Feeding by protists and copepods on the heterotrophic dinoflagellates *Pfiesteria piscicida*, *Stoeckeria algicida*, and *Luciella masanensis*

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ABSTRACT: To investigate interactions between the heterotrophic dinoflagellates *Pfiesteria piscicida*, *Stoeckeria algicida*, and *Luciella masanensis* and their protozoan and metazoan predators, we measured the growth and/or ingestion rates of the heterotrophic dinoflagellate *Oxyrrhis marina*, the ciliate *Strombidinopsis jeokjo*, and the calanoid copepods *Acartia* spp. (*A. hongi* and *A. omorii*) when fed on *P. piscicida*, *S. algicida*, and *L. masanensis*. The maximum growth and ingestion rates of *O. marina* fed on *P. piscicida* (0.66 d⁻¹ and 0.33 ng C predator⁻¹ d⁻¹, respectively) were markedly higher than those of the same predator fed on *S. algicida* (0.22 d⁻¹ and 0.14 ng C predator⁻¹ d⁻¹, respectively) or *L. masanensis* (0.04 d⁻¹ and 0.07 ng C predator⁻¹ d⁻¹, respectively). The maximum growth and ingestion rates of *S. jeokjo* fed on *P. piscicida* and *S. algicida* (1.61 to 1.77 d⁻¹ and 44 to 49 ng C predator⁻¹ d⁻¹, respectively) were much higher than when fed on *L. masanensis* (−0.1 d⁻¹ and 10 ng C predator⁻¹ d⁻¹, respectively). *S. jeokjo* had significantly higher attack ratios (number of attempted captures relative to number of physical contacts between predator and prey) when fed on *P. piscicida* and *S. algicida* (18 to 25%) than on *L. masanensis* (5%). Similarly, successful capture (number of prey ingested relative to number of attempted captures) of *P. piscicida* and *S. algicida* (82 to 87%) was significantly higher than that of *L. masanensis* (2%). *L. masanensis* may have defensive behavior or chemical protection against predation. However, maximum ingestion rates of *Acartia* spp. fed on these dinoflagellate species were similar. In understanding the population dynamics and predator–prey interactions of these 3 closely related dinoflagellate species, it is important to distinguish between predation by protists and by copepods.

KEY WORDS: Graze · Growth · Harmful algal bloom · Ingestion · Marine · Protist · Red tide

INTRODUCTION

Since the small heterotrophic dinoflagellate *Pfiesteria piscicida* (order Peridiniales and family Pfiesteriaceae) was first discovered in the early 1990s (Steidinger et al. 1996), several more dinoflagellate species in the family Pfiesteriaceae, such as *Cryptoperidiniopsis brodyi*, *Luciella atlantis*, *L. masanensis*, *Pseudopfiesteria shumwayae*, and *Stoeckeria algicida*, have been discovered (Jeong et al. 2005b, Litaker et al. 2005, Steidinger et al. 2006, Mason et al. 2007). Both scientists and the public have paid attention to these dinoflagellates because some of the species are harmful to fish (Noga et al. 1996, Glasgow et al. 2001), shellfish (Springer et al. 2002, Shumway et al. 2006), and humans (Schmechel & Koltai 2001). These dinoflagellates often co-occur in estuaries as well as in coastal waters (e.g. Lewitus et al. 2002). Their cell length and width are approximately 5 to 25 μm and 3 to 20 μm, respectively, and the ranges of the cell lengths and widths overlap between species (Jeong et al. 2006). Further, their shapes are very similar, whereas their plate patterns and DNA sequences clearly differ (Steidinger et al. 1996, Jeong et al. 2005b, 2006, Litaker et
al. 2005, Rublee et al. 2005, Marshall et al. 2006). Therefore, it is very difficult to distinguish one species from the others in fixed samples. They feed on prey with the same feeding mechanism, i.e. by using a peduncle (a feeding tube) (Burkholder & Glasgow 1995, Jeong et al. 2005a). An important question arises: do they have similar ecological roles in marine plankton communities? With respect to their ecological roles, their feeding and mortality due to predation are important factors. Recent studies (Jeong et al. 2006, 2007a) have revealed that the kinds of prey species which L. masanensis (previously Lucy) feeds on were very similar to those consumed by P. piscicida. However, the kinds of prey species which P. piscicida and L. masanensis feed on are very different from those consumed by S. algicida, which is known to feed only on the raphidophyte Heterosigma akashiwo and blood cells (Jeong et al. 2005b, 2007a). Also, the growth and ingestion rates of P. piscicida feeding on optimal and suboptimal algal prey and fish blood cells were usually higher than those of L. masanensis feeding on the same prey species (Jeong et al. 2006, 2007a). Therefore, with regard to feeding, P. piscicida, L. masanensis, and S. algicida have different roles.

With regard to mortality due to predation, limited studies have thus far reported on the growth and/or ingestion rates of the predators on Pfiesteria piscicida (Mallin et al. 1995, Stoecker et al. 2000, Stoecker & Gustafson 2002, Gransden & Lewitus 2003, Setèlé et al. 2005, Lewitus et al. 2006); these predators include the naked ciliates Euplotes spp., Strombidium spp., and Strobilidium sp., the tintinnid ciliates Tintinnopsis spp., Tintinnidium spp., Nolaculisus spp., Favella spp., rotifers, and the copepod Acartia tonsa (Stoecker et al. 2000, Stoecker & Gustafson 2002, Gransden & Lewitus 2003, Lewitus et al. 2006). Moreover, no studies have thus far been conducted on predators that feed on Luciella masanensis and Stoeckeria algicida. Therefore, to investigate the mortality rates due to predation for these 2 dinoflagellates and to investigate whether they play ecological roles similar to P. piscicida, we need to measure the numerical (i.e. growth rate) and functional responses (i.e. ingestion rate) of common predators to prey concentration when fed on P. piscicida, S. algicida, and L. masanensis.

Heterotrophic dinoflagellates, ciliates, and copepods are some of the major components in marine planktonic food webs. Heterotrophic dinoflagellates and ciliates are ubiquitous and occasionally dominate within the microzooplankton in terms of abundance and/or biomass, while copepods dominate similarly within the mesozooplankton (Lessard 1991, Jeong 1999, Kimmel & Roman 2004, Turner et al. 2005). These 3 groups sometimes have considerable grazing or predation impact on populations of diverse prey (Verity et al. 1999, Kim & Jeong 2004). Heterotrophic dinoflagellates feed on a variety of prey (for review, see Jeong 1999). Ciliates also feed on diverse prey, including bacteria (e.g. Sherr & Sherr 2002), algae (Montagnes et al. 1996, Kamiyama & Arima 2001), and heterotrophic dinoflagellates (Jeong et al. 2004). However, no studies have been conducted on predator–prey relationships among heterotrophic dinoflagellates, and only a few studies have been conducted on predation by ciliates on heterotrophic dinoflagellates (Stoecker et al. 2000, Gransden & Lewitus 2003, Jeong et al. 2004, Lewitus et al. 2006). The lack of information regarding the interactions among heterotrophic dinoflagellates and between ciliates and heterotrophic dinoflagellates restricts our understanding of the cycling of materials, energy flow, and population dynamics of dominant heterotrophic dinoflagellates in marine food webs. Copepods feed on diverse prey, including algae (e.g. Turner et al. 2005), heterotrophic dinoflagellates, and ciliates (Gifford & Dagg 1991, Jeong et al. 2001, Roman et al. 2006). However, information available on predation by copepods on heterotrophic dinoflagellates is also much less than that for phototrophic dinoflagellate prey. When Pfiesteria piscicida, Stoeckeria algicida, and Luciella masanensis are offered singularly as prey to heterotrophic dinoflagellates, ciliates, and copepods, these predators may display different patterns in their functional responses to the concentration of each prey species.

To explore differential feeding by heterotrophic dinoflagellates, ciliates, and copepods on heterotrophic dinoflagellates, we established cultures of Pfiesteria piscicida, Stoeckeria algicida, and Luciella masanensis as prey, and the heterotrophic dinoflagellate Oxyrrhis marina, the ciliate Strombidinopsis jeokjo, and the calanoid copepods Acartia spp. (A. hongi and A. omorii) as predators, and conducted experiments to measure the growth and/or ingestion rates of each predator feeding on each prey species. These prey and predators often coexist in Masan Bay and/or the coastal waters off Kunsan, Korea. The results of the present study provide a basis for understanding the interactions between P. piscicida, S. algicida, and L. masanensis and their predators and the ecological roles of these 3 heterotrophic dinoflagellates in marine planktonic communities.

**MATERIALS AND METHODS**

**Preparation of experimental organisms.** For the isolation and culture of Pfiesteria piscicida (GenBank Accession number AM231031), Stoeckeria algicida (AJ841809), and Luciella masanensis (AM050344),
Table 1. Isolation and maintenance conditions of the experimental organisms

<table>
<thead>
<tr>
<th>Organism</th>
<th>Location</th>
<th>Time</th>
<th>Temp. (°C)</th>
<th>Salinity (psu)</th>
<th>Prey species</th>
<th>Concentration (cells ml⁻¹)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pfiesteria piscicida</em></td>
<td>Off Incheon</td>
<td>Jul 2005</td>
<td>24.0</td>
<td>25.4</td>
<td><em>Amphidinium carterae</em></td>
<td>20000–30000</td>
<td>Jeong et al. (2006)</td>
</tr>
<tr>
<td><em>Stoeckeria algicida</em></td>
<td>Masan Bay</td>
<td>Jul 2004</td>
<td>24.8</td>
<td>20.6</td>
<td><em>Heterosigma akashiwo</em></td>
<td>30000</td>
<td>Jeong et al. (2005a)</td>
</tr>
<tr>
<td><em>Luciella masanensis</em></td>
<td>Keum Estuary</td>
<td>May 2001</td>
<td>16.0</td>
<td>28.0</td>
<td><em>Amphidinium carterae</em></td>
<td>30000–40000</td>
<td>Jeong et al. (2007a)</td>
</tr>
<tr>
<td><em>Oxyrrhis marina</em></td>
<td>Keum Estuary</td>
<td>May 2001</td>
<td>16.0</td>
<td>27.7</td>
<td><em>Amphidinium carterae</em></td>
<td>8000</td>
<td>Jeong et al. (2003)</td>
</tr>
<tr>
<td><em>Strombidinopsis jeokjo</em></td>
<td>Off Kunsan</td>
<td>Aug 2005</td>
<td>25.1</td>
<td>27.0</td>
<td><em>Prorocentrum micans</em></td>
<td>5000</td>
<td>As in Jeong et al. (2004)</td>
</tr>
</tbody>
</table>

*Pfiesteria piscicida* and *A. omorii* were used for these experiments. *A. omorii* was provided as prey, and a clonal culture of each protistan predator was established by 2 serial single-cell isolations as described in Jeong et al. (2003, 2004). These predator cultures were maintained at 20°C for >1 mo before experiments were conducted.

Table 2. Design of experiments

<table>
<thead>
<tr>
<th>Expt</th>
<th>Prey Species</th>
<th>Predator Species</th>
<th>Prey Density (cells ml⁻¹)</th>
<th>Predator Density (cells ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Pfiesteria piscicida</em></td>
<td><em>Oxyrrhis marina</em></td>
<td>0, 36, 153, 298, 530, 1170, 3500, 8260, 15370</td>
<td>9–361 (363)</td>
</tr>
<tr>
<td>2</td>
<td><em>Stoeckeria algicida</em></td>
<td><em>Oxyrrhis marina</em></td>
<td>0, 48, 190, 428, 976, 2970, 4589, 10,510, 15,940</td>
<td>9–490 (493)</td>
</tr>
<tr>
<td>3</td>
<td><em>Luciella masanensis</em></td>
<td><em>Oxyrrhis marina</em></td>
<td>0, 57, 104, 210, 477, 1170, 3080, 9820, 15,260</td>
<td>14–452 (452)</td>
</tr>
<tr>
<td>4</td>
<td><em>Pfiesteria piscicida</em></td>
<td><em>Strombidinopsis jeokjo</em></td>
<td>0, 42, 65, 162, 388, 924, 1570, 4130, 8200, 15,090</td>
<td>3.5–8.3 (6.9)</td>
</tr>
<tr>
<td>5</td>
<td><em>Stoeckeria algicida</em></td>
<td><em>Strombidinopsis jeokjo</em></td>
<td>0, 40, 85, 170, 488, 846, 1760, 3650, 6020, 11,640</td>
<td>2.8–7.5 (6.6)</td>
</tr>
<tr>
<td>6</td>
<td><em>Luciella masanensis</em></td>
<td><em>Strombidinopsis jeokjo</em></td>
<td>0, 61, 100, 242, 478, 1050, 2160, 5410, 10,680, 17,420</td>
<td>4.4–12.7 (9.2)</td>
</tr>
<tr>
<td>7</td>
<td><em>Pfiesteria piscicida</em></td>
<td><em>Acartia spp.</em></td>
<td>39, 67, 239, 775, 2570, 8560, 21,790</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td><em>Stoeckeria algicida</em></td>
<td><em>Acartia spp.</em></td>
<td>95, 416, 965, 2940, 5350, 8140, 14,460</td>
<td>20</td>
</tr>
<tr>
<td>9</td>
<td><em>Luciella masanensis</em></td>
<td><em>Acartia spp.</em></td>
<td>49, 95, 266, 1020, 2980, 10,870, 27,810</td>
<td>20</td>
</tr>
</tbody>
</table>

*Ind. l⁻¹ for *Acartia spp.; *Values in parentheses are from control bottles; *A. hongi and *A. omorii
capped, and placed on plankton wheels rotating at 0.9 rpm and incubated at 20°C under the illumination of 20 μE m⁻² s⁻¹ on a 14 h light:10 h dark cycle. To monitor the conditions and interaction between predator and prey species, the cultures were periodically removed from the rotating wheels, observed through the surface of the capped bottles with a dissecting microscope, and then returned to the rotating wheels. Once the target prey cells were no longer detectable, three 1 ml aliquots from each bottle were counted using a compound microscope to determine the cell concentrations of *O. marina*, and the cultures were then used to conduct experiments.

For each experiment, the initial concentrations of *Oxyrrhis marina* and a target prey species (each of *Pfiesteria piscicida*, *Stoeckeria algicida*, and *Luciella masanensis*) were established using an autopipette to deliver predetermined volumes of known cell concentrations to the bottles. Triplicate 42 ml PC experiment bottles (mixtures of predator and prey) and triplicate control bottles (prey only) were set up at each predator–prey combination. Triplicate control bottles containing only *O. marina* were also established at 1 predator concentration. To obtain similar water conditions, the water of a predator culture was filtered through a 0.7 μm GF/F filter (Whatman) and then added to the prey control bottles in the same amount as the volume of the predator culture added into the experiment bottles for each predator–prey combination. All the bottles were then filled to capacity with freshly filtered seawater and capped. To determine the actual predator and prey concentrations at the beginning of the experiment, a 5 ml aliquot was removed from each bottle and, after 24 h of incubation, a 10 ml aliquot was removed and fixed with 5% Lugol’s solution; all predator cells and all or >200 prey cells in three to five 1 ml SRCs were enumerated. The bottles were refilled to capacity with freshly filtered seawater, capped, and placed on rotating wheels under the environmental conditions described above.

The specific growth rate of *Oxyrrhis marina* (or *Strombidinopsis jeokjo*), μ (d⁻¹), was calculated as:

\[ \mu (d) = \frac{\ln (P_f/P_0)}{t} \]  \(1\)

where \(P_f\) and \(P_0\) are the concentration of *O. marina* (or *S. jeokjo*) at 0 h and 48 h (or 24 h for *S. jeokjo*), respectively.

Data for *Oxyrrhis marina* (or *Strombidinopsis jeokjo*) growth rates were fitted to a 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 0.5 \(\mu_{max}\). Data were iteratively fitted to the model using DeltaGraph® (Delta Point).

Ingestion and clearance rates were calculated using the equations of Frost (1972) and Heinbokel (1978). The incubation time was the same as that for estimating the growth rate. Ingestion rate data for *Oxyrrhis marina* (or *Strombidinopsis jeokjo*) were fitted to a 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 0.5 \(I_{max}\). For the experiment on the feeding by copepods, initial concentrations of the target prey were established.
using an autopipette, and those of Acartia spp. were obtained by individually transferring the copepods using a Pasteur pipette (Table 2). Triplicate 500 ml PC experiment bottles and triplicate control bottles (prey only) were set up at each predator–prey combination. To determine actual prey densities at the beginning of the experiment, a 20 ml aliquot was removed from each bottle, fixed with 5% Lugol’s solution, and examined with a compound microscope to determine prey abundance by enumerating cells in three 1 ml SRCs. The bottles were filled again to capacity with freshly filtered seawater, capped, and placed on rotating plankton wheels and incubated at 20°C as described above.

After 48 h of incubation, 20 ml aliquots were taken from each bottle and fixed with 5% Lugol’s solution, and the abundance of the target prey was determined by counting all or >300 cells in three 1 ml SRCs. After incubation for 48 h, Acartia spp. were counted. Mortality of Acartia spp. was zero at the end of the incubation. Ingestion and clearance rates were calculated using the equations of Frost (1972) as described above.

**Attack ratio and successful capture.** This experiment was designed to investigate why the growth and ingestion rates of Strombidinopsis jeokjo feeding on Luciella masanensis were much lower than those achieved on a diet of Pfiesteria piscicida or Stoeckeria algicida.

In this experiment the attack ratio (i.e. the number of attempted captures relative to the number of physical contacts between predator and prey) and successful capture ratio (i.e. the number of prey ingested relative to the number of attempted captures) were determined by monitoring the behavior of Strombidinopsis jeokjo in the presence of Pfiesteria piscicida, Stoeckeria algicida, or Luciella masanensis. Attempted captures represented generation of feeding current by undulating cilia. Successful captures were attacks that resulted in the prey being ingested. Additionally, to explore possible adverse effects (i.e. chemical effects) of the extract of L. masanensis, the water of an L. masanensis culture (density = ca. 20 000 cells–1) was filtered through a 0.7 μm GF/F filter and then added into bottles containing the optimal prey species, P. piscicida.

Individual Strombidinopsis jeokjo cells starved for 1 d were transferred to a Petri dish (50 mm in diameter) containing each prey species (Pfiesteria piscicida, Stoeckeria algicida, and Luciella masanensis) and then each predator was tracked under a dissecting microscope until it successfully engulfed a prey cell or until ca. 10 min had elapsed. For each prey species, the number of predator–prey encounters, attempted prey captures, and ingestion of prey were recorded for 10 to 20 S. jeokjo cells (i.e. 10 to 20 replicates).

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**RESULTS**

**Growth rates**

Oxyrrhis marina grew well feeding on Pfiesteria piscicida and Stoeckeria algicida, but failed to grow when fed Luciella masanensis (Fig. 1). The specific growth rates of O. marina consuming P. piscicida and S. algicida increased rapidly with increasing mean prey concentration (ca. 30 to 60 ng C ml⁻¹) but increased only slightly at higher concentrations (Fig. 1A,B). When the
data were fitted to Eq. (2), the maximum specific growth rate ($\mu_{\text{max}}$) of *O. marina* consuming *P. piscicida* (0.66 d⁻¹) was 3 times higher than when fed *S. algicida* (0.22 d⁻¹) (Table 3). The specific growth rates of *O. marina* consuming *L. masanensis* were between –0.08 and 0.04 d⁻¹ (Fig. 1C).

Like the *Oxyrrhis marina* predator, *Strombidinopsis jeokjo* also grew well with *Pfiesteria piscicida* and *Stoeckeria algicida* as prey, but failed to grow when consuming *Luciella masanensis* (Fig. 2A–C). The specific growth rates of *S. jeokjo* when fed *P. piscicida* and *S. algicida* increased rapidly with increasing mean prey concentration (up to ca. 50 ng C ml⁻¹) but increased only slightly at higher concentrations (Fig. 2A,B). Unlike the *O. marina* predator, when the data were fitted to Eq. (2), $\mu_{\text{max}}$ of *S. jeokjo* feeding on *P. piscicida* (1.77 d⁻¹) was slightly higher than when fed *S. algicida* (1.61 d⁻¹). The specific growth rates of *S. jeokjo* feeding on *L. masanensis* were between –0.83 and –0.11 d⁻¹ (Fig. 2C).

**Ingestion and clearance rates**

The ingestion rates of *Oxyrrhis marina* feeding on *Pfiesteria piscicida* and *Stoeckeria algicida* increased rapidly with increasing mean prey concentration < ca. 210 to 260 ng C ml⁻¹, but were saturated at higher concentrations (Fig. 3A,B), while rates when feeding on *Luciella masanensis* increased rapidly with increasing mean prey concentration (up to ca. 65 ng C ml⁻¹) but increased slowly at higher concentrations (Fig. 3C). When the data were fitted to Eq. (3), the maximum ingestion rate ($I_{\text{max}}$) of *O. marina* consuming *P. piscicida* (0.33 ng C predator⁻¹ d⁻¹) was considerably higher than when consuming *S. algicida* (0.14 ng C predator⁻¹ d⁻¹) and much higher than with *L. masanensis* as prey (0.07 ng C predator⁻¹ d⁻¹) (Table 3). The maximum clearance rates of *O. marina* feeding on *P. piscicida* and *S. algicida* (0.34 to 0.61 μl predator⁻¹ h⁻¹) were also much higher than that with *L. masanensis* as prey (0.02 μl predator⁻¹ h⁻¹).

The ingestion rates of *Strombidinopsis jeokjo* feeding on *Pfiesteria piscicida* and *Stoeckeria algicida* increased rapidly with increasing mean prey concentration (ca. 40 to 50 ng C ml⁻¹) but increased slowly at higher concentrations (Fig. 4A,B). The ingestion rates of *S. jeokjo* consuming *Luciella masanensis* increased with increasing mean prey concentration (up to ca. 270 ng C ml⁻¹) but decreased at higher concentrations (Fig. 4C). When the data were fitted to Eq. (3), $I_{\text{max}}$ values of *S. jeokjo* consuming *P. piscicida* and *S. algicida* (44 to 49 ng C predator⁻¹ d⁻¹) were markedly higher than when feeding on *L. masanensis* (10 ng C predator⁻¹ d⁻¹). The maximum clearance rate of *S. jeokjo* consuming *P. piscicida* (15.4 μl predator⁻¹ h⁻¹) was higher than those with *S. algicida* and *L. masanensis* as prey (5.4 to 6.6 μl predator⁻¹ h⁻¹).

The ingestion rate of *Acartia spp.* feeding on *Pfiesteria piscicida* and *Luciella masanensis* increased continuously with increasing mean prey concentration (up to 1300 ng C ml⁻¹) while that resulting from ingestion of *Stoeckeria algicida* increased with increasing mean prey concentration (up to ca. 650 ng C ml⁻¹) but was saturated at the highest concentration (Fig. 5). The highest values among the ingestion rates of *Acartia* spp. feeding on *P. piscicida*, *S. algicida*, and *L. masanensis* were 3850, 3640, and 3490 ng C predator⁻¹ d⁻¹, respectively, at mean prey concentrations of 1050, 650, and 1330 ng C ml⁻¹, respectively. The maximum clear-

<table>
<thead>
<tr>
<th>Figs.</th>
<th>Species</th>
<th>$\mu_{\text{max}}$</th>
<th>$K_{\text{IR}}$</th>
<th>$x'$</th>
<th>$r^2$</th>
<th>$I_{\text{max}}$</th>
<th>$K_{\text{IR}}$</th>
<th>$r^2$</th>
<th>$C_{\text{max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxyrrhis marina</strong></td>
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</tr>
<tr>
<td>1A &amp; 3A</td>
<td><em>Pfiesteria piscicida</em></td>
<td>0.657</td>
<td>21</td>
<td>0.4</td>
<td>0.797</td>
<td>0.334</td>
<td>83</td>
<td>0.876</td>
<td>0.34</td>
</tr>
<tr>
<td>1B &amp; 3B</td>
<td><em>Stoeckeria algicida</em></td>
<td>0.220</td>
<td>64</td>
<td>–4.7</td>
<td>0.528</td>
<td>0.138</td>
<td>89</td>
<td>0.359</td>
<td>0.61</td>
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<tr>
<td>1C &amp; 3C</td>
<td><em>Luciella masanensis</em></td>
<td>0.042*</td>
<td>0.065</td>
<td>132</td>
<td>0.335</td>
<td>0.01</td>
<td></td>
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<tr>
<td><strong>Strombidinopsis jeokjo</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2A &amp; 3A</td>
<td><em>Pfiesteria piscicida</em></td>
<td>1.77</td>
<td>90</td>
<td>23</td>
<td>0.783</td>
<td>43.5</td>
<td>92</td>
<td>0.973</td>
<td>15.4</td>
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<tr>
<td>2B &amp; 3C</td>
<td><em>Stoeckeria algicida</em></td>
<td>1.61</td>
<td>16</td>
<td>36</td>
<td>0.847</td>
<td>49.3</td>
<td>187</td>
<td>0.955</td>
<td>6.6</td>
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<tr>
<td>2C &amp; 4C</td>
<td><em>Luciella masanensis</em></td>
<td>–0.11*</td>
<td>9.8*</td>
<td>5.4</td>
<td></td>
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<tr>
<td><strong>Acartia spp.</strong></td>
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<tr>
<td>5A</td>
<td><em>Pfiesteria piscicida</em></td>
<td>3850*</td>
<td></td>
<td></td>
<td>153</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>5B</td>
<td><em>Stoeckeria algicida</em></td>
<td>3640*</td>
<td></td>
<td></td>
<td>782</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>5C</td>
<td><em>Luciella masanensis</em></td>
<td>3490*</td>
<td></td>
<td></td>
<td>432</td>
<td></td>
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</table>

*Maximum value of mean growth and/or ingestion rates measured at the given prey concentrations.

Table 3. Growth and grazing data for *Oxyrrhis marina*, *Strombidinopsis jeokjo*, and *Acartia* spp. (*A. hongi* and *A. omorii*). Parameters are for numerical and/or functional response from Eqs. (2) & (3) as presented in Figs. 1–4. $\mu_{\text{max}}$ (maximum growth rate, d⁻¹), $K_{\text{IR}}$ (prey concentration sustaining 0.5 $\mu_{\text{max}}$, 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 0.5 $I_{\text{max}}$, ng C ml⁻¹), $C_{\text{max}}$ (maximum clearance rate, μl predator⁻¹ d⁻¹).
Acartia spp. feeding on P. piscicida, S. algicida, and L. masanensis were 153, 782, and 432 μl predator\(^{-1}\) h\(^{-1}\), respectively.

**Attack ratio and successful capture**

Strombidinopsis jeokjo had a significantly higher attack ratio, (number of attempted captures)/(number of physical contacts), when feeding on *Pfiesteria piscicida* (mean ± SE = 25 ± 5%) than when consuming *Luciella masanensis* (5 ± 1%) (1-tailed t-test, p < 0.005), but not significantly higher than with *Stoeckeria algicida* as prey (18 ± 3%) (p > 0.1) (Fig. 6A). *S. jeokjo* also had a significantly higher attack ratio when feeding on *Pfiesteria piscicida* without added filtered water of a *L. masanensis* culture than with added filtered water (9 ± 3%) (1-tailed t-test, p < 0.05).

Similarly, *Strombidinopsis jeokjo* had a significantly higher successful capture ratio, (number of prey...
ingested)/(number of attempted captures), when feeding on *Pfiesteria piscicida* (mean ± SE = 82 ± 6%) than when consuming *Luciella masanensis* (2 ± 2%) (1-tailed t-test, p < 0.005); however, the former ratio was not significantly lower than that for *S. jeokjo* feeding on *Stoeckeria algicida* (87 ± 5%) (p > 0.1) (Fig. 6B).

*S. jeokjo* also had a significantly higher successful capture ratio when feeding on *P. piscicida* without added filtered water of a *L. masanensis* culture than with added filtered water (49 ± 5%) (1-tailed t-test, p < 0.01). In the case of unsuccessful captures, *S. jeokjo* rejected the prey cells delivered to the mouth area.

**DISCUSSION**

**Differential feeding**

The present study reveals that despite the fact that *Pfiesteria piscicida*, *Stoeckeria algicida*, and *Luciella masanensis* belonging to the family Pfiesteriaceae have similar sizes and shapes, they caused different functional responses by *Oxyrrhis marina* and *Strombidinopsis jeokjo*. In particular, while *P. piscicida* and
S. algicida did support positive growth of the protist predators O. marina and S. jeokjo, L. masanensis did not. $I_{max}$ of S. jeokjo feeding on P. piscicida or S. algicida was substantially higher than when fed L. masanensis.

Several possible explanations exist for these prey dependent differences in ingestion rate and growth rate of Strombidinopsis jeokjo. (1) L. masanensis may be less attractive to S. jeokjo as prey compared to P. piscicida and S. algicida. S. jeokjo has much lower attack ratios when fed on L. masanensis than when fed on P. piscicida and S. algicida. Also, S. jeokjo had significantly higher attack and successful capture ratios when fed on P. piscicida without added filtered water of an L. masanensis culture than with added filtered water. This suggests that chemicals excreted from L. masanensis may inhibit feeding by S. jeokjo. Recently, Moeller et al. (2007) reported the nature of toxins produced by P. piscicida (e.g. metal complexes and free radical toxins) by using 5 different analytical methods. L. masanensis may have toxins that are more potent than those of P. piscicida; however, the presence and toxicity of the toxins of L. masanensis should be investigated in future studies. (2) S. jeokjo may have more difficulty in handling L. masanensis than P. piscicida and S. algicida. S. jeokjo has much lower successful capture ratios when fed on the former dinoflagellate prey than when fed on the latter dinoflagellate prey. (3) Some L. masanensis cells were observed to attack S. jeokjo, while no P. piscicida or S. algicida cells attacked their predators. This attack may be partially responsible for the decrease in the ingestion rate of L. masanensis by S. jeokjo at mean prey concentrations >270 ng C ml$^{-1}$ and indicates direct reversal of predator–prey relationships among heterotrophic protists. Only a few other reports have indicated the same; the heterotrophic dinoflagellate Protoperidinium divergens and the mixotrophic dinoflagellate Fragilidium cf. mexicanum were able to feed on each other (Jeong et al. 1997).

To understand the population dynamics of Pfiesteria piscicida, Stoeckeria algicida, and Luciella masanensis, data on the prey species, their growth rates ($k$) and mortality rates due to predation ($g$) should be obtained. P. piscicida and L. masanensis feed on diverse prey species and have several optimal and suboptimal prey species that support their high growth rates, such as fish blood cells, Amphidinium carterae, cryptophytes, and Heterosigma akashiwo (0.8 to 1.8 d$^{-1}$) (Burkholder & Glasgow 1995, 1997, Parrow et al. 2002, Seaborn et al. 2002, Lin et al. 2004, Jeong et al. 2005b, 2006, 2007a). However, S. algicida feeds only on H. akashiwo and blood cells (Jeong et al. 2005a, 2007a). When combining the kind of prey species, growth rates, and mortality rates due to predation, L. masanensis (with many prey species, high $k$ and low $g$) is the most competitive, followed by P. piscicida (with many prey species, high $k$ and high $g$), and finally S. algicida (with a few prey species, high $k$ on a few prey species but zero or low $k$ on many other co-occurring plankton, and high $g$) and the least competitive. L. masanensis is likely to dominate the assemblage of P. piscicida, S. algicida, and L. masanensis when protistan predators are abundant. Gransden & Lewitus (2003) reported that the growth rate of P. piscicida feeding on the cryptophyte Storeatula major was significantly higher than that of Cryptoperidiniopsis sp. and suggested that the euplotid ciliate Euplotes woodruffi had a greater ability to control the population growth of P. piscicida than Cryptoperidiniopsis sp. Therefore, P. piscicida, S. algicida, L. masanensis, and Cryptoperidiniopsis sp. have different ecological niches in marine planktonic food webs.

The results of the present study suggest that the predator with the lowest $I_{max}$ (Oxyrrhis marina) has a clearly different $I_{max}$ on these 3 different prey species (specialist), while the predators with the highest $I_{max}$ (Acartia spp.) have relatively similar $I_{max}$ values on these 3 different prey species (generalists). And the predator with a moderate $I_{max}$ (Strombidinopsis jeokjo)
shows a combination of specialist and generalist. The smallest predator O. marina may detect differences in taste, nutrition, and/or behavior of these 3 different prey species, but the largest predator Acartia spp. may not detect such differences. Feeding by some copepods is affected by some toxic mixotrophic dinoflagellates (e.g. Huntley et al. 1986). However, feeding of Acartia spp. was not affected by any chemicals excreted from Luciella masanensis, which might have inhibited the feeding of S. jeokjo. Also, Acartia tonsa was reported to graze both non-toxic and potentially toxic strains of Pfiesteria piscicida at approximately equal rates (Roman et al. 2006). Therefore, with regard to Acartia spp., the effect of the toxicity of the chemicals excreted from L. masanensis or P. piscicida is likely to be less than that of toxic mixotrophic dinoflagellates.

**Growth and ingestion rates**

Heterotrophic dinoflagellates feed on bacteria (Lessard & Swift 1985, Strom 1991, H. J. Jeong, K. A. Seong, T. H. Kim unpubl. data), algae (Jacobson 1987, Johnson et al. 2003, Menden-Deuer et al. 2005), heterotrophic nanoflagellates (Jeong et al. 2007b), ciliates (Burkholder & Glasgow 1995), eggs and naupliar stages of copepods (Jeong 1994), larvae of bivalves (Burkholder & Glasgow 1997, Springer et al. 2002), the blood of finfish (Burkholder & Glasgow 1997, Parrow et al. 2005, Jeong et al. 2006), and the epidermis, muscle, and gills of finfish (Burkholder & Glasgow 1997). However, prior to the present study, there had been no studies on the feeding by a heterotrophic dinoflagellate species on other heterotrophic dinoflagellate species. To enhance understanding of material cycling and energy flow in marine planktonic food webs, we should consider predator–prey relationships among heterotrophic dinoflagellates because usually several heterotrophic dinoflagellate species coexist (e.g. Jacobson 1987). The $\mu_{max}$ of Oxyrrhis marina feeding on Pfiesteria piscicida (0.66 d$^{-1}$; present study) is lower than that resulting from consumption of Heterosigma akashiwo (1.43 d$^{-1}$; Jeong et al. 2003), Amphidinium carterae (1.17 d$^{-1}$; Jeong et al. 2001), and the diatom Phaeodactylum tricornutum (1.30 d$^{-1}$; Goldman et al. 1989); however, it is comparable to that arising from predation on the phototrophic flagellates Dunaliella tertiolecta and Isochrysis galbana (0.79 d$^{-1}$) or the raphidophyte Fibrocapsa japonica (0.72 d$^{-1}$; Tillmann & Reckermann 2002), when corrected to 20°C using $Q_{10} = 2.8$ (Hansen et al. 1997). The $I_{max}$ of O. marina feeding on P. piscicida (0.33 ng C predator$^{-1}$ d$^{-1}$) is much lower than $I_{max}$ values arising from consumption of all these algal prey species (1.3 to 7 ng C predator$^{-1}$ d$^{-1}$). O. marina may have more difficulty and thus use more energy in capturing the heterotrophic dinoflagellate prey compared to the algal prey. However, P. piscicida as prey for O. marina may be more nutritional than the algal prey.

This is the first study on predation by Strombidinopsis spp. on Pfiesteria piscicida, Stockeria algicida, and Luciella masanensis. The $\mu_{max}$ of Strombidinopsis jeokjo feeding on P. piscicida and S. algicida (1.61 to 1.77 d$^{-1}$) was much higher than that resulting from the consumption of the heterotrophic dinoflagellates Gyrodinium dominans and Oxyrrhis marina (0.54 to 0.59 d$^{-1}$; Jeong et al. 2004) and also clearly higher than that from feeding on the optimal algal prey Cochlodinium polykrikoides and Akashiwo sanguinea (1.41 to 1.53 d$^{-1}$; Jeong et al. 1999), when corrected to 20°C using $Q_{10} = 2.8$ (Hansen et al. 1997). The $I_{max}$ in the present study is the highest observed value for S. jeokjo to date and thus P. piscicida and S. algicida are the optimal prey for culturing S. jeokjo. However, the $I_{max}$ of S. jeokjo feeding on P. piscicida and S. algicida (44 to 49 ng C predator$^{-1}$ d$^{-1}$) is comparable to that of toxic mixotrophic dinoflagellates Cochlodinium polykrikoides and Akashiwo sanguinea (1.41 to 1.53 d$^{-1}$; Jeong et al. 1999), when corrected to 20°C. These results suggest that (1) P. piscicida and S. algicida may sometimes comprise important prey for the growth of large Strombidinopsis species; and (2) P. piscicida and S. algicida are more nutritional as prey for S. jeokjo than G. dominans, O. marina, and algal prey. In natural environments, a higher abundance of S. jeokjo is expected when it co-occurs with P. piscicida and S. algicida compared to co-occurrence with G. dominans, O. marina, and algal prey.

The naked ciliates Euplotes spp., Strombidium spp., and Strobilidium sp. and the tintinnid ciliates Tintinnopsis spp., Tintinnidium spp., Nolaclusilis spp., and Favella spp. also feed on Pfiesteria piscicida (Stoecker et al. 2000, Lewitus et al. 2006). The maximum clearance rate of Strombidinopsis jeokjo feeding on P. piscicida (15 µl predator$^{-1}$ h$^{-1}$) is comparable to that of Tintinnopsis spp. (4 to 19 µl predator$^{-1}$ h$^{-1}$) for P. piscicida (15 µl predator$^{-1}$ h$^{-1}$) or Strombidium spp. (3 to 46 µl predator$^{-1}$ h$^{-1}$; Stoecker et al. 2000, Setellè et al. 2005), when corrected to 20°C using $Q_{10} = 2.8$ (Hansen et al. 1997). Therefore, S. jeokjo may compete with these ciliates for P. piscicida prey in cases where they coexist. To enhance understanding of the interactions between dominant ciliates and P. piscicida, Stoeckeria algicida, and Luciella masanensis in marine environments, it is necessary to explore the numerical and functional responses of the ciliates to the prey concentration when fed on these 3 dinoflagellate prey.
The calanoid copepod *Acartia tonsa* feeds on *Pfiesteria piscicida* (Mallin et al. 1995, Roman et al. 2006). The highest value (64 180 cells predator⁻¹ d⁻¹) among the ingestion rates of *Acartia* spp. (A. hongi and A. omorii) on *P. piscicida* at mean prey concentrations <17 500 cells ml⁻¹ is greater than rates for consumption of both non-toxic and toxic strains of *P. piscicida* (31 920 and 29 760 cells predator⁻¹ d⁻¹, respectively) (Roman et al. 2006). A. hongi or A. omorii are considerably longer than *A. tonsa*. This difference in size among *Acartia* species and variation in the *P. piscicida* strains ingested may be responsible for the difference in ingestion rates.

Only a few studies have reported on predation by copepods on *Pfiesteria piscicida* and other species belonging to the family Pfiesteriaceae. Thus, to enhance understanding of the interactions between these dinoflagellates and copepods in the natural environment, it would be worthwhile to investigate feeding by dominant copepods on *P. piscicida* and other dinoflagellate species.

Microzooplankton occasionally have considerable predation impact on the populations of *Pfiesteria piscicida*, and hence control the prey population; however, metazooplankton, including copepods, usually do not affect the prey populations (Stoecker & Gustafson 2002, Roman et al. 2006). Nevertheless, it is very difficult to measure in situ ingestion rates of *Oxyrrhis marina* or *Strombidinopsis jeokjo* feeding on *Stoeckeria algicida* and/or *Luciella masanensis* or to calculate the predators’ impact by combining the data on their ingestion rates and abundance because (1) differentiating among *P. piscicida*, *S. algicida*, and *L. masanensis* and then measuring their abundances in fixed natural samples remains difficult because advanced methods (such as DNA probes) of identifying *S. algicida* and *L. masanensis* have not been developed yet; (2) ingestion rates of *O. marina* or *S. jeokjo* feeding on each of *P. piscicida*, *S. algicida*, and *L. masanensis* differ greatly from those observed for consumption of the other prey species. Therefore, to enhance understanding of the interactions between *P. piscicida*, *S. algicida*, and *L. masanensis* and their predators at the population level, advanced methods for identifying these 3 dinoflagellates must be developed.

For a long time, species belonging to the family Pfiesteriaceae were probably not easily recognized due to their small size and the difficulty of establishing monoclinal cultures. However, recently, several new species in this family have been discovered (Jeong et al. 2005b, Litaker et al. 2005, Mason et al. 2007). The present study suggests that species in this family may have a distinct ecological niches and may be differentiated from each other based on their prey species, differential growth and ingestion rates (Lin et al. 2004, Jeong et al. 2005a, 2006, 2007) as well as the differential mortality rate due to predation as shown in the present study. Thus, to understand the roles of a newly discovered species in marine planktonic food webs, predator-prey relationships between the species and coexisting prey species, its growth and ingestion rates resulting from feeding on diverse prey, and the numerical and functional responses of the potential predators, in particular, heterotrophic protistans should be investigated simultaneously.

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**LITERATURE CITED**


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