Growth and ingestion rates of heterotrophic dinoflagellates and a ciliate on the mixotrophic dinoflagellate *Biecheleria cincta*

Yeong Du Yoo¹,*, Eun Young Yoon², Kyung Ha Lee³, Nam Seon Kang³ and Hae Jin Jeong³

¹Marine Biology Research Division, Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA 92093-0202, USA
²Advanced Institutes of Convergence Technology, Seoul National University-Gyeonggi Province, Suwon 443-270, Korea
³School of Earth and Environmental Sciences, College of Natural Sciences, Seoul National University, Seoul 151-747, Korea

To explore the interactions between the mixotrophic dinoflagellate *Biecheleria cincta* (previously *Woloszynskia cincta*) and heterotrophic protists, we investigated whether the common heterotrophic dinoflagellates *Gyrodinium dominans*, *Gyrodinium moestrupii*, *Gyrodinium spirale*, *Oxyrrhis marina*, and *Polykrikos kofoidii*, and the ciliate *Strobilidium* sp. were able to feed on *B. cincta*. We also measured growth and ingestion rates of *O. marina* and *Strobilidium* sp. on *B. cincta* as a function of prey concentration. In addition, these rates were measured for other predators at single prey concentrations at which the growth and ingestion rates of *O. marina* and *Strobilidium* sp. were saturated. All grazers tested in the present study were able to feed on *B. cincta*. *B. cincta* clearly supported positive growth of *O. marina*, *G. dominans*, and *Strobilidium* sp., but it did not support that of *G. moestrupii*, *G. spirale*, and *P. kofoidii*. The maximum growth rates of *Strobilidium* sp. and *O. marina* on *B. cincta* (0.91 and 0.49 d⁻¹, respectively) were much higher than that of *G. dominans* (0.07 d⁻¹). With increasing the mean prey concentration, the specific growth rates of *O. marina* and *Strobilidium* sp. on *B. cincta* increased, but either became saturated or slowly increased. The maximum ingestion rate of *Strobilidium* sp. (1.60 ng C predator⁻¹ d⁻¹) was much higher than that of *P. kofoidii* and *O. marina* (0.55 and 0.34 ng C predator⁻¹ d⁻¹) on *B. cincta*. The results of the present study suggest that *O. marina* and *Strobilidium* sp. are effective protistan grazers of *B. cincta*.

**Key Words:** graze; growth; harmful algal bloom; ingestion; protist; red tide

**INTRODUCTION**

Phototrophic dinoflagellates are ubiquitous and sometimes dominate the abundance and biomass of plankton assemblages in marine environments (Porter et al. 1985, Hallegraeff 1993, Lindberg et al. 2005, Jeong et al. 2010a, 2013b). They can sometimes form dense blooms so called red tides or harmful algal blooms in marine ecosystem (Eppley and Horrison 1975, Burkholder et al. 2008, Kang et al. 2013, Park et al. 2013a). Dense blooms dominated by phototrophic dinoflagellates can upset the balance of food webs and cause great loss to the aquaculture and tourist industries in many countries (e.g., Park et al. 2013b). Furthermore, phototrophic dinoflagellates play diverse roles in marine planktonic food webs (Anderson et al. 2002, Yoo et al. 2009, Jeong et al. 2010b, Hansen 2011); they are primary producers (Tillmann et al. 2009), predators feeding on diverse prey items (Park et al. 2006, Berge et al. 2008, Yoo et al. 2010b, Jeong et al. 2012), and, in turn, act as prey for diverse predators (Jeong and Latz...
1994, Jeong 1999, Tillmann 2004, Jeong et al. 2010b, Kim et al. 2013, Yoo et al. 2013b). Therefore, to understand the roles of phototrophic dinoflagellates in the food web, we must collate data on growth and mortality due to predation.

The phototrophic dinoflagellate Biecheleria cincta was previously named Woloszynskia cincta (Siano et al. 2009, Kang et al. 2011). However, this dinoflagellate has since been reclassified and moved from the genus Woloszynskia to the genus Biecheleria because it has genetic and morphological characteristics that more closely resemble Biecheleria, including an apical furrow apparatus formed by a single elongated narrow vesicle extending over the apex from the ventral to the dorsal side of the cell (Moestrup et al. 2009, Kang et al. 2011, Balzano et al. 2012, Luo et al. 2013). In addition, it has chloroplasts and eyespots that are formed by a stack of cisternae containing brick-like material (type E sensu) (Moestrup et al. 2009, Kang et al. 2011). The presence of this species has been reported in the coastal waters of Korea (Kang et al. 2011).

Recently, Kang et al. (2011) discovered that B. cincta, originally thought to be an exclusively phototrophic dinoflagellate, is a mixotrophic dinoflagellate; it feeds on diverse prey such as the haptophyte Isochrysis galbana, the cryptophytes Teleaulax sp. and Rhodomonas salina, the raphidophyte Heterosigma akashiwo, the euglenophyte Eutreptiella gymnastica, and the dinoflagellates Heterocapsa rotundata and Amphidinium carterae. However, to date, there have been no studies on the mortality of B. cincta due to predation. Grazing pressure sometimes plays an important role in controlling populations of phototrophic dinoflagellates (Watras et al. 1985, Turner 2006, Kang et al. 2013, Yoo et al. 2013a). Heterotrophic dinoflagellates and ciliates are the major components of heterotrophic protist communities (Sherr and Sherr 2007, Jeong et al. 2011, Yoo et al. 2013a). They are effective grazers on many phototrophic dinoflagellates (Eppley and Jeong et al. 2011, Yoo et al. 2013). These results provide a basis for understanding the interactions between B. cincta and heterotrophic protists and their population dynamics in marine planktonic food webs.

MATERIALS AND METHODS

Preparation of experimental organisms

For isolation and culture of Biecheleria cincta (Gen-Bank accession No. FR690459), plankton samples collected with water samplers were taken from Shiwha Bay, Korea during June 2009 when the water temperature and salinity were 22.0°C and 29.3, respectively (Table 1). These samples were screened gently through a 154-µm Nitex mesh and placed in 6-well tissue culture plates. A clonal culture of B. cincta was established by two serial single cell isolations as described by Kang et al. (2011).

For the isolation and culture of the heterotrophic dinoflagellate predators Gyrodinium dominans, G. moestrupii, G. spirale, Oxyrrhis marina, and Polykrikos kofoidii, plankton samples collected with water samplers were taken from the coastal waters off Masan, Saemankeum, or Keum estuary, Korea in 2001-2009, and a clonal culture of each species was established by two serial single-cell isolations (Table 1).

For the isolation and culture of Strobilidium sp., plankton samples collected with water samplers were taken from a pier in Shiwha Bay, Korea, during May 2010 when the water temperature and salinity were 17.7°C and 27.8, respectively (Table 1). A clonal culture of Strobilidium sp. (30-50 µm in cell length) was established by two serial single cell isolations as described by Jeong et al. (2008a).

The carbon contents for B. cincta (0.1 ng C per cell), the heterotrophic dinoflagellates, and the ciliate were estimated from the cell volume according to the methods described by Menden-Deuer and Lessard (2000). The cell volume of the predators was estimated using the methods described by Jeong et al. (2008b) and Jeong et al. (2008a).
for O. marina and Strobilidium sp., respectively.

**Feeding occurrence**

Experiment 1 was designed to investigate whether G. dominans, G. moestrupii, G. spirale, O. marina, P. kofoidii, and Strobilidium sp. were able to feed on B. cincta (Table 2). The concentrations of each predator species offered were similar in terms of carbon biomass.

Approximately $4.8 \times 10^5$ B. cincta cells were added to each of two 80 mL polycarbonate (PC) bottles containing G. dominans, G. moestrupii, G. spirale (100-500 cells mL$^{-1}$), P. kofoidii (30 cells mL$^{-1}$), and Strobilidium sp. (30 cells mL$^{-1}$) (final B. cincta prey concentration = ca. 6,000 cells mL$^{-1}$). One control bottle (without prey) was set up for each experiment. The bottles were placed on a plankton wheel rotating at 0.9 rpm and incubated at 20°C under an illumination of 20 µmol photons m$^{-2}$ s$^{-1}$ on a 14:10 h light-dark cycle.

Five milliliters aliquots were removed from each bottle after 1, 2, 6, 24, and 48-h incubation periods and then transferred into 6-well plate chambers (or slide glasses). Approximately 100 cells of predators at different stages of the feeding process in the plate chamber (or slide glasses) were observed under a dissecting microscope (or inverted microscope) at a magnification of ×20-90 (or ×100-630) to determine whether the predators were able to feed on B. cincta. Cells from those predators that contained ingested B. cincta cells were photographed using a digital camera (Zeiss AxioCam HRc5; Carl Zeiss Ltd., Göttingen, Germany) on the microscope at a magnification of ×400-630.

**Table 1.** Isolation and maintenance conditions of the experimental organisms

<table>
<thead>
<tr>
<th>Organism</th>
<th>Location</th>
<th>Date</th>
<th>T</th>
<th>S</th>
<th>Prey species</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gyrodinium dominans (HTD)</td>
<td>Masan Bay</td>
<td>Apr 2007</td>
<td>15.1</td>
<td>33.4</td>
<td>Amphidinium carterae</td>
<td>30,000-40,000</td>
</tr>
<tr>
<td>Gyrodinium moestrupii (HTD)</td>
<td>Off Saemankeum</td>
<td>Oct 2009</td>
<td>21.2</td>
<td>31.0</td>
<td>Alexandrium minutum</td>
<td>8,000-10,000</td>
</tr>
<tr>
<td>Gyrodinium spirale (HTD)</td>
<td>Masan Bay</td>
<td>May 2009</td>
<td>19.7</td>
<td>31.0</td>
<td>Prorocentrum minimum</td>
<td>20,000-30,000</td>
</tr>
<tr>
<td>Oxyrrhis marina (HTD)</td>
<td>Keum Estuary</td>
<td>May 2001</td>
<td>16.0</td>
<td>27.7</td>
<td>Amphidinium carterae</td>
<td>8,000</td>
</tr>
<tr>
<td>Polykrikos kofoidii (HTD)</td>
<td>Masan Bay</td>
<td>Jun 2007</td>
<td>20.2</td>
<td>32.2</td>
<td>Lingulodinium polyedrum</td>
<td>4,000</td>
</tr>
<tr>
<td>Strobilidium sp. (CIL)</td>
<td>Shiwha Bay</td>
<td>May 2010</td>
<td>17.7</td>
<td>27.8</td>
<td>Teleaulax sp.</td>
<td>50,000-60,000</td>
</tr>
<tr>
<td>Biecheleria cincta (MTD)</td>
<td>Shiwha Bay</td>
<td>Jun 2009</td>
<td>22.0</td>
<td>29.3</td>
<td>Heterosigma akashiwo</td>
<td>10,000-15,000</td>
</tr>
</tbody>
</table>

Sampling location and date, water temperature (T, °C), salinity (S, practical salinity units) for isolation, and prey species and concentrations (cells mL$^{-1}$) for maintenance.

HTD, heterotrophic dinoflagellate; CIL, ciliate; MTD, mixotrophic dinoflagellate.

**Table 2.** Design of the experiments

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Species</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Biecheleria cincta</td>
<td>6,000</td>
</tr>
<tr>
<td>2</td>
<td>Biecheleria cincta</td>
<td>20, 60, 130, 510, 1,690, 3,670, 6,420</td>
</tr>
<tr>
<td>3</td>
<td>Biecheleria cincta</td>
<td>20, 80, 240, 850, 2,640, 5,310, 6,930</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Species</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gyrodinium dominans</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>Gyrodinium moestrupii</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>Gyrodinium spirale</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Oxyrrhis marina</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>Polykrikos kofoidii</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Strobilidium sp.</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>Biecheleria cincta</td>
<td>3, 4, 6, 12, 19, 39, 73, (6)</td>
</tr>
<tr>
<td>3</td>
<td>Biecheleria cincta</td>
<td>5, 12, 14, 24, 23, 35, 35, (12)</td>
</tr>
</tbody>
</table>

The numbers in the prey and predator columns are the actual initial densities (cells mL$^{-1}$) of the prey and predator. Values in the parentheses in the predator column are the predator densities in the control bottles. Feeding occurrence of each predator fed Biecheleria cincta is represented by Y (feeding observed).
Effects of prey concentration on growth and ingestion rates

Experiments 2 and 3 were designed to measure the growth, ingestion, and clearance rates of *O. marina* and *Strobilidium* sp. as a function of the prey concentrations when fed on *B. cincta* (Table 2).

A dense culture of ~15,000 cells mL\(^{-1}\) of *B. cincta* grown mixotrophically on the raphidophyte *Heterosigma akashiwo* in the f/2 medium (Guillard and Ryther 1962, Kang et al. 2011) under the illumination of 20 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\) on a 14 : 10 h light : dark cycle: dark cycle was transferred to a 250-mL PC bottle containing the f/2 medium wherein *H. akashiwo* was undetectable. This culture was maintained in the f/2 medium for 2 d under the illumination of 20 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\) on a 14 : 10 h light : dark cycle and then transferred to another 250-mL PC bottle containing filtered seawater. Three 1-mL aliquots from the bottle were examined using a light microscope to determine the concentration of *B. cincta* cells, and the cultures were then used in further experiments.

Furthermore, dense cultures of *O. marina* (or *Strobilidium* sp.) growing on algal prey were transferred into 250-mL PC bottles containing filtered seawater. The bottles were filled to capacity with freshly filtered seawater, capped, and placed on plankton wheels rotating at 0.9 rpm and incubated at 20°C under the illumination of 20 mol photons m\(^{-2}\) s\(^{-1}\) on a 14 : 10 h light : dark cycle.

For each experiment, the initial concentrations of *O. marina* (or *Strobilidium* sp.) and *B. cincta* were established using an autopipette to deliver predetermined volumes of known cell concentrations to the bottles. Triplicate 42-mL PC experimental bottles (mixtures of predator and prey) and triplicate control bottles (prey only) were set up for each predator-prey combination. Triplicate control bottles containing only *O. marina* (or *Strobilidium* sp.) were also established for a single predator concentration. All the bottles were filled to capacity with freshly filtered seawater and capped. To determine the actual predator and prey densities at the beginning of the experiment, a 5-mL aliquot was removed from each bottle, fixed with 5% Lugol’s solution, and then examined under a light microscope to determine predator and prey abundance by enumerating the cells in three 1-mL Sedgewick-Rafter chambers (SRCs). The bottles were refilled to capacity with freshly filtered seawater, capped, and placed on rotating wheels under the conditions described earlier in this section. Dilution of cultures associated with the refilling of bottles was taken into consideration when calculating growth and ingestion rates. A 10-mL aliquot was taken from each bottle after a 48-h incubation period and fixed with 5% Lugol’s solution, and the abundance of *O. marina* (or *Strobilidium* sp.) and *B. cincta* were determined by counting all or >200 cells in three 1-mL SRCs. Prior to taking the subsamples, the conditions of *O. marina* (or *Strobilidium* sp.) and its prey were assessed using a dissecting microscope, as described earlier in this section.

The specific growth rate of *O. marina* (or *Strobilidium* sp.), \(\mu\) (d\(^{-1}\)) was calculated as follows:

\[
\mu = \frac{\ln (G_t/G_0)}{t}
\]

(1)

where \(G_0\) and \(G_t\) are the concentration of *O. marina* (or *Strobilidium* sp.) at time (t) 0 and 2 d, respectively.

Data for *O. marina* (or *Strobilidium* sp.) growth rates were fitted to a modified Michaelis-Menten equation:

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

(2)

where \(\mu_{max}\) is the maximum growth rate (d\(^{-1}\)), \(x\) is prey concentration (cells mL\(^{-1}\) or ng C mL\(^{-1}\)), \(x'\) is threshold prey concentration (the prey concentration where \(\mu = 0\)), and \(K_{GR}\) is the prey concentration sustaining 1/2 \(\mu_{max}\). Data were iteratively fitted to the model using DeltaGraph (Delta Point Inc., Monterey, CA, USA).

Ingestion and clearance rates were calculated using the equations of Frost (1972) and Heinbokel (1978). The incubation time for calculating ingestion and clearance rates was the same as that for estimating growth rate. Data for *O. marina* (or *Strobilidium* sp.) ingestion rates (IR, cells predator\(^{-1}\) d\(^{-1}\) or ng C predator\(^{-1}\) d\(^{-1}\)) were fitted to a modified Michaelis-Menten equation:

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

(3)

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

Comparison of growth and ingestion rates at single prey concentrations

Experiment 4 was designed to compare the growth and ingestion rates of *G. dominans*, *G. moestrupii*, *G. spirale*, and *P. kofoidii* when *B. cincta* was provided at a single prey concentration (Table 3). Growth and ingestion rates of *O. marina* and *Strobilidium* sp. at single prey concen-
trations were obtained in Experiment 2 and 3.

The *B. cincta* culture was prepared as described earlier in this section. In addition, *G. dominans*, *G. moestrupii*, *G. spirale*, and *P. kofoidii* were cultured in the same manner as described earlier in this section.

The initial concentrations of *G. dominans* (or another predator) and *B. cincta* were established using an auto-pipette to deliver predetermined volumes of known cell concentrations to the bottles. Triplicate 42-mL PC experimental bottles containing mixtures of *G. dominans* (or another predator) and *B. cincta*, triplicate prey control bottles containing *B. cincta* only, and triplicate predator control bottles containing only *G. dominans* (or another predator) were set up for *B. cincta*. Next, 5 mL of the f/2 medium was added to all the bottles, which were then filled to capacity with freshly filtered seawater and capped. To determine the predator and prey concentrations at the beginning of the experiment and the prey concentrations after 2 d, a 5-mL aliquot was removed from each bottle and fixed with 5% (v/v) Lugol’s solution; then, all or >200 predator and prey cells from three 1-mL SRCs were enumerated. Prior to taking subsamples, the conditions of *G. dominans* (or other predators) and *B. cincta* were assessed using a dissecting microscope. The bottles were, again, filled to capacity with freshly filtered seawater, capped, and placed on a rotating wheel at 0.9 rpm at 20°C under the illumination of 20 µmol photons m⁻² s⁻¹ on a 14 : 10 h light : dark cycle. The dilution of the cultures associated with the refilling of bottles was taken into consideration when calculating the growth and ingestion rates.

The growth and ingestion rates were measured in the same manner as described for Experiments 2 and 3.

### Gross growth efficiency (GGE)

GGE, defined as grazer biomass produced (+) or lost (−) per prey biomass ingested, was calculated from the estimates of carbon contents per cell based on the cell volume for each mean prey concentration.

### RESULTS

#### Feeding occurrence and growth

It was observed that *G. dominans*, *G. moestrupii*, *G. spirale*, *O. marina*, *P. kofoidii*, and *Strobilidium* sp. fed on *B. cincta* (Table 2, Fig. 1). All predators in the present study fed on prey by engulfing the prey cells.

*B. cincta* clearly supported positive growth rates for *O. marina*, *G. dominans*, and *Strobilidium* sp. but did not support the growth of *G. moestrupii*, *G. spirale*, or *P. kofoidii*.

#### Effects of prey concentrations on growth and ingestion rates

The specific growth rates of *O. marina* on *B. cincta* increased rapidly with increasing mean prey concentration <ca. 12 ng C mL⁻¹ (120 cells mL⁻¹), but became saturated or slowly increased at higher concentrations (Fig. 2). When the data were fitted to Eq. (2), the maximum specific growth rates of *O. marina* was 0.49 d⁻¹. The feeding threshold prey concentration for the growth of *O. marina* was 1.4 ng C mL⁻¹ (14 cells mL⁻¹).

The specific growth rates of *Strobilidium* sp. on *B. cincta* increased rapidly with increasing mean prey concentration <ca. 71 ng C mL⁻¹ (710 cells mL⁻¹), but became slowly increased at higher concentrations (Fig. 3). When the data were fitted to Eq. (2), the maximum specific growth rates of *Strobilidium* sp. was 0.91 d⁻¹. The feeding threshold prey concentration for the growth of *Strobilidium* sp. was 11.8 ng C mL⁻¹ (118 cells mL⁻¹).

The ingestion rates of *O. marina* on *B. cincta* increased rapidly with increasing mean prey concentration <ca. 45 ng C mL⁻¹ (450 cells mL⁻¹), but became saturated or slowly increased at higher concentrations (Fig. 4). When the data were fitted to Eq. (3), the maximum ingestion rates of *O. marina* was 0.35 ng C predator⁻¹ d⁻¹ (3.5 cells predator⁻¹ d⁻¹). The maximum clearance rate of *O. marina* was 1.47 ng C predator⁻¹ d⁻¹ (140 cells predator⁻¹ d⁻¹).
Comparison of growth and ingestion rates at single prey concentrations

When the mean prey concentrations were 480-600 ng C mL⁻¹, the specific growth rate of *Strobilidium* sp. (0.71 d⁻¹) on *B. cincta* was significantly higher than that of *O. marina* (0.44 d⁻¹) or *G. dominans* (0.07 d⁻¹) (p < 0.01, two-tailed t-test). However, the growth rates of *G. moestrupii*, *G. spirale*, and *P. kofoidii* were negative (Table 3).

The ingestion rate of *Strobilidium* sp. (1.60 ng C predator⁻¹ h⁻¹) increased rapidly with increasing mean prey concentration <ca. 236 ng C mL⁻¹ (2,360 cells mL⁻¹), but became slowly increased at higher concentrations (Fig. 5). When the data were fitted to Eq. (3), the maximum ingestion rates of *Strobilidium* sp. was 2.0 ng C predator⁻¹ d⁻¹ (20.0 cells predator⁻¹ d⁻¹). The maximum clearance rate of *Strobilidium* sp. was 1.72 µl predator⁻¹ h⁻¹. GGEs of *Strobilidium* sp. on *B. cincta* at prey concentrations where the ingestion rates increased slowly were 25-32% (Table 4).

**Fig. 1.** Feeding by heterotrophic dinoflagellates (A-D) and a ciliate (E) on the mixotrophic dinoflagellate *Biecheleria cincta*. (A) *Gyrodinium dominans* with an ingested *B. cincta* cell. (B) *Gyrodinium spirale* with an ingested *B. cincta* cell. (C) *Oxyrrhis marina* with several ingested *B. cincta* cells. (D) *Polykrikos kofoidii* with two ingested *B. cincta* cells. (E) *Strobilidium* sp. with two ingested *B. cincta* cells. Arrows indicate ingested prey cells. All photographs were taken using an inverted microscope. Scale bars represent: A-E, 10 µm.
Fig. 2. Specific growth rates of the heterotrophic dinoflagellate *Oxyrrhis marina* on the mixotrophic dinoflagellate *Biecheleria cincta* as a function of mean prey concentration (x). Symbols represent treatment means ± 1 SE. The curves are fitted according to the Michaelis-Menten equation [Eq. (2)] using all treatments in the experiment. Growth rate (d\(^{-1}\)) = 0.492(x - 1.38)/(5.67 + (x - 1.38)), \(r^2 = 0.843\).

Fig. 3. Specific growth rates of the ciliate *Strobilidium* sp. on the mixotrophic dinoflagellate *Biecheleria cincta* as a function of mean prey concentration (x). Symbols represent treatment means ± 1 SE. The curves are fitted according to the Michaelis-Menten equation [Eq. (2)] using all treatments in the experiment. Growth rate (d\(^{-1}\)) = 0.910(x - 11.8)/(34.8 + (x - 11.8)), \(r^2 = 0.911\).

Fig. 4. Ingestion rates of the heterotrophic dinoflagellate *Oxyrrhis marina* on the mixotrophic dinoflagellate *Biecheleria cincta* as a function of mean prey concentration (x). Symbols represent treatment means ± 1 SE. The curves are fitted according to the Michaelis-Menten equation [Eq. (3)] using all treatments in the experiment. Ingestion rate (ng C predator\(^{-1}\) d\(^{-1}\)) = 1.98(x/62.2 + x), \(r^2 = 0.884\).

Fig. 5. Ingestion rates of the ciliate *Strobilidium* sp. on the mixotrophic dinoflagellate *Biecheleria cincta* as a function of mean prey concentration (x). Symbols represent treatment means ± 1 SE. The curves are fitted according to the Michaelis-Menten equation [Eq. (3)] using all treatments in the experiment. Ingestion rate (ng C predator\(^{-1}\) d\(^{-1}\)) = 0.35(x/9.22 + x), \(r^2 = 0.777\).

### Table 4. Growth and grazing data for the *Oxyrrhis marina* and *Strobilidium* sp. on *Biecheleria cincta*

<table>
<thead>
<tr>
<th>Predator</th>
<th>PDV</th>
<th>(\mu_{\text{max}})</th>
<th>(K_{\text{GR}})</th>
<th>(x')</th>
<th>(I_{\text{max}})</th>
<th>(K_{\text{IR}})</th>
<th>(C_{\text{max}})</th>
<th>GGE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Oxyrrhis marina</em> (HTD)</td>
<td>1.1</td>
<td>0.49</td>
<td>5.67</td>
<td>1.38</td>
<td>0.35</td>
<td>9.22</td>
<td>1.47</td>
<td>45-53</td>
</tr>
<tr>
<td><em>Strobilidium</em> sp. (CIL)</td>
<td>11.4</td>
<td>0.91</td>
<td>34.8</td>
<td>11.8</td>
<td>1.98</td>
<td>62.2</td>
<td>1.72</td>
<td>25-32</td>
</tr>
</tbody>
</table>

Parameters are for numerical and/or functional responses from Eqs. (2) and (3), as presented in Figs 2-5. PDV, predator’s volume (x10\(^3\) µm\(^3\)); \(\mu_{\text{max}}\), maximum growth rate (d\(^{-1}\)); \(K_{\text{GR}}\), prey concentration sustaining 1/2 \(\mu_{\text{max}}\) (ng C mL\(^{-1}\)); \(x'\), threshold prey concentration (ng C mL\(^{-1}\)); \(I_{\text{max}}\), maximum ingestion rate (ng C predator\(^{-1}\) d\(^{-1}\)); \(K_{\text{IR}}\), prey concentration sustaining 1/2 \(I_{\text{max}}\) (ng C mL\(^{-1}\)); \(C_{\text{max}}\), maximum clearance rate (µL predator\(^{-1}\) h\(^{-1}\)); GGE, gross growth efficiency, %, of predators feeding on *B. cincta* at the prey concentrations where the ingestion rates were saturated or the 3 highest ingestion rates were achieved; HTD, heterotrophic dinoflagellate; CIL, ciliate.
Growth (GR) and ingestion rates (IR) of heterotrophic sp. were; Om, St, Gd, Gm, Gs, respectively. All heterotrophic protistan predators investi
max as a function of the IR (as shown in Table 3). Pk, Gm, Gs as a single prey concentration where the growth
max 
A
B
C

Fig. 6. Growth (GR) and ingestion rates (IR) of heterotrophic dinoflagellates and the ciliate on the mixotrophic dinoflagellate Biecheleria cincta as a single prey concentration where the growth and ingestion rates of Oxyrrhis marina and Strobilidium sp. were saturated. GR (A) and IR (B) of the predators on B. cincta as a function of predator size (equivalent spherical diameter, ESD, µm). (C) The GR of predators on B. cincta as a function of the IR (as shown in Table 3). The p-values in (A), (B), and (C) were all p > 0.1 (linear regression ANOVA). Gd, Gymnodinium dominans; Gm, Gymnodinium moestrupii; Gs, Gymnodinium spirale; Om, O. marina; Pk, Polykrikos kofoidii; St, Strobilidium sp.

DISCUSSION

Feeding occurrence and growth

To the best of our knowledge, this study is the first report on feeding by heterotrophic protistan predators on B. cincta. All heterotrophic protistan predators investigated in the present study were able to feed on B. cincta by engulfing the cells. These heterotrophic protists commonly occur in many marine environments (Goldman et al. 1989, Yoo et al. 2010a, Jeong et al. 2011, Yoon et al. 2012). Thus, heterotrophic protists should be considered predators of B. cincta in marine food webs.

O. marina, G. dominans, and Strobilidium sp. exhibited positive growth rates when feeding on B. cincta but G. moestrupii, G. spirale, and P kofoidii did not. Thus, during blooms dominated by B. cincta, O. marina, G. dominans, and Strobilidium sp. are likely to be abundant, while G. moestrupii, G. spirale, and P kofoidii may not be present. B. cincta can be a critical prey for selecting dominant species among heterotrophic protistan communities.

Growth and ingestion rates

The growth rates for G. moestrupii, G. spirale, and P kofoidii feeding on B. cincta were negative, while those for O. marina or Strobilidium sp. were relatively high (Table 3). For G. moestrupii, G. spirale, and P kofoidii feeding on B. cincta, their ingestion rates (0.10, 0.04, and 0.55 ng C predator⁻¹ d⁻¹, respectively) were much lower than their carbon contents (0.4, 1.3, and 4.2 ng C cell⁻¹, respectively) (Jeong et al. 2001b, Kim and Jeong 2004, Yoo et al. 2013b). Thus, low ingestion rates for G. moestrupii, G. spirale, and P kofoidii on B. cincta are likely responsible for their negative growth rates. However, growth rates for G. moestrupii, G. spirale, and P kofoidii are high when feeding on algal prey (Jeong et al. 2001b, Kim and Jeong 2004, Yoo et al. 2013b). The maximum growth rates for G. moestrupii, G. spirale, and P kofoidii when feeding on optimal prey (e.g., Alexandrium minutum, Prorocentrum minimum, and Gymnodinium catenatum) are as high as 1.60, 1.13, and 1.12 d⁻¹, respectively (Jeong et al. 2001b, Kim and Jeong 2004, Yoo et al. 2013b). We assume that the ecological niches of G. moestrupii, G. spirale, and P kofoidii may be different from those of O. marina or Strobilidium sp., and competition among these protistan grazers might reduce when feeding on certain prey.

Among the maximum growth (µ_max) and ingestion rates (I_max) of O. marina feeding on diverse prey items, the µ_max of O. marina on B. cincta is similar than that on Azadini-
un cf. poporum (Table 5). However, the \( \mu_{\text{max}} \) of \( O. marina \) on \( B. cincta \) is lower than that on \( A. \) cf. poporum (Potvin et al. 2013). Therefore, the nutritional value of \( B. cincta \) for growth of \( O. marina \) may be greater than that of \( A. \) cf. poporum. The \( \mu_{\text{max}} \) of \( O. marina \) feeding on \( B. cincta \) is lower than that on the other algal prey species except a toxic strain of \( Karlodinium veneficum \), but higher than that on the heterotrophic nanoflagellate \( Cafeteria \) sp. and the heterotrophic dinoflagellates \( Luciella masanensis \) and \( Stoeckeria algicida \) (Table 5). Therefore, \( B. cincta \) is a better prey item for \( O. marina \) than these heterotrophic nanoflagellate and heterotrophic dinoflagellates, but less favorable prey than the other algal prey species, except \( K. veneficum \). The \( I_{\text{max}} \) of \( O. marina \) feeding on \( B. cincta \) is lower than that on the other algal prey species except the mixotrophic dinoflagellate \( Gymnodinium aureolum \), but higher than that on \( Cafeteria \) sp., \( Pfiesteria piscicida \), \( L. masanensis \), and \( S. algicida \) (Table 5). Therefore, the lower ingestion rate of \( O. marina \) feeding on \( B. cincta \) than that on the other algal prey species except one species may be responsible for its lower growth rates, but the higher ingestion rate of \( O. marina \) feeding on \( B. cincta \) than that on the heterotrophic nanoflagellate and heterotrophic dinoflagellates may be responsible for its higher growth rates. \( O. marina \) may capture and ingest \( B. cincta \) with more difficulty than the other algal prey, except some unpalatable ones, but more easily than heterotrophic nanoflagellate and dinoflagellates. Both the \( \mu_{\text{max}} \) and \( I_{\text{max}} \) of \( O. marina \) feeding on diverse prey species was not significantly correlated with the prey's equivalent spherical diameter \( (p > 0.1, \) ANOVA). Moreover, the \( \mu_{\text{max}} \) of \( O. marina \) feeding on diverse prey species was not significantly correlated with the \( I_{\text{max}} \) \( (p > 0.1, \) ANOVA). Therefore, for \( O. marina \), the nutritional value of the different prey species, including \( B. cincta \), may differ.

The \( \mu_{\text{max}} \) of \( Strobilidium \) sp. on \( B. cincta \) is higher than that on \( A. \) cf. poporum and the euglenophyte \( Eutreptiella gymnastica \), although the \( I_{\text{max}} \) of \( Strobilidium \) sp. on \( B. cincta \) is comparable to or lower than that on \( A. \) cf. poporum and \( E. gymnastica \) (Table 5). Therefore, for \( Strobilidium \) sp., \( B. cincta \) may have higher nutritional value than \( A. \) cf. poporum or \( E. gymnastica \).

### Table 5. Comparison of maximum growth and ingestion rates of \( Oxyrrhis marina \) and \( Strobilidium \) spp. on diverse prey species

<table>
<thead>
<tr>
<th>Predator</th>
<th>Prey species</th>
<th>ESD (( \mu ))</th>
<th>( \mu_{\text{max}} )</th>
<th>( I_{\text{max}} )</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>( Oxyrrhis marina ) (HTD)</td>
<td>( Cafeteria ) sp. (HNF)</td>
<td>3.5</td>
<td>0.19</td>
<td>0.3</td>
<td>Jeong et al. (2007b)</td>
</tr>
<tr>
<td></td>
<td>( Phaeodactylum tricornutum ) (DIA)</td>
<td>4.2</td>
<td>1.30</td>
<td>1.9</td>
<td>Goldman et al. (1989)</td>
</tr>
<tr>
<td></td>
<td>( Isochrysis galbana ) (PRY)</td>
<td>5.1</td>
<td>0.80</td>
<td>2.2</td>
<td>Goldman et al. (1989)</td>
</tr>
<tr>
<td></td>
<td>( Dunaliella tertiolecta ) (CHL)</td>
<td>5.1</td>
<td>0.80</td>
<td>1.5</td>
<td>Goldman et al. (1989)</td>
</tr>
<tr>
<td></td>
<td>( Karlodinium veneficum ) NT (MTD)</td>
<td>9.1</td>
<td>0.90</td>
<td>6.4</td>
<td>Adolf et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>( Amphidinium carterae ) (MTD)</td>
<td>9.7</td>
<td>1.17</td>
<td>2.8</td>
<td>Jeong et al. (2001a)</td>
</tr>
<tr>
<td></td>
<td>( Azadinium cf. poporum ) (PTD)</td>
<td>10.0</td>
<td>0.50</td>
<td>5.0</td>
<td>Potvin et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>( Karlodinium veneficum ) T (MTD)</td>
<td>10.5</td>
<td>0.25</td>
<td>2.2</td>
<td>Adolf et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>( Heterosigma akashiwo ) (RAP)</td>
<td>11.5</td>
<td>1.43</td>
<td>1.3</td>
<td>Jeong et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>( Biecheleria cincta ) (MTD)</td>
<td>12.2</td>
<td>0.49</td>
<td>0.91</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>( Eutreptiella gymnastica ) (EUG)</td>
<td>12.6</td>
<td>0.81</td>
<td>2.7</td>
<td>Jeong et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>( Pfiesteria piscicida ) (HTD)</td>
<td>13.5</td>
<td>0.66</td>
<td>0.33</td>
<td>Jeong et al. (2007a)</td>
</tr>
<tr>
<td></td>
<td>( Luciella masanensis ) (HTD)</td>
<td>13.5</td>
<td>0.04*</td>
<td>0.07</td>
<td>Jeong et al. (2007a)</td>
</tr>
<tr>
<td></td>
<td>( Stoeckeria algicida ) (HTD)</td>
<td>13.9</td>
<td>0.22</td>
<td>0.14</td>
<td>Jeong et al. (2007a)</td>
</tr>
<tr>
<td></td>
<td>( Gymnodinium aureolum ) (MTD)</td>
<td>19.4</td>
<td>0.71</td>
<td>0.51</td>
<td>Yoo et al. (2010a)</td>
</tr>
<tr>
<td></td>
<td>( Fibrocapsa japonica ) (RAP)</td>
<td>20.4</td>
<td>0.72</td>
<td>1.2</td>
<td>Tillmann and Reckermann (2002)</td>
</tr>
<tr>
<td>( Strobilidium ) spp. (CIL)</td>
<td>( Azadinium cf. poporum ) (PTD)</td>
<td>10.0</td>
<td>0.64</td>
<td>179</td>
<td>Potvin et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>( Biecheleria cincta ) (MTD)</td>
<td>12.2</td>
<td>0.91</td>
<td>1.98</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>( Eutreptiella gymnastica ) (EUG)</td>
<td>12.6</td>
<td>-0.94*</td>
<td>2.2</td>
<td>Jeong et al. (2011)</td>
</tr>
</tbody>
</table>

Rates are corrected to 20°C using \( Q_{10} = 2.8 \) (Hansen et al. 1997). ESD, equivalent spherical diameter (\( \mu \)m); \( \mu_{\text{max}} \), maximum growth rate (\( \text{d}^{-1} \)); \( I_{\text{max}} \), maximum ingestion rate (ng C predator \( \text{d}^{-1} \)); HTD, heterotrophic dinoflagellate; HNF, heterotrophic nanoflagellate; DIA, diatom; PRY, prymnesiophyte; CHL, chlorophyte; NT, non-toxic; MTD, mixotrophic dinoflagellate; PTD, phototrophic dinoflagellate; T, toxic; RAP, raphidophyte; EUG, euglenophyte; CIL, ciliate.

*The maximum value among the mean growth rates measured at given prey concentrations.
Both growth and ingestion rates of *O. marina* and *Strobilidium* sp. on *B. cincta* are affected by prey concentrations. The threshold prey concentration for growth of *O. marina* on *B. cincta* (1.4 ng C mL$^{-1}$) was lower than that for the growth rate of *Strobilidium* sp. on the same prey (11.8 ng C mL$^{-1}$). Therefore, *O. marina* is likely to survive at low *B. cincta* concentrations but *Strobilidium* sp. is not. The $K_{\text{in}}$ (the prey concentration sustaining $1/2 \mu$) of 5.7 ng C mL$^{-1}$ for *O. marina* feeding on *B. cincta* was also lower than that of *Strobilidium* sp. (34.8 ng C mL$^{-1}$) feeding on the same algal prey. Thus, *O. marina* is likely to grow rapidly at low *B. cincta* concentrations but *Strobilidium* sp. would not. Additionally, the $K_{\text{in}}$ (the prey concentration sustaining $1/2 \mu$) of 9.2 ng C mL$^{-1}$ for *O. marina* feeding on *B. cincta* was also lower than that of *Strobilidium* sp. (62.2 ng C mL$^{-1}$) feeding on the same algal prey. Thus, these results indicate that, at low prey concentrations, the growth and ingestion rates of *O. marina* would respond more readily to changes in prey concentrations than those of *Strobilidium* sp.

We could not estimate the grazing impact by *O. marina* and *Strobilidium* sp. on *B. cincta* in this study because data on the abundance of *B. cincta*, *O. marina*, and *Strobilidium* sp. are not available. Therefore, to understand the population dynamics of *B. cincta* and heterotrophic protists and their interactions, the abundance of *B. cincta* and its predators in natural environments need to be quantified.

**ACKNOWLEDGEMENTS**

We thank Yeong Jong Hwang and Eric Potvin for technical support. This paper was supported by Basic Research Program through the National Research Foundation of Korea (NRF) grant funded by Ministry of Science, ICT and Future Planning (MSICTFP), the Korean Government NRF/MEST (2012R1A6A3A0304333) award to YD Yoo and the NRF grant funded by MICTFP (NRF-2010-0020702) and Mid-career Researcher Program (2012-R1A2A2A01-010987) award to HJ Jeong.

**REFERENCES**


Moestrup, Ø., Lindberg, K. & Daugbjerg, N. 2009. Studies on...


