Research Article

Mixotrophy in the newly described dinoflagellate *Ansanella granifera*: feeding mechanism, prey species, and effect of prey concentration

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Mixotrophic protists play diverse roles in marine food webs as predators and prey. Thus, exploring mixotrophy in phototrophic protists has emerged as a critical step in understanding marine food webs and cycling of materials in marine ecosystem. To investigate the feeding of newly described mixotrophic dinoflagellate *Ansanella granifera*, we explored the feeding mechanism and the different types of species that *A. granifera* was able to feed on. In addition, we measured the growth and ingestion rates of *A. granifera* feeding on the prasinophyte *Pyramimonas* sp., the only algal prey, as a function of prey concentration. *A. granifera* was able to feed on heterotrophic bacteria and the cyanobacterium *Synechococcus* sp. However, among the 12 species of algal prey offered, *A. granifera* ingested only *Pyramimonas* sp. *A. granifera* ingested the algal prey cell by engulfment. With increasing mean prey concentration, the growth rate of *A. granifera* feeding on *Pyramimonas* sp. increased rapidly, but became saturated at a concentration of 434 ng C mL^{-1} (10,845 cells mL^{-1}). The maximum specific growth rate (i.e., mixotrophic growth) of *A. granifera* feeding on *Pyramimonas* sp. was 1.426 d^{-1}, at 20°C under a 14 : 10 h light-dark cycle of 20 μE m^{-2} s^{-1}, while the growth rate (i.e., phototrophic growth) under similar light conditions without added prey was 0.391 d^{-1}. With increasing mean prey concentration, the ingestion rate of *A. granifera* feeding on *Pyramimonas* sp. increased rapidly, but slightly at the concentrations ≥306 ng C mL^{-1} (7,649 cells mL^{-1}). The maximum ingestion rate of *A. granifera* feeding on *Pyramimonas* sp. was 0.97 ng C predator^{-1} d^{-1} (24.3 cells grazer^{-1} d^{-1}). The calculated grazing coefficients for *A. granifera* feeding on co-occurring *Pyramimonas* sp. were up to 2.78 d^{-1}. The results of the present study suggest that *A. granifera* can sometimes have a considerable grazing impact on the population of *Pyramimonas* spp.

Key Words: engulfment; feeding; growth; ingestion; mixotrophy; protist; red tide

INTRODUCTION

Phototrophic dinoflagellates are one of the major components in marine planktonic communities (Smayda 1997, Jeong et al. 2013*a*, 2013*b*, Park et al. 2013*a*). For a long time, these dinoflagellates were treated as phytoplankton, which can survive only by photosynthesis. However, in the last 2 decades, tens of phototrophic dino-

flagellates have been revealed to be mixotrophic (Stoecker 1999, Jeong et al. 2005c, 2010b, 2012, Turner 2006, Burkholder et al. 2008, Kang et al. 2011); they are known to feed on diverse prey, such as heterotrophic bacteria, cyanobacteria, small flagellates, other mixotrophic dinoflagellates, and ciliates (Stoecker et al. 1997, Jeong et al. 1999, 2005b, 2005c, 2005d, 2010a, 2012, Li et al. 1999, Park et al. 2006, Seong et al. 2006, Berge et al. 2008a, 2008b, Glibert et al. 2009, Yoo et al. 2009, Kang et al. 2011). Thus, discovery of mixotrophy in phototrophic dinoflagellates increase the complexity in food webs, but help in better understanding predator-prey relationships and cycling of materials in the food webs (Jeong et al. 2010b). Some mixotrophic dinoflagellates sometimes have considerable grazing impact on populations of prey species (Jeong et al. 2005c). Recently, several new genera and / or species of phototrophic dinoflagellates have been established (Moestrup et al. 2008, 2009a, 2009b, Kang et al. 2010, Jeong et al. 2014b). To understand the eco-physiology of a phototrophic dinoflagellate and its roles in planktonic food webs of the ecosystem, the feeding ability, type of prey, feeding mechanisms, growth and ingestion rates of this phototrophic dinoflagellate need to be explored.

Recently, we found a new mixotrophic dinoflagellate *Ansanella granifera* in Shiwha Bay, Korea (Jeong et al. 2014a). This newly isolated, thin-walled dinoflagellate has a type E eyespot and a single elongated apical vesicle, and it is closely related to species belonging to the family Suessiaceae. *Ansanella granifera* has 10-14 horizontal rows of amphiesmal vesicles, comparable to *Biecheleria* spp. and *Biecheleriopsis adriatica*, but greater in number than in other species of the family Suessiaceae (Montresor et al. 1999, Kremp et al. 2005, Moestrup et al. 2009a, 2009b, Siano et al. 2009, 2010, Kang et al. 2011, Luo et al. 2013, Jeong et al. 2014a, 2014b, Takahashi et al. 2014). Unlike *Biecheleria* spp. and *B. adriatica*, *A. granifera* has grana-like thylakoids (Horiguchi and Pienaar 1994a, 1994b, Moestrup et al. 2009a, 2009b, Jeong et al. 2014a). Further, *A. granifera* lacks a nuclear fibrous connective, which is present in *B. adriatica* (Moestrup et al. 2009b, Jeong et al. 2014a). *B. adriatica* and *A. granifera* also show a morphological difference in the shape of the margin of the cingulum. In *A. granifera*, the cingular margin formed a zigzag line, and in *B. adriatica* a straight line, especially on the dorsal side of the cell (Moestrup et al. 2009b, Jeong et al. 2014a, Takahashi et al. 2014). The main accessory pigment is peridinin. The small subunit (SSU), internal transcribed spacer regions, and large subunit (LSU) rDNA sequences differ considerably from those of the other known genera in the order Suessiales. *A. granifera*

has a 51-base pair fragment in domain D2 of the LSU of ribosomal RNA, which is absent in the genus *Biecheleria*. In the phylogenetic tree based on the SSU and LSU sequences, *A. granifera* belongs to the large clade of the family Suessiaceae, but a small clade containing this dinoflagellate is clearly divergent from other small clades in the family (Jeong et al. 2014a).

We established a clonal culture of *A. granifera* and observed its feeding behavior under high-resolution video-microscopy in order to explore the feeding mechanisms and determine the prey species when diverse algal species were provided. We also conducted experiments to determine the effects of prey concentration on the growth and ingestion rates of *A. granifera* feeding on the prasinophyte *Pyramimonas* sp., the only algal prey, as a function of prey concentration. In addition, we estimated the grazing coefficients attributable to *A. granifera* feeding on *Pyramimonas* sp. using the ingestion rate obtained from the laboratory experiments and the abundances of predators and prey in the field. The abundances of *A. granifera* and *Pyramimonas* sp. were quantified using real-time polymerase chain reaction (PCR). The results of the present study provide a basis for understanding the feeding mechanisms and ecological roles of *A. granifera* in marine planktonic food webs.

MATERIALS AND METHODS

Preparation of the experimental organisms

The phytoplankton species were grown at 20°C in enriched f/2 seawater media under an illumination of 20 μE m^{-2} s^{-1} of cool white fluorescent light on a 14 : 10 h light-dark cycle. The mean equivalent spherical diameter (ESD) ± standard deviation was measured by using an electronic particle counter (Coulter Multisizer II; Coulter Corporation, Miami, FL, USA). *Synechococcus* sp. (Genbank accession Nos. DQ023295; ESD = ca. 1 μm) were grown at 20°C and 30-31 salinity in enriched f/2 seawater media under a 14 : 10 h light-dark cycle with 20 μE m^{-2} s^{-1} of cool white fluorescent light. The heterotrophic bacterial cells that originated from a clonal culture of *A. granifera* were fluorescently labeled (FLB), following the method of Sherr et al. (1987). To remove any aggregated FLB, the FLB were dispersed throughout the medium using a sonicator (Bransoic cleaner 5510E-DTH; Bransoic, Danbury, CT, USA) for 10-30 min and then filtered through 3-μm pore-sized filter (Polycarbonate; Whatman, Dassel, Germany).

Plankton samples were collected with a water sampler

from Shiwha Bay, Korea (37°18′ N, 126°36′ E), during September 2010, when the water temperature and salinity were 21.3°C and 15.6, respectively (Jeong et al. 2014*a*). The samples were filtered gently through a 154-μm Nitex mesh and placed in 6-well tissue culture plates. A clonal culture of *A. granifera* was established following two serial single-cell isolations. As the concentration of *A. granifera* increased, *A. granifera* was subsequently transferred to 32, 270, and 500- mL polycarbonate (PC) bottles containing fresh f/2 seawater media. The bottles were again filled to capacity with freshly filtered seawater, capped, and placed on a shelf at 20°C under 20 μE m^{-2}s^{-1} illumination provided by cool white fluorescent lights in a 14 : 10 h light-dark cycle.

The carbon contents of *A. granifera* (0.11 ng C per cell) and *Pyramimonas* sp. (0.04 ng C per cell) were measured using a CHN Analyzer (vario MICRO; Elementar, Hanau, Germany) and those of the other phytoplankton species were obtained from our previous studies (Jeong et al. 2010*a*, 2011, 2012, Yoo et al. 2010, Kang et al. 2011).

Prey species

Experiment 1 was designed to investigate whether *A. granifera* was able to feed on different target algal species when unialgal diets of diverse algal species were provided (Table 1). The initial concentrations of each algal species offered were similar, in terms of carbon biomass. To confirm that some of the algal species were not ingested by *A. granifera*, additional higher prey concentrations were provided.

A dense culture (ca. 100,000-200,000 cells mL^{-1}) of *A. granifera* grown photosynthetically was transferred to a 1-L PC bottle containing f/2 medium and maintained in f/2 media for 2 d. Three 1-mL aliquots were then removed from the bottle and examined using a compound microscope to determine *A. granifera* concentration.

In this experiment, the initial concentrations of *A. granifera* and each target algal species were determined by using an autopipette to deliver a predetermined volume of culture with a known cell density to the experi-

Table 1. Taxa, sizes, and concentration of prey species offered as food to *Ansanella granifera* in Experiment 1

Species	ESD (± SD)	Initial prey concentration (cells mL^{-1})	Feeding by *A. granifera*
Bacteria			
Heterotrophic bacteria	0.9 (0.3)	7,000,000	**Y**
Synechococcus sp.	1.0 (0.2)	7,000,000	**Y**
Diatoms			
Chaetoceros calcitrans	6.0 (0.2)	150,000	N
Skeletonema costatum	5.9 (1.1)	150,000	N
Prasinophytes			
Pyramimonas sp.	5.6 (0.1)	150,000	**Y**
Prymnesiophytes			
Isochrysis galbana	4.8 (0.2)	150,000	N
Cryptophytes			
Teleaulax sp.	5.6 (1.5)	100,000	N
Rhodomonas salina	8.8 (1.5)	50,000	N
Rhaphidophyte			
Heterosigma akashiwo	11.5 (1.9)	30,000	N
Dinoflagellates			
Heterocapsa rotundata (T)	5.8 (0.4)	100,000	N
Amphidinium carterae (NT)	9.7 (1.6)	30,000	N
Prorocentrum minimum (T)	12.1 (2.5)	15,000	N
Heterocapsa triquetra (T)	15.0 (4.3)	15,000	N
Scrippsiella trochoidea (T)	22.8 (2.7)	7,000	N

The abundances of the predator for each target prey were 5,000 cells mL^{-1}.
ESD, mean equivalent spherical diameter (μm) ± standard deviation (SD) of the mean was measured by an electronic particle counter (Coulter Multisizer II; Coulter Corporation, Miami, FL, USA); Y, *A. granifera* was observed to feed on a living food cell; N, *A. granifera* was observed not to feed on a living food cell; T, thecate; NT, nonthecate. n > 2,000 for each species.

mental bottles. Triplicate 80-mL PC bottles with mixtures of *A. granifera* and the target prey and duplicate predator control bottles containing *A. granifera* only were set up for each target algal species. The bottles were filled to capacity with freshly filtered seawater, capped, and then placed on a vertically rotating plate at 0.9 rpm and incubated at 20°C under a 14 : 10 h light-dark cycle of cool white fluorescent light at 20 µE m^{-2} s^{-1}. After 12, 24, and 48 h, a 5-mL aliquot was removed from each bottle and transferred into a 20-mL bottle. Two 0.1-mL aliquots were placed on slides and then covered with cover-glasses. Under these conditions, the *A. granifera* cells were alive, but almost stationary. The protoplasms of >100 *A. granifera* cells were carefully examined with a compound microscope and / or an epifluorescence microscope (Zeiss-Axiovert 200M; Carl Zeiss Ltd., Göttingen, Germany) at a magnification of ×100-630 to determine whether or not *A. granifera* was able to feed on target prey species. Images of the ingested cells of each target algal species inside *A. granifera* cells were taken using digital cameras mounted on the microscopes at a magnification of ×630-1,000.

For transmission electron microscopy (TEM), each of intact *Pyramimonas* cells *A. granifera* cell grown photosynthetically, and *A. granifera* cell satiated with *Pyramimonas* sp. was transferred to a 50-mL tube and fixed in 4% (v/v) glutaraldehyde in culture medium. After 1.5-2 h, the entire contents of the tube were placed in a 50-mL centrifuge tube and concentrated at 1,610 ×g for 10 min in a Vision Centrifuge (VS-5500; Vision Scientific Co., Bucheon, Korea). The pellet from the tube was then transferred to a 1.5-mL tube and rinsed with 0.2 M sodium cacodylic acid at pH 7.4. After several rinses in the medium, the cells were post-fixed in 1% (w/v) osmium tetroxide in deionized water. The pellet was then embedded in agar. Subsequently, the pellet was dehydrated using a graded ethanol series (i.e., 50, 60, 70, 80, 90, and 100% ethanol, followed by two 100% ethanol steps). The material was embedded in Spurr's low-viscosity resin (Spurr 1969). Sections were obtained using an RMC MT-XL ultramicrotome (Boeckeler Instruments Inc., Tucson, AZ, USA) and stained with 3% (w/v) aqueous uranyl acetate followed by 2% (w/v) lead citrate. The sections were observed using a JEOL-1010 electron microscope (JEOL Ltd., Tokyo, Japan).

Feeding mechanisms

Experiment 2 was designed to investigate the feeding behaviors of *A. granifera* when a unialgal diet of *Pyramimonas* sp., the only algal prey, was provided. The inges-

tion of these prey species by *A. granifera* was observed in Experiment 1. The initial concentrations of predators and prey were the same as described previously.

The initial concentrations of *A. granifera* and the target algal species were established using an autopipette to deliver a predetermined volume of culture with a known cell density to the experimental bottles. One 80-mL PC bottle with a mixture of *A. granifera* and the algal prey was set up for each target algal species. The bottle was filled to capacity with freshly filtered seawater, capped, and then well mixed. After 1-min incubation, a 1-mL aliquot was removed from the bottle and transferred into a 1-mL Sedgewick-Rafter Chamber (SRCs). By monitoring the behavior of >30 unfed *A. granifera* cells for each target prey species under a compound microscope and / or an epifluorescence microscope at a magnification of ×100-630, all of the feeding processes were observed. A series of images showing the feeding process of a *A. granifera* cell was taken using a video analyzing system (Sony DXC-C33; Sony Co., Tokyo, Japan) mounted on an epifluorescence microscope at a magnification of ×100-630.

Growth and ingestion rates

Experiment 3 was designed to investigate the growth and ingestion rates of *A. granifera*. We measured the growth, ingestion, and clearance rates of *A. granifera* feeding on unialgal diet consisting of the optimal prey *Pyramimonas* sp. as a function of prey concentration.

A dense culture (ca. 32,000 cells mL^{-1}) of *A. granifera* growing photosynthetically was transferred into a 1-L PC bottle containing freshly filtered seawater. The culture was transferred into one 1-L PC bottle. Three 1-mL aliquots from the bottle were counted using a compound microscope to determine the cellular concentrations of *A. granifera* in each bottle, and the cultures were then used to conduct experiments.

The initial concentrations of *A. granifera* and *Pyramimonas* sp. were established as described previously. Triplicate 42-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 set up for each predator-prey combination. To ensure similar experimental conditions, the water from *A. granifera* culture was filtered through a 0.7-µm GF/F filter and added to the prey control bottles at similar amounts as the volume of the predator culture added to the experiment bottles for each predator-prey combination. Next, 5 mL of f/2 medium was added to all the bottles, which were then filled

to capacity with freshly filtered seawater and capped. Five milliliters of f/2 medium were added to all bottles, which were then filled to capacity with freshly filtered seawater and capped. To determine the actual initial predator and prey densities (cells mL^{-1}) at the beginning of the experiment (*A. granifera* and *Pyramimonas* sp.: 16/237, 34/476, 51/2818, 49/5418, 96/11104, 291/71050, 525/133630) and after 2 d incubation, 5-mL aliquots were removed from each bottle and fixed with 5% Lugol's solution, and all *A. granifera* cells and all or >300 prey cells in three 1-mL SRCs were enumerated. Prior to taking the subsamples, the condition of *A. granifera* and its prey was assessed under a dissecting microscope. The bottles were filled again to capacity with f/2 medium, capped, placed on a vertically rotating plate rotating at 0.9 rpm, and incubated at 20°C under a 14 : 10 h light-dark cycle with 20 µE m^{-2} s^{-1} of cool white fluorescent light. The dilution of the cultures associated with refilling of the bottles was considered in calculating the growth and ingestion rates.

The specific growth rate of *A. granifera*, µ (d^{-1}), was calculated as follows:

$$\mu = \frac{\text{Ln} (A_t / A_0)}{t} \qquad (1)$$

, where A_0 is the initial concentration of *A. granifera* and A_t is the final concentration after time t. The time period was 2 d.

Data for *A. granifera* growth rate were fitted to the following equation:

$$\mu = \frac{\mu_{max} (x - x')}{K_{GR} + (x - x')} \qquad (2)$$

, where μ_{max} = the maximum growth rate (d^{-1}), x = prey concentration (cells mL^{-1} or ng C mL^{-1}), x' = threshold prey concentration (the prey concentration where µ = 0), and K_{GR} = the prey concentration sustaining 1/2 μ_{max}. Data were iteratively fitted to the model using DeltaGraph (SPSS Inc., Chicago, IL, USA).

Ingestion and clearance rates for 2 d were also calculated using the equations of Frost (1972) and Heinbokel (1978). The incubation times for calculating the ingestion and clearance rates were the same as for estimating the growth rate.

Ingestion rate data were fitted to a Michaelis-Menten equation:

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

, where I_{max} = the maximum ingestion rate (cells predator^{-1} d^{-1} or ng C predator^{-1} d^{-1}), x = prey concentration (cells mL^{-1} or ng C mL^{-1}), and K_{IR} = the prey concentration sustaining 1/2 I_{max}.

Cell volume of *Ansanella granifera*

After the 2-d incubation, the cell length and maximum width of *A. granifera* preserved in 5% acid Lugol's solution (n = 10-30 for each prey concentration) were measured using an image analysis system on images collected with a epifluorescence microscope (AxioVision 4.5; Carl Zeiss Ltd.). The shape of *A. granifera* was estimated to be oval. The cell volume of the preserved *A. granifera* was calculated according to the following equation: volume = 4/3 π [(cell length + cell width) / 4]^3.

Swimming speed

A dense culture (ca. 50,000 cells mL^{-1}) of *A. granifera* growing photosynthetically was transferred into 500-mL PC bottle. An aliquot from the bottle was added to a 50-mL cell culture flask and allowed to acclimate for 30 min. The video camera was focused on one field seen as one circle in a cell culture flask under a dissecting microscope at 20°C and the movement of *A. granifera* cells was then recorded at a magnification of ×40 using a video analyzing system (SV-C660; Samsung, Seoul, Korea) and taken using a CCD camera (KP-D20BU; Hitachi, Tokyo, Japan). The mean and maximum swimming velocities were analyzed for all swimming cells seen for the first 10 min. The average swimming speed was calculated based on the linear displacement of cells in 1 s. during single-frame playback. The swimming speeds of 30 cells were measured.

Potential grazing impact

By combining field data on the abundances of the predators and the target prey with the ingestion rates of the predator on the prey obtained in the present study, we estimated the grazing coefficients attributable to *A. granifera* feeding on co-occurring *Pyramimonas* sp. Data on the abundances of *A. granifera* and the co-occurring *Pyramimonas* sp. used in this estimate were obtained by analyzing the water samples taken from the waters inside and outside Shiwha Bay, Korea in 2010-2013 using real-time PCR.

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

$$g = CR \times PC \times 24 \qquad (4)$$

, where CR (mL predator^{-1} h^{-1}) is the clearance rate of *A. granifera* feeding on a target prey at a prey concentration and PC is a predator concentration (cells mL^{-1}). The CR values were calculated as:
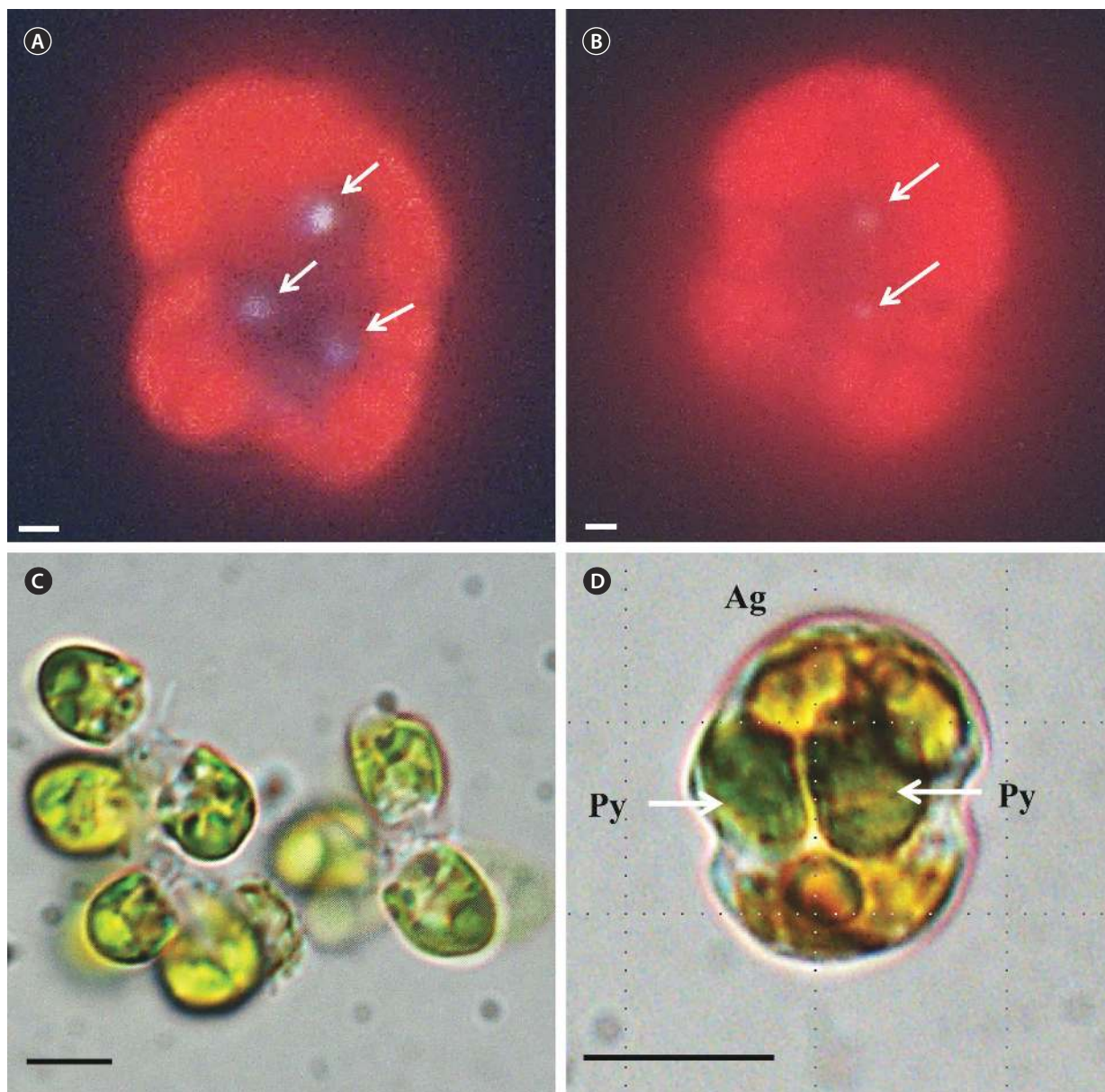
Fig. 1. Feeding by the mixotrophic dinoflagellate *Ansanella granifera* feeding on bacteria and the prasinophyte *Pyramimonas* sp. (A) An *A. granifera* cell with 3 ingested fluorescent-labeled bacteria (arrows). (B) An *A. granifera* cell with 2 ingested cyanobacterium *Synechococcus* sp. (arrows). (C) Unfed *Pyramimonas* cells. (D) An *A. granifera* (Ag) cell with 2 ingested *Pyramimonas* (Py) cells (arrows). Scale bars represent: A & B, 1 μm; C & D, 5 μm.

$$CR = IR / x \qquad (5)$$

, where IR (cells eaten predator^{-1} h^{-1}) is the ingestion rate of *A. granifera* feeding on the target prey and x (cells mL^{-1}) is the prey concentration. These CR values were corrected using $Q_{10} = 2.8$ (Hansen et al. 1997) because the in situ water temperature and the temperature used in the laboratory for this experiment (20°C) were sometimes different.

RESULTS

Prey species

A. granifera was able to feed on heterotrophic bacteria and the cyanobacterium *Synechococcus* sp. (Fig. 1). In addition, among 12 algal prey species (5-23 μm in equivalent spherical diameter) offered, *A. granifera* ingested
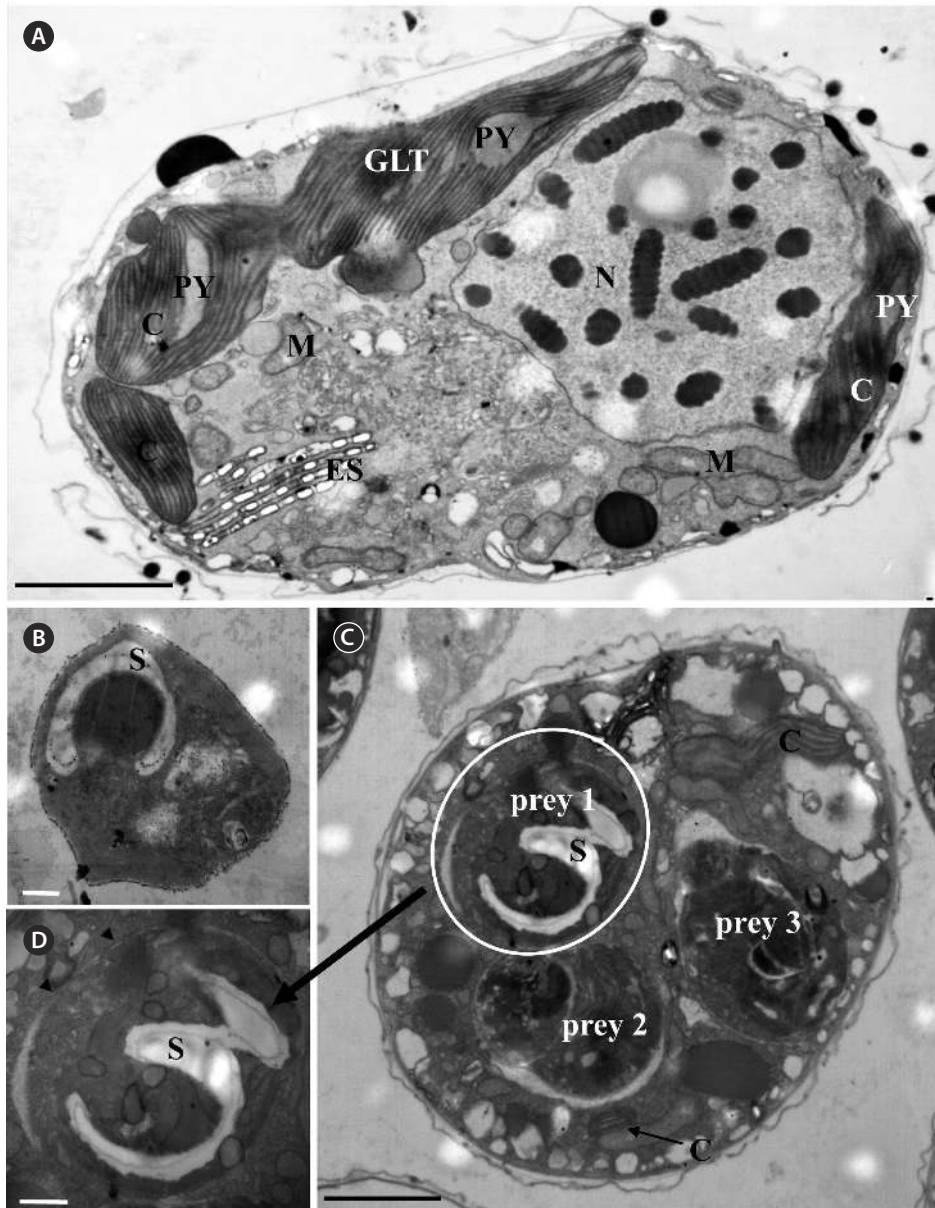
Fig. 2. Transmission electron micrographs (TEM) of *Ansanella granifera* and *Pyramimonas* sp. (A) A unfed *A. granifera* cell showing its chloroplasts (C), eyespot (ES), grana-like thylakoids (GLT), nucleus (N), mitochondria (M), and pyrenoid (PY). (B) Unfed *Pyramimonas* sp. cells showing starch (S). (C) An *A. granifera* cell with 3 ingested *Pyramimonas* sp. cells. (D) Enlarged image of Fig. 2C showing an ingested prey cell having starch (S) inside the food vacuole, arrowheads: food vacuole. Scale bars represent: A & C, 2 μm; B & D, 0.5 μm.

only *Pyramimonas* sp. (Table 1, Figs 1-3). TEM confirmed that *A. granifera* ingested *Pyramimonas* cells (Fig. 2). Intact *Pyramimonas* cells had the pyrenoids surrounded by starch. These pyrenoids with starch were conserved inside the food vacuoles of predator cells. However, it did not feed on the prymnesiophyte *Isochrysis galbana*, the diatoms (*Skeletonema costatum* and *Chaetoceros calcitrans*), cryptophytes (e.g., *Teleaulax* sp. and *Rhodomonas salina*), the raphidophyte *Heterosigma akashiwo*, the na-

ked dinoflagellate (*Amphidinium carterae*), and thecate dinoflagellates (*Heterocapsa rotundata*, *Heterocapsa triquetra*, *Prorocentrum minimum*, and *Scrippsiella trochoidea*) (Table 1).

Feeding mechanisms

A. granifera fed on *Pyramimonas* sp. by engulfment after spinning a prey cell (Fig. 3). *A. granifera* did not try to
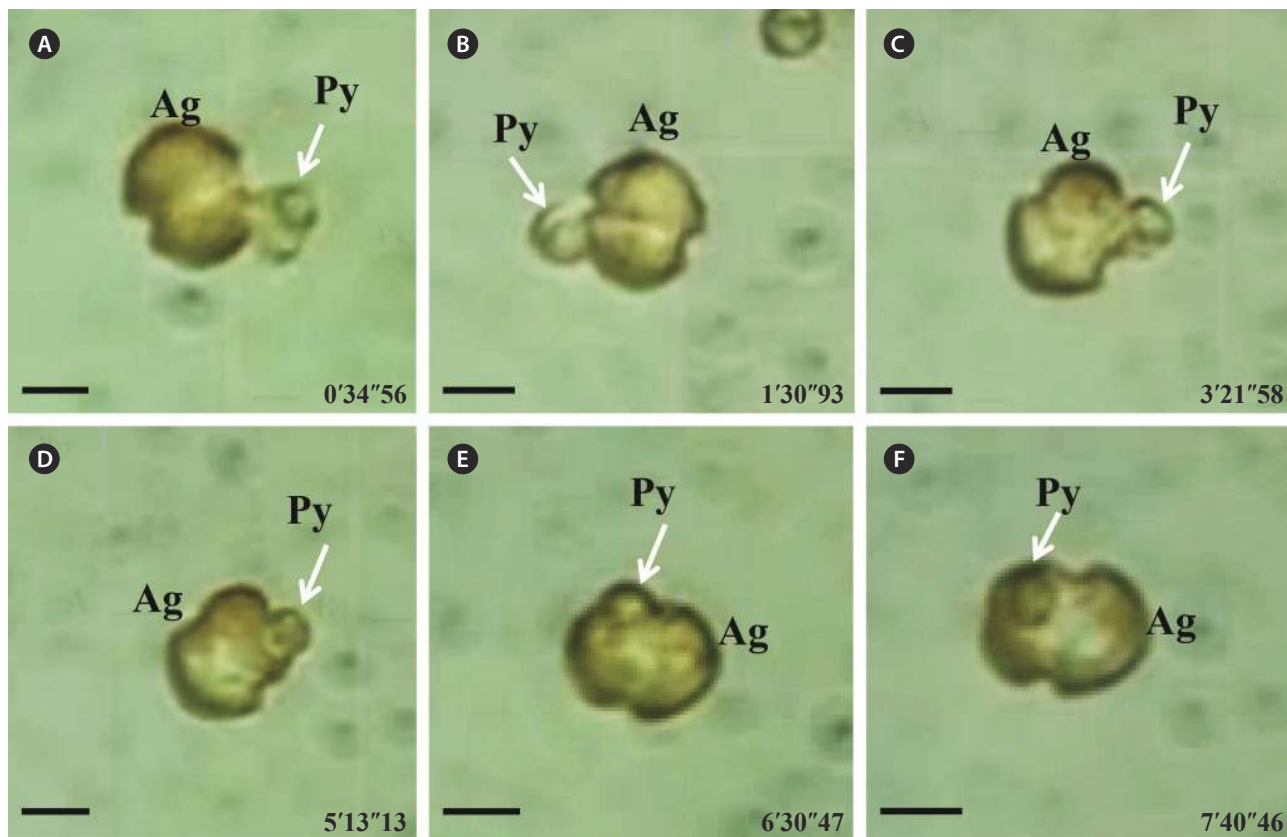
Fig. 3. (A-F) Feeding process of a *Ansanella granifera* cell (Ag) feeding on a *Pyramimonas* sp. cell (Py) by engulfment. The white arrow indicates the prey cell. Scale bars represent: A-F, 5 μm.

attack the other algal species when encountering an algal cell. Furthermore, with the exception of *Pyramimonas* sp., it did not spin around a target cell.

Growth and ingestion rates

With increasing mean prey concentration, the growth rate of *A. granifera* feeding on *Pyramimonas* sp. increased rapidly, but became saturated at a concentration of 434 ng C mL^{-1} (10,845 cells mL^{-1}) (Fig. 4). When the data were fitted to Eq. (2), the maximum specific growth rate (i.e., mixotrophic growth) of *A. granifera* feeding on *Pyramimonas* sp. was 1.426 d^{-1} at 20°C under a 14 : 10 h light-dark cycle of 20 μE m^{-2} s^{-1}, while its growth rate (i.e., phototrophic growth) under similar light conditions without added prey was 0.391 d^{-1}. The K$_{GR}$ (i.e., the prey concentration sustaining 1/2 μ$_{max}$) was 148 ng C mL^{-1} (3,690 cells mL^{-1}).

With increasing mean prey concentration, the ingestion rate of *A. granifera* feeding on *Pyramimonas* sp. increased rapidly, but slightly at concentrations ≥306 ng C mL^{-1} (7,649 cells mL^{-1}) (Fig. 5). When the data were fitted
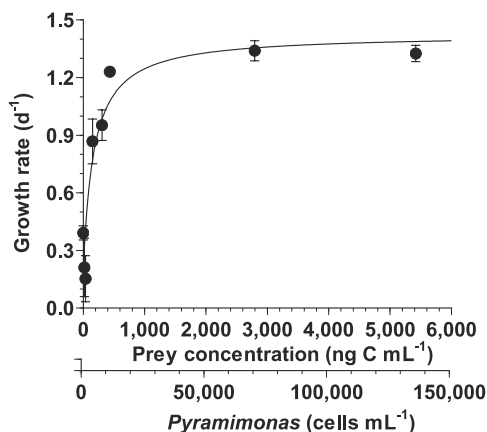

Fig. 4. Specific growth rate of *Ansanella granifera* feeding on *Pyramimonas* sp. as a function of mean prey concentration (x, ng C mL^{-1}). Symbols represent treatment means ± 1 standard error. The curve is fitted by a Michaelis-Menten equation [Eq. (2)] using all treatments in the experiment. Growth rate (d^{-1}) = 1.426 [(x + 22.03) / (147.6 + [x + 22.03])], r^2 = 0.854.
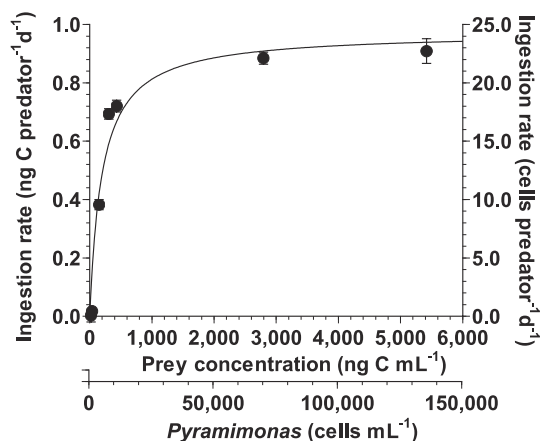
Fig. 5. Ingestion rate of *Ansanella granifera* feeding on *Pyramimonas* sp. as a function of mean prey concentration (x, ng C mL^{-1}). Symbols represent treatment means ± 1 standard error. The curve is fitted by a Michaelis-Menten equation [Eq. (3)] using all treatments in the experiment. Ingestion rate (d^{-1}) = 0.973 [x / (198.3 + x)], r^2 = 0.948.
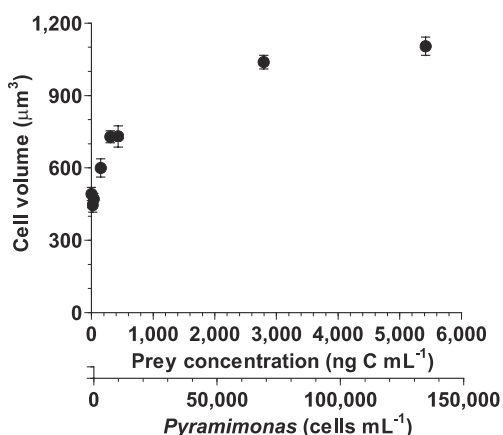

Fig. 6. The cell volume of *Ansanella granifera* feeding on *Pyramimonas* sp. after a 48-h incubation as a function of mean prey concentration. Symbols represent treatment means ± 1 standard error.
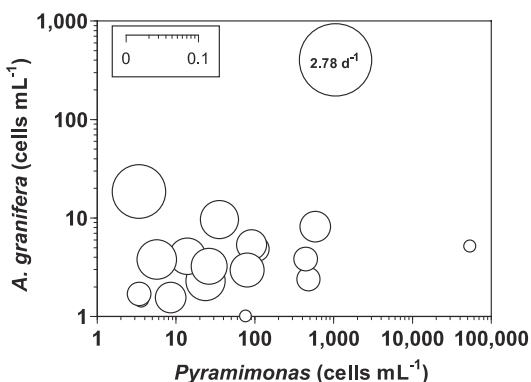

Fig. 7. Calculated grazing coefficients (h^{-1}) attributable to *Ansanella granifera* feeding on natural populations of *Pyramimonas* sp. (see text for calculation) (n = 12).

to Eq. (3), the maximum ingestion rate of *A. granifera* feeding on *Pyramimonas* sp. was 0.973 ng C predator^{-1} d^{-1} (24.3 cells grazer^{-1} d^{-1}) and K$_{IR}$ (the prey concentration sustaining 1/2 I$_{max}$) was 198 ng C mL^{-1} (4,958 cells mL^{-1}). The maximum clearance rate of *A. granifera* feeding on *Pyramimonas* sp. was 0.4 μL grazer^{-1} h^{-1}.

Cell volume

After a 2-d incubation, the mean cell volumes of *A. granifera* fed on *Pyramimonas* sp. at the lowest mean prey concentrations of 19-38 ng C mL^{-1} (446-471 μm^3) was comparable to those that were starved (492 μm^3) (Fig. 6). The cell volume increased rapidly up to 1,039 μm^3 at the mean prey concentration of 2,794 ng C mL^{-1} and then slowly up to 1,104 μm^3 at the mean prey concentration of 5,419 ng C mL^{-1}.

Swimming speed

A. granifera swam with alternating slow moving and very quick moving swims. The average (± standard error, n = 30) and maximum swimming speeds of *A. granifera* in the given conditions were 802 (± 51) and 1,603 μm s^{-1}, respectively.

Potential grazing impact

The grazing coefficients attributable to *A. granifera* feeding on co-occurring *Pyramimonas* sp. in the water samples taken in the waters of Shiwha Bay, Korea in 2010-2013 (n = 20), when the concentrations of *Pyramimonas* sp. and *A. granifera* were 3-53,243 cells mL^{-1} and 1-403 cells mL^{-1}, respectively, were 0.003-2.78 d^{-1} (Fig. 7). The highest grazing coefficient was obtained when the concentration of *Pyramimonas* sp. and *A. granifera* were 1,053 cells mL^{-1} and 403 cells mL^{-1}, respectively. In 11 of 20 samples, the grazing coefficients attributable to *A. granifera* feeding on co-occurring *Pyramimonas* sp. exceeded 0.02 d^{-1}.

DISCUSSION

Feeding mechanisms and prey species

The mixotrophic dinoflagellate *A. granifera* fed on algal prey by engulfment. Similar-sized and shaped mixotrophic dinoflagellates *Biecheleria cincta* (previously *Woloszynskia cincta*), *Gymnodinium aureolum*, *Karlodinium armiger*, and *Paragymnodinium shiwhaense*, along with

the heterotrophic dinoflagellates *Gyrodiniellum shi-whaense*, *Luciella masanensis*, *Pfiesteria piscicida*, and *Stoeckeria algicida*, feed on algal prey using a peduncle (Burkholder et al. 1992, Jeong et al. 2005a, 2006, 2007, 2010a, 2011, Berge et al. 2008a, Yoo et al. 2010, Kang et al. 2011). However, the peduncle is not found inside the protoplasm of *A. granifera* cells. Among the algal prey tested, *A. granifera* was only able to feed on the prasinophyte *Pyramimonas* sp. (5.6 μm in ESD). In general, engulfment-feeding dinoflagellates are able to ingest prey cells that are smaller than themselves, whereas peduncle-feeding dinoflagellates are able to feed on prey cells that are larger than themselves (Jeong et al. 2005a, 2005d, 2010a, Lim et al. 2014). The small size and engulfment feeding mechanism of *A. granifera* may be responsible for its feeding on only small *Pyramimonas* sp. The heterotrophic dinoflagellate *S. algicida* is able to feed only on the raphidophyte *H. akashiwo*, while the sister species, *Stoeckeria changwonensis* is able to feed on diverse prey species (Jeong et al. 2005a, Lim et al. 2014). However, the maximum growth and ingestion rates of *S. algicida* are greater than those of *S. changwonensis*. Therefore, Lim et al. (2014) suggested that diversification of prey items and feeding intensity may be traded during evolution. The maximum growth and ingestion rates of *A. granifera* on *Pyramimonas* sp. are comparable to or greater than those of other dinoflagellate predators (see next section). It will be worthwhile exploring the relationship between the number of prey species and feeding activity of other mixotrophic and heterotrophic dinoflagellates. One or few prey species may limit the period in which these predators appear and cause large fluctuations in their abundance.

Pyramimonas spp. sometimes causes red tides or harmful algal blooms (Bird and Karl 1991, Gradinger 1996, Alonso-Rodríguez et al. 2000, Daugbjerg et al. 2000, Rodriguez et al. 2002, Kang et al. 2013). Prior to this study, *K. armiger* was the only mixotrophic dinoflagellate known to feed on *Pyramimonas* sp. (Berge et al. 2008a, 2008b). *A. granifera* is now one of the two mixotrophic dinoflagellates that have been reported to feed on *Pyramimonas* spp. (Table 2). The heterotrophic dinoflagellates *Gyrodinium dominans*, *Gyrodinium spirale*, and *Oblea rotunda*, and the ciliates *Laboea strobili* and *Strombidinopsis* sp. are known to feed on *Pyramimonas* spp. (Jacobson and Anderson 1986, Stoecker et al. 1988, Putt 1991, Hansen 1992, Nakamura et al. 1995). However, to date, positive growth rates of only *G. dominans*, *K. armiger*, and *A. granifera* have been reported. Therefore, during *Pyramimonas* blooms, *G. dominans*, *K. armiger*, and *A. granifera* may be abundant. To predict the population dynamics of *G. spirale*, *O. rotunda*, *L. strobili*, and *Strombidinopsis* spp. during *Pyramimonas* blooms, it will be worthwhile measuring the growth and ingestion rates of these predators on *Pyramimonas* spp.

Growth and ingestion rates

The maximum growth rate of *A. granifera* feeding on *Pyramimonas* sp. (1.426 d^{-1}) is much greater than that of *K. armiger* feeding on *Pyramimonas orientalis* (0.45 d^{-1}) (Table 2). The maximum ingestion rate of *A. granifera* feeding on *Pyramimonas* sp. (0.973 ng C predator^{-1} d^{-1}) is much greater than that of *K. armiger* on *P. orientalis* (0.02 ng C predator^{-1} d^{-1}). The cell size of *A. granifera* (10.5 μm in ESD) is smaller than that of *K. armiger* (16.7 μm). Therefore, the much higher ingestion rate of *A. granifera* feeding on *Pyramimonas* sp. and smaller cell size may be partially responsible for this greater growth rate.

Table 2. Protistan grazers on *Pyramimonas* spp.

Predator	Prey	ESD	GR	IR	Reference
Mixotrophic dinoflagellates					
Ansanella granifera	*Pyramimonas* sp.	5.6	1.43	0.97	This study
Karlodinium armiger	*P. orientalis*	5.6	0.45	0.02	Berge et al. (2008a, 2008b)
Karlodinium armiger	*P. propulsa*	10.7	NA	NA	Berge et al. (2008a, 2008b)
Heterotrophic dinoflagellates					
Oblea rotunda	*Pyramimonas* sp.	NA	NA	NA	Jacobson and Anderson (1986)
Gyrodinium dominans	*P. parkeae*	10.5	0.03-0.1	NA	Nakamura et al. (1995)
Gyrodinium spirale	*Pyramimonas* sp.	6.6	NA	NA	Hansen (1992)
Ciliates					
Laboea strobila	*Pyramimonas* sp.	NA	NA	NA	Stoecker et al. (1988)
Strombidinopsis sp.	*Pyramimonas* sp.	NA	NA	NA	Putt (1991)

ESD, mean equivalent spherical diameter (μm); GR, growth rate (d^{-1}); IR, ingestion rate (ng C predator^{-1} d^{-1}); NA, not available.

The ratio of the mixotrophic growth rate (1.426 d^{-1}) relative to the autotrophic growth rate (0.391 d^{-1}) of *A. granifera* feeding on *Pyramimonas* sp. at 20°C under a 14 : 10 h light-dark cycle of 20 μE m^{-2} s^{-1}, 3.7, is greater than that of any other mixotrophic dinoflagellates except *K. armiger* on *Rhodomonas baltica*, *Dinophysis acuminata* on *Mesodinium rubrum*, and *B. cincta* (previously *W. cincta*) on *H. akashiwo* (Table 3). Furthermore, the difference between autotrophic and mixotrophic growth rates of *A. granifera* feeding on *Pyramimonas* sp., which is 1.04, is greater than any other mixotrophic dinoflagellate except *P. shiwhaense* on *A. carterae* (Table 3). Therefore, *A. granifera* can acquire growth materials and energy through feeding much more than photosynthesis compared to other mixotrophic dinoflagellates, except a few species. Mixotrophy in *A. granifera* may be a critical strategy in increasing its population.

The maximum growth rate of *A. granifera* feeding on the optimal prey obtained under a 14 : 10 h light-dark cycle of 20 μE m^{-2} s^{-1} (1.426 d^{-1}) is lower than that of *Gymnodinium smaydae* (2.23 d^{-1}) (Lee et al. 2014), but higher than that of any other mixotrophic dinoflagellate so far reported (0.20-1.10 d^{-1}) at diverse light intensities (Table 3). Even though *G. smaydae* has the highest mixotrophic growth rate among dinoflagellates, it cannot grow well photosynthetically. High mixotrophic and autotrophic growth rates enable *A. granifera* to slowly increase its population when the concentration of *Pyramimonas* spp. is low, but rapidly increase when that of *Pyramimonas* spp. is high.

The maximum ingestion rate of *A. granifera* feeding on *Pyramimonas* sp. (0.97 ng C predator^{-1} d^{-1}) is also greater than that of the mixotrophic dinoflagellates *Karlodinium veneficum*, *Gyrodinium galathenum*, *P. shiwhaense*, *Prorocentrum donghaiense*, which are similar in size to *A. granifera* (0.03-0.38 ng C predator^{-1} d^{-1}) (Table 3). *A. granifera* feeds on only *Pyramimonas* sp., while the other mixotrophic dinoflagellates can feed on diverse algal prey species (Li et al. 1999, Jeong et al. 2005*d*, Adolf et al. 2006). Therefore, *A. granifera* may have adapted to feed on *Pyramimonas* sp. unlike other mixotrophic dinoflagellates.

When the data on maximum mixotrophic and ingestion rates of *A. granifera* and other mixotrophic dinoflagellates so far reported were analyzed, the maximum ingestion rates of all mixotrophic dinoflagellates feeding on the optimal prey species were significantly correlated with the size of the predator ($p < 0.01$, a linear regression ANOVA) (Fig. 8). Furthermore, the maximum ingestion rates of engulfment feeders are also significantly correlated with the size of the predator ($p < 0.01$, a linear regression ANOVA), but those of the peduncle feeders were not
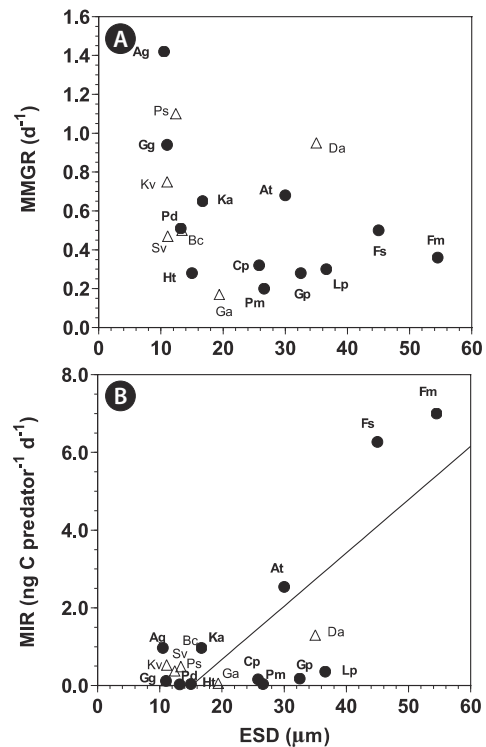

Fig. 8. The maximum mixotrophic growth (MMGR) (A) and ingestion rates (MIR) (B) of mixotrophic dinoflagellates feeding on optimal prey species by engulfment (close circles) and peduncle (open triangles) as a function of the size (equivalent spherical diameters [ESD, μm]) of the predator (see Table 3). The equation of the regression was MIR (ng C predator^{-1} d^{-1}) = 0.1197 × (ESD of predator) - 1.592, $r^2 = 0.579$ for all mixotrophs (n = 18, $p < 0.01$); MIR (ng C predator^{-1} d^{-1}) = 0.1371 × (ESD of predator) - 2.07, $r^2 = 0.604$ for engulfment feeders (n = 16, $p < 0.01$). MIR of the peduncle feeders and MMGR were not significantly correlated with ESD of predators ($p > 0.1$). Ag, *Ansanella granifera*; At, *Amylax triacantha*; Bc, *Biecheleria cincta*; Cp, *Cochlodinium polykrikoides*; Da, *Dinophysis acuminata*; Fm, *Fragilidium* cf. *mexicanum*; Fs, *Fragilidium subglobosum*; Ga, *Gymnodinium aureolum*; Gg, *Gyrodinium galatheanum*; Gp, *Gonyaulax polygramma*; Ht, *Heterocapsa triquetra*; Ka, *Karlodinium armiger*; Kv, *Karlodinium veneficum*; Lp, *Lingulodinium polyedrum*; Pd, *Prorocentrum donghaiense*; Pm, *Prorocentrum micans*; Ps, *Paragymnodinium shiwhaense*; Sv, *Symbiodinium voratum*.

significantly correlated ($p > 0.1$). This suggests that large engulfment feeding mixotrophic dinoflagellates may ingest more prey cells than smaller ones, but peduncle feeding mixotrophic dinoflagellates may not. The maximum mixotrophic growth rates of all mixotrophic dinoflagellates feeding on the optimal prey species were not significantly correlated with the size of the predator ($p > 0.1$, a linear regression ANOVA). Difference in nutritional values of the optimal prey species for each predator and / or growth efficiency may have contributed to the absence of a significant correlation.

Table 3. Optimal prey and maximum mixotrophic growth (MMGR), ingestion (MIR), and clearance rates of each mixotrophic dinoflagellate predator species

Predator	ESD	Optimal prey	ESD	RPP	T	LI	MMGR	AGR	RMAG	(M-A)	MIR	Reference
Engulfment feeding												
Ansanella granifera	10.5	Pyramimonas sp.	5.6	1.9	20	20	1.43	0.39	3.7	1.04	0.97	This study
Gyrodinium galatheanum	11.0	Storeatula major	6.6	2.0	20	372-384	0.94	0.32	2.9	0.62	0.12	Li et al. (1999)
Prorocentrum donghaiense	13.2	Teleaulax sp.	5.6	2.4	20	20	0.51	0.38	1.4	0.14	0.03	Jeong et al. (2005c)
Heterocapsa triquetra	15.0	Teleaulax sp.	5.6	2.7	20	20	0.28	0.18	1.5	0.10	0.04	Jeong et al. (2005c)
Karlodinium armiger[a]	16.7	Rhodomonas baltica	10.7	1.6	15	180	0.65	0.06	10.8	0.59	0.97	Berge et al. (2008b)
Cochlodinium polykrikoides	25.8	Teleaulax sp.	5.6	4.6	20	50	0.32	0.17	2.0	0.16	0.16	Jeong et al. (2004)
Prorocentrum micans	26.6	Teleaulax sp.	5.6	4.8	20	20	0.20	0.11	1.9	0.09	0.04	Jeong et al. (2005c)
Amylax triacantha	30.0	Mesodinium rubrum	22.0	1.4	15	20	0.68	-0.08	-8.5	0.76	2.54	Park et al. (2013b)
Gonyaulax polygramma	32.5	Teleaulax sp.	5.6	5.8	20	50	0.28	0.19	1.5	0.09	0.18	Jeong et al. (2005d)
Lingulodinium polyedrum	36.6	Scrippsiella trochoidea	25.1	1.5	20	50	0.30	0.18	1.7	0.12	0.36	Jeong et al. (2005c)
Fragilidium subglobosum	45.0	Ceratium tripos	59.5	0.8	15	45	0.50	0.16	3.1	0.34	6.27	Hansen and Nielsen (1997)
Fragilidium cf. mexicanum	54.5	Lingulodinium polyedrum	37.9	1.4	22	20	0.36	-0.05	-7.2	0.41	7.00	Jeong et al. (1999)
Peduncle feeding												
Gymnodinium smaydae	10.5	Heterocapsa rotundata	9.5	1.1	20	20	2.23	0.01	>10	1.60	1.60	Lee et al. (2014)
Karlodinium veneficum	11.0	Storeatula major	6.6	2.0	20	250	0.75	0.55	1.4	0.20	0.13	Adolf et al. (2006)
Symbiodinium voratum	11.1	Heterosigma akashiwo	11.5	1.0	20	20	0.47	0.30	1.6	0.17	0.53	Jeong et al. (2012)
Paragymnodinium shiwhaense	12.4	Amphidinium carterae	9.7	1.3	20	20	1.10	-0.22	-4.9	1.32	0.38	Yoo et al. (2010)
Biecheleria cincta	13.4	Heterocsigma akashiwo	11.5	1.2	20	20	0.50	0.04	12.5	0.46	0.49	Kang et al. (2011)
Gymnodinium aureolum	19.4	Teleaulax sp.	5.6	3.5	20	20	0.17	0.12	1.4	0.05	0.06	Jeong et al. (2010a)
Dinophysis acuminata	35.0	Mesodinium rubrum	22.0	1.6	20	60	0.95	0.19	5.0	0.76	1.30	Kim et al. (2008)

ESD, equivalent spherical diameter (m); RPP, ratio of predator to prey ESD; T, temperature (°C); LI, light intensity (μE m^{-2} s^{-1}); MMGR, maximum mixotrophic growth rate (d^{-1}); AGR, autotrophic growth rate (d^{-1}); RMAG, ratio of mixotrophic to autotrophic growth rates; (M-A), (MMGR-AGR); MIR, maximum ingestion rate (ng C predator^{-1} d^{-1}).

[a]Indicates the capability of feeding both peduncle and engulfment. Berge et al. (2008a) suggested that *Karlodinium armiger* fed on *Rhodomonas baltica* mainly by engulfment.

Swimming speed

The average and maximum swimming speeds of *A. granifera* in the given conditions, 802 and 1,603 μm s^{-1}, respectively, are greater than those of any other mixotrophic dinoflagellate. These values are greater than those of the dinoflagellate *Cochlodinium polykrikoides*, the fastest mixotrophic dinoflagellate previously reported (1,063 and 1,449 μm s^{-1}, respectively). Thus, *A. granifera* appears to be the fastest mixotrophic dinoflagellate reported to date. Mixotrophic dinoflagellates can increase their populations by migrating between well-lit surface water and eutrophicated bottom water (Eppley and Harrison 1975, Park et al. 2001). Thus, the ability of fast swimming may enable *A. granifera* to reach depths deeper than those reached by other mixotrophic dinoflagellate in vertical migration. When the thermocline is located deep, *A. granifera* may have an advantage over other competitors in meeting eutrophicated bottom water.

Grazing impact

The grazing coefficients attributable to *A. granifera* feeding on co-occurring *Pyramimonas* sp. obtained in the present study were up to 2.78 d^{-1} (i.e., 93% of the population of *Pyramimonas* sp. was removed by *A. granifera* populations in 1 d). In 11 of 20 samples, the grazing coefficients attributable to *A. granifera* feeding on co-occurring *Pyramimonas* sp. exceeded 0.02 d^{-1} (i.e., ≥2% of the population of *Pyramimonas* sp. was removed by *A. granifera* populations in 1 d). Therefore, *A. granifera* has the potential to sometimes have a considerable grazing impact on populations of co-occurring *Pyramimonas* sp.

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REFERENCES

Adolf, J. E., Stoecker, D. K. & Harding, L. W. Jr. 2006. The balance of autotrophy and heterotrophy during mixotrophic growth of *Karlodinium micrum* (Dinophyceae). J. Plankton Res. 28:737-751.

Alonso-Rodríguez, R., Páez-Osuna, F. & Cortés-Altamirano, R. 2000. Trophic conditions and stoichiometric nutrient balance in subtropical waters influenced by municipal sewage effluents in Mazatlán Bay (SE Gulf of California). Mar. Pollut. Bull. 40:331-339.

Berge, T., Hansen, P. J. & Moestrup, Ø. 2008*a*. Feeding mechanism, prey specificity and growth in light and dark of the plastidic dinoflagellate *Karlodinium armiger*. Aquat. Microb. Ecol. 50:279-288.

Berge, T., Hansen, P. J. & Moestrup, Ø. 2008*b*. Prey size spectrum and bioenergetics of the mixotrophic dinoflagellate *Karlodinium armiger*. Aquat. Microb. Ecol. 50:289-299.

Bird, D. F. & Karl, D. M. 1991. Massive prasinophyte bloom in northern Gerlache Strait. Antarct. J. U. S. 26:152-154.

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.

Burkholder, J. M., Noga, E. J., Hobbs, C. H., Glasgow, H. B. Jr. & Smith, S. A. 1992. New "phantom" dinoflagellate is the causative agent of major estuarine fish kills. Nature 358:407-410.

Daugbjerg, N., Marchant, H. J. & Thomsen, H. A. 2000. Life history stages of *Pyramimonas tychotreta* (Prasinophyceae, Chlorophyta), a marine flagellate from the Ross Sea, Antarctica. Phycol. Res. 48:199-209.

Eppley, R. W. & Harrison, W. G. 1975. Physiological ecology of *Gonyaulax polyedra*, a red water dinoflagellate of southern California. *In* Locicero, V. R. (Ed.) Proc. 1st Int. Conf. Toxic Dinoflagellate Blooms, Massachusetts Science and Technology Foundation, Wakefield, MA, pp. 11-22.

Frost, B. W. 1972. Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnol. Oceanogr. 17:805-815.

Glibert, P. M., Burkholder, J. M., Kana, T. M., Alexander, J., Skelton, H. & Shilling, C. 2009. Grazing by *Karenia brevis* on *Synechococcus* enhances its growth rate and may help to sustain blooms. Aquat. Microb. Ecol. 55:17-30.

Gradinger, R. 1996. Occurrence of an algal bloom under Arctic pack ice. Mar. Ecol. Prog. Ser. 131:301-305.

Hansen, P. J. 1992. Prey size selection, feeding rates and growth dynamics of heterotrophic dinoflagellates with special emphasis on *Gyrodinium spirale.* Mar. Biol.

114:327-334.

Hansen, P. J., Bjørnsen, P. K. & Hansen, B. W. 1997. Zooplankton grazing and growth: scaling within the 2-2,000-μm body size range. Limnol. Oceanogr. 42:687-704.

Hansen, P. J. & Nielsen, T. G. 1997. Mixotrophic feeding of *Fragilidium subglobosum* (Dinophyceae) on three species of *Ceratium*: effects of prey concentration, prey species and light intensity. Mar. Ecol. Prog. Ser. 147:187-196.

Heinbokel, J. F. 1978. Studies on the functional role of tintinnids in the Southern California Bight. I. Grazing and growth rates in laboratory cultures. Mar. Biol. 47:177-189.

Horiguchi, T. & Pienaar, R. N. 1994*a*. *Gymnodinium natalense* sp. nov. (Dinophyceae), a new tide pool dinoflagellate from South Africa. Jpn. J. Phycol. 42:21-28.

Horiguchi, T. & Pienaar, R. N. 1994*b*. Ultrastructure and ontogeny of a new type of eyespot in dinoflagellates. Protoplasma 179:142-150.

Jacobson, D. M. & Anderson, D. M. 1986. Thecate heterotrophic dinoflagellates: feeding behavior and mechanisms. J. Phycol. 22:249-258.

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., Ha, J. H., Yoo, Y. D., Park, J. Y., Kim, J. H., Kang, N. S., Kim, T. H., Kim, H. S. & Yih, W. H. 2007. Feeding by the *Pfiesteria*-like heterotrophic dinoflagellate *Luciella masanensis*. J. Eukaryot. Microbiol. 54:231-241.

Jeong, H. J., Jang, S. H., Moestrup, Ø., Kang, N. S., Lee, S. Y., Potvin, É. & Noh, J. H. 2014*a*. *Ansanella granifera* gen. et sp. nov. (dinophyceae), a new dinoflagellate from the coastal waters of Korea. Algae 29:75-99.

Jeong, H. J., Kim, J. S., Kim, J. H., Kim, S. T., Seong, K. A., Kim, T. H., Song, J. Y. & Kim, S. K. 2005*a*. 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., Lee, K. H., Yoo, Y. D., Kang, N. S. & Lee, K. 2011. Feeding by the newly described, nematocyst-bearing heterotrophic dinoflagellate *Gyrodiniellum shiwhaense*. J. Eukaryot. Microbiol. 58:511-524.

Jeong, H. J., Lee, S. Y., Kang, N. S., Yoo, Y. D., Lim, A. S., Lee, M. J., Kim, H. S., Yih, W., Yamashita, H. & LaJeunesse, T. C. 2014*b*. Genetics and morphology characterize the dinoflagellate *Symbiodinium voratum*, n. sp., (Dinophyceae) as the sole representative of *Symbiodinium* clade E. J. Eukaryot. Microbiol. 61:75-94.

Jeong, H. J., Park, J. Y., Nho, J. H., Park, M. O., Ha, J. H., Seong, K. A., Jeng, C., Seong, C. N., Lee, K. Y. & Yih, W. H. 2005*b*. Feeding by the red-tide dinoflagellates on the cyanobacterium *Synechococcus*. Aquat. Microb. Ecol. 41:131-143.

Jeong, H. J., Shim, J. H., Kim, J. S., Park, J. Y., Lee, C. W. & Lee, Y. 1999. Feeding by the mixotrophic thecate dinoflagellate *Fragilidium* cf. *mexicanum* on red-tide and toxic dinoflagellates. Mar. Ecol. Prog. Ser. 176:263-277.

Jeong, H. J., Yoo, Y. D., Kang, N. S., Lim, A. S., Seong, K. A., Lee, S. Y., Lee, M. J., Lee, K. H., Kim, H. S., Shin, W., Nam, S. W., Yih, W. & Lee, K. 2012. Heterotrophic feeding as a newly identified survival strategy of the dinoflagellate *Symbiodinium*. Proc. Natl. Acad. Sci. U. S. A. 109:12604-12609.

Jeong, H. J., Yoo, Y. D., Kang, N. S., Rho, J. R., Seong, K. A., Park, J. W., Nam, G. S. & Yih, W. 2010*a*. Ecology of *Gymnodinium aureolum*. I. Feeding in western Korean waters. Aquat. Microb. Ecol. 59:239-255.

Jeong, H. J., Yoo, Y. D., Kim, J. S., Kim, T. H., Kim, J. H., Kang, N. S. & Yih, W. 2004. Mixotrophy in the phototrophic harmful alga *Cochlodinium polykrikoides* (Dinophycean): prey species, the effects of prey concentration, and grazing impact. J. Eukaryot. Microbiol. 51:563-569.

Jeong, H. J., Yoo, Y. D., Kim, J. S., Seong, K. A., Kang, N. S. & Kim, T. H. 2010*b*. 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., Lee, K. H., Kim, T. H., Seong, K. A., Kang, N. S., Lee, S. Y., Kim, J. S., Kim, S. & Yih, W. H. 2013*a*. Red tides in Masan Bay, Korea in 2004-2005: I. Daily variations in the abundance of red-tide organisms and environmental factors. Harmful Algae 30(Suppl. 1):S75-S88.

Jeong, H. J., Yoo, Y. D., Lim, A. S., Kim, T. -W., Lee, K. & Kang, C. K. 2013*b*. Raphidophyte red tides in Korean waters. Harmful Algae 30(Suppl. 1):S41-S52.

Jeong, H. J., Yoo, Y. D., Park, J. Y., Song, J. Y., Kim, S. T., Lee, S. H., Kim, K. Y. & Yih, W. H. 2005*c*. Feeding by the phototrophic red-tide dinoflagellates: five species newly revealed and six species previously known to be mixotrophic. Aquat. Microb. Ecol. 40:133-155.

Jeong, H. J., Yoo, Y. D., Seong, K. A., Kim, J. H., Park, J. Y., Kim, S., Lee, S. H., Ha, J. H. & Yih, W. H. 2005*d*. Feeding by the mixotrophic red-tide dinoflagellate *Gonyaulax polygramma*: mechanisms, prey species, effects of prey concentration, and grazing impact. Aquat. Microb. Ecol. 38:249-257.

Kang, N. S., Jeong, H. J., Moestrup, Ø., Shin, W., Nam, S. W., Park, J. Y., de Salas, M. F., Kim, K. W. & Noh, J. H. 2010. Description of a new planktonic mixotrophic dinoflagellate *Paragymnodinium shiwhaense* n. gen., n. sp. from the coastal waters off western Korea: morphology, pig-

ments, and ribosomal DNA gene sequence. J. Eukaryot. Microbiol. 57:121-144.

Kang, N. S., Jeong, H. J., Yoo, Y. D., Yoon, E. Y., Lee, K. H., Lee, K. & Kim, G. 2011. Mixotrophy in the newly described phototrophic dinoflagellate *Woloszynskia cincta* from western Korean waters: feeding mechanism, prey species and effect of prey concentration. J. Eukaryot. Microbiol. 58:152-170.

Kang, N. S., Lee, K. H., Jeong, H. J., Yoo, Y. D., Seong, K. A., Potvin, É., Hwang, Y. J. & Yoon, E. Y. 2013. Red tides in Shiwha Bay, western Korea: a huge dike and tidal power plant established in a semi-enclosed embayment system. Harmful Algae 30(Suppl. 1):S114-S130.

Kim, S., Kang, Y. G., Kim, H. S., Yih, W., Coats, D. W. & Park, M. G. 2008. Growth and grazing responses of the mixotrophic dinoflagellate *Dinophysis acuminata* as functions of light intensity and prey concentration. Aquat. Microb. Ecol. 51:301-310.

Kremp, A., Elbrächter, M., Schweikert, M., Wolny, J. L. & Gottschling, M. 2005. *Woloszynskia halophila* (Biecheler) comb. nov.: a bloom-forming cold-water dinoflagellate co-occurring with *Scrippsiella hangoei* (Dinophyceae) in the Baltic Sea. J. Phycol. 41:629-642.

Lee, K. H., Jeong, H. J., Jang, T. Y., Lim, A. S., Kang, N. S., Kim, J. H., Kim, K. Y., Park, K. & Lee, K. 2014. Feeding by the newly described mixotrophic dinoflagellate *Gymnodinium smaydae*: feeding mechanism, prey species, and effect of prey concentration. J. Exp. Mar. Biol. Ecol. 459:114-125.

Li, A., Stoecker, D. K. & Adolf, J. E. 1999. Feeding, pigmentation, photosynthesis and growth of the mixotrophic dinoflagellate *Gyrodinium galatheanum*. Aquat. Microb. Ecol. 19:163-176.

Lim, A. S., Jeong, H. J., Jang, T. Y., Yoo, Y. D., Kang, N. S., Yoon, E. Y. & Kim, G. H. 2014. Feeding by the newly described heterotrophic dinoflagellate *Stoeckeria changwonensis*: a comparison with other species in the family Pfiesteriaceae. Harmful Algae 36:11-21.

Luo, Z., Yang, W., Xu, B. & Gu, H. 2013. First record of *Biecheleria cincta* (Dinophyceae) from Chinese coasts, with morphological and molecular characterization of the strains. Chin. J. Oceanol. Limnol. 31:835-845.

Moestrup, Ø., Hansen, G. & Daugbjerg, N. 2008. Studies on woloszynskioid dinoflagellates III: on the ultrastructure and phylogeny of *Borghiella dodgei* gen. et sp. nov., a cold-water species from Lake Tovel, N. Italy, and on *B. tenuissima* comb. nov. (syn. *Woloszynskia tenuissima*). Phycologia 47:54-78.

Moestrup, Ø., Lindberg, K. & Daugbjerg, N. 2009*a*. Studies on woloszynskioid dinoflagellates IV: The genus *Biecheleria*

gen. nov. Phycol. Res. 57:203-220.

Moestrup, Ø., Lindberg, K. & Daugbjerg, N. 2009*b*. Studies on woloszynskioid dinoflagellates. V. Ultrastructure of *Biecheleriopsis* gen. nov., with description of *Biecheleriopsis adriatica* sp. nov. Phycol. Res. 57:221-237.

Montresor, M., Procaccini, G. & Stoecker, D. K. 1999. *Polarella glacialis*, gen. nov., sp. nov. (Dinophyceae): Suessiaceae are still alive!. J. Phycol. 35:186-197.

Nakamura, Y., Suzuki, S. & Hiromi, J. 1995. Growth and grazing of a naked heterotrophic dinoflagellate, *Gyrodinium dominans*. Aquat. Microb. Ecol. 9:157-164.

Park, J. G., Jeong, M. K., Lee, J. A., Cho, K. J. & Kwon, O. S. 2001. Diurnal vertical migration of a harmful dinoflagellate, *Cochlodinium polykrikoides* (Dinophyceae), during a red tide in coastal waters of Namhae Island, Korea. Phycologia 40:292-297.

Park, J., Jeong, H. J., Yoo, Y. D. & Yoon, E. Y. 2013*a*. Mixotrophic dinoflagellate red tides in Korean waters: distribution and ecophysiology. Harmful Algae 30(Suppl. 1):S28-S40.

Park, M. G., Kim, M. & Kang, M. 2013*b*. A dinoflagellate *Amylax triacantha* with plastids of the cryptophyte origin: phylogeny, feeding mechanism, and growth and grazing responses. J. Eukaryot. Microbiol. 60:363-376.

Park, M. G., Kim, S., Kim, H. S., Myung, G., Kang, Y. G. & Yih, W. 2006. First successful culture of the marine dinoflagellate *Dinophysis acuminata*. Aquat. Microb. Ecol. 45:101-106.

Putt, M. 1991. Development and evaluation of tracer particles for use in microzooplankton herbivory studies. Mar. Ecol. Prog. Ser. 77:27-37.

Rodriguez, F., Varela, M. & Zapata, M. 2002. Phytoplankton assemblages in the Gerlache and Bransfield Straits (Antarctic Peninsula) determined by light microscopy and CHEMTAX analysis of HPLC pigment data. Deep Sea Res. Part II Top. Stud. Oceanogr. 49:723-747.

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. Mar. Ecol. Prog. Ser. 322:85-97.

Sherr, B. F., Sherr, E. B. & Fallon, R. D. 1987. Use of monodispersed, fluorescently labeled bacteria to estimate *in situ* protozoan bacterivory. Appl. Environ. Microbiol. 53:958-965.

Siano, R., Kooistra, W. H. C. F., Montresor, M. & Zingone, A. 2009. Unarmoured and thin-walled dinoflagellates from the Gulf of Naples, with the description of *Woloszynskia cincta* sp. nov. (Dinophyceae, Suessiales). Phycologia 48:44-65.

Siano, R., Montresor, M., Probert, I., Not, F. & de Vargas, C. 2010. *Pelagodinium* gen. nov. and *P. béii* comb. nov., a

dinoflagellate symbiont of planktonic foraminifera. Protist 161:385-399.

Smayda, T. J. 1997. Harmful algal blooms: their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnol. Oceanogr. 42:1137-1153.

Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26:31-43.

Stoecker, D. K. 1999. Mixotrophy among dinoflagellates. J. Eukaryot. Microbiol. 46:397-401.

Stoecker, D. K., Li, A., Coats, D. W., Gustafson, D. E. & Nannen, M. K. 1997. Mixotrophy in the dinoflagellate *Prorocentrum minimum*. Mar. Ecol. Prog. Ser. 152:1-12.

Stoecker, D. K., Silver, M. W., Michaels, A. E. & Davis, L. H. 1988. Obligate mixotrophy in *Laboea strobila*, a ciliate which retains chloroplasts. Mar. Biol. 99:415-423.

Takahashi, K., Sarai, C. & Iwataki, M. 2014. Morphology of two marine woloszynskioid dinoflagellates, *Biecheleria brevisulcata* sp. nov. and *Biecheleriopsis adriatica* (Suessiaceae, Dinophyceae), from Japanese coasts. Phycologia 53:52-65.

Turner, J. T. 2006. Harmful algae interactions with marine planktonic grazers. *In* Granéli, E. & Turner, J. T. (Eds.) *Ecology of Harmful Algae*. Springer-Verlag, Berlin, pp. 259-270.

Yoo, Y. D., Jeong, H. J., Kang, N. S., Song, J. Y., Kim, K. Y., Lee, G. & Kim, J. 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.

Yoo, Y. D., Jeong, H. J., Kim, M. S., Kang, N. S., Song, J. Y., Shin, W., Kim, K. Y. & Lee, K. 2009. Feeding by phototrophic red-tide dinoflagellates on the ubiquitous marine diatom *Skeletonema costatum*. J. Eukaryot. Microbiol. 56:413-420.