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Feeding by red-tide dinoflagellates on the cyanobacterium *Synechococcus*

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ABSTRACT: We investigated the feeding by 18 red-tide dinoflagellate species on the cyanobacterium *Synechococcus* sp. We also calculated grazing coefficients by combining the field data on abundances of the dinoflagellates *Prorocentrum donghaiense* and *P. micans* and co-occurring *Synechococcus* spp. with laboratory data on ingestion rates obtained in the present study. All 17 cultured red-tide dinoflagellates tested (*Akashiwo sanguinea*, *Alexandrium catenella*, *A. minutum*, *A. tamarense*, *Cochlodinium polykrikoides*, *Gonyaulax polygramma*, *G. spinifera*, *Gymnodinium catenatum*, *G. impudicum*, *Heterocapsa rotundata*, *H. triquetra*, *Karenia brevis*, *Lingulodinium polyedrum*, *Prorocentrum donghaiense*, *P. minimum*, *P. micans*, and *Scrippsiella trochoidea*) were able to ingest *Synechococcus*. Also, *Synechococcus* cells were observed inside the protoplasms of *P. triestinum* cells collected from the coastal waters off Shiwha, western Korea, during red tides dominated by the dinoflagellate in July 2005. When prey concentrations were 1.1 to 2.3×10^6 cells ml^{-1}, the ingestion rates of these cultured red-tide dinoflagellates on *Synechococcus* sp. (1.0 to 64.2 cells dinoflagellate^{-1} h^{-1}) generally increased with increasing size of the dinoflagellate predators (equivalent spherical diameters = 5.2 to 38.2 µm). The ingestion rates of *P. donghaiense* and *P. micans* on *Synechococcus* sp. increased with increasing mean prey concentration, with saturation occurring at a mean prey concentration of approximately 1.1 to 1.4×10^6 cells ml^{-1}. The maximum ingestion and clearance rates of *P. micans* on *Synechococcus* sp. (38.2 cells dinoflagellate^{-1} h^{-1} and 4.3 µl dinoflagellate^{-1} h^{-1}) were much higher than those of *P. donghaiense* on the same prey species (7.7 cells dinoflagellate^{-1} h^{-1} and 2.6 µl dinoflagellate^{-1} h^{-1}). The ingestion rates of red-tide dinoflagellates on *Synechococcus* sp. were comparable to those of the heterotrophic nanoflagellates and ciliates on *Synechococcus* spp., so far reported in the literature. The calculated grazing coefficients attributable to small *Prorocentrum* spp. (*P. donghaiense* + *P. minimum*) and *P. micans* on co-occurring *Synechococcus* spp. were up to 3.6 and 0.15 h^{-1}, respectively. The results of the present study suggest that red-tide dinoflagellates potentially have a considerable grazing impact on populations of *Synechococcus*.

KEY WORDS: Cyanophyte · Grazing · Harmful algal bloom · Ingestion · Marine · Protist · Red tide

INTRODUCTION

The photosynthetic cyanobacterium *Synechococcus* is a ubiquitous prokaryote in marine environments, present from tropical waters to polar waters (Walker & Marchant 1989, Burkill et al. 1993, Chavez et al. 1996, Landry et al. 1996, Vezina & Vincent 1997, Campbell et al. 1998, Zubkov et al. 1998, Sherry & Wood 2001, Liu et al. 2002, Mackey et al. 2002, DiTullio et al. 2003, Maranon et al. 2003). It often dominates the abun-

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dance and/or the primary production of phytoplankton in both coastal and open oceanic waters (Chang et al. 1996, 2003, Karlson et al. 1996, Phlips & Badylak 1996, Agawin et al. 1998, Yahel et al. 1998, Crosbie & Furnas 2001, Arin et al. 2002, Chiang et al. 2002, Stal et al. 2003, Nielsen et al. 2004, Wawrik & Paul 2004). The abundance of *Synechococcus* spp. often exceeds 10^5 cells ml^{-1} and sometimes forms red tides (Lindell & Post 1995, Partensky et al. 1996, 1999, Morel 1997, Andreoli et al. 1999, Diaz & Maske 2000, Uysal 2000, Agawin et al. 2003, Murrell & Lores 2004). In oceanic waters, *Synechococcus* has been thought to be one of the major contributors to CO_2 and nutrient uptake from ambient waters and, in turn, eventually from the atmosphere (Maranon et al. 2003). Therefore, the growth and mortality of *Synechococcus* are important factors in understanding the cycling of the materials in marine planktonic food webs.

There have been many studies on the growth of *Synechococcus*, in particular the effects of iron concentrations on its growth (Wells et al. 1994, Henley & Yin 1998, Timmermans et al. 1998, Yin & Henley 1999). Also, there have been a large number of studies on the mortality of *Synechococcus* due to predation by heterotrophic protists (Campbell & Carpenter 1986, Kuosa 1990, Caron et al. 1991, Strom 1991, Šimek 1997, Dolan & Šimek 1998, 1999, Lewitus et al. 1998, Ochs & Eddy 1998, Christaki et al. 1999, Monger et al. 1999, Rivkin et al. 1999, Cowlishaw 2000, Boenigk et al. 2001, Guillou et al. 2001, Pitta et al. 2001, Quevedo & Anadon 2001, Bettarel et al. 2002, Jochem 2003, Agawin et al. 2004). Many studies on the feeding by heterotrophic protists on *Synechococcus* have suggested that heterotrophic nanoflagellates and ciliates are usually major grazers on *Synechococcus* (Bettarel et al. 2002, Jochem 2003, Worden & Binder 2003, Agawin et al. 2004). Here, an important question concerning the protistan predators on *Synechococcus* arises: Is there any unknown predator which can affect the population dynamics of *Synechococcus*?

Dinoflagellates are ubiquitous protists and sometimes form red-tide patches in coastal (e.g. Jeong 1995) and offshore and/or oceanic waters (e.g. Tyler & Seliger 1978, Tester & Steidinger 1997). Red tides dominated by dinoflagellates can alter the balance of food webs and cause large-scale mortalities of fish and shellfish. Recently, many red-tide dinoflagellates, which had previously been thought to be exclusively autotrophic dinoflagellates, have been revealed to be mixotrophic dinoflagellates (Bockstahler & Coats 1993, Chang & Carpenter 1994, Jacobson & Anderson 1996, Granéli et al. 1997, Stoecker et al. 1997, Stoecker 1999, Skovgaard et al. 2000, Smalley et al. 2003, Jeong et al. 2004, 2005a,b). These dinoflagellates usually co-occur with *Synechococcus* and/or the blooms of these dinoflagellates sometimes succeed those of *Synechococcus* (Taslakian & Hardy 1976, Chavez et al. 1996, Tarran et al. 1999, 2001, Duyl et al. 2002, Murrell & Lores 2004). There is a possibility that red-tide dinoflagellates feed on *Synechococcus*; however, the interactions between red-tide dinoflagellates and *Synechococcus*, in particular possible predator–prey relationships, are still poorly understood.

There have been few studies on the feeding by red-tide dinoflagellates on *Synechococcus* (Legrand et al. 1998). Legrand et al. (1998) reported that *Synechococcus* was not ingested by the red-tide dinoflagellate *Heterocapsa triquetra*. However, we have recently found that some red-tide dinoflagellates, including *H. triquetra*, are able to ingest *Synechococcus*. Therefore, the following basic questions arise: (1) Are most red-tide dinoflagellates able to ingest *Synechococcus*? (2) What is the range of ingestion rates of red-tide dinoflagellates on *Synechococcus*? Are the ingestion rates of red-tide dinoflagellates on *Synechococcus* comparable to those of heterotrophic nanoflagellates and ciliates? (3) What is the potential grazing impact by red-tide dinoflagellates on *Synechococcus*? If most red-tide dinoflagellates are able to ingest *Synechococcus*, we must change conventional views about the planktonic food webs related to *Synechococcus* and to dinoflagellates, and the mechanisms of outbreak and persistence of red tides, etc.

We (1) investigated whether or not 17 cultured red-tide dinoflagellates, having a wide range of morphological properties (size, shape, single or chain forms, thecate or naked, etc.), were able to feed on *Synechococcus*; (2) observed *Prorocentrum triestinum* cells collected from the coastal waters off Shiwha, western Korea, during the red tide dominated by the dinoflagellate in July 2005, to ascertain the feeding by dinoflagellates on *Synechococcus* in natural environments; (3) conducted experiments to determine the ingestion rates of those 17 cultured red-tide dinoflagellates on *Synechococcus* at a single high-prey concentration, and (4) measured the ingestion rates of *Prorocentrum donghaiense* (previously *P. dentatum* in Korean, Chinese, Japanese, and in some United States waters) and *P. micans* on *Synechococcus* as a function of the prey concentration. (5) The ingestion rates of red-tide dinoflagellates on *Synechococcus* were compared to those of heterotrophic nanoflagellates and ciliates reported in the literature. (6) We also estimated the grazing coefficients attributable to small *Prorocentrum* spp. (*P. donghaiense* and *P. minimum*) and *P. micans* on co-occurring *Synechococcus* using our data for ingestion rates obtained from laboratory experiments and the abundances of predator and prey in the field. The results of the present study provide a basis for understanding the interactions between red-tide dinoflagellates and co-occurring *Synechococcus* and their population dynamics.

MATERIALS AND METHODS

Preparation of experimental organisms. *Synechococcus* sp. (SYN, Genbank Accession Number DQ023295, equivalent spherical diameter [ESD] = ca. 1 µm) was grown at 20°C in enriched *f*/2 seawater media (Guillard & Ryther 1962) without silicate, under a 14:10 h light:dark cycle of 20 µE m^{-2} s^{-1} of cool white fluorescent light, while dinoflagellate predators were grown under a 14:10 h light:dark cycle of 30 µE m^{-2} s^{-1} (Table 1). Mean ESDs (± SD) of the dinoflagellates were measured by an electronic particle counter (Coulter Multisizer II, Coulter Corporation). Cultures in their exponential growth phase were used for these feeding experiments.

Feeding occurrence. Expt 1 was designed to investigate whether or not each dinoflagellate species was able to feed on *Synechococcus* (Table 1).

A dense culture of each dinoflagellate predator, maintained in *f*/2 media and growing photosynthetically in its exponential growth phase on shelves and incubated under a 14:10 h light:dark cycle of 30 µE m^{-2} s^{-1}, was transferred to a 1 l polycarbonate (PC) bottle containing freshly filtered seawater. Three 1 ml aliquots were then removed from the bottle and examined using a compound microscope to determine the dinoflagellate concentration.

In this experiment, the initial concentrations of each dinoflagellate predator (2000 to 20 000 cells ml^{-1}) and *Synechococcus* (1 to 2 × 10^6 cells ml^{-1}) were established using an autopipette to deliver a predetermined volume of culture with a known cell density to the experimental bottles. Triplicate 80 ml PC bottles (mixtures of dinoflagellate predator and *Synechococcus*) and triplicate predator control bottles (containing dinoflagellate predator only) were set up at a single prey concentration for each dinoflagellate predator. The bottles were filled to capacity with freshly filtered seawater, capped, and then placed on a shelf at 20°C under the continuous illumination of 30 µE m^{-2} s^{-1}. After 5, 10, 30, and 60 min, and 4 h of incubation, a 10 ml aliquot was removed from each bottle and then fixed with formalin (final conc. = 4%). The fixed aliquots were filtered onto 3 µm pore size, 25 mm PC black membrane filters, and then the concentrated cells on the membranes were observed under an epifluorescent microscope (Olympus BH2, Olympus) with blue light excitation at a magnification of 1000× to determine whether or not each dinoflagellate predator was able to feed on *Synechococcus*. However, ingested *Synechococcus* cells were rarely detectable in the protoplasms of *Akashiwo sanguinea*, *Lingulodinium polyedrum*, and *Scrippsiella trochoidea* under the epifluorescent microscope. Therefore, after conducting the same processes as described above, except for the *Synechococcus* cells being fluorescently labeled using DTAF (Siegler et al. 1989), 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 dinoflagellate body at consecutive intervals of 1 to 2 µm along the *z*-axis. Pictures showing ingested *Synechococcus* cells inside each dinoflagellate predator cell were taken using digital cameras on these microscopes at a magnification of 1000×.

Table 1. Cultured dinoflagellate species used as predators on *Synechococcus* in Expts 1 and 2. Mean equivalent spherical diameter (ESD, µm) (±SD) was measured by an electronic particle counter measured before these experiments; n > 2000 for each species

Predator species	ESD (±SD)
Heterocapsa rotundata	5.8 (0.4)
Prorocentrum minimum	12.1 (2.5)
Prorocentrum donghaiense	13.3 (2.0)
Heterocapsa triquetra	15.0 (4.3)
Alexandrium minutum	16.7 (2.9)
Gymnodinium impudicum	17.8 (2.6)
Karenia brevis	20.3 (1.1)
Scrippsiella trochoidea	22.8 (2.7)
Cochlodinium polykrikoides	25.9 (2.9)
Prorocentrum micans	26.6 (2.8)
Alexandrium tamarense	28.1 (3.1)
Akashiwo sanguinea	30.8 (3.5)
Gonyaulax polygramma	32.5 (3.0)
Alexandrium catenella	32.6 (2.7)
Gymnodinium catenatum	33.9 (1.6)
Gonyaulax spinifera	35.0 (1.3)
Lingulodinium polyedrum	38.2 (3.6)

To observe ingested *Synechococcus* cells inside the protoplasms of dinoflagellate predator cells collected from natural environments, we took water samples using a clean bucket from the surface of the coastal waters off Siwha, Ansan, western Korea, during red tides dominated by *Prorocentrum triestinum* in July 2005. The water samples were poured into 100 ml polyethylene bottles and immediately preserved with formalin (final conc. = 4%). The fixed aliquots were filtered onto 5 µm pore size, 25 mm PC black membrane filters, and then the concentrated cells on the membranes were observed under the epifluorescent microscope with blue light excitation at a magnification of 1000×.

Ingestion rates. Expt 2 was designed to compare the ingestion rates of cultured red-tide dinoflagellates on *Synechococcus* when similar prey concentrations were provided. We provided live *Synechococcus* at initial concentrations of 1.1 to 2.3 × 10^6 cells ml^{-1} for the dinoflagellate predators, because the ingestion rates of *Prorocentrum donghaiense* and *P. micans* on *Synechococcus* were almost saturated at these prey concentrations (see

Figs. 2 & 3). Two different methods were used for these experiments; the first method involved measuring ingestion rates by plotting the numbers of ingested *Synechococcus* cells (seen as orange-colored inclusions under an epifluorescence microscope) inside the protoplasm of a dinoflagellate against incubation time, as in Sherr et al. (1987). This method was used for *Heterocapsa triquetra*, *Karenia brevis*, *P. donghaiense*, and *P. micans* inside which all the ingested prey cells were easily seen and each ingested prey cell was clearly countable. The second method was measuring ingestion rates by comparing concentrations of the dinoflagellate predator and *Synechococcus* between the experimental and control bottles. This method was used for the other dinoflagellate predators inside which all ingested prey cells could not be seen and/or each ingested prey cell was not clearly countable. To assess how close the results from these 2 methods are, ingestion rates of *P. donghaiense* on *Synechococcus* were measured using both of these methods.

A dense culture of each dinoflagellate predator maintained in an *f*/2 medium and growing photosynthetically in its exponential phase under a 14:10 h light:dark cycle of 30 µE m^{-2} s^{-1} for ca. 1 mo was transferred into a 1 l PC bottle. Three 1 ml aliquots from the bottle were counted using a compound microscope, to determine cell concentrations of the dinoflagellate predator, and the cultures were then used to conduct experiments.

For the first method (prey-inclusion method), initial concentrations of the dinoflagellate predator (2000 to 20 000 cells ml^{-1}) and live *Synechococcus* were established using an autopipette to deliver predetermined volumes of known cell concentrations to the bottles. Triplicate 80 ml PC experimental bottles (containing mixtures of predators and prey) and triplicate predator-control bottles (containing predators only) were also established. Then, 20 ml of *f*/2 medium was added to all the bottles, which were then filled to capacity with freshly filtered seawater, capped, and then placed on the shelf. After 1, 5, 10, and 20 min of incubation, 10 ml aliquots were removed from each bottle, transferred into 20 ml vials, and then fixed with formalin (final conc. = 4 %). One 2 ml fixed aliquot was filtered onto 3 µm pore size, PC black membrane filters. Orange-colored inclusions (*Synechococcus* cells) inside the protoplasm of >30 dinoflagellate predator cells on the PC black membrane filters were enumerated under an epifluorescence microscope with blue light excitation. No orange-colored inclusions were observed inside the protoplasm of the dinoflagellate predators in the control bottles. *Prorocentrum* spp. cells in old cultures (>1 mo after being transferred) sometimes contained yellow-colored inclusions, even when prey cells were not provided. Therefore, we used only *Prorocentrum* spp. in cultures of <7 d after being transferred to new medium and ascertained that there were no yellow-colored inclusions seen under an epifluorescence microscope. The bottles were capped, placed on a shelf, and incubated as described above. A linear regression curve for the number of prey cells inside a dinoflagellate predator cell against incubation time was obtained, and then an ingestion rate (prey cells dinoflagellate^{-1} h^{-1}) was calculated by exploration, as in Sherr et al. (1987).

For the second method (bottle-incubation method), the initial concentrations of the dinoflagellate predators and live *Synechococcus* were established using an autopipette, to deliver predetermined volumes of known cell concentrations to the bottles. Triplicate 80 ml PC experimental bottles (containing mixtures of predators and prey), triplicate prey-control bottles (containing prey only), and triplicate predator-control bottles (containing predators only) were also established. Then, 20 ml of *f*/2 medium was added to all the bottles, which were then filled to capacity with freshly filtered seawater, capped, placed on the shelf, and incubated at 20°C under an illumination of 30 µE m^{-2} s^{-1}. To determine the actual initial predator and prey densities (cells ml^{-1}) at the beginning of the experiment and after 6 h incubation, a 5 ml aliquot was removed from each bottle and fixed with 5 % Lugol's solution. All or >300 dinoflagellate predator cells, fixed in Lugol's solution, in three 1 ml Sedgwick–Rafter counting chambers were enumerated. Another 5 ml aliquot was removed from each bottle, fixed with formalin (final conc. = 4 %), and then filtered onto 0.2 µm pore size, PC black membrane filters. Orange-colored prey cells on the PC filter were enumerated under an epifluorescence microscope. Ingestion and clearance rates were calculated using the equations of Frost (1972) and Heinbokel (1978).

Effects of prey concentration. Expt 3 was designed to investigate the ingestion rates of *Prorocentrum donghaiense* and *P. micans* on *Synechococcus* as a function of prey concentration. The actual initial predator (and prey) concentrations were 13 to 16 570 cells ml^{-1} (111 to 2 996 200; 9 prey concentrations) for *P. donghaiense* and 10 to 3260 cells ml^{-1} (131 to 3 438 500; 9 prey concentrations) for *P. micans*. Using the first method (prey-inclusion method), as in Expt 2, triplicate ingestion rates at each prey concentration were obtained. All ingestion rate data were fitted to a Michaelis–Menten equation:

$$IR = \frac{I_{max}(x)}{K_{IR} + (x)} \quad (1)$$

where I_{max} is the maximum ingestion rate (cells dinoflagellate^{-1} h^{-1}), x is the prey concentration (cells ml^{-1}), and K_{IR} is the prey concentration sustaining one-half I_{max}.

Potential grazing impact. We estimated the grazing coefficients (mortality rate due to predation) attributable to small *Prorocentrum* spp. (*P. donghaiense* + *P. minimum*) and *P. micans* on *Synechococcus* by combining field data on abundances of the dinoflagellate predators and *Synechococcus* with ingestion rates of the dinoflagellate predators on *Synechococcus* obtained in the present study. The ingestion rate of *P. minimum* on *Synechococcus* at a certain prey concentration was calculated by multiplying that of *P. donghaiense* by 0.81, because the ingestion rate of *P. minimum* on *Synechococcus* at a prey concentration of 1.83×10^6 cells ml^{-1} (5.9 cells dinoflagellate^{-1} h^{-1}) was 0.81 times lower than that of *P. donghaiense* at the same prey concentration (7.3 cells dinoflagellate^{-1} h^{-1}), calculated using the equation of the regression curve in Fig. 2. Data on the abundances of small *Prorocentrum* spp. (*P. donghaiense* + *P. minimum*), *P. micans*, and the co-occurring *Synechococcus* used in this estimation were obtained from the water samples off Masan (bay waters, in 2004) and at 6 stations 90 to 220 km off Jeju Island (offshore and oceanic waters, in 2003), Korea.

The grazing coefficients (g, h^{-1}) were calculated as:

$$g = CR \times GC \quad (2)$$

where CR (ml dinoflagellate^{-1} h^{-1}) is a clearance rate of an algal predator on a target prey at a prey concentration and GC is a grazer concentration (cells ml^{-1}). CR values were calculated as:

$$CR = IR/PC \quad (3)$$

where IR (cells eaten dinoflagellate^{-1} h^{-1}) is the ingestion rate of the algal predator on the target prey and PC (cells ml^{-1}) is a prey concentration. CRs were corrected using Q_{10} = 2.8 (Hansen et al. 1997), because in situ water temperatures and the temperature used in the laboratory for this experiment (20°C) were sometimes different.

RESULTS

Dinoflagellate predators

All cultured red-tide dinoflagellates tested (*Akashiwo sanguinea*, *Alexandrium catenella*, *A. minutum*, *A. tamarense*, *Cochlodinium polykrikoides*, *Gonyaulax polygramma*, *G. spinifera*, *Gymnodinium catenatum*, *G. impudicum*, *Heterocapsa rotundata*, *H. triquetra*, *Karenia brevis*, *Lingulodinium polyedrum*, *Prorocentrum donghaiense*, *P. minimum*, *P. micans*, and *Scrippsiella trochoidea*) were able to ingest both live and fluorescent-labeled *Synechococcus* (Fig. 1A–Q). Ingested *Synechococcus* cells were usually found aggregated in 1 or 2 areas of the protoplasm of the thecate dinoflagellates *H. triquetra*, *L. polyedrum*, and *S. trochoidea*, while they were widely distributed in the protoplasm of *P. donghaiense* and *P. micans* and the naked dinoflagellates *C. polykrikoides* and *G. catenatum* (Fig. 1).

Synechococcus cells were observed inside the protoplasm of dinoflagellate (*Prorocentrum triestinum*) cells collected from the surface of coastal waters off Shiwha, western Korea, during red tides dominated by the dinoflagellate in July 2005 (Fig. 1R).

Ingestion rates of diverse dinoflagellate predators

The ingestion rate of *Prorocentrum donghaiense* on *Synechococcus*, measured using the second method (bottle-incubation method) at an initial prey concentration of 2.3×10^6 cells ml^{-1} (8.2 cells dinoflagellate^{-1} h^{-1}), was only 10% higher than the ingestion rate at the same prey concentration calculated using the equation of the regression line on the ingestion rates measured using the first method (prey-inclusion method) (7.4 cells dinoflagellate^{-1} h^{-1}) (Table 2, Fig. 2).

When the initial prey concentrations of *Synechococcus* were 1.1 to 2.3 × 10^6 cells ml^{-1}, the ingestion rates of the red-tide dinoflagellates on *Synechococcus*

Table 2. Ingestion rates (means, SEs in parentheses) of the dinoflagellate predators on *Synechococcus*, measured using 2 different methods in Expt 2 (see 'Materials and methods'). BI: bottle-incubation method; PI: prey-inclusion method

Predator species	Method	Initial concentrations of *Synechococcus* (10^6 cells ml^{-1})	Ingestion rate (cells dinoflagellate^{-1} ml^{-1})
Heterocapsa rotundata	BI	1.17 (0.04)	1.0 (0.2)
Prorocentrum minimum	BI	1.83 (0.04)	5.9 (1.2)
Prorocentrum donghaiense	BI	2.25 (0.10)	8.2 (0.4)
Prorocentrum donghaiense	PI	2.25	7.4[a]
Heterocapsa triquetra	PI	1.20 (0.03)	4.4 (0.3)
Alexandrium minutum	PI	1.09 (0.01)	3.2 (2.2)
Gymnodinium impudicum	BI	1.28 (0.02)	14.5 (1.5)
Karenia brevis	BI	1.25 (0.04)	5.0 (0.1)
Scrippsiella trochoidea	BI	1.26 (0.04)	7.1 (1.1)
Cochlodinium polykrikoides	BI	1.08 (0.20)	38.7 (1.1)
Prorocentrum micans	PI	1.38 (0.04)	35.4 (2.1)
Alexandrium catenella	BI	1.89 (0.05)	29.5 (6.7)
Alexandrium tamarense	BI	1.13 (0.05)	13.7 (0.9)
Akashiwo sanguinea	BI	1.90 (0.11)	62.9 (5.4)
Gonyaulax polygramma	BI	1.65 (0.65)	42.4 (2.8)
Gymnodinium catenatum	BI	1.00 (0.04)	30.2 (2.8)
Gonyaulax spinifera	BI	1.14 (0.09)	24.3 (3.5)
Lingulodinium polyedrum	BI	1.53 (0.04)	64.2 (2.2)

[a]7.4 was calculated using the equation of the regression line in Fig. 2

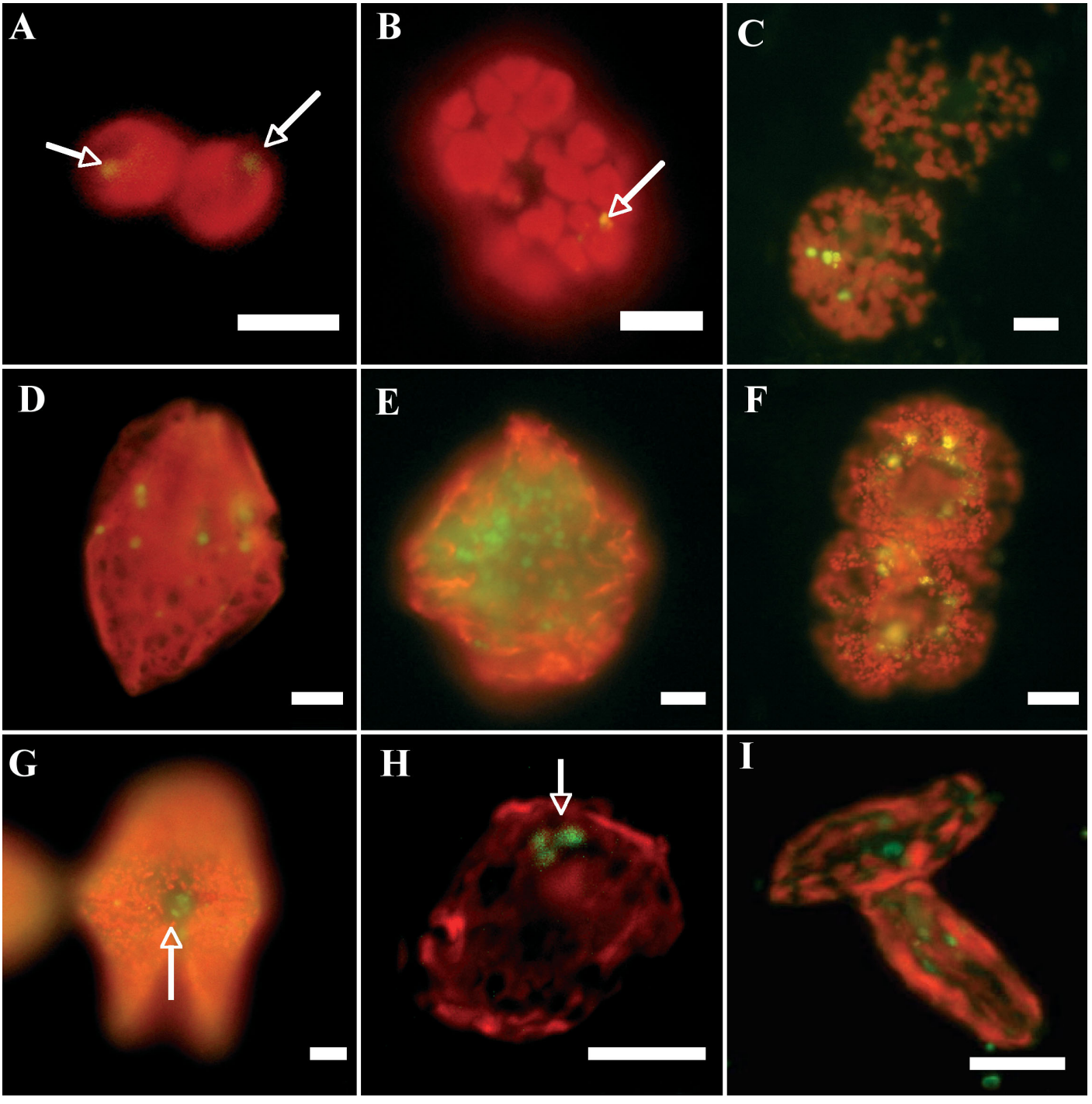

Fig. 1. Feeding by dinoflagellate predators on *Synechococcus*. Cultured cells of the predators and prey: (A) *Heterocapsa rotundata*, (B) *Gymnodinium impudicum*, (C) *Cochlodinium polykrikoides*, (D) *Prorocentrum micans*, (E) *Gonyaulax polygramma*, (F) *Gymnodinium catenatum*, (G) *Akashiwo sanguinea*, (H) *P. minimum*, (I) *P. donghaiense*, (J) *H. triquetra*, (K) *Alexandrium minutum*, (L) *Karenia brevis*, (M) *Scrippsiella trochoidea*, (N) *A. catenella*, (O) *A. tamarense*, (P) *Gonyaulax spinifera*, (Q) *Lingulodinium polyedrum*. (R) Ingested *Synechococcus* cells inside the protoplasm of *P. triestinum* collected from a natural water sample. Scale bars = 5 µm. Arrows indicate ingested prey cells. (A to G) and (R) are photomicrographs showing dinoflagellate predators ingesting live *Synechococcus* cells (seen as orange-colored inclusions), taken using an epifluorescence microscope, and (H to Q) are photomicrographs showing dinoflagellate predators ingesting fluorescently labeled *Synechococcus* cells (seen as green-colored inclusions), taken using a confocal laser scanning microscope

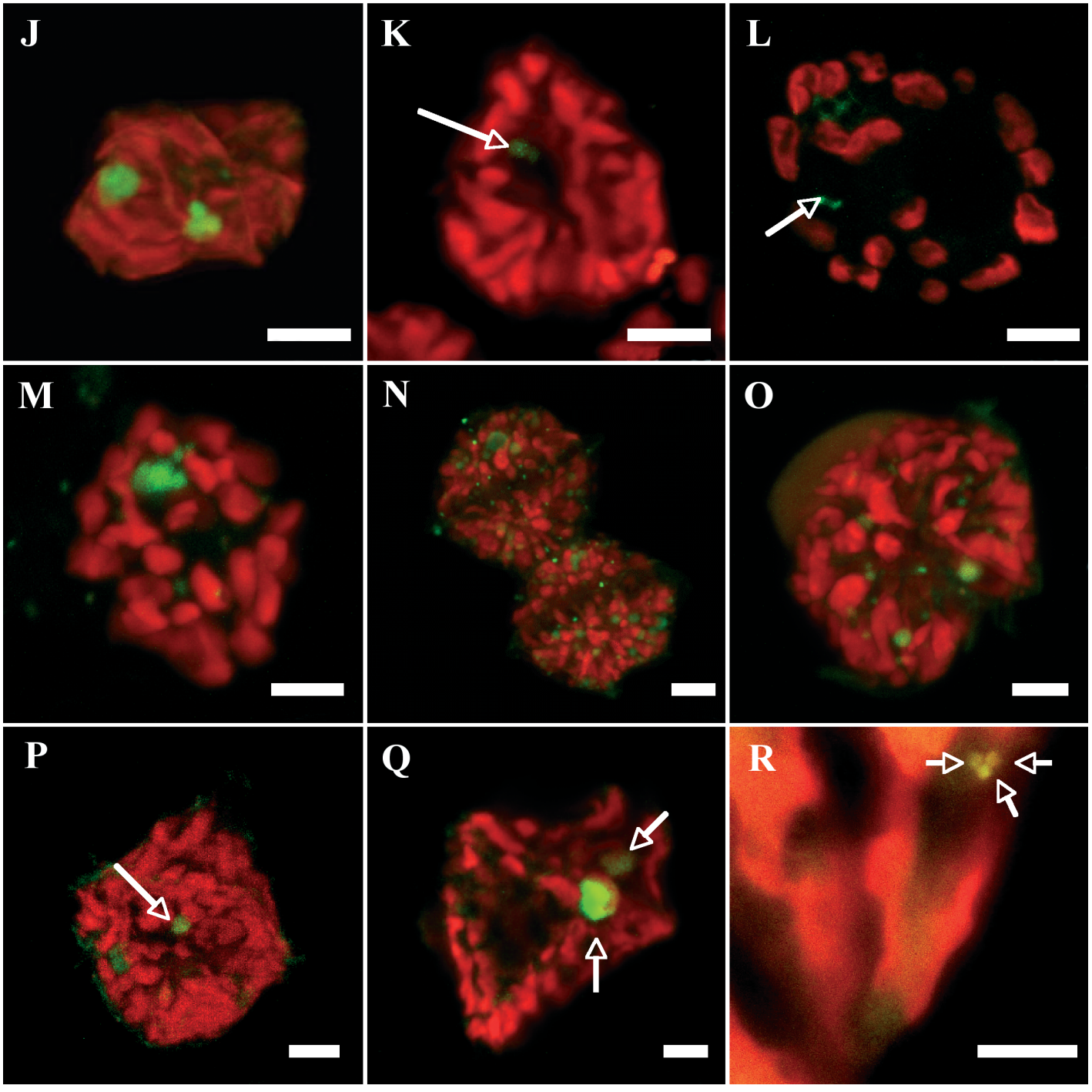

Fig. 1 (continued)

generally increased as the size of the algal predators increased (Table 2). The smallest ingestion rate (1.0 cells dinoflagellate^{-1} h^{-1}) was obtained for the smallest predator *Heterocapsa rodundata* (ESD = 5.2 µm), while the greatest ingestion rate (64.2 cells dinoflagellate^{-1} h^{-1}) was obtained for the largest predator *Lingulodinium polyedrum* (ESD = 38.2 µm).

Effects of prey concentration

The initial concentrations of *Synechococcus* in the experiment on the feeding by *Prorocentrum donghaiense* on *Synechococcus* were 1.1×10^2 to 3.0×10^6 cells ml^{-1}. When being measured using the first method (prey-inclusion method), with increasing *Synechococcus* concentration,

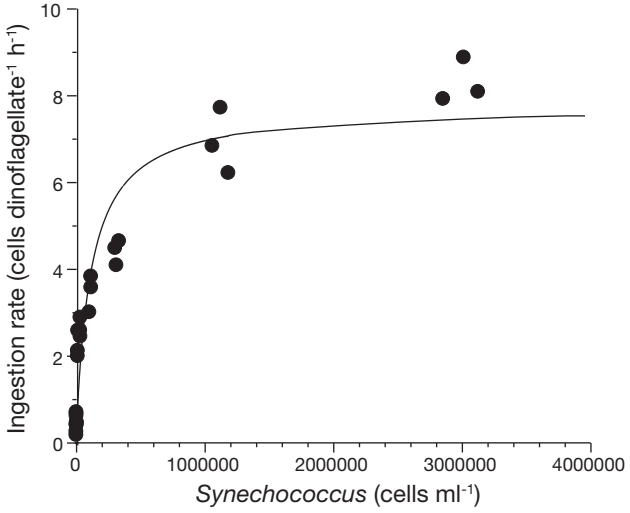

Fig. 2. Ingestion rate (cells dinoflagellate^{-1} h^{-1}) of *Prorocentrum donghaiense* on *Synechococcus* as a function of the initial prey concentration (cells ml^{-1}). Each value of the ingestion rates was calculated by exploration from a linear regression curve on the number of prey cells inside a dinoflagellate predator cell over incubation time (see 'Materials and methods' for calculation). Symbols represent single treatments. The curves were fitted by a Michaelis-Menten equation (Eq. 1) using all treatments in the experiment. Ingestion rate (*IR*, cells dinoflagellate^{-1} h^{-1}) = 7.7 [x/(114 000 + x)], r^2 = 0.909, where x is the prey concentration

the ingestion rate of *P. donghaiense* on *Synechococcus* increased, with saturation at a prey concentration of approximately 1.1×10^6 cells ml^{-1} (Fig. 2). When the data were fitted to Eq. (1), the maximum ingestion rate of *P. donghaiense* on *Synechococcus* was 7.7 cells dinoflagellate^{-1} h^{-1}. The maximum clearance rate of *P. donghaiense* on *Synechococcus* was 2.6 µl dinoflagellate^{-1} h^{-1}.

The initial concentrations of *Synechococcus* in the experiment on the feeding by *Prorocentrum micans* on *Synechococcus* were 1.3×10^2 to 3.4×10^6 cells ml^{-1}. With increasing prey concentration the ingestion rate of *P. micans* on *Synechococcus* increased, with saturation at a prey concentration of approximately 1.4×10^6 cells ml^{-1} (Fig. 3). When the data were fitted to Eq. (1), the maximum ingestion rate of *P. micans* on *Synechococcus* was 38.2 cells dinoflagellate^{-1} h^{-1}. The maximum clearance rate of *P. micans* on *Synechococcus* was 4.3 µl dinoflagellate^{-1} h^{-1}.

Grazing impact

The grazing coefficients attributable to *Prorocentrum donghaiense* on co-occurring *Synechococcus* in Masan Bay, Korea, were 0.1 to 3.6 h^{-1} (i.e. 11 to 98% of a *Synechococcus* population was removed by a population of *P. donghaiense* in 1 h) when the abundances of *P. donghaiense* and *Synechococcus* were 1710 to 55 000 cells ml^{-1} and 550 to 16 130 cells ml^{-1}, respectively (Fig. 4A). The grazing coefficients attributable to *P. donghaiense* on co-occurring *Synechococcus* in the offshore and/or oceanic waters away from Jeju island, Korea, were 0.001 to 0.014 h^{-1} (i.e. 0.1 to 1.5% of a *Synechococcus* population was removed by a population of *P. donghaiense* in 1 h) when the abundances of *P. donghaiense* and *Synechococcus* were 12 to 328 cells ml^{-1} and 70 110 to 203 140 cells ml^{-1}, respectively (Fig. 4A).

The grazing coefficients attributable to *Prorocentrum micans* on co-occurring *Synechococcus* in Masan Bay were 0.04 to 0.15 h^{-1} (i.e. up to 4 to 17% of a *Synechococcus* population was removed by a population of *P. micans* in 1 h) when the abundances of *P. micans* and *Synechococcus* were 100 to 461 cells ml^{-1} and 547 to 9840 cells ml^{-1}, respectively (Fig. 4B).

DISCUSSION

Dinoflagellate predators on *Synechococcus*

All red-tide dinoflagellates tested in the present study were able to ingest *Synechococcus*. Dinoflagellates not tested yet are also likely able to feed on *Synechococcus*. We reported here for the first time that *Alexandrium catenella*, *A. minutum*, *Heterocapsa rotundata* (previously *Katodinium rotundatum*), and

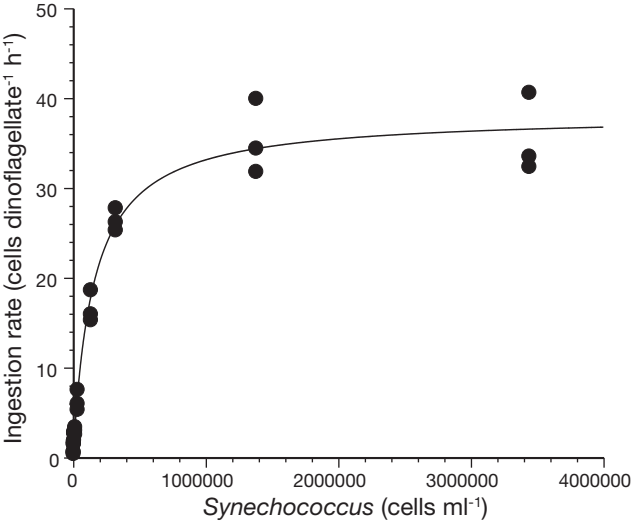

Fig. 3. Ingestion rate (cells dinoflagellate^{-1} h^{-1}) of *Prorocentrum micans* on *Synechococcus* as a function of the initial prey concentration (cells ml^{-1}). Each value of the ingestion rates was calculated as for Fig. 2. Symbols represent single treatments. The curves were fitted by a Michaelis-Menten equation (Eq. 1) using all treatments in the experiment. Ingestion rate (*IR*, cells dinoflagellate^{-1} h^{-1}) = 38.2 [x / (152 000 + x)], r^2 = 0.980, where x is the prey concentration

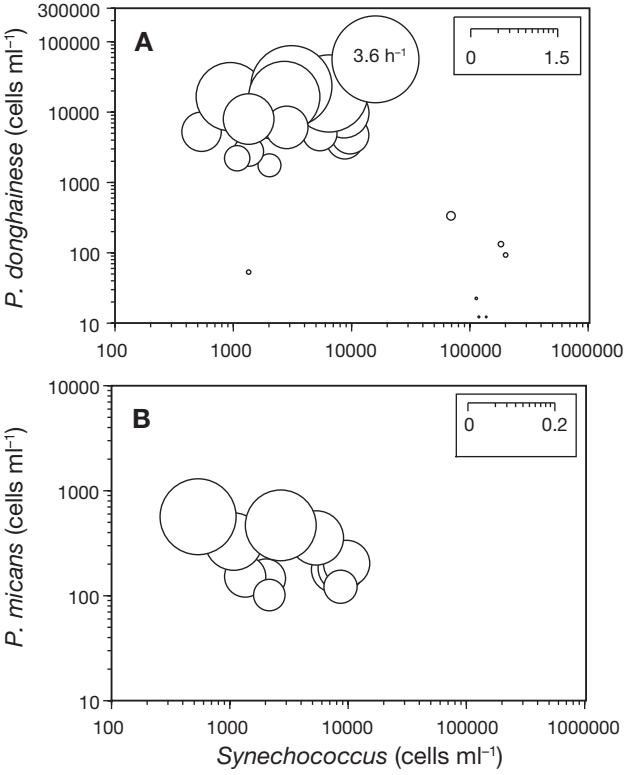

Fig. 4. Calculated grazing coefficients (g) of (A) *Prorocentrum donghaiense* (n = 24) and (B) *P. micans* (n = 11) in relation to the concentration of co-occurring *Synechococcus* (see 'Materials and methods' for calculation). The value of g for *P. donghaiense* was 3.6 h^{-1} when the concentrations of *Synechococcus* and *P. donghaiense* + *P. minimum* were 16 123 and 55 000 cells ml^{-1}. The scale for this g was reduced. Clearance rates, measured under the conditions provided in the present study, were corrected using Q_{10} = 2.8 (Hansen et al. 1997) because *in situ* water temperatures and the temperature used in the laboratory for this experiment (20°C) were sometimes different. The scales of the circles in the inset boxes are g (h^{-1})

Karenia brevis, which had been previously thought to be exclusively autotrophic dinoflagellates, were mixotrophic species. The other dinoflagellates tested in the present study have already been classified as mixotrophic (Bockstahler & Coats 1993, Jacobson & Anderson 1996, Stoecker et al. 1997, Legrand et al. 1998, Jeong et al. 2004, 2005a,b). Feeding by mixotrophic dinoflagellates on *Synechococcus* may be an important factor in marine planktonic communities in the following ways. (1) In food webs, the pathway from one of the most abundant photosynthetic microorganisms in the world's oceans (Ferris & Palenik 1998, Li 1998) to the mixotrophic dinoflagellates is a new discovery. So far, most studies have reported that heterotrophic nanoflagellates and ciliates are responsible for the mortality of *Synechococcus* due to predation. In the future we should take mixotrophic dinoflagellates into consideration as important predators on *Synechococcus*. (2) *Synechococcus* may be too small to be eaten by filter-feeding copepods, while many red-tide dinoflagellates are easily eaten by these grazers (Nival & Nival 1976, Berggreen et al. 1988, Jeong 1995). Therefore, dinoflagellates might be a link between *Synechococcus* and some metazooplankters that are unable to ingest *Synechococcus* directly. (3) Some dinoflagellates such as *K. brevis*, *P. donghaiense*, and *P. minimum* formed red tides in offshore and/or oceanic waters, where the nutrient concentrations were low (Tyler & Seliger 1978, Tester & Steidinger 1997). Because *Synechococcus* are usually abundant in offshore and/or oceanic waters, it may be an important prey source for the red-tide dinoflagellates there. (4) Some studies suggested that besides iron limitation, high microzooplankton grazing pressure could be one of the reasons why the biomass of picophytoplankton (mainly *Synechococcus*) in oceanic waters with high nutrient concentrations remains fairly constant (e.g. Wells et al. 1994). Feeding by mixotrophic dinoflagellates may be another mechanism for limiting the excessive growth of picophytoplankton there.

Ingestion rates

Prior to this present study, there have been no data on the ingestion rate of mixotrophic dinoflagellates on *Synechococcus*. When prey concentrations were 1.1 to 2.3 × 10^6 cells ml^{-1}, ingestion rates of the red-tide dinoflagellates on *Synechococcus* sp. varied from 1 to 64 cells dinoflagellate^{-1} h^{-1}. Data from these studies show that the ingestion rates of 17 cultured red-tide dinoflagellates on *Synechococcus* sp. are positively correlated with the ESDs of the dinoflagellates (Fig. 5). This relationship suggests that the sizes of the algal predators may be an important factor affecting their ingestion rates on *Synechococcus*.

When the carbon content for *Synechococcus* sp., estimated from cell volume (1 μm^3) according to Strathmann (1967), is 0.2 pg C $cell^{-1}$, the maximum ingestion rate of *Prorocentrum donghaiense* on *Synechococcus* sp. (1.5 pg C dinoflagellate^{-1} h^{-1}) was slightly higher than those on a cryptophyte (1.1 pg C dinoflagellate^{-1} h^{-1}), while the maximum ingestion rate of *P. micans* on a *Synechococcus* sp. (7.5 pg C dinoflagellate^{-1} h^{-1}) was much higher than that on a cryptophyte (1.7 pg C dinoflagellate^{-1} h^{-1}) (Jeong et al. 2005b). The maximum ingestion rate of *P. micans* (ESD = 26.6 µm) feeding on a *Synechococcus* sp. under the conditions provided in the present study was slightly higher than that of *Cochlodinium polykrikoides* (ESD = 25.9 µm) on a cryptophyte (6.7 pg C dinoflagellate^{-1} h^{-1}) (Jeong et al. 2004) and the same as that of *Gonyaulax polygramma* (ESD = 32.5 µm) on the cryptophyte (7.5 pg C dinofla-

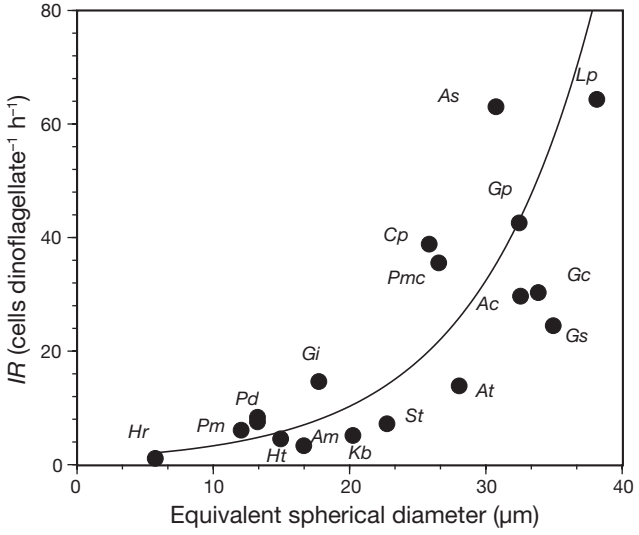

Fig. 5. Ingestion rates (*IR*s) of 17 red-tide dinoflagellates on *Synechococcus* as a function of dinoflagellate size (equivalent spherical diameter, µm). The equation of the regression was *IR* (cells dinoflagellate^{-1} h^{-1}) = $0.991e^{(0.116 \times ESD)}$, r^2 = 0.999 when the equivalent spherical diameters were 5.2 to 38.2 µm. *Ac*: *Alexandrium catenella*; *At*: *A. tamarense*; *Am*: *A. minutum*; *As: Akashiwo sanguinea*; *Cp*: *Cochlodinium polykrikoides*; *Gp*: *Gonyaulax polygramma*; *Gs*: *Gonyaulax spinifera*; *Gc: Gymnodinium catenatum*; *Gi*: *Gymnodinium impudicum*; *Hr*: *Heterocapsa rotundata*; *Ht*: *H. triquetra*; *Kb*: *Karenia brevis*; *Lp*: *Lingulodinium polyedrum*; *Pd*: *P. donghaiense*; *Pmc*: *Prorocentrum micans*; *Pm*: *P. minimum*; *St*: *Scrippsiella trochoidea*

gellate^{-1} h^{-1}) (Jeong et al. 2005a). If the dinoflagellates having similar sizes have similar ingestion rates as shown above, *Synechococcus* may be an optimal prey for *P. micans*.

The maximum ingestion rate of *Prorocentrum donghaiense* on a *Synechococcus* sp. was higher than that of the small heterotrophic nanoflagellate *Picophagus flagellatus* or *Pseudobodo* sp. on *Synechococcus* sp. (Guillou et al. 2001, Christaki et al. 2002), but lower than that of the heterotrophic nanoflagellate *Cafeteria roenbergensis* (Boenigk et al. 2001), when corrected to 20°C using Q_{10} = 2.8 (Hansen et al. 1997) (Table 3). The maximum ingestion rate of *P. micans* on *Synechococcus* was also comparable to that of the ciliate *Uronema* sp., having a volume similar to this dinoflagellate. Therefore, the red-tide dinoflagellates have ingestion rates comparable to the heterotrophic nanoflagellates and ciliates when fed on a *Synechococcus* sp., and thus these grazers may sometimes compete with one another for a *Synechococcus* if they co-occur.

Table 3. Comparison of ingestion and clearance rates in red-tide dinoflagellates (DIN), heterotrophic nanoflagellates (HNF), and ciliates (CIL) when fed on *Synechococcus*. Rates are corrected to 20°C using Q_{10} = 2.8 (Hansen et al. 1997). PDV: predators' volume, as µm^3; I_{max}: maximum ingestion rate, as cells dinoflagellate^{-1} h^{-1}; C_{max}: maximum clearance rate, as mµl dinoflagellate^{-1} h^{-1}

Predator	PDV	I_{max}	C_{max}	Source
Prorocentrum donghaiense (DIN)	1200	7.7	2.6	Present study
Prorocentrum micans (DIN)	9900	38.2	4.3	Present study
Picophagus flagellatus (HNF)	8	0.8	2.8	Guillou et al. (2001)
Pseudobodo sp. (HNF)	14	3.3	13.4	Christaki et al. (2002)
Cafeteria roenbergensis (HNF)	20	15.1	–	Boenigk et al. (2001)
Bodo saltans (HNF)	45	2.0	–	Dolan & Šimek (1998)
Uronema sp. (CIL)	8300	31.0	148.2	Christaki et al. (1999)
Strombidium sulcatum (CIL)	157000	96.0	515.0	Christaki et al. (1999)

Grazing impact

Grazing coefficients (*g*) attributable to *Prorocentrum donghaiense* and *P. micans* on co-occurring *Synechococcus* in Masan Bay, Korea, were up to 3.6 and 0.15 h^{-1}, respectively (i.e. up to 98 and 17% of *Synechococcus* populations were removed by the populations of *P. donghaiense* and *P. micans*, respectively, in 1 h). Therefore, *P. donghaiense* and *P. micans* may sometimes have a considerable grazing impact on populations of co-occurring *Synechococcus* in Masan Bay. However, grazing coefficients attributable to *P. donghaiense* in offshore and/or oceanic waters away from Jeju Island, Korea, were up to 0.014 h^{-1} (i.e. up to 1.5% of a *Synechococcus* population was removed by a population of *P. donghaiense* in 1 h). The removal of 23% of a *Synechococcus* population by a population of *P. donghaiense* per day in offshore and/or oceanic waters may not be absolutely low, but it is relatively much lower than that in Masan Bay. High abundances of *Synechococcus* (70 000 to 203 000 cells ml^{-1}) compared to the abundances of *P. donghaiense* (12 to 328 cells ml^{-1}) in oceanic waters are responsible for these relatively lower grazing coefficients. The maximum concentration of *P. donghaiense* so far reported was 360 000 cells ml^{-1} in the offshore/oceanic waters of the East China Sea (Lu et al. 2002), but in this case *g* could not be calculated, because data on the concentrations of co-occurring *Synechococcus* had not been reported. If the abundances of *P. donghaiense* were 360 000 cells ml^{-1}, *P. donghaiense* could almost eliminate *Synechococcus* in a few minutes at a *Synechococcus* concentration of 203 000 cells ml^{-1}. Some dinoflagellates such as *K. brevis*, *P. donghaiense*, and *P. minimum*, which sometimes form red tides in offshore and/or oceanic waters may have considerable grazing impact on populations of co-occurring *Synechococcus* (Tyler & Seliger 1978, Tester

& Steidinger 1997). However, the grazing rates of some mixotrophic dinoflagellates are known to be affected by light and/or nutrient conditions (Hansen & Nielsen 1997, Steocker et al. 1997, Jeong et al. 1999, Hansen et al. 2000, Jakobsen et al. 2000, Li et al. 2000, Skovgaard et al. 2000, Smalley et al. 2003). Therefore, the grazing impact of dinoflagellate predators on co-occurring *Synechococcus* may also be affected by light and/or nutrient conditions. Also, co-occurring phototrophic plankton cells may affect the grazing impact by dinoflagellate predators on *Synechococcus*, because many dinoflagellate predators have been known to feed on phototrophic plankton (Stoecker et al. 1997, Jeong et al. 1999, 2004, 2005a,b).

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