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 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. 2013a, 2013b, Park et al. 2013a). 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, Gilbert 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.


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 videomicroscopy 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 (Bransonic cleaner 5510E-DTH; Bransonic, 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. 2014a).

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. 2010a, 2011, 2012, Yoo et al. 2010, Kang et al. 2011).

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

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

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.

### 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-
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:

\[ \mu = \frac{\ln (A_t / A_0)}{t} \]  

(1)

where A₀ is the initial concentration of A. granifera and Aₜ 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:

\[ \mu = \frac{\mu_{\text{max}} (x - x')}{K_{GR} + (x - x')} \]  

(2)

where \( \mu_{\text{max}} \) = 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 \( \mu = 0 \)), and \( K_{GR} \) = the prey concentration sustaining 1/2 \( \mu_{\text{max}} \). 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:

\[ IR = \frac{I_{\text{max}} (x)}{K_{IR} + (x)} \]  

(3)

where \( I_{\text{max}} \) = 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 \( I_{\text{max}} \).

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 = \( 4/3 \pi \left( \frac{\text{cell length} + \text{cell width}}{4} \right)^3 \).

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-D208U; 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:

\[ g = CR \times PC \times 24 \]  

(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:
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

\[
CR = \frac{IR}{x}
\]

where IR (cells eaten predator\(^{-1}\) h\(^{-1}\)) is the ingestion rate of \textit{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.

**Fig. 1.** Feeding by the mixotrophic dinoflagellate \textit{Ansanella granifera} feeding on bacteria and the prasinophyte \textit{Pyramimonas} sp. (A) An \textit{A. granifera} cell with 3 ingested fluorescent-labeled bacteria (arrows). (B) An \textit{A. granifera} cell with 2 ingested cyanobacterium \textit{Synechococcus} sp. (arrows). (C) Unfed \textit{Pyramimonas} cells. (D) An \textit{A. granifera} (Ag) cell with 2 ingested \textit{Pyramimonas} (Py) cells (arrows). Scale bars represent: A & B, 1 µm; C & D, 5 µm.
feeding mechanisms

A. granifera fed on Pyramimonas sp. by engulfment after spinning a prey cell (Fig. 3). A. granifera did not try to

Fig. 2. Transmission electron micrographs (TEM) of Ansanella granifera and Pyramimonas sp. (A) An 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 naked 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
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 \(\mu_{max}\)) 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. 3.](image-url) (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.

![Fig. 4.](image-url) 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])], \(r^2 = 0.854\)
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 \(K_{m}\) (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 *Wolszynska cincta*), *Gymnodinium aureolum*, *Karlodinium armiger*, and *Paragymnodinium shiwaense*, along with
the heterotrophic dinoflagellates *Gyrodinium shiwaense*, *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-Rodriguez 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 strobila* 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. strobila*, and *Strombidinopsis* sp. 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.

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

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 dinoflagellate 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*, *Gymnodinium galathenum*, *B. cincta*, *Prorocentrum donghaiense*, and *Karlodinium veneficum* (Jose et al. 2019), 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 other mixotrophic dinoflagellates can feed on diverse algal prey species (Li et al. 1999, Jeong et al. 2005d, 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 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.

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) - 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*; Fs, *Fragilidium subglobosum*; Ga, *Gymnodinium aureolum*; Gg, *Gyrodinium galathenum*; Gp, *Gonyaulax polygramma*; Ht, *Heterocapsa triqueta*; Ka, *Karlodinium armiger*; Kv, *Karlodinium veneficum*; Lp, *Lingulodinium polyedrum*; Ps, *Prorocentrum micans*; Pm, *Paragymnodinium mimonas*; ppmdae, *Pyramimonas* sp.; W. cincta, *W. cincta*; Sv, *Symbiodinium shiwhaense*. Values of the optimal prey species for each predator and/or growth efficiency were also significantly correlated with the size of the predator (p < 0.01, 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

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

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.

ACKNOWLEDGEMENTS

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