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Ingestion of the unicellular cyanobacterium *Synechococcus* by the mixotrophic red tide ciliate *Mesodinium rubrum*

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We explored phagotrophy of the phototrophic ciliate *Mesodinium rubrum* on the cyanobacterium *Synechococcus*. The ingestion and clearance rates of *M. rubrum* on *Synechococcus* as a function of prey concentration were measured. In addition, we calculated grazing coefficients by combining the field data on abundance of *M. rubrum* and co-occurring *Synechococcus* spp. with laboratory data on ingestion rates. The ingestion rate of *M. rubrum* on *Synechococcus* sp. linearly increased with increasing prey concentrations up to approximately 1.9×10^6 cells mL^{-1}, to exhibit sigmoidal saturation at higher concentrations. The maximum ingestion and clearance rates of *M. rubrum* on *Synechococcus* were 2.1 cells $predator^{-1}\ h^{-1}$ and 4.2 nL $predator^{-1}\ h^{-1}$, respectively. The calculated grazing coefficients attributable to *M. rubrum* on co-occurring *Synechococcus* spp. reached 0.04 day^{-1}. *M. rubrum* could thus sometimes be an effective protistan grazer of *Synechococcus* in marine planktonic food webs. *M. rubrum* might also be able to form recurrent and massive blooms in diverse marine environments supported by the unique and complex mixotrophic arrays including phagotrphy on hetrotrophic bacteria and *Synechococcus* as well as digestion, kleptoplastidy and karyoklepty after the ingestion of cryptophyte prey.

Key Words: grazing impact; ingestion; *Mesodinium*; mixotrophy; *Synechococcus*

INTRODUCTION

Mesodinium rubrum Lohmann 1908 is a cosmopolitan species that recurrently forms ciliate red tides in diverse marine environments (Taylor et al. 1971, Lindholm 1985, Crawford 1989, Yih et al. 2013). *M. rubrum* is able to carry out photosynthesis as well as phagotrophic feeding on prey organisms such as cryptophytes (Yih et al. 2004*a*, Johnson and Stoecker 2005, Park et al. 2007, Hansen et al. 2012, 2013) and heterotrophic bacteria (Myung et al. 2006). In turn, *M. rubrum* is known to be an important prey item for many protistan and metazoan grazers at higher trophic level (Sullivan and Gifford 2004, Yih et al. 2004*b*, Liu et al. 2005, Park et al. 2006, Reguera et al. 2012,

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Lee et al. 2014). This species usually co-occurs with bacterioplankton (Powell et al. 2005, Jeong et al. 2013) and *M. rubrum* blooms sometimes succeed those of bacterioplankton (Jeong et al. 2013). Therefore, mixotrophy of *M. rubrum* is most likely a very important phenomenon for the balanced maintenance of healthy marine environments. To understand the ecology of *M. rubrum* in marine food web systems, further exploration on the unique aspects of phagotrophy in *M. rubrum* on several kinds of prey is still desired.

The phototrophic prokaryote *Synechococcus* is a ubiquitous cyanobacterium in marine ecosystem (Johnson and Sieburth 1979, Waterbury et al. 1979, Marañón et al. 2003, Huang et al. 2012) with its cosmopolitan distribution from tropical to polar waters (Walker and Marchant 1989, Burkill et al. 1993, Landry et al. 1996, Powell et al. 2005). *Synechococcus* spp. numerically dominate the abundance of phytoplankton in marine environments (Glibert et al. 2004, Murrell and Lores 2004). In addition, *Synechococcus* sometimes contributes significantly to phytoplankton biomass and primary production in marine ecosystem (Glover et al. 1986, Li 1994, Jeong et al. 2013). It is known to be one of the major contributors to CO_2 and nutrient uptake from the ocean waters (Marañón et al. 2003). Therefore, the growth and mortality of *Synechococcus* are important factors in understanding the cycling of biomaterials in marine microbial food webs.

Several protistan grazers are known to ingest *Synechococcus* (Christaki et al. 1999, 2002, Jeong et al. 2005, 2010, 2012, Apple et al. 2011, Strom et al. 2012). The marine ciliate *M. rubrum* has been found to co-occur with *Synechococcus* spp. in the coastal waters (Lignell et al. 2003, Jeong et al. 2013, Liu et al. 2013). Therefore to better understand *Mesodinium* bacterivory in microbial food webs, we investigated the predator-prey relationships between *M. rubrum* and *Synechococcus*.

We explored whether *M. rubrum* is able to feed on *Synechococcus*. We also measured the ingestion rates of *M. rubrum* on *Synechococcus* as a function of prey concentration. In addition, we estimated grazing coefficients attributable to *M. rubrum* on co-occurring *Synechococcus* using our data for ingestion rates obtained from the laboratory experiments and data on the abundance of *Mesodinium* and *Synechococcus* in the field. The results of the present study provide a basis for improved estimation and understanding the population dynamics of *M. rubrum* in marine ecosystems.

MATERIALS AND METHODS

Preparation of experimental organisms

For isolation and cultivation of *Mesodinium rubrum* strain MR-MAL01 (Table 1) plankton samples were collected from Gomso Bay, Korea, during May 2001 when the water temperature and salinity were 18.0°C and 31.5, respectively. A culture of *M. rubrum* was established by serial single-cell isolations (Yih et al. 2004*a*). The cryptophyte *Teleaulax amphioxeia* strain CR-MAL01 (Yih et al. 2004*a*) was offered as prey of *M. rubrum*. Both *M. rubrum* and *T. amphioxeia* were maintained at 20°C in f/2 medium (Guillard and Ryther 1962) without silicate under continuous illumination of 20 µmol photons m^{-2} s^{-1} of cool white fluorescent light in the walk-in incubator system of the Marine Biology Research and Education Center, Kunsan National University. The phototrophic prokaryote *Synechococcus* strain CC9311 (clade I) (Table 1) was also grown at 20°C in f/2 medium (Guillard and Ryther 1962) without silicate under continuous illumination of 20 µmol photons m^{-2} s^{-1}. This strain has two phycoerythrin proteins (PE I and PE II) (Ong and Glazer 1991).

The equivalent spherical diameter and cell volume of *M. rubrum* (Table 1) was measured using an electron particle counter (Coulter Multisizer II; Coulter Corporation, Miami, FL, USA). The carbon contents for *M. rubrum* was estimated from cell volume according to Menden-Deuer and Lessard (2000). The cell volume and carbon content for *Synechococcus* sp. was adopted from Apple et al. (2011).

Table 1. Origin, strain name, and mean cell volume of the two experimental organisms

Organism	Cell volume	Location	Prey species	References
Synechococcus sp. CC9311	0.6	California current, USA	-	Apple et al. (2011)
Mesodinium rubrum MR-MAL01	5,996	Gomso Bay, Korea	*Teleaulax amphioxeia*	Yih et al. (2004*a*)

Cell volume (µm^3) of *Mesodinium rubrum* was measured by an electronic particle counter.

Prey concentrations (PCs) effects on ingestion and clearance rates

Experiment was designed to measure the ingestion and clearance rates of *M. rubrum* as a function of the PC when fed on *Synechococcus* sp.

We prepared dense cultures of *M. rubrum* (12,000 cells mL^{-1}) and *Synechococcus* sp. that were separately grown phototrophically in f/2 medium (Guillard and Ryther 1962) without silicate under continuous illumination of 20 µmol photons m^{-2} s^{-1}. Three 1 mL aliquots were subsampled from each *M. rubrum* culture for the cell counting under a light microscope (Olympus BH2; Olympus Co., Tokyo, Japan). For the *Synechococcus* cell counting, 5 mL aliquots from each *Synechococcus* culture were removed and then fixed with formalin (final conc. = 4%). The fixed sample was stained using DAPI (final conc. = 1 µM) and then filtered onto 25-mm polycarbonate black membrane filters of 0.2 µm-pore-size. The *Synechococcus* cells on the membranes were observed under an epifluorescence microscope (Olympus BH2; Olympus Co.) with UV light excitation at a magnification of ×1,000.

The initial concentrations of *M. rubrum* and *Synechococcus* were established using a pipette to deliver predetermined volumes of known cell concentration to the bottles. Triplicate 80 mL experimental bottles (containing mixtures of *M. rubrum* and *Synechococcus*), triplicate prey control bottles (containing *Synechococcus* only) and triplicate predator control bottles (containing *M. rubrum* only) were also established. All the bottles were placed on a shelf and incubated at 20°C under illumination of 20 µmol photons m^{-2} s^{-1} of cool white fluorescent light.

After 1-, 10-, 20-, and 30-min incubation periods, 5 mL aliquots were removed from each bottle, and then fixed with formalin. The fixed samples were stained using DAPI and then filtered onto 3 µm-pore-sized polycarbonate white membrane filters. Then, the cells of *M. rubrum* with *Synechococcus* as well as *Synechococcus* inside a *M. rubrum* were enumerated under an epifluorescence microscope with UV, blue, and green-light excitation at a magnification of ×1,000 by scanning the *M. rubrum* body at consecutive intervals of 1 to 2 µm focal depth along the z-axis. We tried to minimize the concentration of heterotrophic bacteria in the *M. rubrum* culture. For the experiments we subsampled *M. rubrum* from the upper thin layer with high density of *M. rubrum* using a siphon, and then diluted to the target concentrations by adding autoclaved seawater to the subsamples. Thus, the initial concentrations of heterotrophic bacteria in the experimental bottles were <16% of *Synechococcus* concentrations.

The ingestion rate (IR; cells $predator^{-1}$ h^{-1}) was calculated by linear regression of the number of *Synechococcus* per *M. rubrum* cell as a function of incubation time as in Sherr et al. (1987).

The clearance rate (CR; mL $predator^{-1}$ h^{-1}) was calculated as:

$$CR = IR / PC \quad (1)$$

, where IR (cells $predator^{-1}$ h^{-1}) is the ingestion rate of the *M. rubrum* predator on the *Synechococcus* prey and PC (cells mL^{-1}) is the prey concentration.

Ingestion and clearance rates were calculated using the equations of Frost (1972) and Heinbokel (1978). Data for IRs (cells $predator^{-1}$ h^{-1}) were fitted to a Michaelis-Menten equation:

$$IR = \frac{I_{max}(x)}{K_{IR} + (x)} \quad (2)$$

, where I_{max} = the maximum ingestion rate (MIR; cells $predator^{-1}$ h^{-1}), x = PC (cells mL^{-1}), and K_{IR} = the PC sustaining 1/2 I_{max}.

Potential grazing impact

We estimated the grazing coefficients attributable to *Mesodinium rubrum* on *Synechococcus* spp. by combining field data on the abundance of *M. rubrum* and *Synechococcus* with the ingestion rates of the *M. rubrum* on *Synechococcus* sp. obtained in the present study. Field data on the abundance of *M. rubrum* and co-occurring *Synechococcus* used in this estimation were originally obtained using the water samples from Masan Bay (2004-2005), Korea (Jeong et al. 2013).

The grazing coefficient (g day^{-1}) was calculated as:

$$g = CR \times GC \times 24 \quad (3)$$

, where CR (mL $predator^{-1}$ h^{-1}) is the clearance rate of a *M. rubrum* predator on *Synechococcus* prey at a given PC and GC is a predator concentration (cells mL^{-1}). CR values 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.

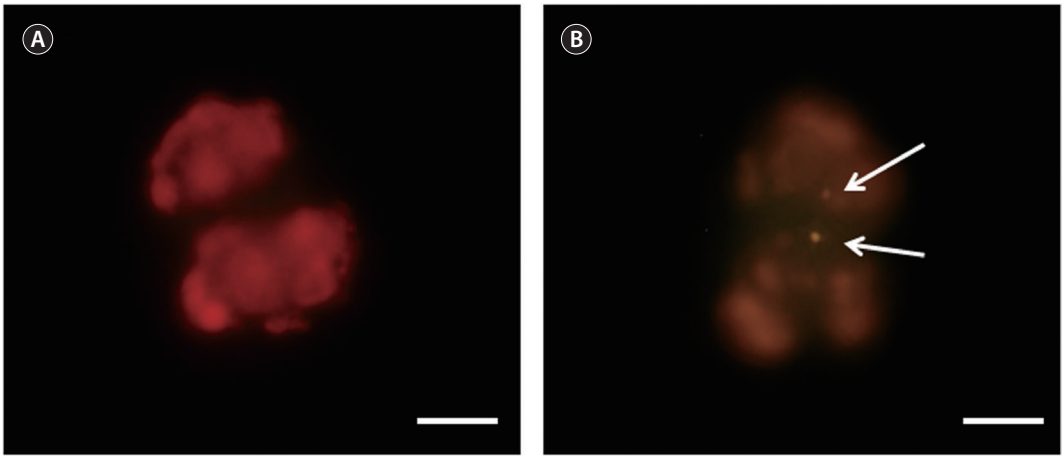

Fig. 1. Epifluorescence images of *Mesodinium rubrum* with ingested prey *Synechococcus* sp. (A) An unfed *M. rubrum* cell under an epifluorescence microscope with green light excitation. (B) *M. rubrum* with two ingested *Synechococcus* cells under an epifluorescence microscope with blue light excitation. Arrows indicate ingested prey cells. Scale bars represents: A & B, 10 μm.

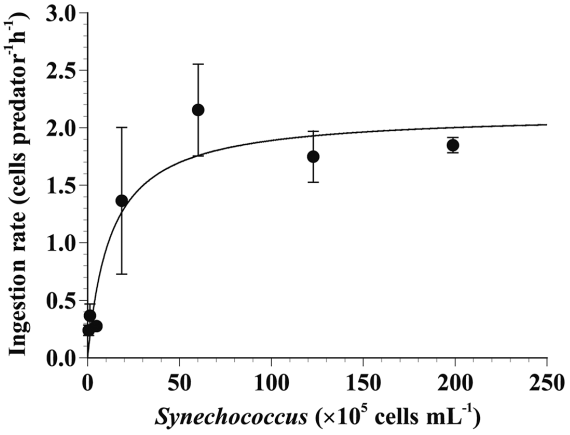

Fig. 2. Specific ingestion rates of the photosynthetic ciliate *Mesodinium rubrum* on *Synechococcus* sp. as a function of mean prey concentration (x). Symbols represent treatment means ± 1 standard error. The curves are fitted by a Michaelis-Menten equation [Eq. (2)] using all treatments in the experiment. Ingestion rate (cells predator^{-1} h^{-1}) = 2.1 [x / (1.2 × 10^6 + x)], r^2 = 0.697.

RESULTS AND DISCUSSION

PCs effects on ingestion and clearance rates

We found that *M. rubrum* ingested *Synechococcus* cells (Fig. 1). This should be the first report of grazing by red tide ciliate *M. rubrum* on *Synechococcus*. *M. rubrum* commonly co-occurred with *Synechococcus* in many marine ecosystems (Lignell et al. 2003, Jeong et al. 2013, Liu et al. 2013). Thus, *M. rubrum* should be considered to be a potentially grazer on *Synechococcus* in marine planktonic food webs.

The specific ingestion rates (SIRs) of *M. rubrum* on *Synechococcus* linearly increased with increasing PCs up to 1.9 × 10^6 cells mL^{-1}, but exhibit sigmoidal saturation at higher concentrations (Fig. 2). When the data were fitted to Eq. (2), the MIR of *M. rubrum* was 2.1 cells predator^{-1} h^{-1} (0.5 pg C predator^{-1} h^{-1}). In addition, *M. rubrum* was able to acquire up to 12.6 pg C from *Synechococcus* daily. The maximum clearance rate of *M. rubrum* on *Synechococcus* was 4.2 nL predator^{-1} h^{-1} (Table 2).

In comparison with MIRs of other red tide organisms when fed on *Synechococcus*, the MIR of *M. rubrum* on *Synechococcus* is quite much lower (Table 3). The maximum of volume SIR (VSIR) of *M. rubrum* was also lower than that of the other predators. *M. rubrum* is also able to feed on heterotrophic bacteria with higher SIR than that for *Synechococcus* (Myung et al. 2006). In addition, *M. rubrum* fed on exclusively cryptophyte prey species when offered a variety of algal prey species (Park et al. 2007, Myung et al. 2013). Thus, such kind of multiple prey species for the phagotrophy of *M. rubrum* might have evoked a type of partitioned ingestion with differential prey preferences.

The ingestion rates of the red tide organisms on *Synechococcus* were affected by the PC. The K_{IR} (the mean PC sustaining 1/2 I_{max} of MIR) of 1.2 × 10^6 cells mL^{-1} for *M. rubrum* feeding on *Synechococcus* was relatively higher than that for other predators (Table 3). Therefore, the ingestion of *M. rubrum* on *Synechococcus* was less sensitive than that of other red tide organisms to the concentration of prey cells under prey-limited conditions.

The MIRs of heterotrophic nanoflagellates, mixotrophic dinoflagellates, heterotrophic dinoflagellates, and ciliates feeding on *Synechococcus* sp. are in general positively correlated with the predator's equivalent spherical diameter ($p < 0.01$, ANOVA) (Table 4, Fig. 3). This relationship suggests that the sizes of the protistan grazers may be an important factor affecting their ingestion rates on *Synechococcus*. However, the MIR of *M. rubrum* on *Synechococcus* is relatively quite lower than that of the other protistan grazers with the exceptions in *Picophagus flagellates*, *Bodo saltans*, and *Gonimonas pacifica*. Furthermore, *M. rubrum* exhibited jumping behavior (Fenchel and Hansen 2006). This jumping behavior of *M. rubrum* may be partially responsible for the minimal ingestion. The MIR of *Ochromonas* sp. feeding on *Synechococcus* was higher than that of the other protistan grazers with the exception *Eutinnuis* sp. (Table 4). In addition, the SIR of *Ochromonas* was higher than that of the other protistan grazers. Thus, *Synechococcus* was the optimal prey for *Ochromonas* sp. not for *M. rubrum* among bacteriovorus protistan grazers.

The MIR of *M. rubrum* on *Synechococcus* provided in the present study was higher than that of the small heterotrophic nanoflagellate *Picophagus flagellates* (0.18 pg C predator^{-1} h^{-1}), *Bodo saltans* (0.12 pg C predator^{-1} h^{-1}) on

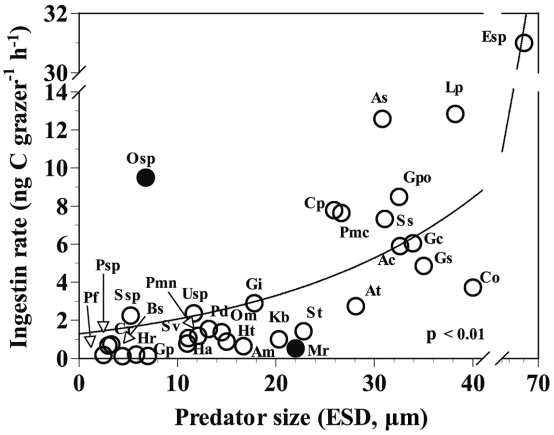

Fig. 3. Ingestion rates of protistan grazers on *Synechococcus* as a function of predator size (equivalent spherical diameter, ESD, µm). The equation of the regression was follows: Ingestion rate (ng C predator^{-1} h^{-1}) = $1.33e^{(0.046 \times ESD)}$, $r^2 = 0.790$. Ac, *Alexandrium catenella*; Am, *A. minutum*; As, *Akashiwo sanguinea*; At, *A. tamarense*; Bs, *Bodo saltans*; Co, *Chattonella ovata*; Cp, *Cochlodinium polykrikoides*; Cr, *Cafeteria roenbergensis*; Esp, *Eutintinnus* sp.; Gc, *Gymnodinium catenatum*; Gi, *G. impudicum*; Gp, *Goniomonas pacifica*; Gpo, *Gonyaulax polygramma*; Gs, *G. spinifera*; Ha, *Heterosigma akashiwo*; Hr, *Heterocapsa rotundata*; Ht, *H. triquetra*; Kb, *Karenia brevis*; Lp, *Lingulodinium polyedrum*; Mr, *Mesodinium rubrum*; Om, *Oxyrrhis marina*; Osp, *Ochromonas* sp.; Pd, *Prorocentrum donghaiense*; Pf, *Picophagus flagellates*; Pmc, *P. micans*; Pmn, *P. minimum*; Psp, *Pseudobodo* sp.; Ss, *Strombidium sulcatum*; Ssp, *Spumella* sp.; St: *Scrippsiella trochoidea*; Sv, *Symbiodinium voratum*; Usp, *Uronema* sp.

Table 2. Comparison of maximum ingestion rates and carbon acquisition in *Mesodinium rubrum* with three different prey species

Prey species	PV	MIR	K_{IR}	MCR	CA	BC	Reference
Synechococcus sp.	0.6	0.5	1.2×10^6	4.2	12.6	1.2	This study
Heterotropic bacteria	0.3	2.8	-	37	66.1	6.2	Myung et al. (2006)
Teleaulax amphioxeia[a]	76.1	1.9	2.7×10^4	-	44.8	5.5	Yih et al. (2004*a*)

PV, prey volume (µm^3); MIR, maximum ingestion rate (pg C predator^{-1} h^{-1}); K_{IR}, the prey concentration sustaining 1/2 MIR (cell mL^{-1}); MCR, maximum clearance rate (nL predator^{-1} h^{-1}); CA, carbon acquired from prey by predator per day (pg C predator^{-1} d^{-1}); BC, acquired carbon as percentage of predator's carbon (%).
[a]The maximum value among the mean ingestion rates measured at given prey concentrations.

Table 3. Comparison of ingestion rates and carbon acquisition of red tide organisms on *Synechococcus* using prey-inclusion method in the literature

Red tide organism	Taxon	PD	MIR	K_{IR}	MCR	VSIR	Reference
Mesodinium rubrum	CIL	5,996	0.5	12	4.2	0.5	This study
Heterosigma akashiwo[a]	RAP	697	0.8	-	300	7.7	Jeong et al. (2010)
Symbiodinium voratum	DIN	720	1.1	3.9	165.4	5.7	Jeong et al. (2012)
Prorocentrum donghaiense	DIN	1,200	1.5	1.1	2,600	5.6	Jeong et al. (2005)
Heterocapsa triquetra	DIN	1,770	0.9	-	-	2.4	Jeong et al. (2005)
Alexandrium minutum	DIN	2,440	0.6	-	-	1.4	Jeong et al. (2005)
Prorocentrum micans	DIN	9,900	7.6	1.5	4,300	5.9	Jeong et al. (2005)
Chattonella ovata[a]	RAP	33,490	3.7	-	100	1.0	Jeong et al. (2010)

PD, predator volume (µm^3); MIR, maximum ingestion rate (pg C predator^{-1} h^{-1}); K_{IR}, mean prey concentration sustaining 1/2 MIR ($\times 10^5$ cells mL^{-1}); MCR, maximum clearance rate (nL predator^{-1} h^{-1}); VSIR, volume specific ingestion rate ($\times 10^{-3}$ h^{-1}); CIL, ciliate; RAP, raphidophyte; DIN, dinophyte.
[a]The maximum value among the mean ingestion rates measured at given prey concentrations.

Synechococcus (Guillou et al. 2001). However, the MIR of *M. rubrum* on *Synechococcus* was lower than that of *Pseudobodo* sp. (0.68 cells predator^{-1} h^{-1}) (Dolan and Šimek 1998, Christaki et al. 2002). Therefore, *M. rubrum* may sometimes compete with the heterotrophic nanoflagellates for the common prey *Synechococcus* if they co-occur.

Impact of *Mesodinium rubum* on prey species

M. rubrum was found to be able to feed on the cyanobacterium *Synechococcus* sp. as well as the cryptophyte *Teleaulax amphioxeia* and heterotrophic bacteria (Yih et al. 2004*a*, Myung et al. 2006). *M. rubrum* grew well when supplied with *T. amphioxeia* (Yih et al. 2004*a*). However, *M. rubrum* did not sustain growth when only heterotrophic bacteria or *Synechococcus* were offered as prey (personal observation, data not shown here). Therefore, *Synechococcus* may not make a critical contribution to the population growth of *M. rubrum* in natural environments, but become supplementary prey.

M. rubrum is known to require both food uptake and photosynthesis for sustainable growth. The prey cells are used as sources for energy, carbon, and nutrients. Accordingly, *M. rubrum* seems to be able to perform photosynthesis using kleptoplastids from a cryptomonad *T. amphioxeia* while taking up heterotrophic bacteria and *Synechococcus* as phosphorus and nitrogen source.

Table 4. Comparison of ingestion rates and carbon acquisition of protistan grazers on *Synechococcus* in the literature

Predator species	Taxon	ESD	MIR	MCR	Reference
Mesodinium rubrum	CIL	22.0	0.53	4.2	This study
Picophagus flagellates	HNF	2.5	0.18	2.5	Guillou et al. (2001)
Pseudobodo sp.	HNF	3.0	0.68	10.9	Christaki et al. (2002)
Cafeteria roenbergensis	HNF	3.3	0.74	-	Boenigk et al. (2001)
Bodo saltans	HNF	4.4	0.12	-	Dolan and Šimek (1998)
Spumella sp.	HNF	5.2	2.25	-	Boenigk et al. (2001)
Ochromonas sp.	HNF	6.8	9.50	-	Boenigk et al. (2001)
Gonimonas pacifica	CRY	7.0	0.13	4.6	Apple et al. (2011)
Heterosigma akashiwo[a]	RAP	11.0	0.78	300	Jeong et al. (2010)
Symbiodinium voratum	MTD	11.1	1.06	165.4	Jeong et al. (2012)
Uronema sp.	CIL	11.7	2.38	148.2	Christaki et al. (1999)
Prorocentrum minimum	MTD	12.1	1.20	-	Jeong et al. (2005)
Prorocentrum donghaiense	MTD	13.2	1.54	2,600	Jeong et al. (2005)
Oxyrrhis marina	HTD	14.5	1.38	42.2	Apple et al. (2011)
Heterocapsa triquetra	MTD	15.0	0.88	-	Jeong et al. (2005)
Alexandrium minutum	MTD	16.7	0.64	-	Jeong et al. (2005)
Gymnodinium impudicum	MTD	17.8	2.90	-	Jeong et al. (2005)
Karenia brevis	MTD	20.3	1.00	-	Jeong et al. (2005)
Scrippsiella trochoidea	MTD	22.8	1.42	-	Jeong et al. (2005)
Cochlodinium polykrikoides	MTD	25.9	7.78	-	Jeong et al. (2005)
Prorocentrum micans	MTD	26.6	7.64	4,300	Jeong et al. (2005)
Alexandrium tamarense	MTD	28.1	2.74	-	Jeong et al. (2005)
Akashiwo sanguinea	MTD	30.8	12.6	-	Jeong et al. (2005)
Strombidium sulcatum	CIL	31.0	7.32	515.0	Christaki et al. (1999)
Gonyaulax polygramma	MTD	32.5	8.48	-	Jeong et al. (2005)
Alexandrium catenella	MTD	32.6	29.50	-	Jeong et al. (2005)
Gymnodinium catenatum	MTD	33.9	6.04	-	Jeong et al. (2005)
Gonyaulax spinifera	MTD	35.0	4.86	-	Jeong et al. (2005)
Lingulodinium polyedrum	MTD	38.2	12.84	-	Jeong et al. (2005)
Chattonella ovata[a]	RAP	40.0	3.72	100.0	Jeong et al. (2010)
Eutintinnuis sp.	CIL	68.0	31.00	219.0	Apple et al. (2011)

ESD, equivalent spherical diameter (μm); MIR, maximum ingestion rate (pg C predator^{-1} h^{-1}); MCR, maximum clearance rate (nL predator^{-1} h^{-1}); CIL, ciliate; HNF, heterotrophic nanoflagellate; CRY, cryptophyte; RAP, raphidophyte; MTD, mixotrophic dinoflagellate; HTD, heterotrophic dinoflagellate.

[a]The maximum value among the mean ingestion rates measured at given prey concentrations.

Grazing impact on *Synechococcus* populations

The grazing coefficients attributable to *M. rubrum* on co-occurring *Synechococcus* spp. in Masan Bay, Korea were affected mostly by the abundance of *M. rubrum* predator. The abundance of *M. rubrum* and *Synechococcus* spp. (n = 40) were 11-933 cells mL^{-1} and 51-39,509 cells mL^{-1}, respectively. Grazing coefficients attributable to *M. rubrum* on co-occurring *Synechococcus* spp. were 0.001 to 0.036 day^{-1} (Fig. 4).

To our knowledge, prior to this study, there had been no reports on the estimation of grazing impact by *Mesodinium* on co-occurring populations of *Synechococcus*. Grazing coefficients derived from studies in Masan Bay in 2004-2005 show that up to 3.6% of *Synechococcus* populations can be removed by *Mesodinium* in approximately 1 day. High mean abundance of *Synechococcus* (3,568 cell mL^{-1}) and relatively low mean abundance of *Mesodinium* (99 cell mL^{-1}) in coastal waters are responsible for the resulted relatively lower grazing coefficients. The results of the present study suggested that *M. rubrum* was not able to control the whole *Synechococcus* populations. However, the ingestion rates of protists are known to be affect by light and nutrition conditions (Jeong et al. 1999, Myung et al. 2006, Berge et al. 2008). Therefore, the lower grazing impact of *Mesodinium* on co-occurring *Synechococcus* may also be affected by light and nutrients conditions as well as the prey availability.

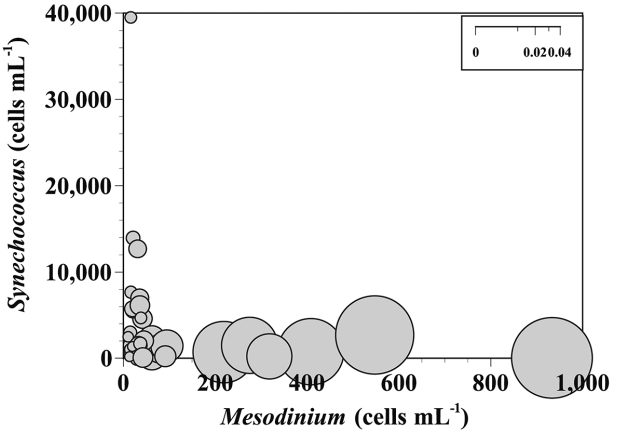

Fig. 4. Calculated grazing coeffienents of *Mesodinium rubrum* (n = 40) in relation to the concentration of co-occurring *Synechococcus* (see text for calculation). Clearance rates measured under the conditions provided in the present study, were corrected using Q_{10} = 2.8 (Hansen et al. 1997) because 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 day^{-1}.

Ecological significance of *Synechococcus* feeding by *Mesodinium rubrum*

Complex mixotrophy in the marine ciliate *Mesodinium rubrum*. Unique status of kleptoplastidy in *M. rubrum* was shown by the highly organized chloroplast-mitochondria complexes from its cryptophyte prey (Johnson et al. 2007, Nam et al. 2012), long-time functioning of the kleptoplastids (Myung et al. 2013), and even the karyoklepty from the ingested cryptophyte prey cells (Johnson et al. 2007). Bacterivory in *M. rubrum* was noteworthy as an alternate source of essential microelements and cell carbon (Myung et al. 2006). Present study confirms that *M. rubrum* also feed on *Synechococcus* cells, one of the most abundant single cell phototrophs in the sea. In the euphotic zone of the oligotrophic open ocean *Synechococcus* spp. predominates the upper euphotic layers, and sometimes perform nitrogen fixation, to be fed into open ocean food web (Phlips et al. 1989, Walker and Marchant 1989). Equipped with quite unique and complex mixotrophic arrays of metabolism such as phagotrphy on hetrotrophic bacteria and *Synechococcus* as well as digestion, kleptoplastidy, and karyoklepty after the ingestion of cryptophyte prey, *M. rubrum* might be able to form recurrent and massive blooms in diverse marine environments (Crawford 1989, Herfort et al. 2011, Yih et al. 2013).

Metabolic importance of *Synechococcus* for *Mesodinium rubrum*. C requirements for the growth of *M. rubrum* can be met by photosynthesis using kleptoplastids from prey cryptophytes as well as C from ingested cryptophytes, heterotrophic bacteria, and autotrophic bacteria like *Synechococcus* spp. (Yih et al. 2004*a*, Myung et al. 2006, 2011, 2013, Park et al. 2007). Maximum contribution to the growth of *M. rubrum* by ingested cryptophytes, heterotrophic and phototrophic bacteria were estimated to reach 5.5, 6.2, and 1.2% body carbon $ciliate^{-1}$ day^{-1}, respectively (Yih et al. 2004*a*, Myung et al. 2006).

New trophic pathways from marine phototrophic prokaryotes. Present study showed that *M. rubrum* is able to feed on phototrophic prokaryotes-the most abundant microorganisms in the ocean (Johnson and Sieburth 1979, Waterbury et al. 1979, Ferris and Palenik 1998). *M. rubrum* has thus long been involved in the newly recognized trophic pathways between diverse marine organisms and *Synechococcus*, which further emphasizes the ecological importance of *M. rubrum* as a new model organism with multiple layers of mixotrophy. Currently, more information about the population dynamics of *M. rubrum* is needed to understand the relative importance

of its *Synechococcus* feeding for the frequent success of the mixotrophic ciliate in the sea.

CONCLUSION

Phagotrophy of the phototrophic ciliate *Mesodinium rubrum* on the cyanobacterium *Synechococcus*, one of the most abundant single cell phototrophs in the sea, was firstly confirmed by the feeding experiment in the present study. By the unique and complex mixotrophic arrays including phagotrophy on heterotrophic bacteria and *Synechococcus* as well as digestion, kleptoplastidy and karyoklepty after the ingestion of cryptophyte prey, thus, *M. rubrum* can form recurrent and massive blooms in diverse marine environments. The new trophic pathway from *Synechococcus* to diverse predators linked by *M. rubrum* might further emphasize the ecological importance of *M. rubrum* as a marine model organism with multiple layers of mixotrophy.

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