Mixotrophy in the newly described dinoflagellate *Alexandrium pohangense*: A specialist for feeding on the fast-swimming ichthyotoxic dinoflagellate *Cochlodinium polykrikoides*

An Suk Lim a, Hae Jin Jeong a,b,*, Ji Hye Kim a, Se Hyeon Jang a, Moo Joon Lee a, Kitack Lee c

a School of Earth and Environmental Sciences, College of Natural Sciences, Seoul National University, Seoul 151-747, Republic of Korea
b Advanced Institutes of Convergence Technology, Suwon, Gyeonggi-do 443-270, Republic of Korea
c School of Environmental Science and Engineering, Pohang University of Science and Technology, Pohang 790-784, Republic of Korea

ARTICLE INFO
Article history:
Received 4 June 2015
Received in revised form 31 July 2015
Accepted 31 July 2015

Keywords:
Grazing
Growth
HAB
Ingestion
Protist
Red tide

ABSTRACT

Newly described *Alexandrium pohangense* is a phototrophic dinoflagellate, and its trophic mode should be explored to understand its roles in marine ecosystems. To investigate feeding of *A. pohangense* and its ecological roles, its trophic mode, prey type, feeding mechanism, functional and numerical responses, and grazing impact were analyzed. Among diverse algal prey tested, it fed only on the fast swimming ichthyotoxic dinoflagellate *Cochlodinium polykrikoides*. *A. pohangense* ingested *C. polykrikoides* cells by engulfment, after immobilizing the prey cell using excreted materials. Thus, *A. pohangense* has an effective mechanism for feeding on fast-swimming prey through prey immobilization. With increasing mean prey concentrations, the specific growth and ingestion rates of *A. pohangense* increased rapidly before saturating at *C. polykrikoides* concentrations of 138 ng C ml⁻¹ (197 cells ml⁻¹) and 99 ng C ml⁻¹ (141 cells ml⁻¹), respectively. The maximum growth rate of *A. pohangense* fed with *C. polykrikoides* was 0.487 d⁻¹, while the growth rate of *A. pohangense* without added *C. polykrikoides* was 0.091 d⁻¹. The maximum ingestion rate of *A. pohangense* on *C. polykrikoides* was 4.99 ng C predator⁻¹ d⁻¹ (7.1 cells predator⁻¹ d⁻¹). The grazing coefficients attributable to *A. pohangense* on co-occurring *C. polykrikoides*, calculated by combining field data on abundance with the ingestion rates obtained in the present study, were 0.09–1.57 d⁻¹, which indicates that *A. pohangense* could have a considerable grazing impact on *C. polykrikoides* populations.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Trophic modes (i.e., exclusively autotrophic, mixotrophic, and heterotrophic) in dinoflagellates are important to understand their ecological roles in marine ecosystems (e.g., Jeong et al., 2010a). In the last two decades, many phototrophic dinoflagellates have been revealed as mixotrophic (Bockstahler and Coats, 1993; Jacobson and Anderson, 1996; Stoecker, 1999; Li et al., 2000; Skovgaard et al., 2000; Park et al., 2006; Berge et al., 2008; Burkholder et al., 2008; Jeong et al., 2010a, 2012; Kang et al., 2011). Mixotrophic dinoflagellates play diverse roles in food webs (Jeong et al., 1999a, 2010a; Gilbert et al., 2009; Hansen, 2011; Sanders, 2011; Seong and Jeong, 2011); they are able to feed on diverse prey items such as bacteria, algae, heterotrophic protists, and metazoans (for examples, see Jeong et al., 2010a). In turn, they serve as prey for various predators (Jeong et al., 1999b, 2008, 2015). The mixotrophic abilities of some dinoflagellates contribute considerably to their growth, and to the formation of red-tide patches (Jeong et al., 2010a, 2015; Lee et al., 2014a). Therefore, the mixotrophic abilities of newly described phototrophic dinoflagellate species should be explored.

Several *Alexandrium* species are toxic (Cembella et al., 2002; Anderson et al., 2012; Yoo et al., 2013), producing diverse phycotoxins such as saxitoxins, goniodomins, and spirolidones (Anderson et al., 2012). Saxitoxins cause paralytic shellfish poisoning and are related to the formation of harmful algal blooms causing large-scale mortality of finfish and shellfish; they also cause diseases in humans (Hallegraeff, 1993; Cembella et al., 2002; Anderson et al., 2012). Moreover, some *Alexandrium* species secrete lytic compounds that kill other protists (Tillmann et al., 2007; Blossom et al., 2012). Therefore, the growth and distribution of *Alexandrium* species are critical factors for scientists, government
officials, and the aquaculture industry. Approximately 30 *Alexandrium* species have been described so far, of which only five species (i.e., *A. minutum*, *A. tamarense*, *A. catenella*, *A. ostenfeldii*, and *A. pseudogonyaulax*) are known to be mixotrophic (*Nygaard and Tobiesen, 1993; Jacobson and Anderson, 1996; Gribble et al., 2005; Jeong et al., 2005a,b; Yoo et al., 2009; Blossom et al., 2012*). Therefore, to understand the ecological roles and the bloom dynamics of newly described *Alexandrium* species, it is necessary to investigate their mixotrophic ability.

*Coccolithus polykrikoides* is a bloom-forming dinoflagellate found in the coastal waters of many countries (*Jeong et al., 2008*; *Mulholland et al., 2009; Tang and Gobler, 2010; Park et al., 2013a*). This dinoflagellate is known to kill fish in aqua-cages by clogging their gills with mucus, thus sometimes causing great losses in the aquaculture industry (*Park et al., 2013b*). Therefore, many countries spend considerable budgets to minimize the loss due to *C. polykrikoides*. Owing to these efforts, the mixotrophic ability, optimal temperature, salinity, light intensity, and interactions with competing species of *C. polykrikoides* have been well documented (*Park et al., 2001; Jeong et al., 2004; Kim et al., 2004; Tang and Gobler, 2010; Gobler et al., 2012; Lim et al., 2014a*). However, information on effective predators of this dinoflagellate is still lacking. The chain-forming *C. polykrikoides* is one of the fastest swimming phototrophic dinoflagellates and may not be easy for potential predators to capture (maximum swimming speed = ~1450 μm s⁻¹; *Jeong et al., 1999a*). Large ciliates, *Strombidinopsis* spp., are effective protistan predators of *C. polykrikoides* (*Jeong et al., 1999b, 2008*). To better understand *C. polykrikoides* bloom dynamics and harmful effects, its mortality rate due to predation should be explored.

Recently, *Alexandrium* sp. was isolated from the waters off Pohang, Korea, during a *Coccolithus polykrikoides* red tide, and a clonal culture was established. Based on morphological and genetic analyses, it was revealed as a new species and described as *Alexandrium pohangense* (*Lim et al., 2015a*). In the present study using this culture, mixotrophic ability, prey type, and feeding mechanisms of this dinoflagellate were investigated. Additionally, the growth and ingestion rates of *A. pohangense* on *C. polykrikoides*, the only prey, as a function of prey concentration, were determined. Furthermore, grazing impact of *A. pohangense* on *C. polykrikoides* populations was estimated using data on the abundance of these two species in the field, and ingestion rates obtained in this study. The results of the present study provided a basis for understanding the interactions between *A. pohangense* and *C. polykrikoides* and their ecological roles in marine ecosystems.

### 2. Materials and methods

#### 2.1. Preparation of experimental organisms

Phytoplankton species used in this study were grown at 20 °C in enriched f/2 seawater media (*Guillard and Ryther, 1962*) under continuous illumination of 100 μE m⁻² s⁻¹ provided by cool white fluorescent lights (*Table 1*). The mean equivalent spherical diameter (ESD) ± standard deviation was measured using an electronic particle counter (*Coulter Multisizer II, Coulter Corporation, Miami, FL, USA*). The carbon content of phytoplankton was estimated from the cell volume according to *Strathmann (1967)*. *Alexandrium pohangense* was isolated from plankton samples collected from waters off Pohang, Korea, in September 2014, when the water temperature and salinity were 23.3 °C and 31.1, respectively (*Lim et al., 2015a*). Only cultures in the exponential growth phase were used.

<table>
<thead>
<tr>
<th>Prey species</th>
<th>ESD (μM)</th>
<th>Initial prey concentration cell ml⁻¹</th>
<th>Effects</th>
<th>Feeding by <em>A. pohangense</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Diatom</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skeletonema costatum</td>
<td>5.9 (1.1)</td>
<td>150,000</td>
<td>N N</td>
<td></td>
</tr>
<tr>
<td>Prymnesiophytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isochrysis galbana</td>
<td>4.8 (0.2)</td>
<td>150,000</td>
<td>I N</td>
<td></td>
</tr>
<tr>
<td>Cryptophytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Teleopsis sp.</td>
<td>5.6 (1.5)</td>
<td>100,000</td>
<td>L N</td>
<td></td>
</tr>
<tr>
<td>Strepatochila major</td>
<td>6.0 (1.7)</td>
<td>50,000</td>
<td>L N</td>
<td></td>
</tr>
<tr>
<td>Rhodomonas salina</td>
<td>8.8 (1.5)</td>
<td>50,000</td>
<td>L N</td>
<td></td>
</tr>
<tr>
<td>Raphidiophytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hetersigmu akashiwo</td>
<td>11.5 (1.9)</td>
<td>30,000</td>
<td>I N</td>
<td></td>
</tr>
<tr>
<td>Mixotrophic dinoflagellates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterocapsa rotundata (T)</td>
<td>5.8 (0.4)</td>
<td>100,000</td>
<td>I N</td>
<td></td>
</tr>
<tr>
<td>Amphidinium carneae (NT)</td>
<td>9.7 (1.6)</td>
<td>30,000</td>
<td>I N</td>
<td></td>
</tr>
<tr>
<td>Prorocentrum minimum (T)</td>
<td>12.1 (2.5)</td>
<td>20,000</td>
<td>I N</td>
<td></td>
</tr>
<tr>
<td>Heterocapsa triqueta (T)</td>
<td>15.0 (1.3)</td>
<td>30,000</td>
<td>I N</td>
<td></td>
</tr>
<tr>
<td>Gymnodinium aureolum (NT)</td>
<td>19.5 (4.9)</td>
<td>3000</td>
<td>I N</td>
<td></td>
</tr>
<tr>
<td>Gyrodinium sp. (NT)</td>
<td>22.8 (2.7)</td>
<td>15,000</td>
<td>I N</td>
<td></td>
</tr>
<tr>
<td>Coccolithus polykrikoids (NT)</td>
<td>25.9 (2.9)</td>
<td>2000</td>
<td>I Y</td>
<td></td>
</tr>
<tr>
<td>Prorocentrum micans (NT)</td>
<td>26.6 (2.8)</td>
<td>3000</td>
<td>I N</td>
<td></td>
</tr>
<tr>
<td>Akashiwo sanguineus (NT)</td>
<td>30.8 (3.5)</td>
<td>1000</td>
<td>I N</td>
<td></td>
</tr>
<tr>
<td>Gymnodinium catenatum (T)</td>
<td>33.9 (1.6)</td>
<td>1000–3000</td>
<td>I N</td>
<td></td>
</tr>
</tbody>
</table>

#### 2.2. Prey type

Experiment 1 was designed to investigate whether *Alexandrium pohangense* was able to feed on potential prey when unialgal diets of diverse microalgal species were provided (*Table 1*). The initial concentrations of each algal species were similar in terms of carbon biomass.

A dense culture of *Alexandrium pohangense* growing photosynthetically in f/2 media at 20 °C under a 14:10 h light-dark (LD) cycle at 100 μE m⁻² s⁻¹ was transferred to a 2-L PC bottle containing f/2 medium. The culture was maintained in f/2 media for 2 d under the same conditions described above. Three 1-mL aliquots were then removed from the bottle and *A. pohangense* densities were determined using a compound light microscope.

To observe the ingestion of eukaryotic algal prey under a light microscope and/or an epifluorescence microscope, the target initial concentrations of *Alexandrium pohangense* and each algal prey species were established as described below (*Table 1*). Triplicate 42-mL PC experimental bottles and predator control bottles were set up for each target algal species. The bottles were filled to capacity with freshly filtered seawater, capped, and then placed on a shelf and incubated at 20 °C under a 14:10 h LD cycle of cool white fluorescent light at 100 μE m⁻² s⁻¹. After 6, 12, 24, and 48 h of incubation, a 5-mL aliquot was subsampled from each bottle. Two tenths to one mL aliquots of the subsample were placed on slides with cover glasses. The protoplasts of ~200 A. pohangense cells was carefully examined using a light microscope and/or epifluorescence microscope at a magnification of 100–400× to
determine whether A. pohangense had fed on the target algal prey species. A. pohangense cells containing the ingested cells were photographed at a magnification of 400–1000× using digital cameras mounted on the microscopes. During these feeding experiments, the cells of all prey species were immobilized, and the cells of four species were lysed after A. pohangense cells were added. To test whether the filtrates of A. pohangense culture affected the behavior of prey species, an A. pohangense culture of approximately 300 cells ml⁻¹ was filtered through 5-µm pores (Millipore co.) and added to the wells of a 6-well plate chamber containing the algal prey species with the same concentrations as in Table 1. In addition, seawater filtered through 5-µm pores was added to the wells of another 6-well plate chamber containing the target algal species as a control. Five min after the filtrates of A. pohangense were added, >200 prey cells were examined using a light microscope to determine whether the target prey cells were immobilized. In addition, 6 h after the filtrates of A. pohangense were added, the water in the wells was examined using a light microscope to determine whether target prey cells were lysed.

2.3. Feeding mechanisms

Experiment 2 was designed to investigate the feeding mechanisms of Alexandrium pohangense on C. polykrikoides dinoflagellates, the only prey species. The initial concentrations of the predator and prey were the same as in Experiment 1.

The initial concentrations of Alexandrium pohangense 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 42-ml PC bottle (mixtures of A. pohangense and C. polykrikoides) was set up. The bottle was filled to capacity with freshly filtered seawater, capped, and then mixed well. After 6, 24, and 48 h of incubation on the shelf, the feeding behavior of >60 A. pohangense cells was monitored using a light microscope and/or epifluorescence microscope at a magnification of 100–630 ×. The feeding processes were observed, from the time a prey cell was captured to the time that it was engulfed by the predator. A series of photographs showing the process of A. pohangense feeding on C. polykrikoides were taken using a video recording system (Sony DXC-C33; Sony Co., Tokyo, Japan) mounted on a light microscope at a magnification of 100–630 ×.

2.4. Prey concentration effects

Experiment 3 was designed to determine the specific growth and ingestion rates of Alexandrium pohangense on C. polykrikoides as a function of prey concentration. A dense culture of A. pohangense maintained in an f/2 medium and growing photosynthetically was transferred to a 1-1 PC bottle. Three 1-ml aliquots from the bottle were examined using a compound microscope to determine A. pohangense cell concentrations. 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 established. To ensure similar water conditions, we filtered the water of a predator culture through a 0.7-µm GF/F filter, and added this to the prey control bottles in the same amount as the volume of the predator culture added into the experimental bottles for each predator-prey combination. We added 5 ml f/2 medium to all the bottles, which were then filled to capacity with freshly filtered seawater and capped. To determine the actual predator and algal prey concentrations at the start of the experiment and after 2 d, a 5-ml aliquot was removed from each bottle and fixed with 5% Lugol’s solution; all or >200 predator and prey cells in triplicate 1-ml Sedgewick-Rafter chambers were then enumerated. The bottles were refilled to capacity with filtered seawater, capped, and placed on the shelf because C. polykrikoides has negative growth on the rotating wheel. The dilution of the cultures associated with refilling the bottles was considered when calculating the growth and ingestion rates.

The specific growth rate of Alexandrium pohangense was calculated as follows:

$$\mu = \frac{\ln(A_t - A_0)}{t_1 - t_0}$$

where \(A_0\) is the initial concentration of A. pohangense and \(A_t\) is the final concentration after time \(t\) (in this instance, \(t = 2 d\)).

Data for Alexandrium pohangense growth rate were fitted to the following equation:

$$\mu = \frac{\mu_{\text{max}}(x - x^\circ)}{K_r(x - x^\circ)}$$

where \(\mu_{\text{max}}\) is the maximum growth rate (d⁻¹), \(x\) is prey concentration (cells ml⁻¹ or ng C ml⁻¹), \(x^\circ\) is threshold prey concentration (i.e., the prey concentration where \(\mu = 0\)), and \(K_r\) is the prey concentration sustaining \(\frac{1}{2}\mu_{\text{max}}\). Data were iteratively fitted to the model using DeltaGraph® software (SPSS Inc., Chicago, IL, USA).

Ingestion and clearance rates were 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:

$$IR = \frac{I_{\text{max}}(x)}{K_r + (x)}$$

2.5. Potential grazing impact

By combining field data on the abundance of Alexandrium spp. and C. polykrikoides with the ingestion rates of Alexandrium pohangense on C. polykrikoides obtained in the present study, we estimated the grazing coefficients attributable to A. pohangense and the co-occurring C. polykrikoides. Data on the abundance of Alexandrium spp. and the co-occurring C. polykrikoides used in this estimate were obtained by counting the water samples taken from the waters off Pohang, Korea when C. polykrikoides red tide patches were observed in 2014. The morphological features of A. pohangense under the light microscope were difficult to distinguish from those of other Alexandrium spp., the abundance of Alexandrium spp. having size and shape similar to A. pohangense was used for the calculation.

The grazing coefficients (g, d⁻¹) were calculated as:

$$g = CR \times A \times 24$$

where CR (ml predator⁻¹ h⁻¹) is the clearance rate of Alexandrium pohangense feeding on C. polykrikoides at a prey concentration, and A is Alexandrium spp. concentration (cells ml⁻¹). The CR values were calculated as:

$$CR = \frac{IR}{x}$$

where IR (cells eaten predator⁻¹ h⁻¹) is the ingestion rate of A. pohangense on C. polykrikoides, and \(x\) (cells ml⁻¹) is C. polykrikoides concentration. Since the laboratory experiments were performed at 20 °C, these CR values were corrected using \(Q_{10} = 2.8\) (Hansen et al., 1997).
2.6. Swimming speed

To measure the swimming speed of *Alexandrium pohangense*, a culture of *A. pohangense* (ca. 500 cells ml\(^{-1}\)) growing autotrophically under a 14:10 h LD cycle of 100 μE m\(^{-2}\) s\(^{-1}\) in f/2 media was transferred into a 50-ml cell culture flask and allowed to acclimate for 30 min. The video camera focused on one field seen as a circle in a cell culture flask under a dissecting microscope at 20 °C and swimming of *A. pohangense* cells was then recorded at a magnification of 20× using a video recording system (Samsung, SV-C660, Seoul, Korea) and taken using a CCD camera (Hitachi, HP-D20BU, Tokyo, Japan). Swimming of all cells seen for the first 10 min (\(n = 20\)) was analyzed and the swimming speed of each cell was calculated based on the linear displacement of the cell during single-frame playback.

3. Results

3.1. Prey type and feeding mechanisms

Among the phytoplankton species offered, *Alexandrium pohangense* fed exclusively on the mixotrophic dinoflagellate *Cochlodinium polykrikoides* (Fig. 1; Table 1), and did not even try to attempt to feed on other phytoplankton species except for *C. polykrikoides*

Fig. 1. The process by which *Alexandrium pohangense* cells feed on *C. polykrikoides* cells. (A) An intact 4-celled *C. polykrikoides* (Cp) chain. (B) A disrupted chain of Cp after *A. pohangense* cells (final concentration = ca. 300 cells ml\(^{-1}\)) were added. (C–H) The process of an Ap cell engulfing a Cp cell. (H) The Ap cell with completely ingested Cp cell. (I) An Ap cell engulfing one of two Cp cells in a chain. (J) The Ap cell engulfing the other Cp cell of the chain. (K) The Ap cell with two ingested Cp cells. Given numbers in (C–H) indicate elapsed time (min:sec). Arrows indicate Cp cells. Scale bars = 20 μm for (A and B) and 10 μm for (C–K). Predators and prey are the same cells in C–H and I–K.
Chains consisting of four *Cochlodinium polykrikoides* cells were broken into one or two cells and became immobilized within 30 min after *Alexandrium pohangense* cells (final concentration = ~300 cells ml\(^{-1}\)) were added to water containing *C. polykrikoides* chains (Fig. 1A and B). An *A. pohangense* cell was able to engulf either one (Fig. 1C–H) or two cells in a row through the sulcus (Fig. 1I–K). The time (mean ± standard error, n = 6) for a prey cell to be completely engulfed and fed on by *A. pohangense* after contact was made was 118 ± 17 s (Supplementary Video 1). The breakage of the *C. polykrikoides* chains and immobilization of the cells was also observed within 30 min when prey cells were exposed to the filtrates of a culture containing approximately 300 *A. pohangense* cells ml\(^{-1}\).

*Alexandrium pohangense* immobilized cells of most of the tested phytoplankton species within 5 min of being added, even though they did not feed on them (Table 1). Additionally, the cryptophytes *Teleaulax* sp., *Rhodomonas salina*, *Storeatula major*, and the naked ciliate *Mesodinium rubrum* were completely lysed within 6 h after *A. pohangense* cells were added (Fig. 2). The immobilization of the cells of most phytoplankton species within 5 min and lysis of the four species within 6 h were also observed after target prey cells were exposed to the filtrates from a culture containing approximately 300 *A. pohangense* cells ml\(^{-1}\).

### 3.2. Growth and ingestion rates of Alexandrium pohangense on Cochlodinium polykrikoides

*A. pohangense* grew well feeding on *Cochlodinium polykrikoides*. With increasing mean prey concentration, the specific growth rates of *A. pohangense* increased rapidly before saturating at a *C. polykrikoides* concentration of 138 ng C ml\(^{-1}\) (197 cells ml\(^{-1}\)) (Fig. 3). When the data were fitted to Eq. (2), the maximum specific growth rate (i.e., mixotrophic growth) of *A. pohangense* fed on *C. polykrikoides* at 20 °C under a 14:10 LD cycle of 100 μE m\(^{-2}\) s\(^{-1}\) was 0.487 d\(^{-1}\), while that without *C. polykrikoides* (i.e., autotrophic growth) was 0.091 d\(^{-1}\). The *K*\(_{GR}\) (i.e. the prey concentration sustaining ½*μ*\(_{max}\)) was 11.3 ng C ml\(^{-1}\) (16 cells ml\(^{-1}\)).

With increasing mean prey concentration, the ingestion rates of *Alexandrium pohangense* increased rapidly at a *Cochlodinium polykrikoides* concentration of 99 ng C ml\(^{-1}\) (141 cells ml\(^{-1}\)), but slowly at higher prey concentrations (Fig. 4). When the data were fitted to Eq. (3), the maximum ingestion rate of *A. pohangense* on *C. polykrikoides* was 4.99 ng C predator\(^{-1}\) d\(^{-1}\) (7.1 cells predator\(^{-1}\) d\(^{-1}\)) and *K*\(_{IR}\) (the prey concentration sustaining ½*I*\(_{max}\)) was 30.0 ng C predator\(^{-1}\) d\(^{-1}\) (42.8 cells predator\(^{-1}\) d\(^{-1}\)). The maximum clearance rate of *A. pohangense* on *C. polykrikoides* was 3.2 μl predator\(^{-1}\) h\(^{-1}\).

#### 3.3. Potential grazing impact

The calculated grazing coefficients attributable to *Alexandrium pohangense* on co-occurring *Cochlodinium polykrikoides* in the waters of Yongil Bay, Pohang, Korea in 2014 were 0.089–1.567 d\(^{-1}\) when the abundances of *A. pohangense* and *C. polykrikoides* were 1–13 cells ml\(^{-1}\) and 3–259 cells ml\(^{-1}\), respectively (Fig. 5).

#### 3.4. Swimming speed

The average (±SE, n = 20) and maximum swimming speeds of *A. pohangense* were 218 (±12) and 340 μm s\(^{-1}\), respectively.

---

**Fig. 2.** (A) An intact *Rhdomonas salina* (*Rs*) cell without Ap and (B) a lysed Rs cell with added culture of Ap. (C) An intact *Teleaulax* sp. cell (*Tel*) and (D) lysed Tel cells with added culture of Ap. Scale bars = 10 μm for A–D.
4. Discussion

4.1. Prey species and feeding mechanisms

This study clearly shows that *Alexandrium pohangense* is a mixotrophic dinoflagellate. *A. pohangense* was able to feed only on *Cocchlodinium polykrikoides* among the diverse phytoplankton species tested in this study. *A. pohangense* is the first reported mixotrophic dinoflagellate predator for *C. polykrikoides*. Interestingly, the prey type that *A. pohangense* was able to feed on was different from that of *A. minutum*, *A. tamarensense*, *A. catenella*, *A. ostenfeldii*, and *A. pseudogonyaulax*, whose mixotrophic abilities were revealed (Table 2); *A. tamarensense* was not able to feed on *C. polykrikoides* (Jeong et al., 2005a). However, *A. minutum*, *A. tamarensense*, *A. catenella*, *A. ostenfeldii*, and *A. pseudogonyaulax* were able to feed on cryptophytes, dinoflagellates, ciliates, and even diatoms, which *A. pohangense* was unable to feed on (Nygaard and Tobiesen, 1993; Jacobson and Anderson, 1996; Jeong et al., 2005a,b; Yoo et al., 2009; Blossom et al., 2012). Therefore, the ecological niche of *A. pohangense* is likely to be different from that of other *Alexandrium* spp. Several studies suggested that protistan predators and prey have specific recognition relationships such as chemosensory mechanisms (Hansen and Calado, 1999; Wootton et al., 2007; Roberts et al., 2011; Sieg et al., 2011). Therefore, *A.
pohangense may have receptors and ligands involved in recognizing prey species that are different from those of the other Alexandrium species, and it would be beneficial to analyze these cell surface components and mechanisms related to prey species recognition at a molecular level.

The maximum swimming speed of *Alexandrium pohangense* (340 μm s⁻¹) measured in the present study was much lower than that of *Cochlodinium polykrikoides*, one of the fastest swimming phototrophic dinoflagellates (~1450 μm s⁻¹; Jeong et al., 1999a). Theoretically, a slow-swimming *A. pohangense* cell may not be able to capture a fast-swimming *C. polykrikoides* cell. Furthermore, *A. pohangense* does not have toefilaments or nematocysts used to anchor swimming prey cells as do *Gymnodinium aureolum* and *Paragymnodinium shiwaeae* (Jeong et al., 2010b; Yoo et al., 2010). Interestingly, *A. pohangense* splits a chain of *C. polykrikoides* into one or two cells, and then immobilizes and engulfs the *C. polykrikoides* cells through the sulcus. Therefore, *A. pohangense* has an effective mechanism to capture and ingest very fast-swimming cells. Some other *Alexandrium* species cause immobilization and/or lysis (Tillmann and John, 2002; Tillmann et al., 2007; Blossom et al., 2012; A. pseudogonyaulax produces a mucous trap to immobilize some algal species (Blossom et al., 2012); A. ostenfeldii lyzes some heterotrophic and autotrophic protists (Tillmann et al., 2007). However, these studies suggested that known toxins such as saxitoxins and spirolides were not involved in this immobilization activity (for examples, see Tillmann and John, 2002). *A. pohangense* does not have putative sxtA saxitoxin genes (Lim et al., 2015a). Therefore, the lytic substrates of *A. pohangense* may not be saxitoxins and thus it is necessary to identify them. The filtrates of *A. pohangense* cultures containing approximately 300 cells ml⁻¹ immobilized the cells of all tested target prey species within 5 min and lysed the cells of four species within 6 h. Thus, *A. pohangense* cells may excrete substances into the ambient waters that cause immobilization or lysis of prey cells.

Ma et al. (2011) reported that the lytic compounds of *Alexandrium tamarensense* showed a high affinity toward brassicasterol, a sterol component of cell membranes. Interestingly, *Rhodomonas salina* and *Storeatula major*, which were lysed by *A. pohangense*, have brassicasterol as a major sterol (>90% of the cell membrane) (Adolf et al., 2006; Ma et al., 2011). Therefore, lytic compounds of *A. pohangense* may be related to brassicasterol. In addition, karlotoxins produced by *Karlodinium veneficum* (previously *K. micrum*) were suggested to cause lysis of *Oxyrrhis marina*, which possesses 5,22-cholestadien-24β-methyl-3β-ol (brassicasterol) and 5-cholesten-3β-ol (cholesterol) are the major membrane sterols of the cell membranes (Deed and Place, 2006). Therefore, the relationships between lytic compounds and sterols among protists should be explored.

### 4.2. Growth and ingestion rates and contribution of mixotrophy

The maximum growth rate (i.e., mixotrophic growth) of *Alexandrium pohangense* on *Cochlodinium polykrikoides* (0.49 d⁻¹) is higher than that of *Alexandrium catenella* on the optimal prey, cryptophyte *Teleaulax acuta* or *Alexandrium pseudogonyaulax* on *Heterocapsa rotundata* (0.06–0.32 d⁻¹) even though the sizes of these predators are similar (Table 3). Additionally, the ratio of mixotrophic to autotrophic growth rates (RMAG) of *A. pohangense* on *C. polykrikoides* (4.9) is greater than that of other *Alexandrium* predators on the optimal prey species (0.8–1.5). Furthermore, the RMAG of *A. pohangense* engulfment feeding is the greatest among the reported engulfment-feeding mixotrophic dinoflagellates except *Karlodinium armiger* (Table 3; Fig. 6). Therefore, mixotrophy may provide *A. pohangense* an advantage in forming red tide patches over these competitors.

The maximum ingestion rate of *Alexandrium pohangense* on *Cochlodinium polykrikoides* (4.99 ng C predator⁻¹ d⁻¹) is highest among engulfment-feeding mixotrophic dinoflagellates except *Fragilidium* spp. (Table 3). *A. pohangense* is smaller than *Fragilidium* spp.; thus, the smaller size of *A. pohangense* may be partially responsible for the lower ingestion rate compared to *Fragilidium* spp. However, the maximum ingestion rate of *A. pohangense* is much greater than that of *Gonyaulax polygramma* or *Amylax triacantha*, which are similar in size to *A. pohangense*. Therefore, size may not be a critical factor affecting maximum ingestion rates of the engulfment-feeding mixotrophic dinoflagellates. *Fragilidium* spp. are able to feed voraciously on diverse dinoflagellates, including the heterotrophic species *Proteoperidinium* cf. *divergens* (Jeong et al., 1997, 1999a; Skovgaard et al., 2000). It is possible that the ingestion rates of engulfment-feeding mixotrophic dinoflagellates are intrinsic characteristics of the genus or species.

**Table 3**

Optimal prey, maximum mixotrophic growth rate (MMGR), autotrophic growth rate (AGR), and maximum ingestion rates (MIR) of each engulfment-feeding mixotrophic-dinoflagellate predator species.

<table>
<thead>
<tr>
<th>Predator</th>
<th>ESD</th>
<th>Optimal prey</th>
<th>ESD</th>
<th>T</th>
<th>LI</th>
<th>MMGR</th>
<th>AGR</th>
<th>RMAG</th>
<th>MIR</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alexandrium pohangense</em></td>
<td>32.0</td>
<td>Cochlodinium</td>
<td>25.8</td>
<td>20</td>
<td>100</td>
<td>0.49</td>
<td>0.10</td>
<td>4.9</td>
<td>4.99</td>
<td>(1)</td>
</tr>
<tr>
<td><em>Anasella granifera</em></td>
<td>10.5</td>
<td>Pyramimonas sp.</td>
<td>5.6</td>
<td>20</td>
<td>20</td>
<td>1.43</td>
<td>0.39</td>
<td>0.7</td>
<td>2</td>
<td>(2)</td>
</tr>
<tr>
<td><em>Prorocentrum donghaiense</em></td>
<td>13.2</td>
<td>Teleaulax sp.</td>
<td>5.6</td>
<td>20</td>
<td>20</td>
<td>0.51</td>
<td>0.38</td>
<td>1.4</td>
<td>0.03</td>
<td>(3)</td>
</tr>
<tr>
<td><em>Heterocapsa triqueta</em></td>
<td>15.0</td>
<td>Teleaulax sp.</td>
<td>5.6</td>
<td>20</td>
<td>20</td>
<td>0.28</td>
<td>0.18</td>
<td>1.5</td>
<td>0.04</td>
<td>(4)</td>
</tr>
<tr>
<td><em>Alexandrium minutum</em></td>
<td>16.7</td>
<td>Teleaulax acuta</td>
<td>15</td>
<td>17</td>
<td>11</td>
<td>0.11</td>
<td>0.13</td>
<td>0.8</td>
<td>(4)</td>
<td></td>
</tr>
<tr>
<td><em>Karlodinium armiger</em></td>
<td>16.7</td>
<td>Rhodomonas baltica</td>
<td>10.7</td>
<td>15</td>
<td>180</td>
<td>0.65</td>
<td>0.06</td>
<td>10.8</td>
<td>0.97</td>
<td>(5)</td>
</tr>
<tr>
<td><em>Cochlodinium polykrikoides</em></td>
<td>25.8</td>
<td>Teleaulax sp.</td>
<td>5.6</td>
<td>20</td>
<td>50</td>
<td>0.32</td>
<td>0.17</td>
<td>2.0</td>
<td>0.16</td>
<td>(6)</td>
</tr>
<tr>
<td><em>Prorocentrum micans</em></td>
<td>26.6</td>
<td>Teleaulax sp.</td>
<td>5.6</td>
<td>20</td>
<td>20</td>
<td>0.20</td>
<td>0.11</td>
<td>1.9</td>
<td>0.04</td>
<td>(3)</td>
</tr>
<tr>
<td><em>Amylax triacantha</em></td>
<td>30.0</td>
<td>Mesodinium rubrum</td>
<td>22.0</td>
<td>15</td>
<td>20</td>
<td>0.68</td>
<td>0.08</td>
<td>2.54</td>
<td>(7)</td>
<td></td>
</tr>
<tr>
<td><em>Gonyaulax polygramma</em></td>
<td>32.5</td>
<td>Teleaulax sp.</td>
<td>5.6</td>
<td>20</td>
<td>50</td>
<td>0.28</td>
<td>0.19</td>
<td>1.5</td>
<td>0.18</td>
<td>(8)</td>
</tr>
<tr>
<td><em>Alexandrium catenella</em></td>
<td>32.6</td>
<td>Teleaulax acuta</td>
<td>15</td>
<td>17</td>
<td>09</td>
<td>0.06</td>
<td>0.06</td>
<td>1.0</td>
<td>(4)</td>
<td></td>
</tr>
<tr>
<td><em>Alexandrium pseudogonyaulax</em></td>
<td>35.4</td>
<td>Heterocapsa rotundata</td>
<td>5.8</td>
<td>15</td>
<td>120</td>
<td>0.32</td>
<td>0.22</td>
<td>1.5</td>
<td>(4)</td>
<td></td>
</tr>
<tr>
<td><em>Lingulodinium polyedrum</em></td>
<td>36.6</td>
<td>Scruppsella trochoidea</td>
<td>25.1</td>
<td>20</td>
<td>50</td>
<td>0.30</td>
<td>0.18</td>
<td>1.7</td>
<td>0.36</td>
<td>(3)</td>
</tr>
<tr>
<td><em>Fragilidium subglobosum</em></td>
<td>45.0</td>
<td>Ceratium tripos</td>
<td>59.5</td>
<td>15</td>
<td>45</td>
<td>0.50</td>
<td>0.16</td>
<td>3.1</td>
<td>6.27</td>
<td>(9, 10)</td>
</tr>
<tr>
<td><em>Fragilidium cf. mexicanum</em></td>
<td>54.5</td>
<td>Lingulodinium polyedrum</td>
<td>37.9</td>
<td>22</td>
<td>29</td>
<td>0.36</td>
<td>0.05</td>
<td>7.00</td>
<td>(10, 11)</td>
<td></td>
</tr>
</tbody>
</table>

ESD, equivalent spherical diameter (μm); 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 rate; MIR, maximum ingestion rate (ng C predator⁻¹ d⁻¹).

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

(1) This study, (2) Lee et al. (2014b), (3) Jeong et al. (2005a), (4) Blossom et al. (2012), (5) Berge et al. (2008), (6) Jeong et al. (2004), (7) Park et al. (2013c), (8) Jeong et al. (2005c), (9) Hansen and Nielsen (1997), (10) Jeong et al. (2010a), (11) Jeong et al. (1999a).
4.3. Grazing impact

Prior to this study, only a few large ciliates and heterotrophic dinoflagellates, among protists, have been reported to feed on Cochlodinium polykrikoides (Jeong et al., 1999b, 2005d, 2006, 2007, 2008; Cho, 2006; Lim et al., 2014b). For field samples, the grazing impact of these heterotrophic protists on co-occurring C. polykrikoides has not yet been analyzed. The grazing coefficients (g) attributable to A. pohangense on co-occurring C. polykrikoides calculated in the present study can be up to 1.57 d⁻¹ (i.e., up to 79% of the C. polykrikoides populations were removed by A. pohangense in a day). Therefore, A. pohangense could have a considerable grazing impact on populations of co-occurring C. polykrikoides. C. polykrikoides has often caused huge red tides in the coastal waters of many countries and caused massive fish mortality and economic loss (Gobler et al., 2003; Mulholland et al., 2009; Park et al., 2013b; Lim et al., 2014a, 2015b). Therefore, A. pohangense may help to decrease fish mortality due to C. polykrikoides red tides.

Acknowledgements

We thank Kyung Ha Lee, Sung Yeon Lee, and Ji Eun Kwon for technical supports. This work was supported by the National Research Foundation of Korea Grant funded by the Korea Government/MICTFP (NRF - 2015-M1A5A1041806), the Korea Meteorological Administration Research and Development program under grant (CATER2014-6010), and Management of marine organisms causing ecological disturbance and harmful effect Program of Korea Institute of Marine Science and Technology Promotion (KIMST) award to HJ.[SS]

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhal.2015.07.010.

References
