

# Active ingestion of fluorescently labeled bacteria by mesopelagic heterotrophic nanoflagellates in the East Sea, Korea

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**ABSTRACT:** To determine whether heterotrophic nanoflagellates (HNF) in the mesopelagic actively ingest bacteria and whether HNF grazing rates comprise a significant fraction of the mesopelagic bacterial production, we measured uptake rates of fluorescently labeled bacteria (FLB) by mesopelagic HNF and bacterial production in the East Sea, 5 times from April 1996 to September 1997. Although bacterial abundance and production were much lower in the mesopelagic than in the epipelagic zone, HNF clearance rates and volume-specific HNF clearance rates in both layers were usually within the same range, 1 to 11 nl HNF<sup>-1</sup> h<sup>-1</sup> and 0.1 to 1 × 10<sup>6</sup> h<sup>-1</sup>, respectively. Two samples in the mesopelagic showed somewhat increased clearance rates (14 to 16 nl HNF<sup>-1</sup> h<sup>-1</sup>) and volume-specific HNF clearance rates (up to 2.6 × 10<sup>6</sup> h<sup>-1</sup>). Fractions of actively grazing HNF were not statistically different in the 2 layers (epipelagic: 33 to 98 %, mesopelagic: 22 to 79 %) in most cruises. HNF grazing rates in the mesopelagic (0.1 to 1.1 × 10<sup>6</sup> cells l<sup>-1</sup> h<sup>-1</sup>) were either major fractions of or often greater than bacterial production, suggesting that HNF grazing on bacteria was the major fate of mesopelagic bacterial production at the study site.

**KEY WORDS:** Heterotrophic nanoflagellates · Grazing · FLB · Mesopelagic zone · The East Sea

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## INTRODUCTION

Bacteria in the mesopelagic have been recognized as playing major roles in organic matter fluxes originating in the euphotic zone (Cho & Azam 1988, 1995, Simon et al. 1992). However, significantly less information is available about the fate of bacteria in the mesopelagic zone (Turley 1991). Viruses are thought to be significant in regulating attached bacteria in the mesopelagic (Proctor & Fuhrman 1991). However, free-living bacteria comprise the majority of bacterial abundance in the mesopelagic (Cho & Azam 1988, 1995). Recently, Steward et al. (1996) presented evidence that viruses may cause substantial mortality of mesopelagic

bacterioplankton, but other sources may contribute as well. Several studies reported the ubiquitous occurrence of heterotrophic nanoflagellates (HNF) in the mesopelagic (Pomeroy & Johannes 1968, Taylor et al. 1986, Cho & Azam 1988, Steward et al. 1996). However, nothing is known about their grazing impact on bacterioplankton. Because HNF are known as major grazers of marine bacteria in epipelagic environments (Sanders et al. 1992, Peters 1994), they may be also important bacterial grazers, controlling the growth of bacterioplankton communities in the mesopelagic.

The goals of this study were to examine whether HNF in the mesopelagic zone actively ingest bacteria, to quantify the fraction of the mesopelagic bacterial production consumed by HNF, and to determine factors that control bacteria-HNF interactions in the mesopelagic zone.

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## MATERIALS AND METHODS

**Study area and sample collection.** During 5 cruises in the East Sea (Fig. 1), seawater samples were collected with 10 l Niskin bottles mounted on a CTD-rosette from 0, 10, 20, 30, 50, 75, 100, 200 and 500 m depths. In April 1996, an additional sample was taken at a depth of 180 m. Samples collected from a depth interval of 200 to 500 m are regarded as mesopelagic samples. As the euphotic depth was in most cases shallower than 50 m depth in the East Sea, our choice seemed to fit the concept of mesopelagic zone (Young 1983). Each sample was used for determining HNF abundance, HNF grazing rates on bacteria, bacterial abundance and bacterial production.

**HNF abundances, grazing rates of HNF, and fractions of active HNF.** Samples for measurements of HNF abundance were fixed immediately with alkaline Lugol solution (final conc. of 0.5%) and borate-buffered formalin (final conc. of 3%; Sherr et al. 1989, Rassoulzadegan 1991). Five to 15 ml of seawater was stained with primulin (final conc. of  $250 \mu\text{g ml}^{-1}$ ), and collected on a black  $0.4 \mu\text{m}$  polycarbonate filter (25 mm diameter) at a vacuum not exceeding 100 mm Hg (Caron 1983). Primulin-stained cells were visualized and enumerated at  $\times 1000$  magnification with UV exci-

tation using an epifluorescence microscope (Olympus BX60 with U-MWU filter set, BP330-385 excitation filter, DM400 dichroic mirror, and BA420 barrier filter). Heterotrophic flagellates fluoresced bluish-white under UV excitation, while autotrophic flagellates were distinguished from heterotrophs by red autofluorescence under blue excitation (Olympus filter set U-MWB, BP450-480 excitation filter, DM500 dichroic mirror, and BA515 barrier filter). To calculate biovolume of HNF, both the short and long axes of HNF were measured, and a minimum of 100 microscopic fields or 30 cells were counted.

Measurements of HNF grazing rates on bacteria were made using fluorescently labeled bacteria (FLB). FLB were prepared by staining natural bacterial samples collected 3 to 5 d prior to each cruise from coastal waters in the East Sea with 5-(4,6-dichlorotriazin-2-yl) aminofluorescein (DTAF) according to Sherr et al. (1987). FLB uptake experiments were carried out in duplicate in 250 ml polycarbonate bottles presoaked in 10% (vol/vol) HCl and copiously rinsed with deionized water. To 250 ml samples, ca 1 ml of the FLB stock was added. The added concentration of FLB was between 5 to 31% of bacterial abundance. Grazing experiments were conducted in the dark, at *in situ* temperature, and atmospheric pressure. Thirty ml of subsamples were collected at 0, 10, 20 and 30 min, fixed, and kept refrigerated until microscopic examinations. Subsamples were primulin-stained and filtered, and at least 100 microscopic fields were counted. The filters were first observed under UV excitation at a magnification of  $\times 1000$ . When an HNF was located, the incident light was changed to blue, which allowed us to count the FLB contained within the HNF. The FLB fluoresced a bright apple green under blue light.

We calculated the number of ingested FLB  $\text{l}^{-1} \text{h}^{-1}$  from the slope of ingested FLB  $\text{l}^{-1}$  versus incubation time for each bottle. Average values of ingested FLB  $\text{l}^{-1} \text{h}^{-1}$  were calculated for duplicate bottles. Bacterivory rates were measured over the linear portion of the uptake curve, and the uptake was linear for ca 30 min. The coefficient of variation ( $= 100 \times \text{SD}/\text{mean}$ ) of the slopes from duplicate bottles ranged similarly between the euphotic zone (0.1 to 45%; mean  $\pm$  SD =  $15 \pm 15\%$ ) and the mesopelagic zone (1 to 47%; mean  $\pm$  SD =  $18 \pm 18\%$ ) except 2 mesopelagic data of 51 and 63%.

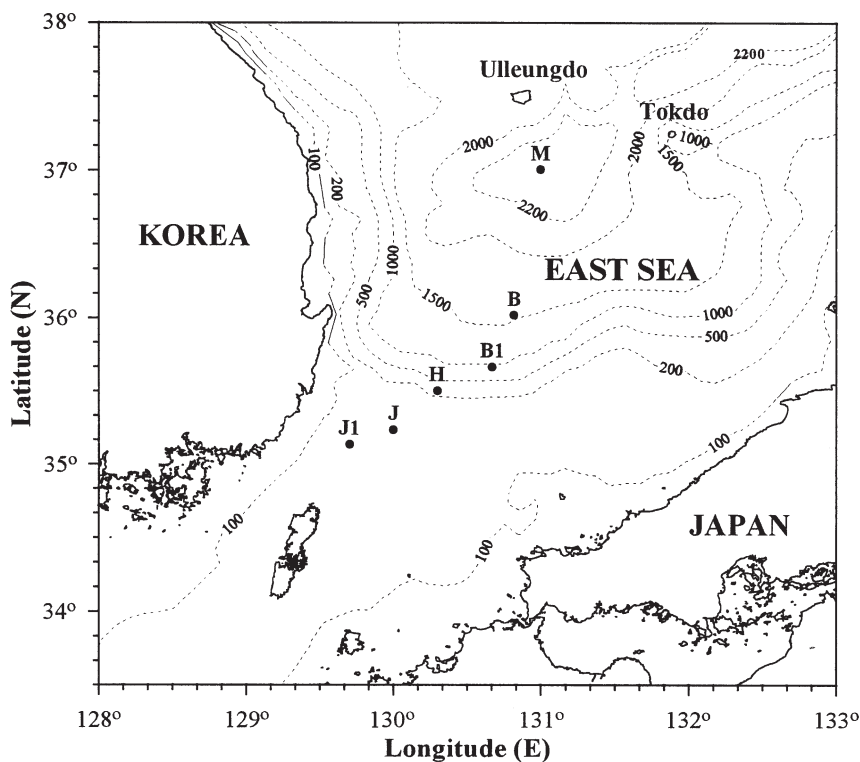


Fig. 1. Map of study area and sampling stations (M, B, B1, H, J, J1) in East Sea. Depth contour lines are shown in meters

Grazing rates were calculated by multiplying the ratio of bacterial abundances to FLB by the mean values of ingested FLB  $l^{-1} h^{-1}$ . Ingestion rates were calculated by dividing grazing rates by HNF abundances. Clearance rates were calculated by dividing ingestion rates by bacterial abundances.

The validity of our estimates of ingestion rates was tested by comparing the standard protocol estimates (the method of Sherr et al. 1987) with estimates according to a new protocol proposed by González (1999). It has been shown that ingestion rates can be calculated from the fraction of HNF with ingested FLB during an FLB uptake experiment and the ratio of FLB to bacteria (Eq. 3 in González [1999]), assuming that the distributions of bacteria and FLB ingested by HNF follow Poisson distributions. Also, to find out if the percentage of actively grazing HNF is low in the mesopelagic, estimates of the fraction of HNF actively grazing on bacteria were calculated according to the method of González (1999). Actively grazing HNF are defined as those ingesting any bacteria (total bacteria = natural bacteria + FLB) during uptake experiments. The fraction of active HNF ( $F_{\text{active}}$ ) over a time period was estimated as:  $F_{\text{active}} = 1 - [1/\exp(I/t)]$ , where  $I$  is the ingestion rate on total bacteria by HNF and  $t$  is the incubation time (González 1999).

**Bacterial abundance and production.** Samples for measurements of bacterial abundance were fixed with 0.2  $\mu\text{m}$  filtered, borate-buffered formalin (final conc. of 2%). Bacteria, stained with DAPI (4',6-diamidino-2-phenylindole; final conc. of 1  $\mu\text{g ml}^{-1}$ ) and collected on black 0.2  $\mu\text{m}$  polycarbonate filters, were counted under UV excitation using an epifluorescence microscope (Porter & Feig 1980). The biovolumes of bacteria and FLB were determined by microphotographs and slides projected onto a paper screen. Projected slides of fluorescent beads of known diameter (0.4 and 1.0  $\mu\text{m}$ , Polysciences Inc.) were used for calibration (Moran et al. 1991). Bacterial production was measured by the  $^{14}\text{C}$ -leucine incorporation method (Simon & Azam 1989, Ducklow et al. 1992). Ten to 30 ml of samples (in triplicate) were dispensed into sterile polypropylene tubes, and  $^{14}\text{C}$ -leucine (sp. act. = 315  $\text{mCi mmol}^{-1}$ ) was added to the tubes at 10 nM (final conc.). All tubes were incubated in the dark for 1 to 2 h at *in situ* water temperature and atmospheric pressure. Formalin-killed samples (final conc. of 2%) served as blanks. After incubation,  $^{14}\text{C}$ -leucine incorporation was stopped by adding 2% formalin. The samples were extracted with ice-cold trichloroacetic acid (TCA, final conc. of 5%) for 15 min. The extracted samples were kept cool, filtered onto 0.45  $\mu\text{m}$  cellulose nitrate filters (Whatman), and rinsed 3 times with 5 ml of 5% ice-cold TCA and subsequently 3 times with 5 ml of 80% ice-cold ethanol. The filters were placed into scintilla-

tion vials and dissolved in 1 ml of ethyl acetate. Ten ml of liquid scintillation cocktail (Lumagel<sup>®</sup>) was added and the samples were radioassayed. The incorporated radioactivity was converted to cell number using a conversion factor of  $0.18 \times 10^{18}$  cells  $\text{mol}^{-1}$  leucine incorporated (Ducklow et al. 1992).

**Other analyses.** Water temperature was measured by a CTD system (SBE911, Seabird). The euphotic depth was estimated by multiplying the Secchi depth by 2.7. Linear regression, multiple regression and Student's *t*-test analyses were performed using SPSS for Windows (Version 8.0: SPSS Inc. 1997). To test whether the relationships between 2 variables in the euphotic, lower epipelagic and mesopelagic zones were identical, a multiple regression model with group variables (e.g.  $Z_1$  and  $Z_2$ , each employing the values of 0 and 1) and interaction terms ( $Z_1X$  and  $Z_2X$ ) was analyzed. We assigned different values to  $Z_1$  and  $Z_2$  for the different zones (i.e. euphotic: 0 and 0, lower epipelagic: 1 and 0, mesopelagic: 0 and 1, respectively). The adopted model is:

$$Y = \beta_0 + \beta_1X + \beta_2Z_1 + \beta_3Z_2 + \beta_4Z_1X + \beta_5Z_2X$$

where  $Y$  is the dependent and  $X$  the independent variable, and  $Z_1$  and  $Z_2$  are group variables representing zones. We removed insignificant coefficients by a stepwise regression method and then obtained the best model which described the regression functions for each zone (Neter et al. 1996).

## RESULTS

### Vertical profiles of temperature, bacteria and HNF variables

In the surface layer, water temperature ranged from 10.4 to 25.7°C, and decreased roughly linearly down to 100 m (Fig. 2A). A steep decline from 16.8 to 1.3°C was recorded between 100 and 200 m. Temperature variations at these depths seemed to be associated not only with seasonal variations but also with intense upwelling. In July 1996 at 100 m depth at Stn B, water temperature was 2.7°C compared to 9.2°C in April 1996 and 13.1°C in May 1996. At depths of 200 to 500 m, water temperature varied from 0.4 to 6.4°C. The euphotic depth ranged from 27 m (at Stn J in March 1997) to 81 m (at Stn M in September 1997) and was shallower than 50 m in 21 out of 23 measurements. Bacterial abundance in the euphotic zone ranged from  $0.2 \times 10^9$  cells  $l^{-1}$  to  $1.8 \times 10^9$  cells  $l^{-1}$ , and was sometimes as low as that at 200 m depth (Fig. 2B). Bacterial abundance decreased with increasing depth from  $0.2$  to  $1.2 \times 10^9$  cells  $l^{-1}$  at 100 m to  $0.1$ – $0.5 \times 10^9$  cells  $l^{-1}$  at 200 m, and  $0.1$  to  $0.2 \times 10^9$  cells  $l^{-1}$  at 500 m depth. Bacterial produc-

tion generally showed a rapid decline below the mixed layer, and then gradually decreased further below this (Fig. 2C). The values ranged from  $0.5 \times 10^6$  to  $37 \times 10^6$  cells  $l^{-1} h^{-1}$  in the euphotic zone and from  $0.1 \times 10^6$  to  $4 \times 10^6$  cells  $l^{-1} h^{-1}$  in the mesopelagic zone.

HNF abundance in the euphotic zone varied by an order of magnitude from  $0.4 \times 10^6$  to  $7.5 \times 10^6$  HNF  $l^{-1}$ , while in the mesopelagic zone it varied from  $0.1$  to  $0.4 \times 10^6$  HNF  $l^{-1}$ , with decreasing variation in HNF abundance with increasing depth (Fig. 2D). HNF ingestion

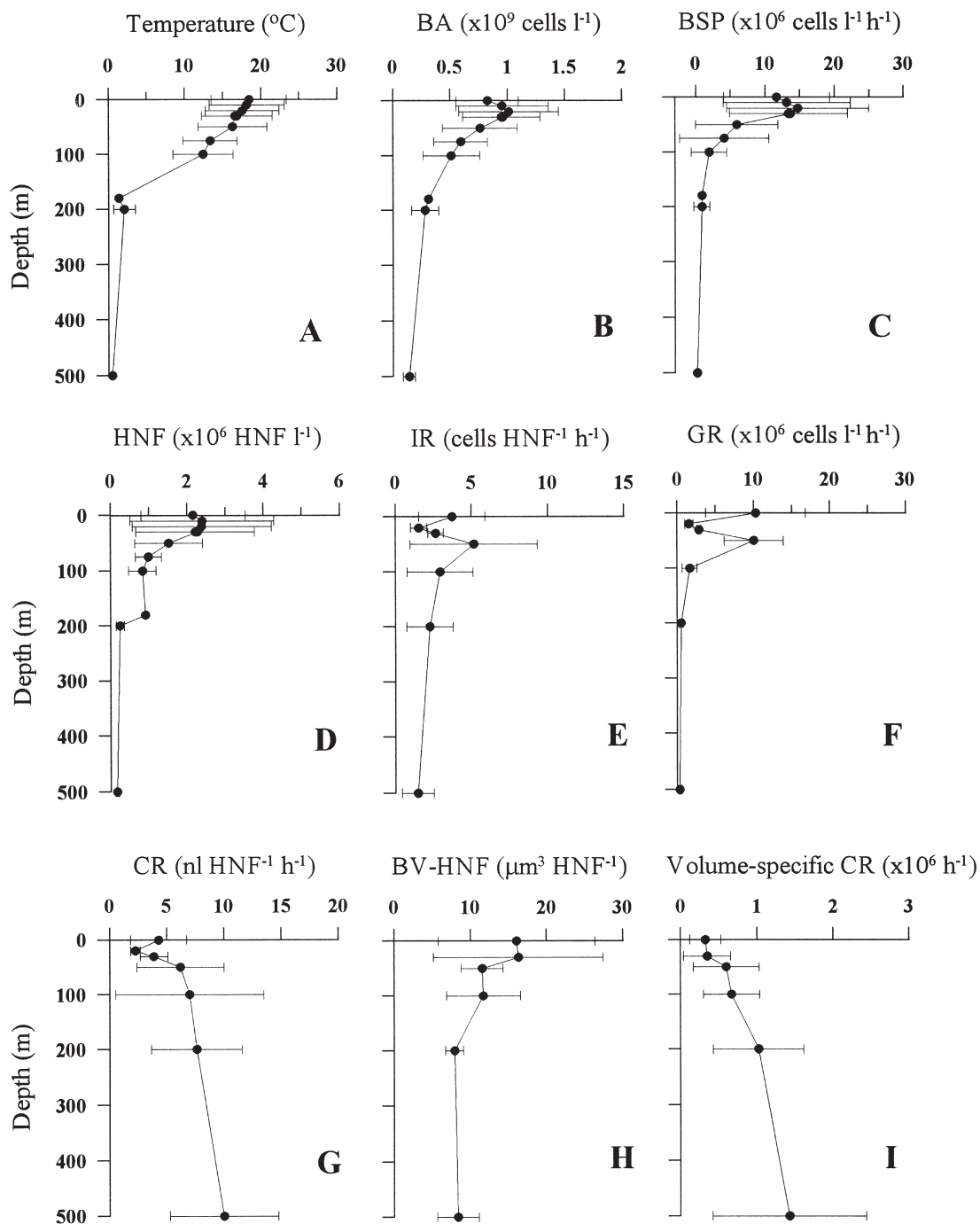


Fig. 2. Depth-profiles of means of (A) water temperature, (B) bacterial abundance (BA), (C) bacterial secondary production (BSP), (D) heterotrophic nanoflagellate (HNF) abundance, (E) ingestion rates (IR), (F) grazing rates (GR), (G) clearance rates (CR), (H) cell-biovolume (BV) of HNF, and (I) volume-specific CR in the East Sea. Data are means  $\pm$  1 SD from April 1996, May 1996, July 1996, March 1997, July 1997 and September 1997 cruises combined. When bars are not shown, 1 SD is less than size of symbol

rates in the euphotic zone ranged from 1 to 8 cells  $\text{HNF}^{-1} \text{h}^{-1}$ , and in the mesopelagic zone from 1 to 5 cells  $\text{HNF}^{-1} \text{h}^{-1}$  (Fig. 2E). Subsurface maxima of HNF ingestion rates were usually observed at 50 to 100 m depth, except on July 1997 when a surface maximum was found. HNF grazing rates in the euphotic zone varied widely from  $0.8 \times 10^6$  to  $23.3 \times 10^6$  cells  $\text{l}^{-1} \text{h}^{-1}$ , and in the mesopelagic zone varied from 0.1 to  $1.1 \times 10^6$  cells  $\text{l}^{-1} \text{h}^{-1}$  (Fig. 2F). They were consistently higher in the euphotic zone than in the mesopelagic zone in all cruises. HNF clearance rates in both the euphotic and mesopelagic zones had a similar range of 1 to 11  $\text{nl HNF}^{-1} \text{h}^{-1}$  (Fig. 2G), but often (2 out of 13 measurements) high clearance rates (14 to 16  $\text{nl HNF}^{-1} \text{h}^{-1}$ ) were found in the mesopelagic. Mean biovolumes of HNF ranged from 5 to 48  $\mu\text{m}^3$  in the euphotic zone, and those in the mesopelagic from 6 to 11  $\mu\text{m}^3$  (Fig. 2H). Mean biovolumes of FLB and marine bacteria were comparable, ranging from 0.07 to 0.20  $\mu\text{m}^3$  and from 0.05 to 0.34  $\mu\text{m}^3$ , respectively (data not shown). Only in May 1996 was the mean biovolume of FLB (0.11  $\mu\text{m}^3$ ) larger than that of bacteria (0.07  $\mu\text{m}^3$ , see 'Discussion'). Volume-specific HNF clearance rates in the euphotic and mesopelagic zones were within a similar range (0.1 to  $1.0 \times 10^6 \text{h}^{-1}$ ). Two samples showed high volume-specific clearance rates ( $1.8$  to  $2.6 \times 10^6 \text{h}^{-1}$ ) in the mesopelagic zone (Fig. 2I). In the lower epipelagic zone (i.e. between the euphotic zone and the mesopelagic zone), in most cases the above variables had values intermediate to those of the euphotic and mesopelagic zones (Fig. 2).

### Comparisons of HNF variables in euphotic and mesopelagic zones

HNF ingestion rates did not seem to be statistically different between the euphotic and mesopelagic zones on any cruise (Table 1). However, HNF clearance rates often seemed to be higher in the mesopelagic zone than in the euphotic zone in April 1996, March 1997 and September 1997 (Table 1). In other cruises, HNF clearance rates were statistically not different between the 2 zones. Volume-specific clearance rates were not different between the mesopelagic zone and the euphotic zone in May and July 1996 and March 1997 (Table 1). Thus, HNF ingestion rates seemed to be similar in both zones, but clearance rates and volume-specific clearance rates were either similar or sometimes enhanced in the mesopelagic zone.

### Relationships between HNF and bacteria in the mesopelagic zone

Grazing rates were only significantly correlated with bacterial abundance ( $r^2 = 0.48$ ,  $p = 0.008$ ; see Fig. 3A) and water temperature ( $r^2 = 0.40$ ,  $p = 0.020$ ; not shown in Fig. 3). Grazing rates in most cases were either higher than bacterial production or a large fraction thereof (see Fig. 3B). Bacterial abundance and HNF abundance were weakly ( $r^2 = 0.16$ ,  $p < 0.06$ ) correlated (see Fig. 3C). Multiple regression analysis with group variables showed that the relationships between graz-

Table 1. Heterotrophic nanoflagellates (HNF). Comparison of ingestion rates, clearance rates, and volume-specific clearance rates measured in euphotic and mesopelagic zones of East Sea. Values are mean  $\pm$  1SD, with numbers in parentheses. Student's *t*-test assessed whether mean values were different between 2 zones; *p*-values  $< 0.05$  represent significant differences between 2 zones. nd: not determined

Date	Ingestion rates (cells $\text{HNF}^{-1} \text{h}^{-1}$ )			Clearance rates ( $\text{nl HNF}^{-1} \text{h}^{-1}$ )			Volume-specific clearance rates ( $\times 10^6 \text{h}^{-1}$ )		
	Euphotic	Mesopelagic	<i>p</i>	Euphotic	Mesopelagic	<i>p</i>	Euphotic	Mesopelagic	<i>p</i>
9–14 Apr 1996	2.7 $\pm$ 1.0 (4)	1.6 (1)	nd	2.9 $\pm$ 1.0 (4)	5.8 (1)	nd	0.15 $\pm$ 0.03 (4)	1.02 (1)	nd
27–31 May 1996	5.6 $\pm$ 1.9 (4)	2.6 $\pm$ 1.9 (4)	0.075	6.5 $\pm$ 2.5 (4)	8.7 $\pm$ 6.3 (4)	0.538	0.47 $\pm$ 0.23 (4)	1.25 $\pm$ 1.02 (4)	0.185
23–30 Jul 1996	4.5 $\pm$ 2.7 (3)	1.5 $\pm$ 0.9 (2)	0.246	6.0 $\pm$ 1.6 (3)	7.0 $\pm$ 4.4 (2)	0.802	0.42 $\pm$ 0.16 (3)	0.82 $\pm$ 0.51 (2)	0.459
10–16 Mar 1997	2.2 $\pm$ 1.0 (4)	2.7 $\pm$ 1.5 (3)	0.622	3.3 $\pm$ 1.0 (4)	11.7 $\pm$ 2.1 (3)	0.001	0.38 $\pm$ 0.22 (4)	1.36 $\pm$ 0.62 (2)	0.254
22 Sep –1 Oct 1997	1.5 $\pm$ 0.5 (3)	1.0 $\pm$ 0.5 (3)	0.261	2.3 $\pm$ 0.4 (3)	6.2 $\pm$ 1.6 (3)	0.014	nd	nd	nd

ing rates and HNF abundance in the euphotic, lower epipelagic and mesopelagic zones were identical (Fig. 3D, Table 2). For other variables, slopes of the relationships in each zone were the same (Fig. 3, Table 2).

### Fractions of active HNF

The fractions of active HNF during the incubation time (called hereafter % active HNF) varied in the

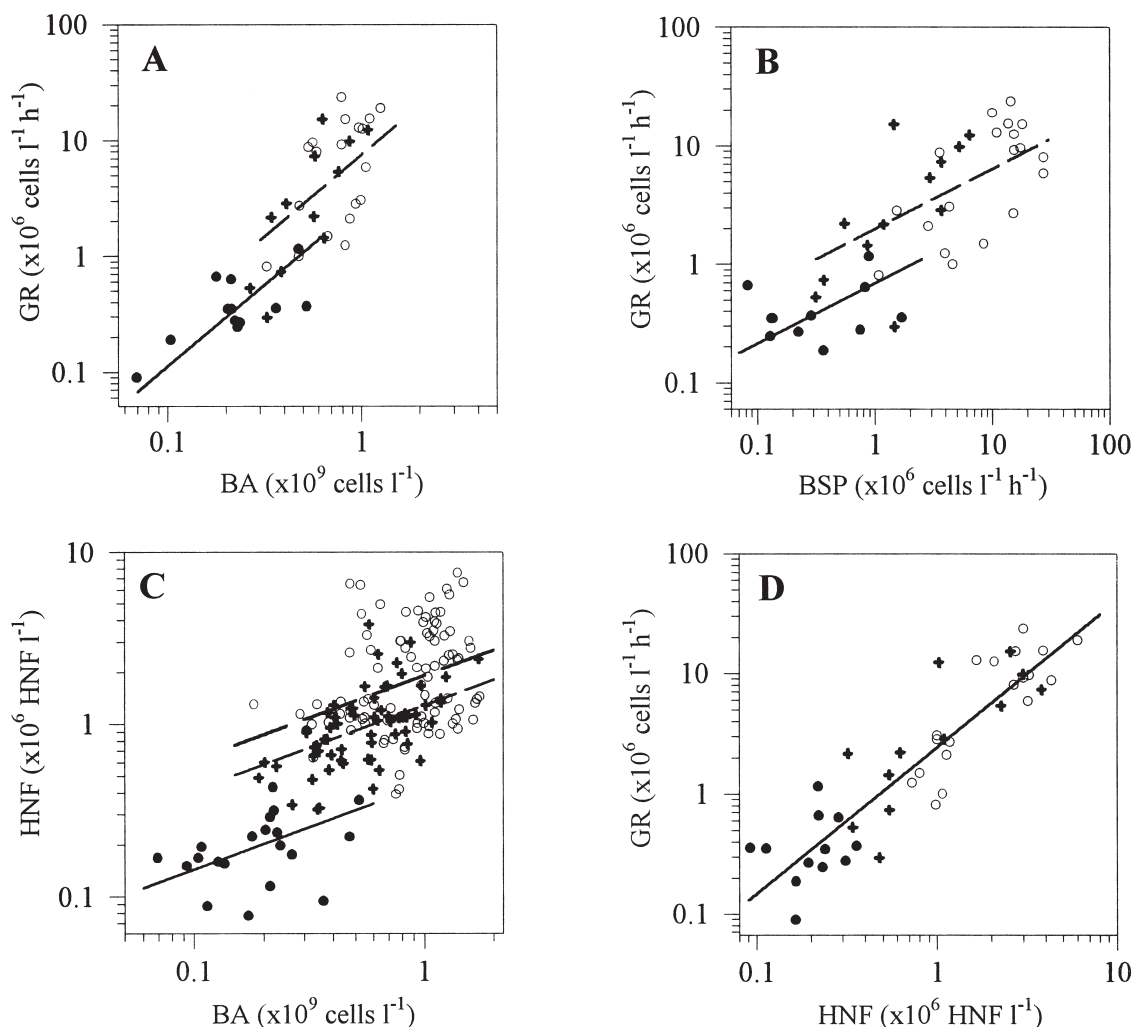


Fig. 3. Scatter plots of (A) bacterial abundance (BA) vs grazing rate (GR), (B) bacterial secondary production (BSP) vs GR, (C) BA vs heterotrophic nanoflagellate (HNF) abundance, and (D) HNF abundance vs GR. Samples were from euphotic (○), lower epipelagic (◆), and mesopelagic (●) zones of the East Sea. Regression lines represent best equations for each zone

Table 2. Heterotrophic nanoflagellates (HNF). Best models selected by stepwise regression method (significance level,  $\alpha = 0.05$ ), coefficients of determination ( $r^2$ ),  $F$ -statistics, and  $p$ -values. Analyses tested whether relationships between 2 variables in euphotic, lower epipelagic and mesopelagic zones were identical. Adopted model was:  $Y = \beta_0 + \beta_1 X_1 + \beta_2 Z_1 + \beta_3 Z_2 + \beta_4 Z_1 X + \beta_5 Z_2 X$ . We assigned different values to  $Z_1$  and  $Z_2$  for different zones (euphotic: 0, 0; lower epipelagic: 1, 0; mesopelagic: 0, 1). Common-log transformed data were used for each variable

Y variable	X variable	Selected model	$r^2$	$F$	$p$
HNF abundance	Bacterial abundance	$Y = 0.28 + 0.49X - 0.17Z_1 - 0.63Z_2$	0.62	94.2	<0.0001
Grazing rates	Bacterial production	$Y = 0.30 + 0.51X - 0.46Z_2$	0.68	44.5	<0.0001
Grazing rates	Bacterial abundance	$Y = 0.88 + 1.41X - 0.42Z_2$	0.72	54.8	<0.0001
Grazing rates	HNF abundance	$Y = 0.39 + 1.22X$	0.81	180.5	<0.0001

Table 3. Percentages of active heterotrophic nanoflagellates (HNF) in euphotic and mesopelagic zones, estimated according to González (1999). Values are mean  $\pm$  1 SD (range; n). Student's *t*-test assessed whether mean values were different between 2 zones; *p*-values <0.05 represent significant differences between 2 zones. nd: not determined

Date	% active HNF		<i>p</i>
	Euphotic zone	Mesopelagic zone	
9–14 Apr 1996	68 $\pm$ 17 (49–86; 4)	40 (–; 1)	nd
27–31 May 1996	91 $\pm$ 9 (78–98; 4)	55 $\pm$ 25 (4)	0.057
23–30 Jul 1996	84 $\pm$ 12 (77–98; 3)	43 $\pm$ 11 (35–51; 2)	0.032
10–16 Mar 1997	63 $\pm$ 21 (33–77; 4)	65 $\pm$ 20 (42–79; 3)	0.944
22 Sep –1 Oct 1997	50 $\pm$ 12 (37–60; 3)	34 $\pm$ 10 (22–41; 3)	0.142

mesopelagic from 22 to 79% and in the euphotic zone from 33 to 98% (Table 3). Actively grazing HNF often comprised similar portions in both the mesopelagic and the euphotic zones. In July 1996, the % active HNF were significantly different between the euphotic and mesopelagic zones: An average of 84% of the HNF community in the euphotic zone ingested bacteria compared to 43% in the mesopelagic (Table 3). Percentages of active HNF showed significant relationship with clearance rates both in the euphotic and

mesopelagic zones (Fig. 4), but with different slopes and *y*-axis intercepts. However, clearance rates did not correlate (*p* > 0.05) with total active HNF abundance in either zone (data not shown).

### DISCUSSION

Unexpectedly, no significant differences were observed between ingestion rates in the euphotic and mesopelagic zones in the present study. Further, HNF clearance rates and volume-specific HNF clearance rates were generally in the same ranges in the 2 zones and often higher in the mesopelagic zone. This is intriguing because slow growth (11 to 89 d doubling time calculated on the basis of bacterial abundance and production) and low abundance of mesopelagic bacteria ( $0.1$  to  $0.5 \times 10^9$  cells  $l^{-1}$ ) seemed to present unfavorable conditions for the ingestion of bacteria by HNF (Peters 1994). In seawater samples incubated for 30 to 60 h, HNF increased their clearance rates (15 to 20  $nl$  HNF $^{-1}$  h $^{-1}$ ) at the optimal conditions of maximum bacterial abundance, and under unfavorable conditions with minimum bacterial abundance (i.e. at starving status) reduced their clearance rates (5 to 10  $nl$  HNF $^{-1}$  h $^{-1}$ ) (Andersen & Fenchel 1985). Thus, reduced clearance rates of HNF would be expected in the mesopelagic, but our result did not agree with this prediction. This phenomenon might suggest innately high clearance rates by mesopelagic HNF, and maybe different species occur in the mesopelagic. The high clearance rate in mesopelagic HNF would be advantageous to HNF survival during conditions of low abundance and slow growth of bacteria in the mesopelagic.

Another surprising observation was that similar percentages of active HNF were frequently found in both pelagic zones. Actively ingesting HNF during the incubation period occurred persistently in the mesopelagic samples, with a range of 22 to 79% of HNF assemblages comparable to 33 to 98% active HNF values found in the euphotic zone of the East Sea and 11 to 99% active HNF in marine samples (González 1999). These results indicate that mesopelagic HNF are still active and survive in the mesopelagic. Furthermore, a ca 4-fold variation in % active HNF (Fig. 4) suggests that mesopelagic HNF may adapt their physiological state to the changing conditions in the mesopelagic. Also, close relationships between HNF clearance rates and % active HNF for both the euphotic and mesopelagic zones indicate that with more active members in the HNF assem-

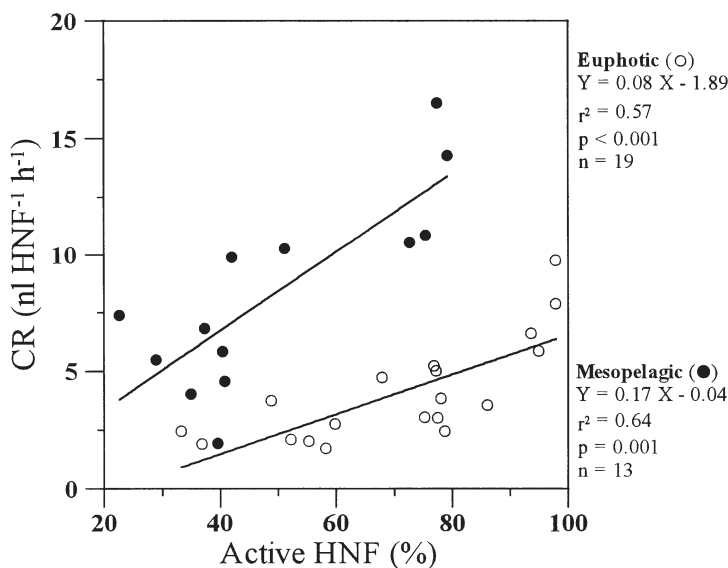


Fig. 4. Scatter plots of clearance rates (CR) vs percentage active HNF in samples from euphotic (○) and mesopelagic (●) zones of East Sea

blages, HNF increased clearance rates in both zones. This is striking, because the measurements in each pelagic zone were from different seasons, depths and prey conditions, presumably encompassing diverse HNF assemblages. Varying their clearance rates according to the fractions of actively grazing HNF available in the assemblages would enable HNF to adjust their consumption of bacteria in the mesopelagial.

Similar rates of HNF clearance and comparable percentages of active HNF in both pelagic zones indicate that low HNF grazing rates in the mesopelagial would not be due to low levels of activity by mesopelagic HNF and low % of active HNF, but to low abundance and flux of bacteria to mesopelagic HNF. Consistently, the ratios of HNF grazing to bacterial production were larger than 1 in 67% of the samples (Fig. 3B). Such high ratios in the mesopelagial suggest that mesopelagic HNF may consume other particles and bacterial production exported from the euphotic zone. Alternatively, bacterial production may be underestimated. However, the possibility of underestimating bacterial production would be remote (see below). Thus, in general, HNF seem to be the major cause of bacterial mortality and to overwhelmingly control the growth of bacteria in the mesopelagial in the study area, and presumably in other oceanic systems as well.

Regression analyses of HNF variables with other variables showed that only HNF grazing on bacteria in the mesopelagic zone depended significantly on bacterial abundance and water temperature. The dependence of HNF grazing on bacterial abundance and water temperature is consistent with other HNF grazing model available in the literature (Vaqué et al. 1994). However, HNF grazing on bacteria was not significantly correlated with bacterial production and HNF abundance in the mesopelagic zone. Analyses of a multiple regression model with group variables and interaction terms showed that responses of HNF grazing rates to changes in HNF abundance, bacterial abundance and bacterial production are similar in the euphotic, lower epipelagic and mesopelagic zones (Fig. 3, Table 2). This suggests to us that significantly similar relationships between HNF grazing rates and bacterial production and HNF abundance exist in the mesopelagial, and that the lack of significant relationships in the mesopelagial would be due to the small number of samples in our study. Probably, dependence of HNF grazing on bacterial production would also hold in the mesopelagial, and large-growing bacteria would be grazed selectively by HNF (González et al. 1990, 1993) in the mesopelagial.

The above scenarios could be affected if we underestimated bacterial production or overestimated HNF grazing in the mesopelagic samples. It is unlikely that bacterial production was underestimated: a conversion factor of  $1.8 \times 10^{17}$  cells (mol leucine incorporated)<sup>-1</sup> for

<sup>14</sup>C-leucine incorporation seems reasonable since conversion factors obtained empirically for the euphotic samples were close to this (data not presented), and since extracellular isotope dilution of leucine would be negligible due to undetectable leucine concentration in the mesopelagic zone (Park et al. 1995). Were we to use lower conversion factors for the mesopelagic zone (i.e. no intracellular isotope dilution of leucine: Simon et al. 1992), the conclusion of major control of mesopelagic bacteria by HNF would not be affected. Probably, underestimation of bacterial production could come from ingestion and subsequent digestion of bacteria by HNF during the measurements. Using values and relationship in the literature on carbon-based gross growth efficiency (ca 20 to 40%: Sherr et al. 1987, González et al. 1993) of HNF and the temperature dependence of digestion rate (Sherr et al. 1988), we would calculate that <2% of bacterial production was underestimated for 1 to 2 h incubation (data not shown; also, M. Simon pers. comm.).

Short-term uptake experiments of FLB are rarely applied to oligotrophic aquatic environments (Christaki et al. 1999, González 1999) since such an approach often lacks sensitivity because of their low bacterial abundance (Sherr et al. 1993). Recently, González (1999) proposed a new protocol for estimating HNF grazing rates from fractions of HNF with ingested FLB, and showed an excellent match with estimates using the standard protocol (according to the method of Sherr et al. [1987]). Furthermore, he anticipated that the new protocol would be useful for aquatic environments with low bacterial abundance. For our mesopelagic as well as our euphotic samples, we obtained optimum correspondence at a slope of 1:1 (Fig. 5) as did González (1999). Thus, the short-term uptake experiments using FLB in our mesopelagic samples seemed effective. The use of different biovolumes of FLB than those of *in situ* marine bacteria might affect our grazing rates, since it is known that HNF clearance rates are strictly related to bacterial biovolume (González et al. 1990). Thus, biovolume-corrections must be made for measured grazing rates when the mean biovolumes of FLB differ significantly from those of marine bacteria. González (1996) proposed that HNF could be highly effective size-selective grazers for small bacteria (<0.1  $\mu\text{m}^3$ ), and that HNF clearance rates depend on bacterial biovolume in a Monod-like relationship. Since the range of bacterial biovolumes in our study was 0.05 to 0.34  $\mu\text{m}^3$ , use of the proposed model was inviting. However, the clearance rates from this study were much higher than the maximum clearance rate of HNF reported by González (1996), suggesting that direct use of such relationship requires further investigation. Nevertheless, relative biovolume-corrections could be and were approximated: the cor-



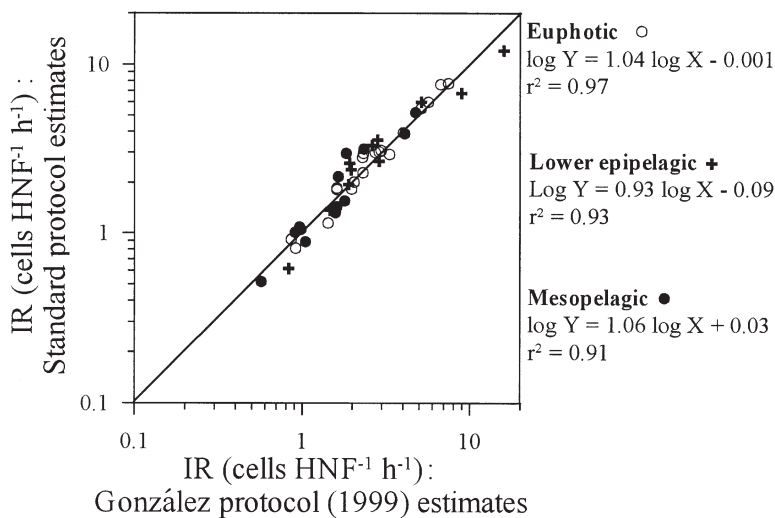


Fig. 5. Comparison of ingestion rates obtained experimentally from FLB (fluorescently labeled bacteria) method of Sherr et al. (1987) and those calculated from fractions of HNF with ingested FLB according to González (1999). Data were from euphotic (○), lower epipelagic (+) and mesopelagic (●) zones of East Sea. Line represents 1:1 slope

rections did not affect our general conclusions, although changes in the HNF grazing to bacterial production ratios were substantial. After biovolume-correction, the ratios of HNF grazing to bacterial production in the mesopelagic ranged from 0.12 to 4.47, and ratios > 1.0 occurred in 5 out of 12 samples.

In the present study, we assumed that growth rates of mesopelagic bacteria and grazing rates of mesopelagic HNF might not be influenced by incubating mesopelagic samples at atmospheric pressure. There were but few studies available in the literature regarding pressure effects on mesopelagic bacteria. Turley (1993) showed that leucine incorporation by bacteria attached to sinking particles collected from 200 m depth did not differ at 1 and 100 atm pressure. However, this result might not be directly applicable to our free-living bacteria samples. Recently, Tholosan et al. (1999) reported that pressure effects on bacterial production measured by  $^3\text{H}$ -thymidine incorporation seemed to be first evident at 800 m depth. It thus seems that incubation of our mesopelagic samples at atmospheric pressure did not cause a serious underestimation of bacterial production. However, no information is available about pressure effects on mesopelagic HNF grazing rates. To confirm the above scenario, further work using non-decompressed samples for HNF grazing rates is needed.

Although our conclusions await future confirmation, our results showing active mesopelagic HNF and high grazing pressure by HNF on mesopelagic bacteria have important implications for biogeochemical cycles and

microbial food webs in the mesopelagic. Solubilization of a sinking particles mediated by particle-associated bacteria (Smith et al. 1992) provides dissolved organic matter for the growth of free-living bacteria (Cho & Azam 1988). The large-scale production of bacteria would be dominantly channeled to HNF in the mesopelagic, and mesopelagic HNF would respire bacterial organic carbon and regenerate nutrients in a tightly coupled manner, since upon exposure to food-bacteria starved flagellates, irrespective of starvation period, are known to instantly increase respiration within a few minutes of feeding (Fenchel 1982). In addition, mesopelagic HNF would be a link between bacteria and microzooplankton, completing the microbial loop (Azam et al. 1983) in the mesopelagic zone. The survival and significant potential growth of mesopelagic HNF would support the proposed interaction between HNF and aggregates in the mesopelagic (Lochte

1991), and thereby the contribution of mesopelagic HNF to decomposition and fragmentation of aggregates during their settlement through the mesopelagic.

In conclusion, HNF in the mesopelagic zone seem to survive well the low abundance and production of bacteria in this zone, and to exert dominant grazing forces on mesopelagic bacteria at the study site. The HNF would thus play an important role in biogeochemical cycles and energy flow via microbial food-webs in the mesopelagic. The flow of energy through the mesopelagic microbial loop may be limited by availability of bacteria.

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