Bacterivory by co-occurring red-tide algae, heterotrophic nanoflagellates, and ciliates

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ABSTRACT: We investigated feeding of natural populations of red-tide algae, heterotrophic nanoflagellates (HNFs), and ciliates (<30 µm in cell length) on natural populations of marine bacteria (mostly heterotrophic bacteria) in diverse Korean waters (Masan Bay, Jinhae Bay, Shiwha Bay, Keum River estuary, and the open coastal waters off Yeosu) during red tides in 2004 to 2005. To explore the functional responses of the dominant red-tide algae to bacteria, we also measured the ingestion rates of the dinoflagellates *Cochlodinium polykrikoides*, *Heterocapsa rotundata*, *H. triquetra*, and *Prorocentrum minimum* as well as the raphidophytes *Chattonella ovata* and *Heterosigma akashiwo* as a function of bacterial concentration in the laboratory. During red tides, ingestion rates of the red-tide algae *C. polykrikoides*, *H. rotundata*, *H. triquetra*, *H. akashiwo*, *P. minimum*, and *P. triestinum* on natural populations of bacteria in Korean waters (1.2 to 20.6 cells alga^{-1} h^{-1}) were comparable to those of co-occurring HNFs (0.7 to 39.4 cells HNF^{-1} h^{-1}), but much lower than those of co-occurring ciliates (15 to 713 cells ciliate^{-1} h^{-1}). However, the combined grazing coefficients attributable to the dominant algal predators on natural populations of bacteria during red tides (0.04 to 1.71 d^{-1}) were usually higher than those attributable to co-occurring HNFs (0.01 to 0.20 d^{-1}) or ciliates (0.00 to 0.72 d^{-1}). With increasing mean prey concentration, the ingestion rates of *C. polykrikoides*, *H. rotundata*, *H. triquetra*, *P. minimum*, *C. ovata*, and *H. akashiwo* on bacteria rapidly increased at prey concentrations of ca. 5 to 10×10^6 cells ml^{-1}, and slowly increased or became saturated at higher prey concentrations. The maximum ingestion and clearance rates of *C. polykrikoides*, *H. rotundata*, *H. triquetra*, *P. minimum*, *C. ovata*, and *H. akashiwo* on bacteria (6 to 25 cells alga^{-1} h^{-1} and 1.0 to 4.5 nl alga^{-1} h^{-1}) were comparable to those so far reported in the literature of HNFs on bacteria. The results of the present study suggest that, potentially, red-tide algae can have a considerable grazing impact on populations of bacteria during red tides and are sometimes the most effective protistan predators of marine bacteria.

KEY WORDS: Bacteria · Graze · Harmful algal bloom · Ingestion · Marine · Protist · Red tide

INTRODUCTION

Marine bacteria are one of the major components in marine ecosystems (e.g. Azam et al. 1983), and they sometimes dominate marine plankton biomasses or particulate organic carbon (Cole et al. 1988, Cho & Azam 1990). Heterotrophic nanoflagellates (HNFs) and ciliates are also major components of marine food webs (McManus & Fuhrman 1988, Bloem & Bar-Gilissen 1989, Patterson & Larsen 1991). It has been reported that HNFs and/or small ciliates are major predators of marine bacteria and occasionally control

the populations of marine bacteria (Fenchel 1982, Azam et al. 1983, Sieburth 1984). However, it has been suggested that microorganisms other than HNFs and/or ciliates may sometimes affect the populations of marine bacteria (e.g. Vaque et al. 2002).

Recently, many red-tide algae have been revealed to be mixotrophic (Bockstahler & Coats 1993, Chang & Carpenter 1994, Jacobson & Anderson 1996, Granéli et al. 1997, Stoecker et al. 1997, Legrand et al. 1998, Stoecker 1999, Skovgaard et al. 2000, Smalley et al. 2003, Jeong et al. 2004, 2005a,b,c). They are capable of obtaining nutrition by photosynthesis and/or predation. If red-tide algae ingest bacteria in a significantly large number, they may compete with HNFs and ciliates for bacterial prey, and the grazing impact by the algae on bacteria may make a considerable contribution to the total grazing impact of protistan predators on bacteria. However, there have been few studies on the feeding of red-tide algae on marine bacteria (Nygaard & Tobiesen 1993, Jeong et al. 2005a), although there have been many studies on the interactions between red-tide algae and algicidal bacteria (Kim et al. 1998, Doucette et al. 1999, Imai et al. 2001, Skerratt et al. 2002, Mayali & Azam 2003). Nygaard & Tobiesen (1993) reported that some prymnesiophyte species, the raphidophyte *Heterosigma akashiwo* and the dinoflagellates *Karlodinium micrum* (previously *Gyrodinium galatheanum*) and *Alexandrium tamarense* were able to ingest bacteria, but *H. akashiwo* and *K. micrum* did not ingest bacteria when the phosphate concentrations were high (batch cultures, 0.33 to 1.56 µM). Jeong et al. (2005a) reported that several red-tide dinoflagellates were able to ingest the cyanobacterium *Synechococcus* sp. However, the feeding and grazing impacts of red-tide algae on bacteria in marine ecosystems remain poorly understood. In the interactions between red-tide algae and marine bacteria, the following questions may arise. (1) Are red-tide algae able to ingest marine bacteria in waters where phosphate and/or nitrate plus nitrite concentrations are high? Many algal species have formed red tides in coastal, bay, and estuarine waters where nutrient concentrations are relatively high (usually >10 µM nitrate plus nitrite and >1 µM phosphate) (e.g. Jeong et al. 2005b). Therefore, it is worthwhile to investigate the feeding of diverse red-tide algal species on marine bacteria in eutrophic waters. (2) Do red-tide algae have considerable grazing impact on the natural populations of marine bacteria? In addition, are the ingestion rates and grazing impact of red-tide algae on the natural populations of co-occurring bacteria comparable to those of HNFs and/or ciliates, considered to be the major predators of bacteria?

There have been no reports on comparisons of the grazing impact by co-occurring red-tide algae, HNFs, and ciliates on the natural populations of marine bacteria. (3) What are the functional responses of red-tide algae to bacteria? Further, are the maximum ingestion and clearance rates of red-tide algae on bacteria comparable to those of HNFs and ciliates?

In the present study, we (1) investigated the ingestion rate and grazing impact by the natural populations of co-occurring red-tide algae, HNFs, and ciliates on the natural populations of marine bacteria in several eutrophic Korean waters (Masan Bay, Jinhae Bay, Shiwha Bay, Keum River Estuary, and the open coastal waters off Yeosu) during red tides in 2004 to 2005; (2) examined the functional responses of the red-tide dinoflagellates *Cochlodinium polykrikoides*, *Heterocapsa rotundata*, *H. triquetra*, and *Prorocentrum minimum* and the red-tide raphidophytes *Chattonella ovata* and *Heterosigma akashiwo*, which were the dominant species during the red tides when fed on bacteria in the laboratory; and (3) compared the maximum ingestion and clearance rates of red-tide algae on marine bacteria with those of HNFs and ciliates. The results of the present study provide a basis for understanding the interactions between red-tide algae and co-occurring marine bacteria and possible competition among red-tide algae, HNFs, and ciliates for bacterial prey.

MATERIALS AND METHODS

Feeding of protistan predators on bacteria in Korean waters. Water samples were taken from the surface in Masan Bay, Jinhae Bay, Shiwha Bay, Keum River Estuary, and the open coastal waters off Yeosu, Korea, by using water samplers during red tides from December 2004 to August 2005 (Fig. 1). Water temperatures and salinities in the surface waters were measured using a YSI 30 (YSI), and pH and dissolved oxygen (DO) were measured using pH-11 (Schott Handy—Lab) and Oxi 197i (WTW), respectively (Table 1). Water samples for analyzing nutrient concentrations were gently filtered through GF/F filters and stored frozen at –20°C until nitrate plus nitrite and phosphate concentrations were measured using a nutrient autoanalyzing system (Bran and Luebbe TRAACS 2000). The water samples were transported into the laboratories within 10 to 60 min.

In order to determine the bacterial and HNF abundances, aliquots of the water samples were poured into 100 ml polyethylene bottles and preserved with glutaraldehyde (final conc. = 1% v/v). Three to twelve 1 ml fixed aliquots were stained with 4′6′-diamidino-2-phenylindole (DAPI, final conc. = 1 µM) and then filtered onto 0.2-µm-pore-sized polycarbonate (PC) black membrane filters. Bacteria were enumerated under an epifluorescent microscope with UV light excita-
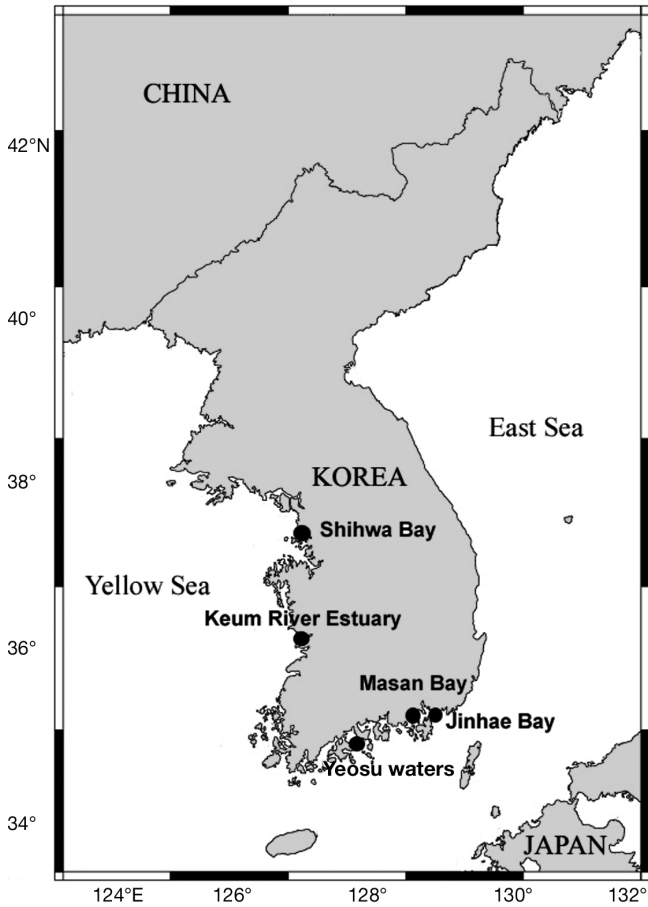
Fig. 1. Map of the study areas (Masan Bay, Jinhae Bay, Shiwha Bay, Keum River Estuary, and the open coastal waters off Yeosu, Korea)

tion (Porter & Feig 1980). Additionally, three 1 to 5 ml fixed aliquots were stained with DAPI and then filtered onto polycarbonate (PC) black membrane filters of 0.2 μm pore sized. HNFs were also enumerated under an epifluorescent microscope with UV light excitation. HNFs could be distinguished from autotrophic nanoflagellates that exhibit orange-colored autofluorescence with blue light excitation. Aliquots of the water samples for counting red-tide algae and ciliates were poured into 500 ml polyethylene bottles and preserved with acidic Lugol's solution. After thorough mixing, >500 algal cells in three 1 ml Sedgwick–Rafter counting chambers (SRCs) and >20 ciliate cells in 3 to 11 SRCs were counted under an inverted microscope with standard transmitted illumination.

The samples for the feeding experiments were screened gently through a 30 μm Nitex mesh and placed in 270 ml PC bottles. Then, 3 to 4 d prior to feeding experiments, bacterial cells collected from the same site were fluorescently labeled by the method of

Sherr et al. (1987). These experiments revealed that the fluorescently labeled bacteria (FLB) were mostly seen as rods (a cylinder) and rarely spherical. We measured the longest (length) and the shortest axes (width) of 23 to 27 FLB cells for each field experiment under an epifluorescent microscope, as described in Lee & Fuhrman (1987), and then calculated the volume according to the following equation: volume = $[\pi (3L - W)/3 \times (W/2)^2]$ for a rod (cylinder) and $4/3 \times [\pi R^3]$ for a sphere, where L is length, W is width, and R is radius, as in Lee (1993). The size of fluorescent beads (0.47 μm, size data supplied by the manufacturer, Polyscience) was also measured to calibrate our results. The volumes of FLB and ratios of the actual initial abundance of FLB to the abundances of natural population of bacteria are shown in Table 2. FLB were added to duplicate (Keum River Estuary and Jinhae Bay) or triplicate (Shiwha Bay, Masan Bay, and Yeosu waters). One control bottle (without FLB) was set up for each experiment. The bottles were placed on shelves and incubated at a temperature equivalent to that of the water temperature at the sampling site under continuous illumination of 30 μE m^{-2} s^{-1} of cool white fluorescent light.

After 1, 5, 10, 20, and 30 min incubation, 10 ml aliquots were removed from each bottle, transferred into 20 ml vials, and then fixed with borate-buffered formalin (final conc. = 3%). The fixed samples were stained using DAPI (final conc. = 1 μM) and then filtered onto PC black membrane filters of 0.2 μm pore size. Green inclusions (FLB) inside the protoplasm of 50 to 70 cells of red-tide algae and 10 to 30 cells each of HNFs and ciliates on the PC black membrane filters were enumerated under an epifluorescent microscope with blue light excitation. The ingestion rate (cells predator^{-1} h^{-1}) was then calculated by linear regression of the number of FLBs per predator cell as a function of incubation time after Sherr et al. (1987).

We estimated the grazing coefficients attributable to red-tide algae, HNFs, and ciliates on bacteria by combining the data on abundances of the predators and bacteria with ingestion rates of the predators on bacteria obtained in the present study. The grazing coefficients (g, h^{-1}) were calculated as follows:

$$g = CR \times GC \qquad (1)$$

where CR (ml predator^{-1} h^{-1}) is the clearance rate of a predator on bacteria at a given prey concentration and GC is the predator concentration (cells ml^{-1}). CRs were calculated as follows:

$$CR = IR/PC \qquad (2)$$

where IR (cells predator^{-1} h^{-1}) is the ingestion rate of the predator on bacteria and PC (cells ml^{-1}) is the prey concentration.

Table 1. Water temperature, salinity, and nutrient conditions for incubation of bacteria and their predators during red tides in Korean waters in 2004–2005. Data on nutrients are mean ± SE

Site	Date (dd/mm/yy)	Temp. (°C)	Salinity (psu)	$NO_2 + NO_3$ (µM)	PO_4 (µM)
Keum Estuary	20/05/05	21.5	17.5	72.3 ± 7.0	0.6 ± 0.2
Shiwha Bay	14/07/05	24.0	22.4	223.2 ± 14.1	8.9 ± 0.0
Masan Bay	25/12/04	10.1	30.5	21.5 ± 1.8	3.0 ± 0.5)
	26/12/04	9.8	30.6	11.6 ± 1.4	1.1 ± 0.1
	09/06/05	23.8	28.7	59.7 ± 2.4	2.2 ± 0.1
Jinhae Bay	23/06/05	27.4	30.2	70.7 ± 1.4	5.1 ± 0.3
	24/06/05	28.1	30.8	72.2 ± 6.7	3.9 ± 0.2
	25/06/05	28.0	30.3	44.4 ± 2.7	2.6 ± 0.1
Yeosu waters (Stn 1)	05/08/05	23.3	32.4	1.0 ± 0.1	1.5 ± 0.2
(Stn 2)	05/08/05	23.9	32.6	1.2 ± 0.1	1.2 ± 0.2
(Stn 3)	05/08/05	25.7	32.4	1.1 ± 0.0	1.4 ± 0.3

Table 2. Volume of FLBs (mm^3) and bacterial abundance (10^6 cells ml^{-1}). Data are means ± SE, except those in parentheses (n). Natural: populations of bacteria; FLB: fluorescently labeled bacteria; Abundance ratio: actual initial abundances of FLBs to the abundances of natural populations of bacteria (%). Ranges of length and width of FLBs used were 0.35–1.22 and 0.61–2.70 µm, respectively

Site	Date (dd/mm/yy)	Volume of FLBs	Natural	FLB	Abundance ratio
Keum Estuary	20/05/05	0.33 ± 0.03 (27)	3.5 ± 0.2	3.1 ± 0.3	89 ± 10 (3)
Shiwha Bay	14/07/05	0.37 ± 0.04 (27)	6.5 ± 0.2	2.5 ± 0.2	38 ± 3 (3)
Masan Bay	25/12/04	0.39 ± 0.05 (23)	0.8 ± 0.0	0.3 ± 0.0	24 ± 4 (9)
	26/12/04		1.0 ± 0.0	0.2 ± 0.0	
	09/06/05		12.9 ± 1.1	2.0 ± 0.1	
Jinhae Bay	23/06/05	0.26 ± 0.02 (23)	6.5 ± 0.5	2.1 ± 0.1	21 ± 3 (9)
	24/06/05		13.0 ± 0.3	1.6 ± 0.1	
	25/06/05		8.0 ± 0.7	1.5 ± 0.1	
Yeosu waters					
(Stn 1)	05/08/05	0.39 ± 0.05 (27)	2.5 ± 0.2	1.8 ± 0.2	46 ± 8 (9)
(Stn 2)	05/08/05		7.2 ± 1.2	1.7 ± 0.1	
(Stn 3)	05/08/05		8.5 ± 0.5	3.6 ± 0.8	

Culture of red-tide algae. The red-tide algae used in the present study were grown at 20°C in enriched *f*/2 seawater media (Guillard & Ryther 1962) without silicate, under a 14 h light:10 h dark cycle with 30 µE m^{-2} s^{-1} of cool white fluorescent light. The mean equivalent spherical diameters (ESDs, mean ±SD) of the algae were measured using an electronic particle counter (Coulter Multisizer II, Coulter Corporation). Carbon contents of the red-tide algae were estimated from cell volumes according to Strathmann (Strathmann 1967). Cultures that were in their exponential growth phase were used for these feeding experiments. The algal cultures were confirmed to be uniprotist cultures without contaminating amoebae or HNFs under a compound microscope at a magnification of ×1000.

Effects of prey concentration. These experiments were designed to investigate the ingestion rates of *Cochlodinium polykrikoides*, *Heterocapsa rotundata*, *H. triquetra*, *Prorocentrum minimum*, and *Heterosigma akashiwo*, the most dominant red-tide species during the present field study, on bacteria as a function of prey concentration. *Chattonella ovata* was also chosen as a target species, because it sometimes forms red-tide patches in some of these field study areas (our unpubl. data).

One or 2 d prior to this experiment, the bacterial cells that originated from a non-axenic culture of the target red-tide alga were fluorescently labeled according to Sherr et al. (1987). The volumes of FLB and ratios of the actual initial abundances of FLB to the abundances of non-FLB bacteria are shown in Table 3. Carbon contents of bacteria were estimated from cell volumes by using the formula: pg C $cell^{-1}$ = 0.12 $V^{0.7}$ (Simon & Azam 1989, recalculated by Norland 1993), where V is the cell volume (µm^3) of the bacteria.

A dense culture of each red-tide alga maintained in an *f*/2 medium in exponential phase was transferred into a 1 l PC bottle. Three 1 ml aliquots from the bottle were counted using a compound microscope to determine the cell concentrations of the red-tide alga as described above.

The mean actual initial predator and prey concentrations are shown in Table 3. Triplicate 270 ml PC experimental bottles (containing mixtures of predator and prey) and triplicate predator control bottles (containing predator only) were also established; 20 ml of *f*/2 medium were added to all the bottles, which were then filled to capacity with freshly filtered seawater, capped, placed on a shelf, and then incubated at 20°C under continuous illumination of 30 µE m^{-2} s^{-1} of cool white fluorescent light. After 1, 5, 10, 20, and 30 min incubation, 10 ml aliquots were removed from each bottle, transferred into 20 ml vials, and then fixed with borate-buffered formalin (final conc. = 3%). The fixed samples were stained using DAPI (final conc. = 1 µM) and then filtered onto 3-µm-pore-sized PC white membrane filters. FLBs inside a red-tide alga were enumerated under an epifluorescent microscope with blue light excitation. The ingestion rates of the red-tide alga on bacteria were determined as de-

Table 3. Volume of FLBs (µm³), abundance ratios (actual initial abundances of FLBs to the abundances of non-FLB bacteria, %), and mean actual initial concentrations of predator and prey. Data are means ± SE, except those in parentheses (n)

| Red tide alga | Volume | Abundance ratios | Concentration (×10⁴ cells ml⁻¹) | | | |
| | | | Predator | | Prey | |
			Min.	Max.	Min.	Max.
Chattonella ovata	0.52 ± 0.09 (23)	28 ± 9 (15)	0.100 ± 0.02 (3)	0.241 ± 0.009 (3)	38 ± 4.1 (3)	570 ± 130 (3)
Cochlodinium polykrikoides	0.18 ± 0.02 (27)	19 ± 4 (15)	0.047 ± 0.004 (3)	0.227 ± 0.005 (3)	55 ± 2.8 (3)	1300 ± 210 (3)
Heterocapsa rotundata	0.32 ± 0.04 (22)	17 ± 5 (18)	0.234 ± 0.015 (3)	0.406 ± 0.024 (3)	65 ± 4.8 (3)	2100 ± 79 (3)
Heterocapsa triquetra	0.35 ± 0.04 (22)	38 ± 12 (18)	0.200 ± 0.002 (3)	0.485 ± 0.033 (3)	64 ± 0.7 (3)	2300 ± 32 (3)
Heterosigma akashiwo	0.30 ± 0.03 (38)	11 ± 3 (18)	0.221 ± 0.013 (3)	0.781 ± 0.146 (3)	95 ± 6.1 (3)	8500 ± 99 (3)
Prorocentrum minimum	0.48 ± 0.09 (22)	17 ± 3 (21)	0.066 ± 0.005 (3)	0.128 ± 0.003 (3)	33 ± 0.85 (3)	900 ± 14 (3)

scribed above. Additionally, at the beginning of the experiment, a 1 ml fixed aliquot was stained with DAPI and then filtered onto 0.2-µm-pore-sized PC black membrane filters. Bacteria (both FLB and non-FLB) outside the red-tide algal cells were also enumerated under an epifluorescent microscope with UV light excitation for non-FLBs and blue light excitation for FLBs. After subsampling, the bottles were capped, placed on a shelf, and incubated again as described above.

All ingestion rate data were fitted to a Michaelis–Menten equation:

$$IR = \frac{I_{max}}{K_{IR}(x)} \qquad (3)$$

where I_{max} is the maximum ingestion rate (cells predator⁻¹ h⁻¹), x is prey concentration (cells ml⁻¹), and K_{IR} is the prey concentration sustaining 1/2 I_{max}.

Two to 6 ml aliquots fixed with formalin at the end of the experiment were stained using DAPI (final conc. = 1 µM) and then filtered onto 3-µm-pore-sized, 25 mm PC white membrane filters. The concentrated cells on the membranes were observed under a confocal laser scanning microscope (CLSM, Carl Zeiss-LSM510) at a magnification of ×1000 by scanning the algal body at consecutive intervals of 1 to 2 µm along the *z*-axis. Pictures showing ingested bacterial cells inside each algal predator cell were taken using digital cameras on a confocal laser scanning microscope and/or an epifluorescent microscope at a magnification of ×1000. Additionally 8 red-tide algal species (*Akashiwo sanguinea*, *Alexandrium catenella*, *Amphidinium carterae*, *Gonyaulax polygramma*, *Gymnodinium catenatum*, *G. impudicum*, *Lingulodinium polyedrum*, and *Scrippsiella trochoidea*) were observed to investigate whether or not each algal species was able to feed on marine bacteria. Approximately 10⁸ FLB cells were placed into a 270 ml PC bottle for each target alga. After 10, 20, and 30 min incubation, 10 ml aliquots were removed from the bottle, transferred into 20 ml vials, and then fixed with formalin. The fixed samples

were stained using DAPI, filtered, and then the concentrated cells on the membranes were observed under a confocal laser scanning microscope as described above.

RESULTS

Feeding by protistan predators on marine bacteria in Korean waters

We measured the ingestion rates of natural populations of co-occurring red-tide algae, HNFs, and ciliates (<30 µm in cell length) on natural populations of marine bacteria during red tides dominated by the dinoflagellate *Heterocapsa rotundata* in the Keum River estuary (20 May 2005); the dinoflagellate *Prorocentrum minimum* in Shiwha Bay (14 July 2005); *P. minimum* and *H. triquetra* (25 to 26 December 2004), the raphidophyte *Heterosigma akashiwo*, and the euglenophyte *Eutreptiella* sp. (9 June 2005) in Masan Bay; *P. minimum* and *H. akashiwo* in Jinhae Bay (23 to 25 June 2005); and the dinoflagellate *Cochlodinium polykrikoides* in the open coastal waters off Yeosu (5 August 2005; Table 4).

During all field experiments, the water temperature ranged (mean ± SE, n) from 9.8 to 28.1°C (22.3 ± 2.0, 11), while the salinity ranged from 17.5 to 32.6 psu (28.9 ± 1.5, 11; Table 1). The range (mean ± SE, n) of the nitrate plus nitrite concentration was 1.0 to 223.2 µM (52.6 ± 19.6, 11), while that of the phosphate concentration was 0.6 to 9.0 µM (2.9 ± 0.8, 11).

During the field experiments, the range of the mean (±SE, n = 3 to 12) abundances of bacteria was from 0.8 (±0.01) to 13.0 (±1.1) × 10⁶ cells ml⁻¹ (Table 2). The minimum bacterial abundance was observed in Masan Bay in December 2004, while the maximum bacterial abundance was observed at the same location in June 2005 (Table 2). The mean abundances of *Heterocapsa rotundata* and/or *H. triquetra*, *Prorocentrum minimum* and/or *P. triestinum*, *Heterosigma akashiwo*, and *Cochlodinium polykrikoides* ranged from 200–37 640,

Table 4. Ingestion of marine bacteria by red-tide algae, heterotrophic nanoflagellates (HNFs), and ciliates (<30 μm) in Korean waters during red tides in 2004–2005. Data are mean ± SE

Location	Date (dd/mm/yy)	Predator	Abundance (ind. ml^{-1})	Ingestion rate (cells ind.$^{-1}$ h^{-1})	Clearance rate (nl ind.$^{-1}$ h^{-1})	Grazing coefficient (d^{-1})
Keum estuary	20/05/05	*Heterocapsa rotundata*	37 640 ± 1380	2.2 ± 0.1	0.6 ± 0.0	0.569 ± 0.02
		Heterocapsa triquetra	8 410 ± 290	2.6 ± 0.4	0.7 ± 0.1	0.151 ± 0.024
		HNFs	2 800 ± 330	6.3 ± 3.9	1.8 ± 1.1	0.120 ± 0.075
		Ciliates	4.2 ± 0.5	19 ± 4	5.4 ± 1.1	0.001 ± 0.000
Shiwha Bay	14/07/05	*Prorocentrum triestinum*	20 650 ± 970	7.0 ± 0.9	1.1 ± 0.1	0.498 ± 0.097
		Heterosigma akashiwo	550 ± 60	9.0 ± 1.2	1.4 ± 0.2	0.020 ± 0.003
		HNFs	1 400 ± 320	7.1 ± 1.9	1.1 ± 0.3	0.046 ± 0.006
		Ciliates	6.0 ± 0.6	107 ± 43	17 ± 7	0.003 ± 0.002
Masan Bay	25/12/04	*Prorocentrum minimum*	3 760 ± 130	2.0 ± 0.5	2.7 ± 0.6	0.238 ± 0.100
		Heterocapsa triquetra	280 ± 10	3.5 ± 0.7	4.6 ± 0.9	0.024 ± 0.001
		HNFs	420 ± 40	1.7 ± 0.5	2.2 ± 0.7	0.029 ± 0.003
		Ciliates	1.8 ± 0.3	43 ± 7	57 ± 9	0.002 ± 0.001
	26/12/04	*Prorocentrum minimum*	8 480 ± 550	2.8 ± 1.2	2.7 ± 1.1	0.702 ± 0.305
		Heterocapsa triquetra	200 ± 30	3.5 ± 1.0	3.4 ± 1.0	0.019 ± 0.007
		HNFs	760 ± 140	2.2 ± 0.4	2.1 ± 0.4	0.036 ± 0.013
		Ciliates	1.3 ± 0.3	219 ± 91	211 ± 88	0.008 ± 0.004
	09/06/05	*Heterosigma akashiwo*	3 530 ± 290	8.5 ± 1.5	0.7 ± 0.1	0.046 ± 0.005
		Eutreptiella sp.	1 530 ± 270	13.2 ± 2.1	1.0 ± 0.2	0.039 ± 0.010
		HNFs	2 560 ± 40	15.4 ± 2.9	1.2 ± 0.2	0.086 ± 0.006
		Ciliates	270 ± 60	473 ± 181	36 ± 14	0.174 ± 0.115
Jinhae Bay	23/06/05	*Prorocentrum minimum*	18 290 ± 1 410	3.9 ± 0.9	0.6 ± 0.1	0.260 ± 0.063
		Heterosigma akashiwo	16 980 ± 770	2.7 ± 0.1	0.4 ± 0.0	0.166 ± 0.005
		HNFs	970 ± 30	7.6 ± 0.4	1.2 ± 0.1	0.027 ± 0.001
		Ciliates	24 ± 7	150 ± 44	23 ± 7	0.013 ± 0.004
	24/06/05	*Heterosigma akashiwo*	59 670 ± 2 430	7.8 ± 1.5	0.6 ± 0.1	0.857 ± 0.166
		Prorocentrum minimum	30 120 ± 760	15.3 ± 5.3	1.2 ± 0.4	0.850 ± 0.297
		HNFs	2 420 ± 440	38 ± 2	2.9 ± 0.1	0.169 ± 0.008
		Ciliates	970 ± 105	137 ± 40	11 ± 3	0.245 ± 0.071
	25/06/05	*Heterosigma akashiwo*	39 510 ± 4 880	4.9 ± 1.4	0.6 ± 0.2	0.581 ± 0.168
		Prorocentrum minimum	7 440 ± 900	5.1 ± 0.2	0.6 ± 0.0	0.113 ± 0.003
		HNFs	600 ± 30	6.4 ± 1.9	0.8 ± 0.2	0.011 ± 0.003
		Ciliates	1 380 ± 140	144 ± 29	18 ± 4	0.596 ± 0.120
Yeosu waters (Stn 1)	05/08/05	*Cochlodinium polykrikoides*	1 300 ± 50	2.9 ± 0.5	1.2 ± 0.2	0.043 ± 0.003
		HNFs	2 570 ± 410	4.1 ± 0.3	1.7 ± 0.1	0.098 ± 0.012
		Ciliates	5.4 ± 0.5	64 ± 1	26 ± 1	0.003 ± 0.000
(Stn 2)	05/08/05	*Cochlodinium polykrikoides*	1 520 ± 160	7.9 ± 1.1	1.1 ± 0.2	0.040 ± 0.010
		HNFs	7 710 ± 990	6.4 ± 0.6)	0.9 ± 0.1	0.174 ± 0.017
		Ciliates	33.8 ± 3.5	159 ± 26	22 ± 4	0.017 ± 0.005
(Stn 3)	05/08/05	*Cochlodinium polykrikoides*	2 330 ± 390	9.3 ± 0.9	1.1 ± 0.1	0.067 ± 0.002
		HNFs	4 870 ± 150	6.3 ± 0.8	0.7 ± 0.1	0.076 ± 0.005
		Ciliates	3.6 ± 0.4	323 ± 93	38 ± 11	0.002 ± 0.000

3760–30 120, 550–59 670, and 1300–2330 cells ml^{-1}, respectively (n = 3, see Table 4 for SEs). The mean abundances of HNFs ranged from 420 to 7710 cells ml^{-1} (n = 3, see Table 4 for SEs), while that of ciliates ranged from 1 to 1380 cells ml^{-1} (n = 3 to 11, see Table 4 for SEs). The HNFs were most abundant during the red tides dominated by *C. polykrikoides* in the open coastal waters off Yeosu in August 2005. On the other hand, the ciliates were abundant during the red tide dominated by *P. minimum* and *H. akashiwo* in Jinhae Bay in June 2005.

During field experiments, the dominant red-tide algae *Cochlodinium polykrikoides*, *Eutreptiella* sp., *Heterocapsa rotundata*, *H. triquetra*, *Heterosigma akashiwo*,

Prorocentrum minimum, and *P. triestinum* were observed to ingest FLBs (Fig. 2). The red-tide dinoflagellates *P. donghaiense* and *P. micans* were also observed to contain bacterial cells inside their protoplasm, even though bacterial abundances were relatively low. No green inclusions were observed inside the protoplasm of the predators in the control bottles (without FLB).

During field experiments the mean (±SE, n) of the ingestion rates of all dominant red-tide algae on natural populations of bacteria obtained in the present study was 6.1 bacteria alga^{-1} h^{-1} (±0.6, 49; range = 1.2 to 20.6 bacteria alga^{-1} h^{-1}; Table 4). The mean ingestion rates (range, mean ± SE, n) of natural populations of bacteria
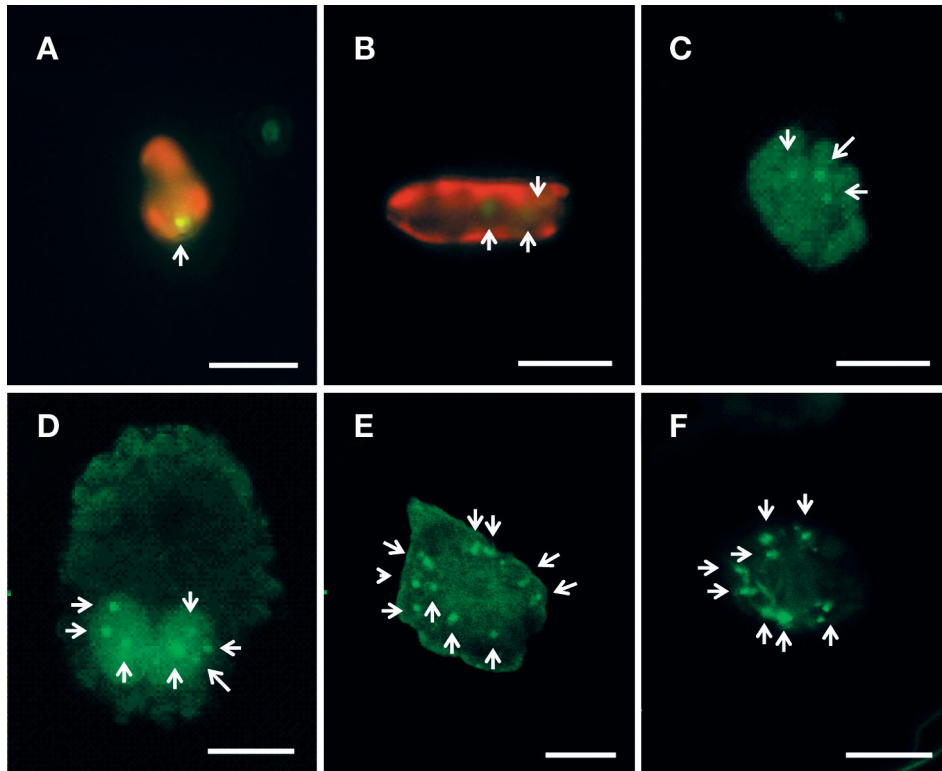
Fig. 2. Feeding by red-tide algae on bacteria: (A) *Heterocapsa rotundata*, (B) *Prorocentrum donghaiense*, (C) *Heterosigma akashiwo*, (D) *Chattonella ovata*, (E) *Gonyaulax polygramma*, and (F) *P. minimum*. Scale bars: 10 µm. Arrows: ingested prey cells, which are fluorescently labeled bacteria. Panels are photographs taken using (A,B) an epifluorescent microscope, (C–F) a confocal laser scanning microscope

by *Heterocapsa rotundata* and/or *H. triquetra* (2.1 to 5.6 bacteria alga^{-1} h^{-1}, 3.1 ± 0.4, 10), *Prorocentrum minimum* and/or *P. triestinum* (1.2 to 20.6 bacteria alga^{-1} h^{-1}, 5.6 ± 1.3, 15), *Heterosigma akashiwo* (2.6 to 11.4 bacteria alga^{-1} h^{-1}, 6.9 ± 0.9, 12), *Cochlodinium polykrikoides* (2.0 to 10.5 bacteria alga^{-1} h^{-1}, 6.7 ± 1.1, 9), and *Eutreptiella* sp. (10.0 to 17.3 bacteria alga^{-1} h^{-1}, 13.2 ± 2.1, 3) were significantly different ($p < 0.01$, ANOVA). The mean ingestion rate of *H. rotundata* and/or *H. triquetra* was significantly lower than that of the other red-tide algae ($p < 0.01$, 1-tailed *t*-test), except *P. minimum* and/or *P. triestinum* ($p > 0.05$), while the mean ingestion rate of *Eutreptiella* sp. was significantly higher than that of the other red-tide algae ($p < 0.05$ to 0.001). However, the mean ingestion rates of *P. minimum* and/or *P. triestinum*, *H. akashiwo*, and *C. polykrikoides* were not significantly different from one another ($p > 0.1$, 1-tailed *t*-test).

The mean ingestion rates (range, mean ± SE, n) of all HNFs (0.7 to 39.4 bacteria HNF^{-1} h^{-1}, 8.5 ± 1.7, 29) and all ciliates on natural populations of bacteria obtained in the present study (15 to 713 bacteria ciliate^{-1} h^{-1}, 175 ± 32, 29) were significantly higher than the mean ingestion rate of all red-tide algae ($p < 0.05$ for HNFs and $p < 0.005$ for ciliates, 1-tailed *t*-test).

During field experiments the mean (±SE, n) clearance rate of all red-tide algae on natural populations of bacteria obtained in the present study was 1.5 nl alga^{-1} h^{-1} (±0.2, 49; range = 0.4 to 6.4 nl alga^{-1} h^{-1}; Table 4). The clearance rates (range, mean ± SE, n) of *Heterocapsa rotundata* and/or *H. triquetra* (0.6 to 6.4 nl alga^{-1} h^{-1}, 2.7 ± 0.7, 10), *Prorocentrum minimum* and/or *P. triestinum* (0.4 to 5.0 nl alga^{-1} h^{-1}, 1.6 ± 0.3, 15), *Heterosigma akashiwo* (0.4 to 1.8 nl alga^{-1} h^{-1}, 0.8 ± 0.1, 12), *Cochlodinium polykrikoides* (0.8 to 1.5 nl alga^{-1} h^{-1}, 1.1 ± 0.1, 9), and *Eutreptiella* sp. (0.8 to 1.3 nl alga^{-1} h^{-1}, 1.0 ± 0.2, 3) on natural populations of bacteria were significantly different ($p < 0.05$, ANOVA). The mean clearance rate of *H. rotundata* and/or *H. triquetra* was significantly greater than that of *H. akashiwo* ($p < 0.005$, 1-tailed *t*-test), or *C. polykrikoides* ($p < 0.05$), while it was not significantly greater than that of *P. minimum* and/or *P. triestinum* ($p > 0.1$).

The mean clearance rate (range, mean ± SE, n) of all HNFs (0.5 to 3.1 nl HNF^{-1} h^{-1}, 1.5 ± 0.2, 29) was not significantly higher than that of all red-tide algae ($p > 0.1$ 1-tailed *t*-test), while that of all ciliates (4 to 385 nl ciliate^{-1} h^{-1}, 46 ± 13, 29) was significantly higher than that of all red-tide algae ($p < 0.005$; Table 4).

The mean (range, mean ± SE, n) grazing coefficient of the natural population of bacteria attributable to all red-tide algae during the red tides (0.012 to 1.146 d^{-1}, 0.244 ± 0.044, 49) was significantly greater than that of all HNFs (0.008 to 0.196 d^{-1}, 0.077 ± 0.011, 29) or all ciliates (0.000 to 0.716 d^{-1}, 0.087 ± 0.033, 29) (p < 0.005 for both HNFs and ciliates, 1-tailed t-test; Table 4). However, the mean grazing coefficient attributable to all HNFs was not significantly different from that attributable to all ciliates (p > 0.1, 2-tailed t-test).

Data from this study show that the ingestion rates of the dominant red-tide algae (n = 49), HNFs (n = 29), and ciliates (n = 29) on the natural populations of bacteria were positively correlated with the bacterial concentrations (p < 0.001 for red-tide algae and HNFs and p < 0.05 for ciliates, linear regression ANOVA) in Korean waters (Fig. 3). However, the ingestion rates of the dominant red-tide algae (n = 49) showed no clear correlation with nitrate plus nitrite or phosphate concentrations (p > 0.1; Fig. 4).

Effects of prey concentration

With increasing initial prey concentration, the ingestion rates of *Heterocapsa rotundata*, *Heterosigma akashiwo*, *Prorocentrum minimum*, *H. triquetra*, *Cochlo-*

dinium polykrikoides, and *Chattonella ovata* on bacteria increased rapidly at the prey concentrations of ca. 5 to 10×10^6 cells ml^{-1}, and increased slowly or reached saturation at higher prey concentrations (Fig. 5, Table 5). When the data were fitted to Eq. (3), the maximum ingestion rates of red-tide algae on bacteria were 12.2 cells alga^{-1} h^{-1} for *H. rotundata*, 11.7 for *H. akashiwo*, 21.9 for *P. minimum*, 6.0 for *H. triquetra*, 17.4 for *C. polykrikoides*, and 24.5 for *C. ovata* (Table 5).

The percentage of daily acquired carbon from bacteria to the body carbon of the smallest alga, *Heterocapsa rotundata*, was the greatest (76.1%), while that of the largest alga, *Chattonella ovata*, was the smallest (1.4%; Table 5).

The maximum clearance rates of bacteria by red-tide algae were 1.4 nl alga^{-1} h^{-1} for *Heterocapsa rotundata*, 2.6 for *Heterosigma akashiwo*, 2.3 for *Prorocentrum minimum*, 1.3 for *H. triquetra*, 1.0 for *Cochlodinium polykrikoides*, and 4.5 for *Chattonella ovata*, respectively (Table 5). The maximum volume-specific clearance rates were 1.4×10^4 h^{-1} for *H. rotundata*, 3.3×10^3 for *H. akashiwo*, 2.5×10^3 for *P. minimum*, 730 for *H. triquetra*, 110 for *C. polykrikoides*, and 130 for *C. ovata*, respectively.

Besides *Cochlodinium polykrikoides*, *Heterocapsa rotundata*, *H. triquetra*, *Prorocentrum donghaiense*, *P. micans*, *P. minimum*, *P. triestinum*, *Chattonella ovata*, and *Heterosigma akashiwo*, all red-tide algae tested in the present study (*Akashiwo sanguinea*, *Alexandrium catenella*, *Amphidinium carterae*, *Gonyaulax polygramma*, *Gymnodinium catenatum*, *Gymnodinium impudicum*, *Lingulodinium polyedrum*, and *Scrippsiella trochoidea*) were observed to contain FLBs inside the protoplasm (under confocal laser scanning microscope or epifluorescent microscope; Fig. 2). The ingested FLB cells were found aggregated in 1 or 2 areas of the protoplasm of some *C. ovata* cells, as shown in Fig. 2D, but they were widely distributed in other *C. ovata* cells (n = 30).

DISCUSSION

Feeding by protistan predators on marine bacteria in Korean waters

The present study simultaneously measured the ingestion rates of the natural populations of marine bacteria by the dominant red-tide algae, HNFs, and ciliates in several locations of Korean waters and compared the calculated grazing impacts of
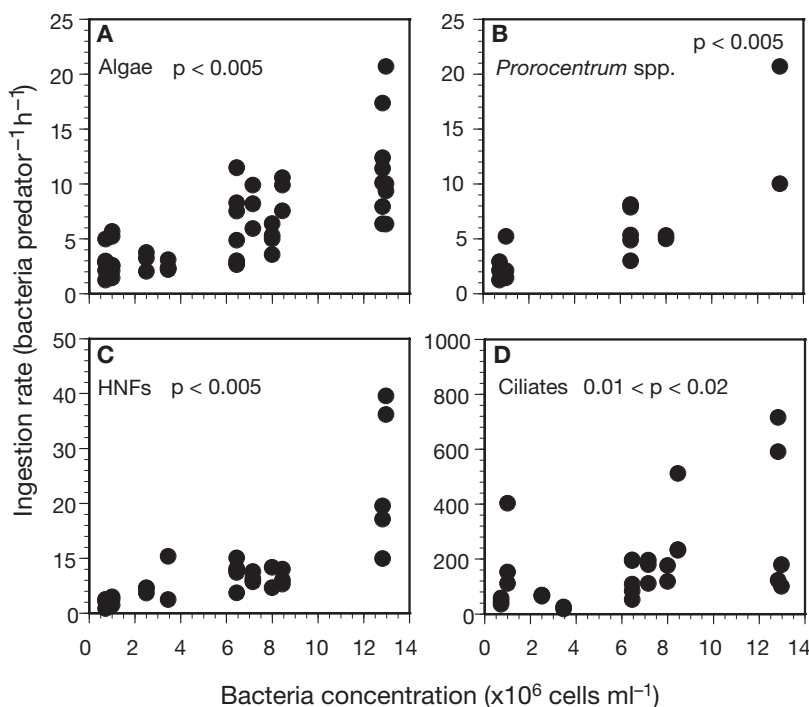

Fig. 3. Ingestion rates (IR, cells predator^{-1} h^{-1}) of: (A) all red-tide algae, (B) *Prorocentrum minimum*/*P. triestinum*, (C) heterotrophic nanoflagellates (HNFs), and (D) ciliates, on natural populations of marine bacteria as a function of bacterial concentration, as in Tables 2 & 4. Note different scales on *y*-axes
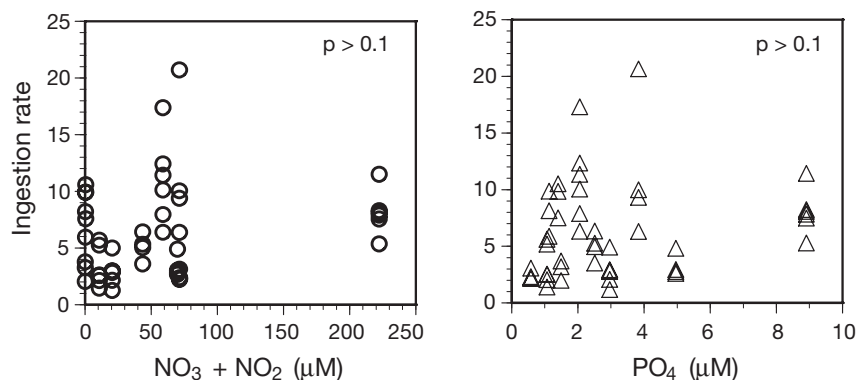
Fig. 4. Ingestion rates (IR, cells predator^{-1} h^{-1}) of all red-tide algae on natural populations of marine bacteria as a function of nitrate plus nitrite (NO$_3$ + NO$_2$) and phosphate (PO$_4$) concentrations, as in Tables 1 & 4

these 3 protistan predator groups. So far, there have been many studies comparing the ingestion rates and/or grazing impact of HNFs and ciliates on marine bacteria (Epstein 1997, Posch et al. 1999, Boenigk & Novarino 2004), but no studies have compared these parameters among co-occurring red-tide algae, HNFs, and ciliates.

Based on comparison of the grazing coefficients on the natural populations of marine bacteria attributable to the dominant red-tide algae, HNFs, and ciliates during red-tides, the results of the present study suggest that the red-tide algae *Heterocapsa rotundata/H. triquetra*, *Heterosigma akashiwo*, *Prorocentrum minimum/P. triestinum*, and *Cochlodinium polykrikoides* are the most (7 of 11 field experiments) or the second most (4 of 11) effective protistan predators of marine bacteria in Korean waters. The combined grazing coefficients of the natural populations of marine bacteria attributable to the dominant red-tide algae (1 or 2 species for each field experiment; see Table 4) ranged (mean ± SE, n) from 0.04 to 1.71 d^{-1} (0.48 ± 0.27, 11) (i.e. 4 to 82% [mean 38%] of the bacterial populations were removed by red-tide algal populations in 1 d). Therefore, with the exception of *C. polykrikoides*, red-tide algae can have considerable potential grazing impact on the populations of marine bacteria in Korean waters during red-tides; thus, we must consider the grazing by red-tide algae on bacteria when estimating the total mortality rates of marine bacteria due to grazing. However, during the absence of red tides, the abundances of red-tide algae were <10% of those measured in the present study (our unpubl. data). Under these circumstances, the calculated grazing impact of red-tide algae on populations of marine bacteria could be negligible.

All dominating red-tide algae in the study areas were able to feed on bacteria when nitrate plus nitrite and phosphate concentrations were high (1 to 223 μM and 0.6 to 9.0 μM, respectively). Also, the ingestion rates of the dominant red-tide algae on natural populations of bacteria were not affected by nitrate plus nitrite or phosphate concentrations. Nygaard & Tobiesen (1993) reported that *Heterosigma akashiwo* did not ingest bacteria at high phosphate concentrations (batch cultures, 0.3 to 1.6 μM), while our data show that *H. akashiwo* ingested bacteria when the phosphate concentrations were 2.2 to 3.9 μM. This feeding by *H. akashiwo* on bacteria when phosphate concentrations were high was confirmed in our laboratory experiment. Different strains of this red-tide alga may yield such contrasting results. Different methods might also cause this discrepancy; Nygaard & Tobiesen (1993) used only radioactively labeled bacteria for the experiments on the uptake of bacteria by *H. akashiwo* and dinoflagellate predators, while we used FLBs that were directly observed under an epifluorescent microscope. It is therefore worthwhile to explore the effects of phosphate concentrations on the feeding by diverse strains of *H. akashiwo* on marine bacteria.

The ingestion rates of HNFs on natural populations of bacteria in Korean waters (1 to 39 cells HNF^{-1} h^{-1}) are comparable to those measured in Chesapeake Bay (4 to 25 cells HNF^{-1} h^{-1}; McManus & Fuhrman 1988), on the shelf off Cape Hatteras, North Carolina (5 to 65 cells HNF^{-1} h^{-1}; Sherr et al. 2002), and in the Weddell Sea (undetected to 67 cells HNF^{-1} h^{-1}; Vaque et al. 2002). The ingestion rates of ciliates on the natural populations of bacteria in Korean waters (15 to 713 cells ciliate^{-1} h^{-1}) were higher than those measured in Tidal creek (1 to 200 cells ciliate^{-1} h^{-1}; Albright et al. 1987). However, these rates were comparable to those in Mejillonse Bay (270 to 663 cells ciliate^{-1} h^{-1}; Vargas & Gonzalez 2004). Therefore, the ingestion rates of HNFs and ciliates measured in the present study were comparable to those in the literature.

There is a possibility that bacteria are ingested by HNFs and these HNFs were, in turn, ingested by red-tide algae in these field experiments, although the incubation time was 30 min. Some large red-tide algae were observed to ingest HNFs (our unpubl. data), but an additional intensive study is necessary to quantify the importance of this 2-step process.

Prey concentration effects on ingestion rates

Prior to the present study, there had been no study on the functional responses by red-tide algae on marine bacteria. In general, with increasing bacterial concentration, the ingestion rates of red-tide algae increased rapidly at the prey concentrations of ca. 5 to 10 × 10^6 cells
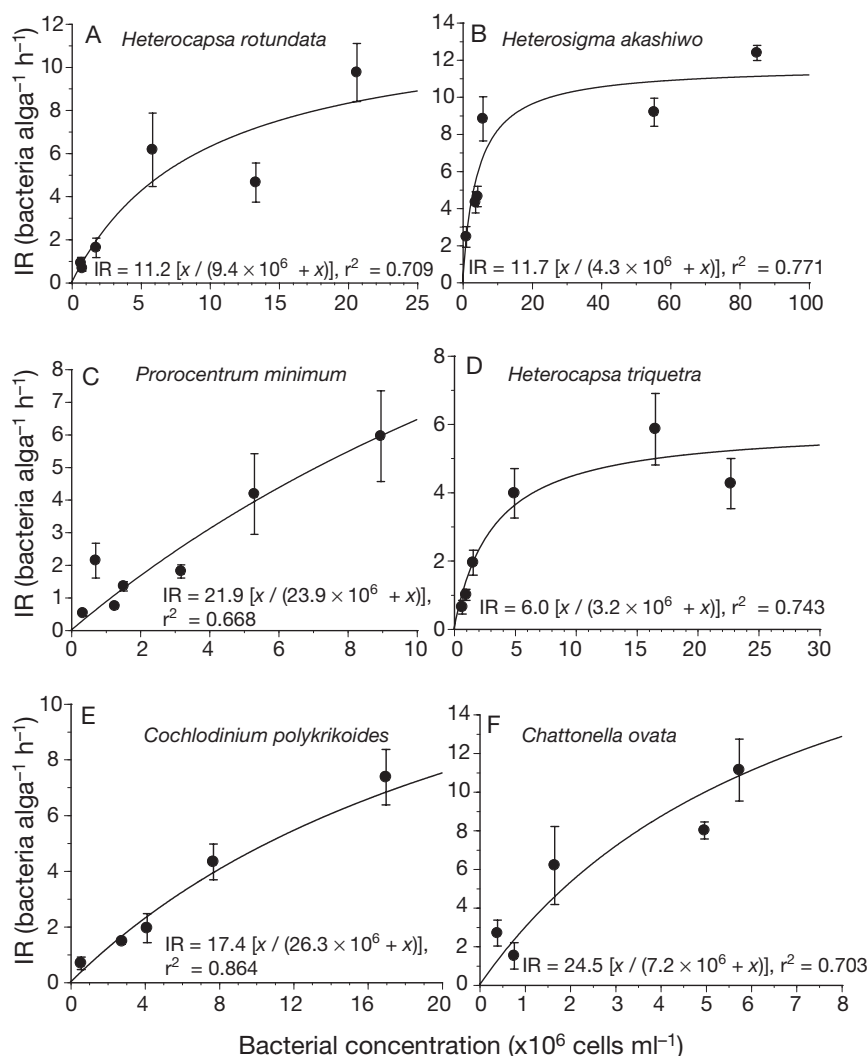
Fig. 5. Ingestion rates (IR, cells alga^{-1} h^{-1}) of red-tide algae on bacteria as a function of the initial prey concentration (cells ml^{-1}, x). (A) *Heterocapsa rotundata*, (B) *Heterosigma akashiwo*, (C) *Prorocentrum minimum*, (D) *Heterocapsa triquetra*, (E) *Cochlodinium polykrikoides*, and (F) *Chattonella ovata*. Each IR value was calculated by exploration from a linear regression curve on the number of prey cells inside an algal predator cell over incubation time (see 'Materials and methods' for calculation). Symbols represent treatment means (±1 SE). Curves were by fitted by a Michaelis–Menten equation (Eq. 3) using all treatments in the experiment. Note different scales on axes

ml^{-1}, but increased slowly or became saturated at higher prey concentrations. The bacterial concentrations in Korean waters in the present study ranged from 7.6×10^5 to 1.3×10^7 cells ml^{-1}, but most bacterial concentrations ranged from 5 to 10×10^6 cells ml^{-1}. Therefore, even a small change in bacterial concentrations may give rise to a marked effect on the ingestion rates of red-tide algae on natural populations of marine bacteria in Korean waters. There is a possibility that the addition of FLB into natural populations of marine bacteria would result in overestimation of the ingestion rates of red-tide algae on

marine bacteria. When the ingestion rates of the dominant red-tide algae on the natural populations of marine bacteria are calculated by using the equations in Fig. 5 along with the abundances of bacteria and added FLB in Table 3, the rates obtained without FLB were theoretically lower than those with FLB by 34 % [(5.0 − 3.3)/5.0 × 100] for *Heterocapsa rotundata*, 13 to 22 % for *H. triquetra*, 3 to 11 % for *Heterosigma akashiwo*, 7 to 26 % for *Prorocentrum minimum/P. triestinum*, and 15 to 38 % for *Cochlodinium polykrikoides*. It is occasionally difficult to determine the abundances of natural populations of bacteria just prior to adding FLB, and, thus, the ratios of the actual initial abundances of FLB to the abundances of the natural population of bacteria could be wide. Therefore, when the FLB method is used to measure the ingestion rates of red-tide algae on the natural population of bacteria, the possibility of overestimation should be considered based on the data on ingestion rates as a function of bacterial concentration.

Data from these studies show that the maximum ingestion rates of 6 red-tide algae on bacteria are not significantly affected by the ESDs of these algae (p > 0.1, linear regression ANOVA), although the largest red-tide alga, *Chattonella ovata*, has the maximum ingestion rate (Fig. 6). In field experiments, the mean ingestion rates of the dominant red-tide algae *Heterosigma akashiwo*, *Prorocentrum minimum* and/or *P. triestinum*, and *Cochlodinium polykrikoides* were not significantly different, although their sizes were different. Therefore, the size of the red-tide algae may not be an important factor affecting the ingestion rates of bacteria by the red-tide algae tested in the present study.

The smallest red-tide alga, *Heterocapsa rotundata*, was able to acquire 76 % of its body carbon from bacteria in a day. Assuming that the growth efficiency of *H. rotundata* feeding exclusively on bacteria is 30 to 40 %, this alga is capable of dividing once per 3 to 4 d. Bacteria may support the positive growth of small red-tide algae and the formation of red-tide patches. However, it is possible that bacteria do not support the growth of the large red-tide algae *H. triquetra*, *Cochlodinium polykrikoides*, or *Chattonella ovata*, because they can obtain only <4 % of their body carbon from bacteria in a day. The maximum ingestion rates of marine

Table 5. Ingestion rates and carbon acquisition of algal predators on marine bacteria as a function of bacterial concentration. Parameters are for the functional response from Eq. (3) as presented in Fig. 5. CPA: carbon content per algal cell (pg C cell^{-1}); CPB: carbon content per bacterial cell used in each experiment (pg C cell^{-1}); I_{max}: maximum ingestion rate (cells alga^{-1} h^{-1}; parentheses: pg C alga^{-1} h^{-1}); C_{max}: maximum clearance rate (nl alga^{-1} h^{-1}); AC: carbon acquired from bacteria by an algal predator per day (pg C predator^{-1} d^{-1}); % Body carbon: acquired carbon as percentage of predator's body carbon; DIN: dinoflagellate; RAP: raphidophyte. CPA was estimated from cell volume according to Strathmann (Strathmann 1967), and CPB, according to Simon & Azam (1989), recalculated by Norland (1993)

	Species	CPA	CPB	I_{max}	C_{max}	AC	% Body carbon
Fig. 5A	*Heterocapsa rotundata* (DIN)	20	0.052	12.2 (0.63)	1.4	15.2	76.1
Fig. 5B	*Heterosigma akashiwo* (RAP)	110	0.049	11.7 (0.57)	2.6	13.8	12.5
Fig. 5C	*Prorocentrum minimum* (DIN)	130	0.067	21.9 (1.47)	2.3	35.2	27.1
Fig. 5D	*Heterocapsa triquetra* (DIN)	220	0.055	6.0 (0.33)	1.3	7.9	3.6
Fig. 5E	*Cochlodinium polykrikoides* (DIN)	930	0.035	17.4 (0.61)	1.0	14.6	1.6
Fig. 5F	*Chattonella ovata* (RAP)	2870	0.070	24.5 (1.72)	4.5	41.2	1.4

bacteria by *H. triquetra* and *C. polykrikoides* (0.3 and 0.6 pg C alga^{-1} h^{-1}, respectively) are only ca. 20 and 10% of those of the same algal predators on an unidentified cryptophyte (ESD = 5.6 µm; 1.6 and 6.7 pg C alga^{-1} h^{-1}, respectively; Jeong et al. 2004, 2005b). *H. triquetra* and *C. polykrikoides* are likely to obtain more carbon from the cryptophyte than from bacteria in the event that both prey items are abundant. Low maximum volume-specific clearance rates of *H. triquetra* and *C. polykrikoides* on marine bacteria (110 to 730 h^{-1}) also suggest that bacteria are not suitable prey for the growth of these algae, but they may be considered as supplementary prey.

The maximum ingestion rates of the red-tide algae on marine bacteria under the laboratory conditions provided in the present study (6 to 25 cells alga^{-1} h^{-1}) are comparable to those of the HNFs *Jakoba libera* and *Pteriodomonas danica* (4 to 10 cells HNF^{-1} h^{-1}; Eccleston-Parry & Leadbeater 1994, Zubkov & Sleigh 1995). However, they are considerably lower than those shown by the ciliate *Uronema* sp. (150 to 380 cells ciliate^{-1} h^{-1}; Alonso et al. 2000). The maximum clearance ingestion rates of the red-tide algae on marine bacteria under the laboratory conditions provided in the present study (1.0 to 4.5 nl alga^{-1} h^{-1}) are comparable to those of the HNFs *Codosiga gracilis*, *J. libera*, *P. danica*, and *Paraphysomonas imperforata* (1.0 to 4.0 nl HNF^{-1} h^{-1}; Eccleston-Parry & Leadbeater 1994, Zubkov & Sleigh 1995). However, they are considerably lower than those shown by the ciliate *Uronema* sp. (50 to 560 nl ciliate^{-1} h^{-1}; Alonso et al. 2000). Therefore, the red-tide algae tested in the present study may sometimes compete with several HNF species for bacterial prey, if they co-occur.

We measured the ingestion rates of the red-tide algae on bacteria at a light intensity of 30 µE m^{-2} s^{-1}. The ingestion rates of the mixotrophic dinoflagellates *Fragilidium subglobosum* and *Karlodinium micrum* increased continuously or increased and then were saturated with increasing light intensity up to ca. 100 µE m^{-2} s^{-1} (Hansen & Nielsen 1997, Li et al. 2000, Skovgaard et al. 2000),

while those of *F.* cf. *mexicanum* (20 to 200 µE m^{-2} s^{-1}; Jeong et al. 1999) and *Prorocentrum minimum* (between 6 and 100% incident light intensity; Stoecker et al. 1997) were not significantly affected by light intensity under the provided conditions. Therefore, the effect of light intensity on feeding in red-tide algae may depend on the species; thus, further studies using diverse red-tide algal species are necessary.

Ecological importance

The results of the present study are ecologically important for planktonic communities in the following ways. (1) All 17 red-tide algal species tested in the field and laboratory in the present study ingested bacteria. Most red-tide algae may be able to feed on marine bacteria. (2) During some red tides, the dominant red-
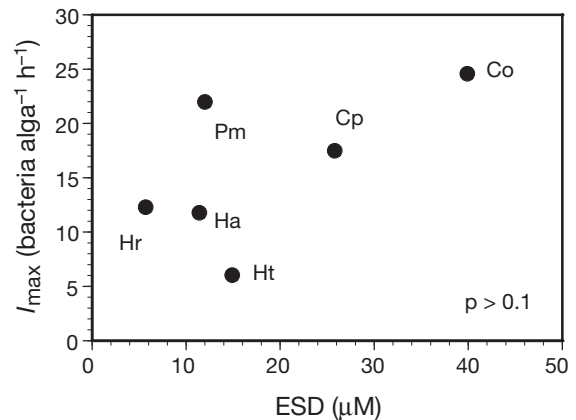

Fig. 6. Maximum ingestion rates (I_{max}) of 6 red-tide algae on bacteria, as a function of algal predator size (equivalent spherical diameters, ESD). Co: *Chattonella ovata*; Cp: *Cochlodinium polykrikoides*; Ha: *Heterosigma akashiwo*; Hr: *Heterocapsa rotundata*; Ht: *Heterocapsa triquetra*; Pm: *Prorocentrum minimum*

tide algae are the most effective predators of marine bacteria among protistan predators (algae, HNFs, and ciliates). As described above, the importance of red-tide algae as predators of bacteria, relative to HNFs and ciliates, depends on the algal species and abundances. The possible high grazing impact of red-tide algae may be partially responsible for lower bacterial grazing rates than those expected by calculations based on the assumption that HNFs are the main consumers of bacteria (e.g. Vaque et al. 2002). (3) Some red-tide algae are prey for ciliates (Jeong et al. 1999), but they can sometimes compete with the ciliates for bacterial prey. (4) Bacteria may be too small to be ingested by the filter-feeding copepods, while many red-tide algae are eaten by copepods (Nival & Nival 1976, Berggreen et al. 1988, Jeong 1995). Therefore, red-tide algae might be a link between bacteria and some zooplankters that are unable to directly ingest bacteria. (5) Usually, the populations of bacteria increase when phytoplankton biomass increases, because the dissolved organic matter from phytoplankton may enhance the growth of bacteria (e.g. Kamiyama et al. 2000). However, uncoupling between abundances of bacteria and phytoplankton has sometimes been observed (e.g. Bird & Karl 1999). Grazing by red-tide algae on bacteria may be partially responsible for this uncoupling. If an alga that is capable of feeding on bacteria is the causative species for a bloom (i.e. mixotrophic dinoflagellates, nanoflagellates, and microflagellates) and if grazing impact by the alga on bacteria is considerable, the abundance of bacteria may be rapidly reduced during algal blooms. The population dynamics of marine bacteria and phytoplankton in red tides may depend on whether the causative species are mixotrophic dinoflagellates (able to feed on bacteria) or exclusively autotrophic diatoms (unable to feed on bacteria).

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