1})
May 15, 1996	Surface	17.8±2.0 (12.0–21.0; 16)	20±1 (18–20; 16)	104±14 (84–134; 16)	8.9±1.3 (6.3–10.4; 13)	12.0±2.6 (8.5–17.1; 14)	133±33 (81–180; 12)	6.3±2.8 (2.8–10.5; 16)	59±33 (12–102; 16)	0.81±0.18 (0.68–1.11; 5)
	Hypoxic	7.8±1.1 (7.0–8.5; 2)	27±1 (26–27; 2)	5±1 (4–5; 2)	2.8±1.7 (1.6–3.9; 2)	4.5±0.3 (4.3–4.7; 2)	203±132 (110–297; 2)	2.1±0.4 (1.9–2.4; 2)	16±5 (12–19; 2)	–[1]
August 13, 1996	Surface	29±2 (27–31; 12)	14±1 (12–15; 12)	57±68 (9–250; 12)	9.2±4.0 (5.7–19.5; 12)	–	–	6.1±1.6 (3.3–9.0; 12)	49±21 (13–94; 12)	0.40±0.26 (0.14–0.78; 5)
	Hypoxic	19±7 (11–27; 7)	21±6 (15–27; 7)	8±4 (6–16; 7)	5.2±2.1 (3.0–8.4; 7)	–	–	2.8±1.1 (1.6–4.9; 7)	19±14 (8–49; 7)	0.09 (–; 1)

[1]Data not available.

Table 2. Comparisons of the ratios of heterotrophic nanoflagellate (HNF) abundance (HNFA) to bacterial abundance (BA), ratios of HNF biomass (HNFC) to bacterial biomass (BOC), bacterial biovolume, and HNF biovolume measured in the surface and hypoxic zones of Lake Shihwa in May and August, 1996. Values are mean ± 1SD (range; n). Students *t*-test was used to test whether mean values are different between the two zones and between two dates. p vlaues (Bonferroni correction α/4) are represented

Date	HNFA/BA (×10^{-5})			HNFC/BOC			Bacterial Biovolume (μm^3 $cell^{-1}$)			HNF Biovolume (μm^3 HNF^{-1})		
	Surface	Hypoxic	p	Surface	Hypoxic	p	Surface	Hypoxic	p	Surface	Hypoxic	p
May, 1996	74±34 (27–129; 13)	89±41 (60–118; 2)	1.000	0.26±0.19 (0.05–0.58; 10)	0.20±0.04 (0.17–0.23; 2)	1.000	0.22±0.08 (0.10–0.33; 11)	0.27±0.06 (0.23–0.31; 2)	1.000	41±10 (17–57; 16)	33±5 (29–36; 2)	0.841
August, 1996	70±20 (46–103; 12)	55±13 (42–77; 7)	0.318	0.22±0.07 (0.13–0.32; 7)	0.12±0.07 (0.06–0.25; 7)	0.069	0.20±0.06 (0.13–0.32; 7)	0.27±0.09 (0.13–0.38; 7)	0.352	36±10 (13–48; 12)	31±19 (18–71; 7)	1.000
p	1.000	1.000	–	1.000	0.612	–	1.000	1.000	–	0.961	1.000	–

Such HNF would survive in the hypoxic waters.

Further, similar virus-to-bacteria ratios were found in hypoxic (203±132) and surface (133±33) waters (Table 1). The high abundances of viruses and similar virus-to-bacteria ratios in hypoxic and surface waters suggest that viruses might be an active member of microbial trophodynamics (Bratbak *et al.* 1992) in hypoxic water as well. Even if we consider that our direct virus counts are possibly overestimates compared to TEM virus counts (1.2—7.1 fold, Hennes and Suttle 1995), our virus-to-bacteria ratios would be still high. In a eutrophic Lake Plussee, virus-to-bacteria ratio was *ca.* 7—9 from hypoxic samples (Weinbauer and Höfle 1998). In spite of the significant changes of physico-chemical conditions (*i.e.* increa-ses of temperature and decreases of salinity) in whole water-column from May to August, similar structures of bacteria and HNF abundances (presumably virus too) were maintained between surface and hypoxic waters. Thus, it seems that trophic functions of microbes in hypoxic water of Lake Shihwa would be alike to those observed in surface water.

Although only one measurement of HNF grazing rates (2.1×10^7 bacteria $l^{-1}h^{-1}$) on bacteria was available in hypoxic water during the study, it still supports our contention of active microbial interactions in hypoxic water. Our HNF ingestion rate of 9 bacteria $HNF^{-1}h^{-1}$ (Table 1) is within the range of 2—17 bacteria $HNF^{-1}h^{-1}$ determined by a method of fluorescently labelled bacteria for HNF of microaerophilic water of Lake Vechten (Bloem and Bar-Glissen 1989) and indicates that HNF would be a significant consumer of bacteria in hypoxic water.

In conclusion, similar abundance structures of bacteria, heterotrophic nanoflagellate (HNF), and viruses were found in hypoxic and surface waters of Lake Shihwa. In hypoxic water, an energy flow from organic matter to bacteria to HNF is suggested to actively operate as in surface oxic layer. It remains to test whether our observations are widely found in

coastal hypoxic waters, and what regulates the flow of energy via microbial loop in hypoxic waters.

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