

Accepted by JEM

JEONG---Mixotrophy in raphidophytes

**REVIEW**

**Mixotrophy in Red-Tide Algae Raphidophytes**

**HAE JIN JEONG**

*School of Earth and Environmental Sciences, College of Natural Sciences, Seoul National  
University, Seoul 151-747, R.O. Korea*

Corresponding Author: H.J. Jeong – Telephone number: +82-2-880-6746; FAX number: +82-2-874-9695; Email: [hjjeong@snu.ac.kr](mailto:hjjeong@snu.ac.kr)

**ABSTRACT.** Marine raphidophytes are common red-tide organisms that are distributed worldwide. They are known to be harmful to other plankton and fish and have often caused large-scale fish mortality in many countries. Thus, the population dynamics of raphidophytes is a critical concern for scientists, the aquaculture industry, and government officers from many countries. Raphidophyte growth and mortality should be investigated to understand bloom dynamics. Raphidophytes were thought to be exclusively autotrophic organisms. However, several recent studies revealed that raphidophytes are able to feed on heterotrophic and autotrophic bacteria, i.e., raphidophytes are mixotrophic algae. Further, high-resolution video microscopy revealed the mechanism by which raphidophytes feed on bacteria, which involves capturing the prey cells in the mucus excreted by mucocysts and engulfing the cells through mucocysts. These discoveries may influence the conventional view on both raphidophyte bloom dynamics and plankton energy flow and carbon cycling. In the present study, I review prey, feeding mechanisms, and ingestion rates of mixotrophic marine raphidophytes. In addition, I examine the ecological significance of raphidophyte mixotrophy.

**Key Words:** Chattonella, Feeding, Food web, Fibrocapsa, Graze, Harmful algal bloom, Heterosigma, Red tide, Trophic interactions.

Raphidophytes are photosynthetic organisms belonging to the Stramenopile lineage (also called Heterokont) in the Chromalveolate group (Keeling 2009). Thus, they contain secondary plastids of red algal origin. *Chattonella* spp., *Fibrocapsa japonica*, and *Heterosigma* spp. are all common marine raphidophytes that are distributed worldwide, excluding arctic waters (Demir et al. 2008; Edvardsen and Imai 2006; Menden-Deuer, Fredrickson, and Strom 2008; Smayda 1998). These genera are known to be harmful to other plankton (Clough and Strom 2005; Graham and Strom 2010; Uye and Takamatsu 1990) and fish (Hiroishi et al. 2005; Marshall et al. 2003) and have frequently caused large-scale fish mortality in many countries (Bourdelaïs et al. 2002; Honjo 1993; Imai et al. 1996; MacKenzie 1991). However, some raphidophyte species serve as prey for several mixotrophic and heterotrophic dinoflagellates (Jeong et al. 2003, 2005d, 2006; Tillmann and Reckermann 2002; Yoo et al. 2010). Thus, raphidophytes have diverse ecological roles in marine ecosystems.

There have been many reports on mixotrophy in protists (Burkholder, Glibert, and Skelton 2008; Jeong 2010a; Sanders 1991; Stoecker 1999). However, before Nygaard and Tobiesen (1993), no one had reported on mixotrophy in raphidophytes. Raphidophytes were previously considered as exclusively autotrophic algae and thus were regarded as phytoplankton. By using isotope-labeled bacteria, Nygaard and Tobiesen (1993) first reported that the raphidophyte *Heterosigma akashiwo* was capable of ingesting heterotrophic bacteria. However, this finding generated significant debate because negative controls were not established. Many scientists also argued that isotope signals from *H. akashiwo* might have originated from isotope-labeled bacteria sticking to the the surface of the algae. However, Seong et al. (2006) and Jeong et al. (2010c) recently used several direct observation methods (fluorescent labeling of bacteria, confocal microscopy, transmission electron microscopy (TEM), and video microscopy) to show

that the raphidophytes *Chattonella* spp., *H. akashiwo* and *Fibrocapsa* sp. can feed on heterotrophic and autotrophic bacteria. These findings may alter conventional thinking on both energy flow and carbon cycling in the marine planktonic community and bloom dynamics in raphidophytes. This paper reviews several properties of raphidophyte species: feeding abilities, type of prey, feeding mechanisms, ingestion rates, and the effect of grazing on prey populations. Finally, I will provide insight into the ecological role of raphidophytes in the marine planktonic community.

**Feeding ability of raphidophyte species.** Thus far, the raphidophytes *Chattonella ovata*, *C. subsalsa*, *Heterosigma akashiwo*, and *Fibrocapsa japonica* have been found to feed on prey (Jeong et al. 2010c; Nygaard and Tobiesen 1993; Seong et al. 2006). *F. japonica* was rarely observed to feed on the cyanobacterium *Synechococcus* sp., while the other raphidophytes were frequently observed feeding on this prey (Jeong et al. 2010c). The size of *F. japonica* is between that of *C. ovata* and *H. akashiwo*. Therefore, predator size alone does not indicate feeding patterns. Based on sequence analysis of the small subunit ribosomal RNA of raphidophyceae in the phylogenetic tree, *F. japonica* is ancestral to *Chattonella* spp. and *Heterosigma* spp. (Bowers et al. 2006). Compared to *Chattonella* spp. and *H. akashiwo*, *F. japonica* may have fewer enzymes involved in prey recognition and/or digestion of *Synechococcus*. Thus, from an evolutionary perspective, *C. ovata*, *C. subsalsa*, and *H. akashiwo* may represent expanded raphidophyte feeding abilities. To test this proposed relationship, it will be necessary to investigate the genomes and proteomes of these three raphidophytes.

**Types of prey on which each raphidophyte species feeds.** Among the various types of prey—including heterotrophic bacteria, cyanobacteria, and diverse-sized algae — only bacteria (including cyanobacteria) are fed upon by *C. ovata*, *C. subsalsa*, *H. akashiwo*, and *F. japonica*

(Table 1). *C. ovata* and *H. akashiwo* also are able to feed on microspheres 0.2--2  $\mu\text{m}$  in size, but not on microspheres  $\geq 3 \mu\text{m}$  (Table 1). *F. japonica* feeds on a smaller size range of microspheres (0.2--1.2  $\mu\text{m}$ ), but not on microspheres  $\geq 2 \mu\text{m}$ . Jeong et al. (2010c) suggested that for *C. ovata* and *H. akashiwo*, which engulf prey cells/microspheres via mucocysts (see next subsection for details), the upper size limit of prey would be ca. 2  $\mu\text{m}$ . Based on TEM serial section analysis, the mucocyst openings of *C. ovata* are ca. 3  $\mu\text{m}$ , while those in *H. akashiwo* are ca. 2  $\mu\text{m}$ . Thus, Jeong et al. (2010c) suggested that mucocyst size in these raphidophytes may be a critical factor in the upper size limit of edible prey. *C. ovata* uses a mucocyst to engulf a single *Synechococcus* sp. cell within 7--20 s of initial prey attachment to the mucus, while *H. akashiwo* engulfs a single *Synechococcus* sp. cell within 40--75 s (Jeong et al. 2010c). *C. ovata* may engulf the prey cell more easily than *H. akashiwo*, at least partially due to its larger mucocyst opening.

Co-occurring mixotrophic dinoflagellates (MTDs) having sizes similar to raphidophytes have been known to feed on diverse prey items ranging from heterotrophic bacteria to much larger heterotrophic prey (Burkholder, Glibert, and Skelton 2008; Jeong et al. 2005b, 2010b). Jeong et al. (2010c) suggested that the raphidophytes may be a less-flexible type of red-tide organism compared to MTDs in terms of prey types and feeding behaviors. In natural environments with an abundant bacterial population, raphidophytes may compete with co-occurring MTDs. However, in environments with fewer bacteria relative to abundant microalgae, and with conditions unfavorable to photosynthesis, MTDs, which are able to feed on a diverse size range of algae, may have a mixotrophic growth advantage over raphidophytes.

**Feeding mechanisms of raphidophytes.** A variety of feeding mechanisms have been reported for flagellated protists, including MTDs (Berge, Hansen, and Moestrup 2008; Hansen and Calado 1999; Jeong et al. 2004, 2005b, 2005c, 2010a; Skovgaard 1996; Stoecker 1999; Yoo

et al. 2010), nanoflagellates (Boenigk and Arndt 2000; Boenigk et al. 2002) and mixotrophic raphidophytes (Jeong et al. 2010c). MTDs ingest algae by direct engulfment or with their peduncles following anchoring or by using trichocysts and/or nematocysts. In direct engulfment, MTDs engulf algae prey through use of the sulcus or apical horns (e.g. Jeong et al. 2005c). Nanoflagellates either intercept and engulf prey cells in feeding currents or raptorially capture and engulf prey (Boenigk and Arndt 2000; Boenigk et al. 2002). Raphidophytes possess two potential means to engulf prey cells (Hara and Chihara 1987): a funnel-shaped groove with two flagella; and mucocyst pores. Recently, however, raphidophytes were shown to engulf *Synechococcus* prey through mucocysts after capturing several prey cells simultaneously in mucocyst-secreted mucus (Jeong et al. 2010c). Hence, the original function of the mucus may have been to capture small prey, and the suffocation of fish due to the mucus during red tides may be a side effect ( Jeong et al. 2010c). This mechanism of feeding on bacterial prey differs from most other heterotrophic nanoflagellates and the heterotrophic dinoflagellates (HTDs) *Oxyrrhis marina* and *Gyrodinium* spp., which intercept and then ingest a single heterotrophic bacterial cell drawn in by flagella-driven feeding currents (Boenigk and Arndt 2000, Jeong et al. 2008). However, several other flagellates, including *Euglena* spp. and *Amphidinium* spp., are known to have mucocysts (Maranda & Shimizu 1996; Zimba, Rowan, and Triemer 2004). Therefore, it is possible that these flagellates may, like raphidophytes, capture prey in mucus with subsequent engulfment by mucocysts. This possibility is worthy of further investigation while searching for additional mucus-feeding flagellates.

**Ingestion and clearance rates.** The earliest detection of feeding in *H. skashiwo* used a radioactive isotope method to determine ingestion rates of 113 heterotrophic bacteria grazer<sup>-1</sup> h<sup>-1</sup> in conditions of low nutrient concentration and zero (no ingestion) at high nutrient concentration

(Nygaard and Tobiesen 1993, Table 2). However, in their study engulfment of living bacteria and/or fluorescent-labeled bacteria (FLB) by *H. akashiwo* was not tested. Seong et al. (2006) clearly demonstrated the presence of ingested FLB in *H. akashiwo* in conditions of high nutrient concentration. Additionally, TEM (Fig. 1--6) confirms inclusion of heterotrophic bacteria by *C. ovata* and *H. akashiwo*. Seong et al. (2006) reported maximum ingestion rates of heterotrophic bacteria by *H. akashiwo* and *C. ovata* to be 11.7 and 24.5 cells grazer<sup>-1</sup> h<sup>-1</sup>, respectively, in conditions of high nutrient concentration. Using the equation given by Seong et al. (2006) to adjust for the bacterial concentrations of 1.52 and 7.25 x 10<sup>6</sup> cells ml<sup>-1</sup> used by Nygaard and Tobiesen (1993), the calculated *H. akashiwo* ingestion rates were 3.1 and 7.3 cells grazer<sup>-1</sup> h<sup>-1</sup>, respectively (Table 2). These recalculated ingestion rates are in turn higher and lower than those determined at low and high nutrient concentrations by Nygaard and Tobiesen (1993). Different methods and different strains of *H. akashiwo* might be at the root of these different results, though additional factors such as light intensity and food concentrations could also affect feeding rates.

The percentage of heterotrophic bacterial carbon acquired daily by *H. akashiwo* and *C. ovata* to use as body carbon was 12.5% and 1.4%, respectively (Seong et al. 2006). Thus, heterotrophic bacteria may contribute to the positive growth of relatively small *H. akashiwo*, but may not support growth of the relatively large *C. ovata*. The abundance of heterotrophic bacteria in coastal or estuarine waters in which the red-tide raphidophytes often co-occur is usually  $\geq 10^6$  cells ml<sup>-1</sup> (Ramaiah & Furuya 2002; HJJ., unpubl. data). Thus, raphidophytes may frequently acquire some of the body carbon necessary for growth from heterotrophic bacteria. This may be one of mechanisms used by raphidophytes to maintain or increase their population size despite low nutrient concentration conditions.

The maximum ingestion rates by *H. akashiwo* and *C. ovata* of marine heterotrophic bacteria (12--25 cells grazer<sup>-1</sup> h<sup>-1</sup>) are comparable to the MTD ingestion rates of *Cochlodinium polykrikoides*, *Heterocapsa rotundata*, and *Prorocentrum minimum* (12--22 cells grazer<sup>-1</sup> h<sup>-1</sup>) and to the HTD rates of *Gyrodinium cf. guttula* and *Pfiesteria piscicida* (14--23 cells grazer<sup>-1</sup> h<sup>-1</sup>; Table 3). However, they are considerably lower than the rate exhibited by HTD *O. marina* (71 cells grazer<sup>-1</sup> h<sup>-1</sup>). These data suggest that the maximum ingestion rates by *H. akashiwo* and *C. ovata* of marine heterotrophic bacteria are comparable to the rates exhibited by all dinoflagellates thus far examined, with the exception of *O. marina*. However, the raphidophyte and dinoflagellate maximum ingestion rates of marine heterotrophic bacteria do not correlate with the equivalent spherical diameters (ESD) of the algal predators (linear regression ANOVA,  $p > 0.1$ ; Fig. 7). The maximum ingestion rates of heterotrophic nanoflagellates (HNF) and ciliates have wide ranges (2--88 cells grazer<sup>-1</sup> h<sup>-1</sup> for HNFs and 37--525 cells grazer<sup>-1</sup> h<sup>-1</sup> for ciliates) (Alonso et al. 2000; Epstein 1997; Epstein and Shiaris 1992; Hondeveld et al. 1992; Kemp 1988). Thus, the maximum raphidophyte ingestion rates of marine heterotrophic bacteria fall in the range of HNF rates, but are considerably lower than most ciliate ingestion rates. Ciliate filter feeding is likely to be a more efficient mechanism to gather and ingest bacterial cells than raphidophyte mucus feeding. However, raphidophyte ingestion rates at the population scale may sometimes be greater than ciliate rates due to a higher abundance of raphidophyte grazers than ciliate grazers (see subsection on grazing impact), particularly during raphidophyte-dominated blooms.

The maximum clearance rates by *H. akashiwo* and *C. ovata* of marine heterotrophic bacteria are 2.6--4.5 nl grazer<sup>-1</sup> h<sup>-1</sup> (Seong et al. 2006), which are higher than clearance rates of MTDs *C. polykrikoides*, *H. rotundata*, and *P. minimum* (1.0--2.3 nl grazer<sup>-1</sup> h<sup>-1</sup>), but are an order of magnitude lower than HTDs *O. marina*, *G. cf. guttula*, and *P. piscicida* (11.4--31.3 nl grazer<sup>-1</sup>

$\text{h}^{-1}$ ; Table 3). In addition, the maximum volume-specific clearance rates by *H. akashiwo* and *C. ovata* feeding on heterotrophic bacteria ( $130\text{--}3,300 \text{ h}^{-1}$ ) were one or more orders of magnitude lower than the clearance rates by *O. marina*, *G. cf. guttula*, and *P. piscicida* feeding on bacteria ( $24,000\text{--}67,000 \text{ h}^{-1}$ ). This suggests that in environments low in bacteria, raphidophytes are more efficient than MTDs at feeding on heterotrophic bacteria, but less efficient than HTDs. The mucus excreted by several mucocysts along the raphidophyte body may function as a sticky net to enable easy capture of heterotrophic bacterial cells even in areas of low bacterial concentration. However, the HTD feeding mechanism of intercepting and engulfing heterotrophic bacteria in feeding currents may be more efficient than the mucus entrapment feeding mechanism used by the raphidophytes noted here.

Jeong et al. (2010c) measured the ingestion rates of *H. akashwio* and *C. ovata/C. subsalsa* on *Synechococcus* sp. as a function of prey concentrations. With increasing prey concentration up to  $2.5 \times 10^6 \text{ cells ml}^{-1}$  (*H. akashwio*) and  $5.5 \times 10^6 \text{ cells ml}^{-1}$  (*C. ovata/C. subsalsa*), ingestion rates of both species increased linearly. Thus, it is impossible to directly compare the maximum ingestion rates of *H. akashwio* and *C. ovata/C. subsalsa* on *Synechococcus* sp. to those reported for grazers from other taxonomic groups. To make comparisons of ingestion rates by raphidophytes to those of other taxonomic groups, I calculated ingestion rates of the raphidophytes or the other grazers at the same prey concentrations by interpolation using the equation in Jeong et al. (2010c). Ingestion rates of raphidophytes on *Synechococcus* were compared to graers of similar size (Table 4). The calculated ingestion rate by *H. akashwio* of *Synechococcus* sp. at a prey concentration of  $1.83 \times 10^6 \text{ cells ml}^{-1}$  ( $2.2 \text{ cells grazer}^{-1} \text{ h}^{-1}$ ) was considerably lower than that of *P. minimum* at the same prey concentration ( $5.2 \text{ cells grazer}^{-1} \text{ h}^{-1}$ ; Table 4). In addition, the calculated ingestion rate by *C. ovata* of *Synechococcus* sp. at a prey

concentration of  $1.53 \times 10^6$  cells  $\text{ml}^{-1}$  ( $8.7$  cells grazer $^{-1}$  h $^{-1}$ ) was also much lower than that of *Lingulodinium polyedrum* at the same prey concentration ( $64.2$  cells grazer $^{-1}$  h $^{-1}$ ). Therefore, raphidophytes may ingest *Synechococcus* prey cells at a lower rate than the similar-sized and potentially co-occurring MTDs. Unfortunately, the feeding mechanisms used by *P. minimum* and *L. polyedrum* to prey on *Synechococcus* have not yet been explored. However, based on the comparison data obtained in this study, I suggest that the feeding mechanisms of *P. minimum* and *L. polyedrum* are likely different and more efficient than the raphidophyte mechanisms when feeding upon *Synechococcus*.

The highest ingestion rate by *C. ovata/C. subsalsa* on *Synechococcus* sp. (ca.  $18.6$ -- $20.5$  cells grazer $^{-1}$  h $^{-1}$ ) obtained by Jeong et al. (2010c) was higher than the ingestion rates of the small HNFs *Picophagus flagellatus*, *Pseudobodo* sp., *Cafeteria roenbergensis*, and *Bodo saltans* on *Synechococcus* spp. ( $0.8$ -- $13.6$  cells grazer $^{-1}$  h $^{-1}$ ; Boenigk et al. 2001; Christaki et al. 2002; Dolan and Simek 1998; Guillou et al. 2001) and comparable to the ingestion rate of the ciliate *Uronema* sp. ( $31$  cells grazer $^{-1}$  h $^{-1}$ ; Christaki et al. 1999). The highest reported ingestion rate of *Synechococcus* by *H. akashiwo* (ca.  $3$ -- $4$  cells grazer $^{-1}$  h $^{-1}$ ; Jeong et al. 2010c) lies between that of *Pseudobodo* sp. and *C. roenbergensis* ingestion rates. However, the ingestion rates by HNFs and ciliates were obtained at prey concentrations of  $0.1$ -- $1.0 \times 10^6$  cells  $\text{ml}^{-1}$ , considerably lower than the prey concentrations at which the highest ingestion rates by *H. akashiwo* and *C. ovata/C. subsalsa* of *Synechococcus* sp. were obtained ( $2.5$ -- $5.5 \times 10^6$  cells  $\text{ml}^{-1}$ ). When ingestion rates of *H. akashiwo* and *C. ovata/C. subsalsa* on *Synechococcus* sp. were calculated at the prey concentrations where rates of HNFs and ciliates were obtained experimentally, the comparison of relative rates changed. For example, when ingestion rates by the raphidophytes on *Synechococcus* sp. were calculated as described above at the prey concentration used for *P.*

*flagellatus* ( $0.12 \times 10^6$  cells  $\text{ml}^{-1}$ ), calculated ingestion rates by *H. akashiwo* on *Synechococcus* sp. ( $0.3$  cells grazer $^{-1}$  h $^{-1}$ ) was lower than that of *P. flagellatus* ( $0.7$  cells grazer $^{-1}$  h $^{-1}$ ; Guillou et al. 2001), while the rate by *C. ovata/C. subsalsa* on *Synechococcus* sp. ( $1.5$  cells grazer $^{-1}$  h $^{-1}$ ) was higher (Table 5). Similar results were obtained with another HNF *Pseudobodo* sp. (Table 5). In addition, the calculated ingestion rates for *H. akashiwo* and *C. ovata/C. subsalsa* on *Synechococcus* sp. at the prey concentration of  $0.21 \times 10^6$  cells  $\text{ml}^{-1}$  used for *Uronema* sp. ( $0.2$  and  $1.2$  cells grazer $^{-1}$  h $^{-1}$ , respectively) are much lower than that of the ciliate ( $31$  cells grazer $^{-1}$  h $^{-1}$ ; Christaki et al. 1999). Thus, at *Synechococcus* prey concentrations of  $0.12$ -- $0.21 \times 10^6$  cells  $\text{ml}^{-1}$ , *C. ovata/C. subsalsa* and *H. akashiwo* may ingest fewer prey cells than HNFs or ciliates. The impact each grazer has on a population of a certain prey species is usually proportional to the individual ingestion rate and the abundance of the grazer. Thus, a lower ingestion rate does not necessarily indicate a lower population impact on prey. To explore the relative contribution of each grazer on the total *Synechococcus* population, field data on the abundance of grazers and co-occurring *Synechococcus* are required, as well as data on individual ingestion rates measured in natural environments or the laboratory.

**Grazing impact.** Based on grazing coefficients from natural populations of marine heterotrophic bacteria attributable to the dominant red-tide algae, HNFs, and ciliates, Seong et al. (2006) reported that *Heterosigma akashiwo* was the most effective protistan predator of marine heterotrophic bacteria (2 out of 5 field experiments) or the second most (1 out of 5) in Korean waters containing *H. akashiwo* ( $>550$  cells  $\text{ml}^{-1}$ ). The grazing coefficients of the natural populations of marine bacteria attributable to *H. akashiwo* ranged from  $0.02$  d $^{-1}$  to  $0.857$  d $^{-1}$  [i.e., 2--58% bacterial population was removed by a population of *H. akashiwo* in 1 day]. Therefore, *H. akashiwo* has considerable potential grazing impact on marine heterotrophic bacterial populations

in Korean waters during red-tide domination. Seong et al. (2006) also reported that the maximum grazing coefficient by *H. akashiwo* on bacterial populations in Korean waters was comparable to that of *Prorocentrum minimum* ( $0.850 \text{ d}^{-1}$ ), slightly greater than the ciliate grazing coefficient ( $0.596 \text{ d}^{-1}$ ), but much greater than the HNF coefficient ( $0.160 \text{ d}^{-1}$ ). Hence, when making an assessment of grazing impact on bacterial populations, raphidophyte grazers should be taken into consideration, particularly during blooms.

Jeong et al. (2010c) reported that the grazing coefficients attributable to *H. akashiwo* on co-occurring *Synechococcus* in Masan Bay and Shiwaha Bay, Korea were as high as  $1.238 \text{ d}^{-1}$  (i.e., as much as 71% *Synechococcus* population was removed by a *H. akashiwo* population in 1 day). This maximum grazing coefficient attributable to *H. akashiwo* on co-occurring *Synechococcus* is lower than that attributed to MTD *P. micans* on co-occurring *Synechococcus* in Masan Bay, Korea, [up to  $3.6 \text{ d}^{-1}$ , i.e., as much as 98% *Synechococcus* population was removed by *P. micans* population, in 1 d; Jeong et al. (2005a)]. However, removal of 71% of *Synechococcus* population by *H. akashiwo* in 1 day is still quite high. Thus, it was suggested that *H. akashiwo* may sometimes have considerable grazing impact on populations of co-occurring *Synechococcus* in Masan and Shiwaha Bays during red-tide domination (Jeong et al. 2010c). The abundance of *H. akashiwo* sometimes exceeds  $100,000 \text{ cells ml}^{-1}$  in other red-tide waters (Nagasaki et al. 1996). Therefore, to understand population dynamics of *Synechococcus* spp. in red-tide waters dominated by *H. akashiwo*, the grazing impact by the raphidophyte on the cyanobacteria should be assessed. However, grazing coefficients obtained during non-red-tide periods showed that *H. akashiwo* likely has a much lower grazing impact on the populations of marine heterotrophic bacteria in Korean waters at those times.

Jeong et al. (2010a) combined the results of Jeong et al. (2005c) and Seong et al. (2006) to

propose a possible mechanism for the outbreak and/or persistence of offshore or oceanic red-tides dominated by MTDs; many MTDs are able to feed on *Synechococcus* sp. and heterotrophic bacteria. Therefore, if the MTDs feed on nitrogen-fixing cyanobacteria (Mitsui et al. 1986; Philips, Zeman, and Hansen 1989) and on heterotrophic bacteria that often have high phosphorus (P) : nitrogen (N) ratios (Tezuka 1990), the MTDs are then able to obtain nitrogen and phosphorus simultaneously to support their own growth in offshore or oceanic waters. This hypothesis may also apply to raphidophytes because they are able to feed on both heterotrophic bacteria and cyanobacteria.

**Ecological importance.** Raphidophyte mixotrophy is ecologically important to planktonic communities for the following reasons (Jeong et al. 2010c; Seong et al. 2006): 1) Raphidophytes were previously believed to be exclusively autotrophic flagellates and were as such treated as phytoplankton; however, they have since been discovered to function as mixotrophic flagellates. Therefore, to understand raphidophyte population dynamics, their feeding on prey should be taken into consideration. In particular, their mixotrophy may affect the outbreak, persistence, and decline of raphidophyte-dominated red tides. 2) In the marine planktonic food web, raphidophytes are able to feed on both heterotrophic and autotrophic bacteria, among the most abundant heterotrophic and photosynthetic microorganisms in all the oceans (Ferris and Palenik 1998; Li 1998). Raphidophyte bacterivory has created newly recognized trophic pathways and predator-prey relationships between bacteria. Previously, several studies have reported some algicidal bacteria activity that killed raphidophytes (e.g. Imai et al. 2001). Thus, raphidophytes and bacteria could interchangeably be predators or prey, and their role can be reversed at anytime in marine environments. Consequently, raphidophyte mixotrophy creates more complexity in marine planktonic webs. To explore population dynamics of raphidophytes, the abundance of bacteria

should also be monitored. 3) Calculated or measured grazing coefficients on the natural populations of marine heterotrophic bacteria and/or *Synechococcus* spp. attributable to the dominant protistan grazers showed that, among red-tide algae, HNFs, and ciliates, raphidophytes are sometimes the most effective protistan predators of marine heterotrophic bacteria, and raphidophytes sometimes have considerable grazing impact on co-occurring heterotrophic and autotrophic bacterial populations. Thus, raphidophytes should be taken into consideration as important predators on heterotrophic and autotrophic bacteria. 4) Raphidophytes are only able to feed on bacteria, while MTDs can feed on larger algal prey as well as bacteria. Hence, these two taxonomic groups play different roles in the marine planktonic food web, which may cause a separation in the ecological niches of raphidophytes and MTDs and may contribute additional explanation of Hutchinson's "paradox of the plankton" (Hutchinson 1961).

#### ACKNOWLEDGMENTS

I thank Dr. Robert Sanders for comments on the manuscript and Kyeong Ah Seong, Yeong Du Yoo, Nam Seon Kang, Jae Seong Kim for technical support. I thank the International Society of Protistologists for support to present this paper. This work was also supported by the National Research Foundation of Korea Grant funded by the Korea Government/MEST (NRF-C1ABA001-2010-0020700) and Ecological Disturbance Program of KIMST award to HJ Jeong.

#### LITERATURE CITED

Alonso, M. C., Rodriguez, V., Rodriguez, J. & Borrego, J. J. 2000. Role of ciliates, flagellates and bacteriophages on the mortality of marine bacteria and on dissolved-DNA concentration in laboratory experimental systems. *J. Exp. Mar. Biol. Ecol.*, **244**:239--252.

- Berge, T., Hansen, P. J. & Moestrup, O. 2008. Feeding mechanism, prey specificity and growth in light and dark of the plastidic dinoflagellate *Karlodinium armiger*. *Aquat. Microb. Ecol.*, **50**:279--288.
- Boenigk, J. & Arndt, H. 2000. Comparative studies on the feeding behavior of two heterotrophic nanoflagellates: the filter-feeding choanoflagellate *Monosiga ovata* and the raptorial-feeding kinetoplastid *Rhynchomonas nasuta*. *Aquat. Microb. Ecol.*, **22**:243--249.
- Boenigk, J., Matz, C., Jürgens, K. & Arndt, H. 2001. The influence of preculture conditions and food quality on the ingestion and digestion process of three species of heterotrophic nanoflagellates. *Microb. Ecol.*, **42**:168--176.
- Boenigk, J., Matz, C., Jürgens, K. & Arndt, H. 2002. Food concentration-dependent regulation of food selectivity of interception-feeding bacterivorous nanoflagellates. *Aquat. Microb. Ecol.*, **27**:195--202.
- Bourdelaïs, A. J., Tomas, C. R., Naar, J., Kubanek, J. & Baden, D. G. 2002 New fish-killing alga in coastal Delaware produces neurotoxins. *Environ. Health. Perspect.*, **110**:465--470.
- Bowers, H.A., Tomas, C.R., Tengs, T., Kempton, J.W., Lewitus, A.J. & Oldach D.W. 2006. Raphidophyceae (Chadefaud ex Silva) systematics and rapid identification: sequence analyses and realtime PCR assays. *J. Phycol.*, **42**:1333--1348.
- Burkholder, J.M., Glibert, P.M. & Skelton, H.M. 2008. Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. *Harmful Algae*, **8**:77--93.
- Christaki, U., Jacquet, S., Dolan, J. R., Vaulot, D. & Rassoulzadegan, F. 1999. Growth and grazing on *Prochlorococcus* and *Synechococcus* by two marine ciliates. *Limnol. Oceanogr.*, **144**:52--61.
- Christaki, U., Courties, C., Karayanni, H., Giannakourou, A., Maravelias, C., Kormas, K. A. &

- Lebaron, P. 2002. Dynamic characteristics of *Prochlorococcus* and *Synechococcus* consumption by bacterivorous nanoflagellates. *Microb. Ecol.*, **43**: 341--352.
- Clough, J. & Strom, S. 2005. Effects of *Heterosigma akashiwo* (Raphidophyceae) on protist grazers: laboratory experiments with ciliates and heterotrophic dinoflagellates. *Aquat. Microb. Ecol.*, **39**:121--134.
- Demir, E., Coyne, K. J., Doblin, M. A., Handy, S. M. & Hutchins, D. A. 2008. Assessment of microzooplankton grazing on *Heterosigma akashiwo* using a species-specific approach combining quantitative real-time PCR (QPCR) and dilution methods. *Microb. Ecol.*, **55**:583--594.
- Dolan, J. R. & Simek, K. 1998. Ingestion and digestion of an autotrophic picoplankter, *Synechococcus*, by a heterotrophic nanoflagellate, *Bodo saltans*. *Limnol. Oceanogr.*, **43**: 1740--1746.
- Edvardsen, B. & Imai, I. 2006. The ecology of harmful prymnesiophytes and raphidophytes. In: Granéli, E & Turner, J. T. (ed.), *The Ecology of Harmful Algae*. Springer, Berlin. p. 67--80.
- Epstein, S. S. 1997. Microbial food webs in marine sediments. I. Trophic interactions and grazing rates in two tidal flat communities. *Microb. Ecol.*, **34**:188--198.
- Epstein, S. S. & Shiaris, M. P. 1992. Rates of microbenthic and meiobenthic bacterivory in a temperate muddy tidal flat community. *Appl. Environm. Microbiol.*, **58**:2426--2431.
- Ferris, M.J. & Palenik, B. 1998. Niche adaptation in ocean cyanobacteria. *Nature*, **396**:226--228.
- Graham, S. L. & Strom, S. L. 2010. Growth and grazing of microzooplankton in response to the harmful alga *Heterosigma akashiwo* in prey mixtures. *Aquat. Microb. Ecol.*, **59**:111--124.
- Guillou, L., Jacquet, S., Chretiennot-Dinet, M.-J. & Vaulot, D. 2001. Grazing impact of two small heterotrophic flagellates on *Prochlorococcus* and *Synechococcus*. *Aquat. Microb.*

- Ecol.*, **26**:201--207.
- Hansen, P. J. & Calado, A. J. 1999. Phagotrophic mechanisms and prey selection in free-living dinoflagellates. *J. Euk. Microbiol.*, **46**:382--389.
- Hara, Y. & Chihara, M. 1987. Morphology, ultrastructure and taxonomy of the raphidophycean alga *Heterosigma akashiwo*. *J. Plankton Res.*, **100**:151--163.
- Hiroishi, S., Okada, H., Imai, I. & Yoshida, T. 2005. High toxicity of the novel bloom-forming species *Chattonella ovata* (Raphidophyceae) to cultured fish. *Harmful Algae*, **4**:783--787
- Hondeveld, B. J. M., Bak, R. P. M. & van Duyl, F. C. 1992. Bacterivory by heterotrophic nanoflagellates in marine sediments measured by uptake of fluorescently labeled bacteria. *Mar. Ecol. Prog. Ser.*, **89**:63--71.
- Honjo, T. 1993. Overview on bloom dynamics and physiological, ecology of *Heterosigma akashiwo*. In: Smayda, T. J. & Shimizu, Y. (ed.), Toxic phytoplankton blooms in the sea. Elsevier, New York. p. 33--41.
- Hutchinson, G.E. 1961. The paradox of the plankton. *American Naturalist*, **95**:137--145.
- Imai, I., Sunahara, T., Nishikawa, T., Hori, Y., Kondo, R. & Hiroishi, S. 2001. Fluctuations of the red tide flagellates *Chattonella* spp. (Raphidophyceae) and the algicidal bacterium *Cytophaga* sp. in the Seto Inland Sea, Japan. *Mar. Biol.*, **138**:1043--1049.
- Imai, I., Itakura, S., Matsuyama, Y. & Yamaguchi, M. 1996. Selenium requirement for growth of a novel red tide flagellate *Chattonella verruculosa* (Raphidophyceae) in culture. *Fish. Sci.*, **62**:834--835.
- Jeong, H. J., Yoo, Y. D., Kim, J. S., Seong, K. A., Kang, N. S. & Kim, T.H. 2010a. Growth, feeding, and ecological roles of the mixotrophic and heterotrophic dinoflagellates in marine planktonic food webs. *Ocean Sci. J.*, **45**:65--91.

- Jeong, H. J., Yoo, Y. D., Kim, J. S., Kim, T. H., Kim, J. H., Kang, N. S. & Yih, W. H. 2004. Mixotrophy in the phototrophic harmful alga *Cochlodinium polykrikoides* (Dinophyceae): prey species, the effects of prey concentration and grazing impact. *J. Eukaryot. Microbiol.*, **51**: 563--569.
- Jeong, H. J., Kim, J. S., Kim, J. H., Kim, S. T., Seong, K. A., Kim, T. H., Song, J. Y. & Kim, S. K. 2005d. Feeding and grazing impact by the newly described heterotrophic dinoflagellate *Stoeckeria algicida* on the harmful alga *Heterosigma akashiwo*. *Mar. Ecol. Prog. Ser.*, **295**:69--78.
- Jeong, H. J., Yoo, Y. D., Kang, N. S., Rho, J. R., Seong, K. A., Park, J. W., Nam, G. S. & Yih, W. H. 2010b. Ecology of *Gymnodinium aureolum*. I. Feeding in western Korean waters. *Aquat. Microb. Ecol.*, **59**:239--255.
- Jeong, H. J., Yoo, Y. D., Park, J. Y., Song, J. Y., Kim, S. T., Lee, S. H., Kim, K. Y. & Yih, W. H. 2005b. Feeding by the phototrophic red-tide algal predators: five species newly revealed and six species previously known to be mixotrophic. *Aquat. Microb. Ecol.*, **40**:133--150.
- Jeong, H. J., Ha, J. H., Park, J. Y., Kim, J. H., Kang, N. S., Kim, S., Kim, J. S., Yoo, Y. D. & Yih, W. H. 2006. Distribution of the heterotrophic dinoflagellate *Pfiesteria piscicida* in Korean waters and its consumption of mixotrophic dinoflagellates, raphidophytes, and fish blood cells. *Aquat. Microb. Ecol.*, **44**:263--278.
- Jeong, H. J., Park, J. Y., Rho, J. H., Park, M. O., Ha, J. H., Seong, K. A., Jeng, C., Seong, C. N. & Yih, W. H. 2005a. Feeding by the red-tide dinoflagellates on the cyanobacterium *Synechococcus*. *Aquat. Microb. Ecol.*, **41**:131--143.
- Jeong, H. J., Seong, K. A., Kang, N. S., Yoo, Y. D., Nam, S. W., Park, J. Y., Shin, W. G., Glibert, P. M. & Johns, D. 2010c. Feeding by raphidophytes on the cyanobacterium *Synechococcus*.

- Aquat. Microb. Ecol.*, **58**:181--195.
- Jeong, H. J., Yoo, Y. D., Seong, K. A., Kim, J. H., Park, J. Y., Kim, S. H., Lee, S. H., Ha, J. H. & Yih, W. H. 2005c. Feeding by the mixotrophic algal predator *Gonyaulax polygramma*: mechanisms, prey species, the effects of prey concentration, and grazing impact. *Aquat. Microb. Ecol.*, **38**:249--257.
- Jeong, H. J., Kim, J. S., Yoo, Y. D., Kim, S. T., Kim, T. H., Park, M. G., Lee, C. H., Seong, K. A., Kang, N. S. & Shim, J. H. 2003. Feeding by the heterotrophic dinoflagellate *Oxyrrhis marina* on the red-tide raphidophyte *Heterosigma akashiwo*: a potential biological method to control red tides using mass-cultured grazers. *J. Eukaryot. Microbiol.*, **50**:274--282.
- Jeong, H. J., Seong, K. A., Yoo, Y. D., Kim, T. H., Kang, N. S., Kim, S., Park, J. Y., Kim, J. S., Kim, G. H. & Song, J. Y. 2008. Feeding and grazing impact by small marine heterotrophic dinoflagellates on heterotrophic bacteria. *J. Eukaryot. Microbiol.*, **55**:271--288.
- Keeling, P. J. 2009. Chromalveolates and the Evolution of Plastids by Secondary Endosymbiosis. *J. Eukaryot. Microbiol.*, **56**:1--8.
- Kemp, P. F. 1988. Bacterivory by benthic ciliates: significance as a carbon source and impact on sediment bacteria. *Mar. Ecol. Prog. Ser.*, **49**:163--169.
- Li, W. K. W. 1998. Annual average abundance of heterotrophic bacteria and *Synechococcus* in surface ocean waters. *Limnol. Oceanogr.*, **43**:1746--1753.
- MacKenzie, L. 1991. Toxic and noxious phytoplankton in Big Glory Bay, Stewart Island, New Zealand. *J. Appl. Phycol.*, **3**:19--34.
- Maranda, L & Shimizu, Y. 1996. *Amphidinium operculatum* var. nov. *gibbosum* (dinophyceae), a free-swimming marine species producing cytotoxic metabolites. *J. Phycol.*, **32**:873--879.
- Marshall, J. -A., Nichols, P. D., Hamilton, B., Lewis, R. J. & Hallegraeff, G. M. 2003.

- Ichthyotoxicity of *Chattonella marina* (Raphidophyceae) to damselfish (*Acanthochromis polycanthus*): the synergistic role of reactive oxygen species and free fatty acids. *Harmful Algae*, **2**:273--281.
- Menden-Deuer, S., Fredrickson, K.A. & Strom, S.L. 2008. Physical and biological drivers of HAB formation: Rates of formation, persistence and decline of a *Heterosigma akashiwo* event in East Sound, Washington, USA. Abstract of 13th International Conference on Harmful Algae, Hong Kong.
- Mitsui, A., Cao, S., Takahashi, A. & Arai, T. 1986. Growth synchrony and cellular parameters of the unicellular nitrogen-fixing marine cyanobacterium, *Synechococcus* sp. strain Miami BG 043511 under continuous illumination. *Physiol. Plant.*, **69**:1--8.
- Nagasaki, K., Itakura, S., Imai, I., Nakagiri, S. & Yamaguchi, M. 1996. The disintegration process of a *Heterosigma akashiwo* (raphidophyceae) red tide in northern Hiroshima Bay, Japan, during the summer of 1994. In: Yasumoto, T., Oshima, Y. & Fukuyo, Y. (ed.), Harmful and toxic algal blooms. UNESCO. p. 251--254.
- Nygaard, K. & Tobiesen, A. 1993. Bacterivory in algae: A survival strategy during nutrient limitation. *Limnol. Oceanogr.*, **38**:273--279.
- Phlips, E. J., Zeman, C. & Hansen, P. 1989. Growth, photosynthesis, nitrogen fixation and carbohydrate production by a unicellular cyanobacterium, *Synechococcus* sp. (Cyanophyta). *J. Applied Phycol.*, **1**:137--145.
- Ramaiah, N. & Furuya, K. 2002. Seasonal variations in phytoplankton composition and transparent exopolymer particles in a eutrophicated coastal environment. *Aquat. Microb. Ecol.*, **30**:69--82.
- Sanders, R. W. 1991. Mixotrophic Protists in Marine and Freshwater Ecosystems. *J. Protozool.*,

38:76--81.

- Seong, K. A., Jeong, H. J., Kim, S., Kim, G. H. & Kang, J. H. 2006. Bacterivory by co-occurring red-tide algae, heterotrophic nanoflagellates, and ciliates on marine bacteria. *Mar. Ecol. Prog. Ser.*, **322**:85--97.
- Skovgaard, A. 1996. Engulfment of *Ceratium* spp. (Dinophyceae) by the thecate photosynthetic dinoflagellate *Fragilidium subglobosum*. *Phycologia*, **35**:490--499.
- Smayda, T. J. 1998. Ecophysiology and bloom dynamics of *Heterosigma akashiwo* (raphidophyceae). In: Anderson, D. M., Cembella, A. D. & Hallegraeff, G. M. (ed.), *Physiological ecology of harmful algal blooms*. p. 113--131.
- Stoecker, D. K. 1999. Mixotrophy among dinoflagellates. *J. Eukaryot. Microbiol.*, **46**:397--401.
- Tezuka, Y. 1990. Bacterial regeneration of ammonium and phosphate as affected by the carbon: nitrogen: phosphorus ratio of organic substrates. *Microb. Ecol.*, **19**:227--238.
- Tillmann, U. & Reckermann, M. 2002. Dinoflagellate grazing on the raphidophyte *Fibrocapsa japonica*. *Aquat. Microb. Ecol.*, **26**:247--257.
- Uye, S.-I. & Takamatsu, K. 1990. Feeding interactions between planktonic copepods and red-tide flagellates from Japanese coastal waters. *Mar. Ecol. Prog. Ser.*, **59**:97--107.
- Yoo, Y. D., Jeong, H. J., Kang, N. S., Song, J. Y., Kim, K. Y., Lee, K. T. & Kim, J. H. 2010. Feeding by the newly described mixotrophic dinoflagellate *Paragymnodinium shiwhaense*: feeding mechanism, prey species, and effect of prey concentration. *J. Eukaryot. Microbiol.*, **57**:145--158.
- Zimba, P. V., Rowan, M. & Triemer R (2004) Identification of euglenoid algae that produce ichthyotoxin(s). *J. Fish Diseases*, **27**:115--117.

Table 1. Feeding occurrence by the raphidophytes *Chattonella* spp. (CHA), *Heterosigma akashiwo* (HAT), and *Fibrocapsa japonica* (FIB). Y – A raphidophyte species was observed to feed on a living food cell or bead; N – A raphidophyte species was observed not to feed on a living food cell or bead. NA – Not tested. Mean equivalent spherical diameter (ESD,  $\mu\text{m}$ )  $\pm$  standard deviation of the mean. Reference: (1) Nygaard and Tobiesen (1993). (2) Seong et al. (2006). (3) Jeong et al. (2010c).

Species	ESD ( $\pm$ SD)	CHA	HAT	FIB	Reference
<b>Bacteria</b>					
Heterotrophic bacteria	0.9 (0.3)	Y	Y	NA	(1), (2)
<i>Synechococcus</i> sp.	1.0 (0.2)	Y	Y	Y	(3)
<b>Diatoms</b>					
<i>Skeletonema costatum</i>	5.9 (1.1)	N	N	N	(3)
<b>Prymnesiophytes</b>					
<i>Isochrysis galbana</i>	4.8 (0.2)	N	N	N	(3)
<b>Cryptophytes</b>					
<i>Teleaulex</i> sp.	5.6 (1.5)	N	N	N	(3)
<i>Rhodomonas salina</i>	8.8 (1.5)	N	N	N	(3)
<b>Mixotrophic dinoflagellates</b>					
<i>Heterocapsa rotundata</i>	5.8 (0.4)	N	N	N	(3)

<i>Amphidinium carterae</i>	9.7 (1.6)	N	N	N	(3)
<i>Prorocentrum minimum</i>	12.1 (2.5)	N	N	N	(3)
<i>Heterocapsa triquetra</i>	15.0 (4.3)	N	N	N	(3)
<i>Scrippsiella trochoidea</i>	22.8 (2.7)	N	N	N	(3)
<i>Cochlodinium polykrikoides</i>	25.9 (2.9)	N	N	N	(3)
<i>Prorocentrum micans</i>	26.6 (2.8)	N	N	N	(3)
<i>Akashiwo sanguinea</i>	30.8 (3.5)	N	N	N	(3)
<i>Gonyaulax polygramma</i>	32.5 (5.4)	N	N	N	(3)
<i>Alexandrium tamarense</i>	32.6 (2.7)	N	N	N	(3)
<i>Lingulodinium polyedrum</i>	38.2 (3.6)	N	N	N	(3)

### **Beads**

Beads (0.2 $\mu\text{m}$ )	0.2	Y	Y	Y	(3)
Beads (0.5 $\mu\text{m}$ )	0.5	Y	Y	Y	(3)
Beads (1.2 $\mu\text{m}$ )	1.2	Y	Y	Y	(3)
Beads (2 $\mu\text{m}$ )	2	Y	Y	N	(3)
Beads (3 $\mu\text{m}$ )	3	N	N	N	(3)
Beads (6 $\mu\text{m}$ )	6	N	N	N	(3)
Beads (7 $\mu\text{m}$ )	7	N	N	N	(3)
Beads (8 $\mu\text{m}$ )	8	N	N	N	(3)

---

Table 2. Maximum ingestion rates ( $I_{\max}$ , cells grazer<sup>-1</sup> h<sup>-1</sup>) of the raphidophyte *Heterosigma akashiwo* on marine heterotrophic bacteria as a function of bacterial concentrations or the ingestion rates (IR, cells grazer<sup>-1</sup>h<sup>-1</sup>) at given prey concentrations (\*). The calculated ingestion rates (IRCal, cells grazer<sup>-1</sup> h<sup>-1</sup>) at the given prey concentration, obtained by interpolation using the equation in Seong et al. (2006) {i.e. IR = 11.7 [pc / (4.3 × 10<sup>6</sup> + pc)], where pc is bacterial prey concentration}. DEP: Nutrients depleted. ENR: Nutrients enriched. Reference: (1) Nygaard and Tobiesen (1993). (2) Seong et al. (2006).

Strain	Nutrients	Prey concentration (x 10 <sup>6</sup> )	IR	IRCal	$I_{\max}$	Reference
<i>Heterosigma akashiwo</i> 1	DEP	7.25	113*			(1)
<i>H. akashiwo</i> 1	ENR	1.52	0*			(1)
<i>H. akashiwo</i> 2	ENR	7.25		7.3		(1) & (2)
<i>H. akashiwo</i> 2	ENR	1.52		3.1		(1) & (2)
<i>H. akashiwo</i> 2	ENR				11.7	(2)

Table 3. Ingestion and clearance rates of the raphidophyte (RAP), mixotrophic dinoflagellate (MTD), and heterotrophic dinoflagellate (HTD) predators on marine heterotrophic bacteria. ESD: Equivalent Spherical Diameter ( $\mu\text{m}$ ).  $I_{\text{max}}$  (maximum ingestion rate, cells grazer<sup>-1</sup> h<sup>-1</sup>; pg C grazer<sup>-1</sup> h<sup>-1</sup> in parenthesis),  $C_{\text{max}}$  (maximum clearance rate, nl grazer<sup>-1</sup> h<sup>-1</sup>). Reference: (1) Seong et al. (2006). (2) Jeong et al. (2005a). (3) Jeong et al. (2008).

Species	Taxon	ESD	$I_{\text{max}}$	$C_{\text{max}}$	Reference
<i>Heterosigma akashiwo</i>	RAP	11.0	11.7	2.6	(1)
<i>Chattonella ovata</i>	RAP	40.0	24.5	4.5	(1)
<i>Heterocapsa rotundata</i>	MTD	4.8	12.2	1.4	(2)
<i>Prorocentrum minimum</i>	MTD	12.1	21.9	2.3	(2)
<i>Heterocapsa triquetra</i>	MTD	15.0	6.0	1.3	(2)
<i>Cochlodinium polykrikoides</i>	MTD	23.6	17.4	1.0	(2)
<i>Pfiesteria piscicida</i>	HTD	13.5	13.7	11.4	(3)
<i>Oxyrrhis marina</i>	HTD	15.6	71.3	31.3	(3)
<i>Gyrodinium cf. guttula</i>	HTD	20.0	23.2	16.1	(3)

Table 4. Comparison in ingestion rates (IRCal, cells grazer<sup>-1</sup> h<sup>-1</sup>) of the raphidophytes *Chattonella ovata* (40.0 µm in Equivalent Spherical Diameter, ESD) and *Heterosigma akashiwo* (11.0 µm) and similar sized red-tide dinoflagellates *Prorocentrum minimum* (12.1 µm) and *Lingulodinium polyedrum* (38.2 µm) on marine cyanobacterium *Synechococcus* sp. at the prey concentrations of 1.83 x 10<sup>6</sup> and 1.53 x 10<sup>6</sup> cells ml<sup>-1</sup>, obtained by interpolation using the equation in Jeong et al. (2010c) {i.e. IR (Ingestion rate, cells grazer<sup>-1</sup> h<sup>-1</sup>) = 5.66 x 10<sup>-6</sup> x pc for *C. ovata* and IR = 1.18 x 10<sup>-6</sup> x pc for *H. akashiwo*, where pc is cyanobacterial prey concentration}. Reference: (1) Jeong et al. (2010c). (2) Jeong et al. (2005a)

Species	ESD	Prey concentration (x 10 <sup>6</sup> )	IR	IRCal	Reference
<i>Heterosigma akashiwo</i>	11.0	1.83		2.2	(1)
<i>Prorocentrum minimum</i>	12.1	1.83	5.2		(2)
<i>Lingulodinium polyedrum</i>	38.2	1.53	64.2		(2)
<i>Chattonella ovata</i>	40.0	1.53		8.7	(1)

Table 5. Comparison in ingestion rates (IRCal, cells grazer<sup>-1</sup> h<sup>-1</sup>) of the raphidophytes *Chattonella ovata* and *Heterosigma akashiwo* and the heterotrophic nanoflagellates *Picophagus flagellatus* and *Pseudobodo* sp. and the ciliate *Uronemna* sp. on marine cyanobacteria *Synechococcus* spp. at the prey concentrations of 0.27 x 10<sup>6</sup> and 0.12 x 10<sup>6</sup> cells ml<sup>-1</sup>, obtained by interpolation using the equation in Jeong et al. (2010c). ESD (Equivalent Spherical Diameter, μm), IR (Ingestion rate, cells grazer<sup>-1</sup> h<sup>-1</sup>). Reference: (1) Jeong et al. (2010c). (2) Guillou et al. (2001). (3) Christaki et al. (2002). (3) Christaki et al. (1999).

Species	ESD	Prey concentration (x 10 <sup>6</sup> )	IR	IRCal	Reference
<i>Heterosigma akashiwo</i>	11.0	0.27		0.3	(1)
<i>Chattonella ovata</i>	40.0	0.27		1.5	(1)
<i>Picophagus flagellatus</i>	5	0.27	0.7		(2)
<i>Heterosigma akashiwo</i>	11.0	0.50		0.6	(1)
<i>Chattonella ovata</i>	40.0	0.50		2.7	(1)
<i>Pseudobodo</i> sp.	5	0.50	2.7		(3)

<continued>

---

Species	ESD	Prey concentration (x 10 <sup>6</sup> )	IR	IRCal	Reference
<i>Heterosigma akashiwo</i>	11.0	0.12		0.2	(1)
<i>Chattonella ovata</i>	40.0	0.12		1.2	(1)
<i>Uronema</i> sp.	5	0.12	31.0		(3)

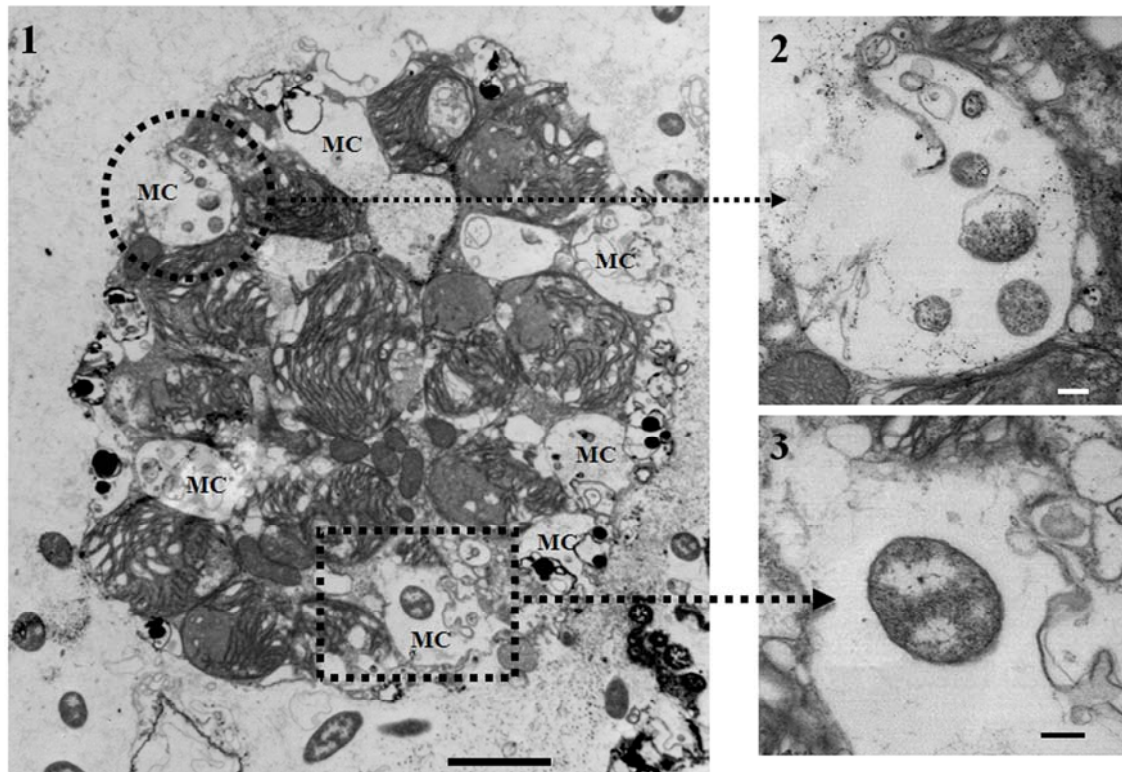
---

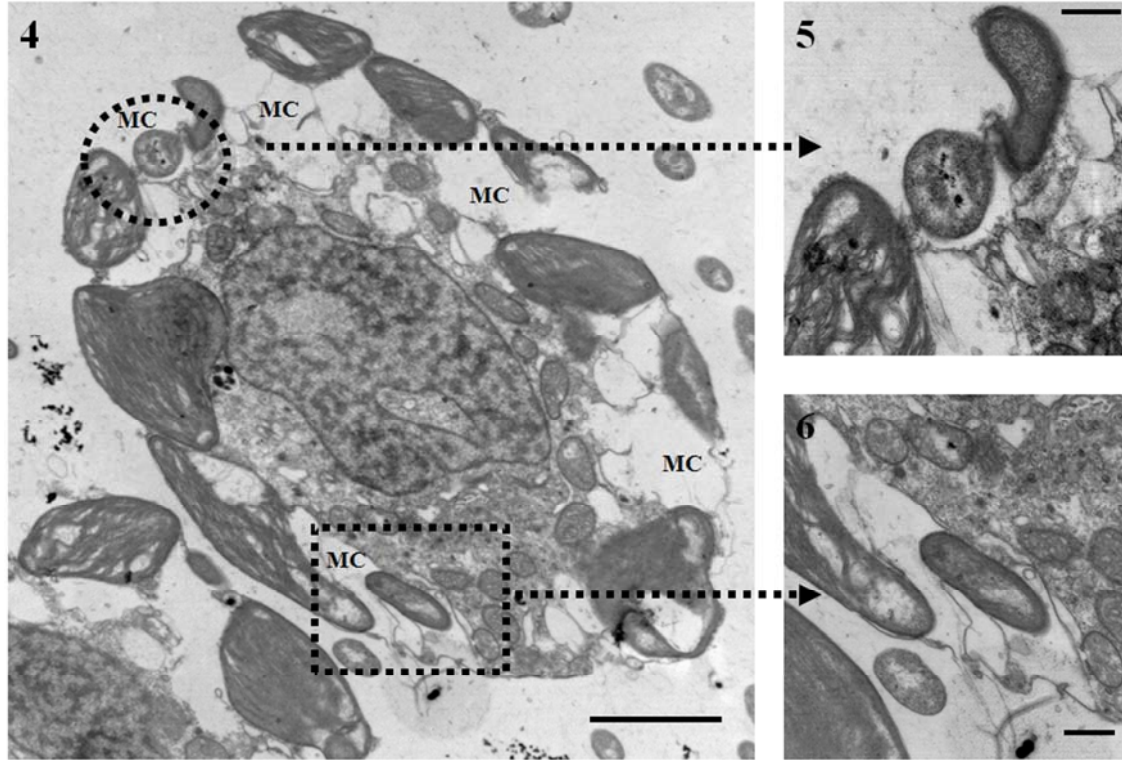
### Figure Legends

Fig. **1--3**. Transmission electron micrographs (TEM) of *Chattonella ovata* fed on heterotrophic bacterial cells. **1**. A *C. ovata* cell with food vacuoles containing one (inside the dashed box) and several single heterotrophic bacterial cells (inside the dashed circle). **2, 3**. Enlarged from Fig. 1 for single heterotrophic bacterial cells inside the food vacuoles. MC: mucocyst. Scale bars = 2  $\mu\text{m}$  for Fig. 1 and 0.2  $\mu\text{m}$  for Fig. 2, 3.

Fig. **4--6**. Transmission electron micrographs (TEM) of *Heterosigma akashiwo* fed on single heterotrophic bacterial cells. **4**. A *H. akashiwo* cell with 2 food vacuoles containing one single heterotrophic bacterial cell in each vacuole (inside the dashed box and circle). **5, 6**. Enlarged from Fig. 4 for single heterotrophic bacterial cells inside the food vacuoles. MC: mucocyst. Scale bars = 2  $\mu\text{m}$  for Fig. 4 and 0.2  $\mu\text{m}$  for Fig. 5, 6.

Fig. **7**. The ingestion rate of the raphidophyte (closed circles), mixotrophic dinoflagellate (closed triangles), and heterotrophic dinoflagellate (open squares) feeding on marine heterotrophic bacteria as a function of grazer size (equivalent spherical diameter,  $\mu\text{m}$ ) as in Table 3. The  $p$  value is  $> 0.1$  [linear regression analysis of variance (ANOVA)]. Co: *Chattonella ovata*, Cp: *Cochlodinium polykrikoides*, Gg: *Gyrodinium cf. guttula*, Ha: *Heterosigma akashiwo*, Hr: *Heterocapsa rotundata*, Ht: *H. triquetra*, Om: *Oxyrrhis marina*, Pb: *Protoperidinium bipes*, Pp: *Pfiesteria piscicida*.





7

