INTRODUCTION

The heterotrophic dinoflagellate *Pfiesteria piscicida* is known to form a bloom that is harmful to finfish (Burkholder et al. 1992, Glasgow et al. 2001) and shellfish (Springer et al. 1996). *P. piscicida* has been observed in many countries (Rublee et al. 2004, 2005); therefore, many sectors of the community around the globe are concerned about the appearance of *P. piscicida* in their coastal waters. However, its detailed distribution, morphological variations, and DNA sequences of isolates have been reported for only a few countries (Steidinger et al. 1996, 2001, Rublee et al. 1999, 2001a,b, 2005, Burkholder et al. 2004).
The DNA sequences (small subunit [SSU], internal transcribed spacers [ITS] 1 and 2, 5.8 S rDNA, and large subunit [LSU]) of all USA isolates of *Pfiesteria piscicida* from several locations (e.g. GenBank accession numbers AF330600 to AF330620) were identical (summarized by Rublee et al. 2005), but differed slightly from that of a Norwegian isolate (GenBank accession number AY033488; Jakobsen et al. 2002). To determine the presence of *P. piscicida* in the waters of a region, and to explore the genetic relationships among isolates of *P. piscicida* from different locations in a region, their DNA sequences should be analyzed and compared to those of the USA and Norway isolates.

*Pfiesteria piscicida* feeds on prey using a peduncle after anchoring the prey by a tow filament (Burkholder & Glasgow 1995), and is thus able to feed on diverse prey species within a wide size range: bacteria (Burkholder & Glasgow 1995, 1997), cyanophytes (Burkholder & Glasgow 1997, Glasgow et al. 1998), cryptophytes (Burkholder & Glasgow 1997, Glasgow et al. 1998, Lewitus et al. 1999, Seaborn et al. 1999, 2001, Burkholder et al. 2001b, Parrow et al. 2001, Feinstein et al. 2002, Gransden & Lewitus 2003, Lin et al. 2004), chrysophytes (Burkholder & Glasgow 1997), diatoms (Glasgow et al. 1998, Burkholder et al. 2001b, Burkholder & Glasgow 1995, 1997, Parrow et al. 2001, Seaborn et al. 2001), autotrophic dinoflagellates (Burkholder & Glasgow 1997), ciliates (Burkholder & Glasgow 1995), larvae of bivalves (Burkholder & Glasgow 1997, Springer et al. 2002), the blood of finfish (Burkholder & Glasgow 1997, Glasgow et al. 1998), and the epidermis, muscle, and gills of finfish (Burkholder & Glasgow 1997) are known to be eaten. It has not yet been investigated whether mixotrophic dinoflagellates and/or raphidophytes are eaten by *P. piscicida*; this is, however, likely, because many mixotrophic dinoflagellate and raphidophyte species co-occur with *P. piscicida*, and these algae often provide a large amount of materials and energy to heterotrophic protistan grazers (e.g. Stoecker et al. 1981, Jeong & Latz 1994, Kamiyama & Arima 2001, Tillmann 2004).

Growth and/or ingestion rates of *Pfiesteria piscicida* have been reported for only a few prey items (Glasgow et al. 1998, Parrow et al. 2001, Seaborn et al. 2001, Feinstein et al. 2002, Lin et al. 2004); growth rates were reported when fed the cyanobacterium *Synechococcus* sp., the cryptophytes *Rhodomonas* spp. and *Cryptomonas* spp., and/or the diatom *Thalassiosira weissflogii*, whereas ingestion rates were reported when fed *Rhodomonas* spp. and/or the blood of fish or humans. Therefore, the limited number of prey species tested has constrained our understanding of the ecology of *P. piscicida* in marine ecosystems. Moreover, to date Lin et al. (2004) is the only report on the growth and ingestion rates of *P. piscicida* as a function of prey concentration, in which the growth and grazing rates on *Rhodomonas* sp. of *P. piscicida* isolated from USA waters is provided.

The overall objectives of the present study were to investigate the distribution of *Pfiesteria piscicida* in Korean waters, and to understand the feeding of Korean isolates on co-occurring mixotrophic dinoflagellates and raphidophytes and on fish blood. To investigate the distribution of *P. piscicida* in Korean waters, we took water samples from several locations along the coasts of the Korean peninsula, established several monoclonal cultures of *P. piscicida* and *Pfiesteria*-like dinoflagellates (PLDs), and then analyzed the morphology and DNA sequence of each isolate. In particular, in order to compare the DNA sequences of Korean isolates with those of the USA and Norway isolates, we analyzed ITS 1 and ITS 2, 5.8 S rDNA, LSU, and SSU. To understand the ecology of *P. piscicida* in Korean waters, we also examined (1) the kinds of prey on which a Korean isolate fed (prey species available: 12 mixotrophic dinoflagellates, 2 raphidophytes, 2 cryptophytes, 1 diatom species, and 3 different blood cells); (2) the growth and ingestion rates of *P. piscicida* fed 9 edible algal prey species at single prey concentrations; and (3) the numerical and functional responses of *P. piscicida* when fed the dinoflagellate *Amphidinium carterae*, an unidentified cryptophyte species (equivalent spherical diameter, ESD = 5.6 μm), the cryptophyte *Rhodomonas salina*, the raphidophyte *Heterosigma akashiwo*, and perch blood cells in the laboratory. Finally, we estimated grazing coefficients of *P. piscicida* on algal prey, by combining field data on the abundance of *P. piscicida* (and PLDs) and co-occurring dominant algae with laboratory data on ingestion rates obtained in the present study.

Based on the maximum growth and ingestion rates of *Pfiesteria piscicida* when fed diverse prey species, we determined the optimal prey supporting the maximum growth rate of *P. piscicida* when fed diverse prey species, or higher than that obtained when fed *Rhodomonas* sp., which has been known to be an optimal prey so far (Parrow et al. 2001, Lin et al. 2004). To understand the similarity in feeding between Korean and USA isolates of *P. piscicida*, the maximum growth and ingestion rates of the Korean isolate fed on *R. salina* (obtained in the present study) were compared to those of the USA isolate fed on *Rhodomonas* sp. The maximum growth and grazing rates of the Korean isolate fed on algal prey (obtained in the present study) were also compared to literature values of other heterotrophic and mixotrophic protists fed on the same prey. The results
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of the present study provide a basis for understanding the distribution of _P. piscicida_ in Korean waters, interactions between _P. piscicida_ and bloom-forming algal species, and the potential for _P. piscicida_ to influence the population dynamics of algal prey.

**MATERIALS AND METHODS**

**Isolation and culture of _Pfiesteria piscicida_.** Using water samplers, plankton samples were collected from the surface waters off Busan, Incheon, Kunsan, Kwangyang, Masan, and Yeosu, Korea, in July 2005 (Fig. 1, Table 1). Those samples for the culture of living organisms were screened gently through a 154 μm Nitex mesh and placed in three 270 ml polycarbonate (PC) bottles. The first bottle was enriched with a mixture comprising an unidentified cryptophyte (5.6 μm in size), _Rhodomonas salina_, and _Amphidinium carterae_, and the second bottle was enriched with perch blood cells. The third bottle was not enriched and served as a control. The bottles were transported within 8 h to one of 2 laboratories (Seoul National University or Kunsan National University) by temperature-controlled cars (20 ± 2°C). Once in the laboratory, bottles were placed on plankton wheels rotating at 0.9 rpm, and were incubated at 20°C under an illumination of 20 μE m⁻² s⁻¹ provided by cool white fluorescent light on a 14:10 h light:dark cycle. Each day, bottles were removed from the rotating wheels and examined under dissecting microscopes. Three to 7 d later, aliquots of enriched water were transferred to 6-well tissue culture plates, and monoclonal cultures of _P. piscicida_ from each location were established via 2 serial single cell isolations. As concentrations of _P. piscicida_ increased, these predators were sequentially transferred to 32, 80, 270, and 500 ml PC bottles containing _A. carterae_. Bottles were again filled to capacity with freshly filtered seawater, capped, and placed on a rotating wheel as described above. Once dense cultures of _P. piscicida_ (ca. 10 000 cells ml⁻¹) were obtained, they were transferred to 500 ml PC bottles containing _A. carterae_ (ca. 20 000 to 30 000 cells ml⁻¹). The carbon content of _P. piscicida_ was estimated from cell volume according to Menden-Deuer & Lessard (2000).

Additionally, each sample for counting was transferred into a 100 ml polyethylene (PE) bottle and preserved in 50% Bouin’s solution (final conc.), into a 20 ml scintillation vial and preserved in 1% glutaraldehyde (final conc.), into a 20 ml scintillation vial and fixed in 4% formalin (final conc.), and into a 500 ml PE bottle and preserved in 5% Lugol’s solution (final conc.). The abundances of phototrophic plankton and heterotrophic protists were determined by enumerating cells in two 1 ml Sedgwick-Rafter chambers (SRCs).

**Morphology of _Pfiesteria piscicida_.** Distilled water was added to a culture of each isolate of _P. piscicida_ satiated with perch blood cells (final salinity: ca. 10 psu). The culture was fixed in 0.5% v/v formalin, and cells were stained with a drop of 10 μg ml⁻¹ calcoflour white M2R before examination under an epifluorescence microscope.

For scanning electron microscopy (SEM), a 5 ml aliquot of a dense culture of _Pfiesteria piscicida_ was fixed using a cold double-fixation method modified from Steidinger et al. (1989). Fixed cells were rinsed 3 to 5 times with distilled water, dehydrated through an ethanol series, and finally critical-point-dried with carbon dioxide. Filters were mounted on a stub and coated with gold-palladium. Cells were viewed with a Jeol JSM-840 SEM and photographed with a Polaroid camera.

**DNA extraction, PCR amplification, sequencing, and data analysis of _Pfiesteria piscicida_.** For DNA extraction, cells of each isolate of _P. piscicida_ starved for 2 d were collected by filtering approximately 50 ml culture onto a 25 mm, 3 μm pore size PC membrane. Genomic DNA was extracted with an UltraClean Soil DNA Kit (Mobio) following the manufacturer’s instructions.

![Fig. 1. Collection locations of _Pfiesteria piscicida_ in Korea: Busan, Incheon, Kunsan, Kwangyang, Masan, and Yeosu](image-url)
Extracted DNA was divided into at least 5 PCR tubes, and more than 5 independent PCR reactions were performed for each isolate. The SSU, ITS 1, 5.8 S, ITS 2, and partial LSU rDNA were amplified using dinoflagellate universal primers and ITS2R (forward: 5’-CAACCTGGTATCTCGCCAGT-3’; reverse: 5’-TCCCTGTTCATTGCGGCATTAC-3’) according to the method of Litaker et al. (2003). A 20 μl PCR was mixed with the following reactants: 1× PCR buffer with 1.5 mM MgCl₂, 0.2 mM dNTP, 0.5 μM of each primer, 5 U Taq DNA polymerase (Bioneer), and 200 ng template DNA. PCR cycles were performed in a UVgeneflex Thermal cycler (UVITEC) under the following conditions: 1 initial cycle of 3 min at 94°C; a series of 35 cycles of 30 s at 92°C, 45 s at 55°C, and 3 min at 72°C; and 1 extension cycle at 72°C for 10 min. PCR products were cloned into the pGEM-T EASY vector (Promega). The cloned materials (>3 clones for each independent PCR) were incubated overnight in liquid LB media at 37°C. Plasmids were extracted from these incubated materials using the AccuPrep Plasmid Extraction Kit (Bioneer). The presence of inserts into plasmids was ascertained by addition of EcoRI restriction endonuclease (Promega) to extracted plasmids. To determine the sequences of the internal fragments of the inserts, the internal primers G17F (5’-ATA CCG TCC TAG TCT TAA CC-3’) and G18R (5’-GCA TCA TCCCTGTTCATTGCGGCATTAC-3’) were used according to the method of Litaker et al. (2003). Sequencing of SSU, ITS 1, 5.8 S, ITS 2, and LSU rDNA was performed using an ABI PRISM 3700 DNA Analyzer (Applied Biosystems). All sequences were aligned using the Contig-Express alignment program (InforMax).

Preparation of prey. Phototrophic cells were grown at 20°C in enriched f/2 seawater media (Guillard & Ryther 1962) without silicate, with continuous illumination of 20 μE m⁻² s⁻¹ provided by cool white fluorescent light. Only cultures in the exponential growth phase were used for feeding experiments. The carbon contents of Heterosigma akashiwo (0.1 ng C cell⁻¹, n > 2000), Rhodomonas salina (0.056 ng C cell⁻¹, n > 2000), and an unidentified cryptophyte (0.017 ng C cell⁻¹, n > 2000) were estimated from cell volume according to Strathmann (1967).

Perch blood cells were obtained from adults by cutting the fin of the tail (ca. 50 cm). The carbon content for perch blood cells (0.009 ng C cell⁻¹, n > 2000) and Amphidinium carterae (0.07 ng C cell⁻¹, n > 2000) was measured using a CHN analyzer (CM 5012 CO₂, Coulometer, UIC).

Expt 1 — Prey species consumed by Pfiesteria piscicida. Expt 1 was designed to investigate whether P. piscicida was able to feed on each target prey species (Table 2). The initial concentrations of each prey species offered were similar in terms of carbon biomass. To confirm no ingestion of some prey species by P. piscicida, additional higher prey concentrations were provided. Mean prey size for each species (n > 2000) was measured by an electronic particle counter (Coulter Multisizer II, Coulter Corporation).

A dense culture of Pfiesteria piscicida (strain PPIC0507), which had been growing on Amphidinium carterae and then starved for 1 d, was transferred to a 1 l PC bottle containing freshly filtered seawater. Three 1 ml aliquots were then removed from the bottle and examined using a compound microscope to determine the concentration of P. piscicida.

In this experiment, initial concentrations of Pfiesteria piscicida and each target prey species were established using an autotipette to deliver a predetermined volume of culture of known cell density to the experimental bottles. Duplicate 42 ml PC bottles (mixtures of P. piscicida and target prey) and duplicate predator control bottles (P. piscicida only) were set up for each target prey species. The bottles were filled to capacity with freshly filtered seawater, capped, and placed on a rotating wheel at 0.9 rpm and incubated at 20°C under an illumination of 20 μE m⁻² s⁻¹ provided by cool white fluorescent light on a 14:10 h light-dark cycle. After 6, 12, 24, and 48 h incubation, a 5 ml aliquot was removed from each bottle and transferred into a 6-well plate chamber. Bottles were again filled to capacity with fresh f/2 medium, capped, and placed on the rotating wheel. For each target species, the feeding behavior of >10 P. piscicida cells that were capturing prey cells and >30 unfed P. piscicida cells was then observed under a dissecting microscope at a magnification of 50 to 90×, to determine whether P. piscicida was able to feed on the target prey species.

Expt 2 — Comparison of growth and ingestion rates at single prey concentrations. Expt 2 was designed to compare the growth and ingestion rates of Pfiesteria piscicida when feeding on mixotrophic dinoflagellates (Amphidinium carterae, Heterocapsa rotundata, Cocchidiuninum polykrikoides, Akashiwo sanguinea, and Gymnodinium catenatum), raphidophytes (Chattonella ovata and Heterosigma akashiwo), cryptophytes (Rhodomonas salina and an unidentified cryptophyte), and perch blood cells at single prey concentrations. These prey species were shown to be consumed by P. piscicida in Expt 1.

One day before initiation of Expt 2, a dense culture (ca. 10 000 cells ml⁻¹) of Pfiesteria piscicida (strain PPIC0507) growing on Amphidinium carterae (20 000 to 30 000 cells ml⁻¹) was transferred into a 500 ml PC bottle, and cells were then starved to minimize possible residual growth. The abundances of P. piscicida and its prey were determined by enumerating the cells present in three 1 ml SRCs.
Initial concentrations of *Pfiesteria piscicida* and each target prey were established using an autopipette to deliver predetermined volumes of known cell concentrations into the bottles. Triplicate 42 ml PC experiment bottles (containing mixtures of predator and prey) and triplicate control bottles (containing prey only) were set up for each predator–prey combination. Triplicate control bottles containing only *P. piscicida* were also established at 1 predator concentration. To ensure similar water conditions, water from predator cultures was filtered through a 0.7 μm GF/F filter, and the same volume was added to prey control bottles as those added to experiment bottles for each predator–prey combination. Next, 10 ml of f/2 medium was added to all bottles, which were then filled to capacity with freshly filtered seawater and capped. To determine actual predator and prey concentrations at the beginning of the experiment and after 1 d (perch blood cells) or 2 d (algal prey) of incubation, a 5 µl aliquot was removed from each bottle and fixed with 5% Lugol's solution, and all or >200 predator and prey cells in three 1 ml SRCs were enumerated. Prior to taking subsamples, the condition of *P. piscicida* and prey was assessed under a dissecting microscope. The bottles were filled again to capacity with freshly filtered seawater, capped, and placed on a rotating wheel at 0.9 rpm at 20°C under an illumination of 20 µE m–2 s–1 provided by cool white fluorescent light on a 14:10 h light:dark cycle. Dilution of cultures that resulted from the refilling of bottles was considered when calculating growth and ingestion rates.

The specific growth rate (μ, d–1) of *Pfiesteria piscicida* was calculated as:

$$\mu = \frac{\ln \left( L_t / L_0 \right)}{t}$$  \hspace{1cm} (1)

where $L_0$ = initial concentration of *Pfiesteria piscicida*, and $L_t$ = final concentration after time $t$ ($t = 1$ d for perch blood cells; 2 d for algal prey). Approximately 20% of blood cells were observed to shrink after 1 d.

The ingestion and clearance rates were calculated using the equations of Frost (1972) and Heinbokel (1978). Incubation times for the calculation ingestion and clearance rates were the same as those for estimation of growth rate.

**Expt 3 — Effects of prey concentration.** Expt 3 was designed to measure the growth, ingestion, and clearance rates of *Pfiesteria piscicida* (strain PPIC0507) as a function of prey concentration when fed *Amphidinium carterae*, *Heterosigma akashiwo*, *Rhodomonas salina*, an unidentified cryptophyte, and perch blood cells. We chose *A. carterae*, *H. akashiwo*, and the unidentified cryptophyte as prey species because they were the dominant algal species when *P. piscicida* and PLDs were abundant in coastal waters off Masan, Busan, and Yeosu in 2004-05. We also chose *R. salina* because *Rhodomonas* sp. was previously identified to be an optimal prey for *P. piscicida* (Parrow et al. 2001, Lin et al. 2004), and perch blood cells because (1) they were observed to support maximum growth of *P. piscicida* in our preliminary test and (2) perch is one of the most common commercial fish cultivated in the coastal waters of Korea.

One day before each experiment with target prey species was started, a dense culture (ca. 10 000 cells ml–1) of *Pfiesteria piscicida* growing on prey species was transferred into two 500 ml PC bottles containing low (for 3 to 4 lower prey concentrations in Expt 3) and medium concentrations (for 4 to 5 medium and higher prey concentrations) of the same prey species. This was done to maximize any possible residual growth resulting from ingestion of prey during batch culture. The maintaining concentration of each prey, and its concentrations in the low and medium treatments, are as follows. Perch blood cells: ca. 300 000 cells ml–1, low 10 000 cells ml–1, medium 50 000 cells ml–1; *Amphidinium carterae*: ca. 20 000 to 30 000 cells ml–1, low 500 cells ml–1, medium 5000 cells ml–1; *Rhodomonas salina*: ca. 40 000 to 50 000 cells ml–1, low 500 cells ml–1, medium 6000 cells ml–1; *Heterosigma akashiwo*: ca. 20 000 to 30 000 cells ml–1, low 500 cells ml–1, medium 5000 cells ml–1; unidentified cryptophyte: ca. 70 000 to 90 000 cells ml–1, low 500 cells ml–1, medium 4000 cells ml–1. All experiments were initiated when prey cells were not detectable in ambient waters. The abundances of *P. piscicida* and its target prey were determined by enumerating the cells present in three 1 ml SRCs.

Expt 3 was set up in the same manner as Expt 2. The range of mean actual prey (and predator) concentrations (±SE, n = 3) in the experimental bottles at the beginning of the experiment were 430 (3) to 896 660 (29 370) cells ml–1 [and 13 (0.3) to 160 (2.3) cells ml–1] for the blood cell, 90 (5) to 45 740 (770) cells ml–1 [and 16 (0.3) to 160 (3.8)] for *Amphidinium carterae*, 90 (5) to 45 740 (270) cells ml–1 [and 13 (1) to 180 (9)] for *Rhodomonas salina*, 90 (2) to 30 140 (1010) cells ml–1 [and 15 (1) to 190 (2)] for *Heterosigma akashiwo*, and 340 (42) to 126 780 (5270) cells ml–1 [and 14 (3) to 150 (3)] for a cryptophyte. The actual predator concentration in the control bottles containing only *Pfiesteria piscicida* for all these experiments was 140 to 170 cells ml–1. Bottles were incubated as in Expt 2.

Growth rate, μ, and ingestion rates of *Pfiesteria piscicida* when fed each target prey were calculated as in Expt 2. Data for the growth rate of *P. piscicida* were fitted to the Michaelis-Menten equation:

$$\mu = \frac{\mu_{\text{max}} (x-x')}{K_{GR} + (x-x')}$$  \hspace{1cm} (2)
where $\mu_{\text{max}}$ = maximum growth rate (d$^{-1}$), $x$ = prey concentration (cells ml$^{-1}$ or ng C ml$^{-1}$), $x'$ = threshold prey concentration (prey concentration where $\mu = 0$), and $K_{\text{IR}}$ = prey concentration sustaining $\frac{1}{2} \mu_{\text{max}}$. Data were iteratively fitted to the model using DeltaGraph (DeltaPoint).

Ingestion and clearance rates were also calculated as in Expt 2. Ingestion rate (IR) data for *Pfiesteria piscicida* were fitted to a Michaelis-Menten equation:

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

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

**Cell volume of Pfiesteria piscicida.** After incubation, cell length and maximum width of *P. piscicida* preserved in 5% acid Lugol's solution (n = 30 for each prey concentration) were measured using an image analysis system on images collected with a compound microscope (Image-Pro Plus 4.5, Media Cybernetics). The shape of *P. piscicida* was estimated as an oval. The cell volume of preserved *P. piscicida* was calculated as:

$$\text{Volume} = \frac{4}{3}\pi[(\text{cell length} + \text{cell width})/4]^3 \quad (4)$$

**Gross growth efficiency.** Gross growth efficiency (GGE), defined as predator biomass produced (+) or lost (-) per prey biomass ingested, was calculated from estimates of carbon content per cell based on cell volume for each mean prey concentration.

**Potential grazing effect.** By combining field data on abundances of the predator and target prey with the ingestion rates of the predator on prey obtained in the present study, we estimated the grazing coefficients attributable to *Pfiesteria piscicida* on co-occurring *Amphidinium* spp., *Heterosigma akashiwo*, and cryptophytes that supported high growth of the predator in Expts 2 and 3. Measuring the abundance of *P. piscicida* in fixed natural samples is difficult because PLDs usually co-occur with *P. piscicida*, and distinguishing between the morphology of *P. piscicida* and PLDs is very difficult. Therefore, we assumed that (1) ingestion rates of the co-occurring algae do not vary between PLDs and *P. piscicida*, and (2) ingestion rates of other *Amphidinium* species and cryptophytes by *P. piscicida* are the same as those of *A. carterae* and the unidentified cryptophyte observed in the present study.

Data on abundances of *Pfiesteria piscicida* and co-occurring *Amphidinium* spp., *Heterosigma akashiwo*, and cryptophytes used in this estimate were obtained from water samples collected from coastal waters off Masan (2004-05), Busan (2005), Kwangyang (2005), and Yeosu (2005).

Grazing coefficients (g, h$^{-1}$) were calculated as:

$$g = CR \times PC \quad (5)$$

where CR = clearance rate of a target prey by *Pfiesteria piscicida* at a given prey concentration (ml predator$^{-1}$ h$^{-1}$), and PC = predator concentration (cells ml$^{-1}$). CR was calculated as:

$$CR = \frac{\text{IR}}{x} \quad (6)$$

where IR = ingestion rate of target prey by *Pfiesteria piscicida* (cells ingested predator$^{-1}$ h$^{-1}$), and $x$ = prey concentration (cells ml$^{-1}$). CR were corrected using $Q_{01} = 2.8$ (Hansen et al. 1997), because *in situ* water temperatures and the temperature used in the laboratory for this experiment (20°C) were sometimes different.

**RESULTS**

**Morphology and gene sequence of Korean isolates of Pfiesteria piscicida**

*Pfiesteria piscicida* was observed in coastal waters off Busan, Incheon, Kunsan, Kwangyang, Masan, and Yeosu, Korea, in July 2005 when water temperature and salinity were 21.4 to 26.8°C and 11.8 to 30.4 psu, respectively (Fig. 1, Table 1).

Biflagellated cells of Korean isolates of *Pfiesteria piscicida* are oval and have a markedly impressed cingulum. Thin plates are arranged in a Kofoidian series of *P. piscicida* isolated from waters off Busan (strain PPBS0507), Kunsan (PPKS0507), Yeosu (PPYS0507), Incheon (PPIC0507), Kwangyang (PPKY0507), and Masan (PPMS0507) were almost identical.

The ranges (mean ± SE, n = 30) of cell length and width of live biflagellated cells of *Pfiesteria piscicida* (strain PPIC0507) satiated with *Heterosigma akashiwo* were 9.1 to 17.0 μm (13.5 ± 0.3 μm) and 6.4 to 14.2 μm (11.0 ± 0.3 μm), respectively, whereas those of live biflagellated cells starved for 2 d (n = 30) were 5.2 to 10.1 μm (7.7 ± 0.2 μm) and 4.3 to 7.6 μm (6.1 ± 0.2 μm), respectively. The ratio of cell length to cell width of live biflagellated cells starved for 2 d (mean ± SE = 1.3 ± 0.03; range = 1.1 to 1.5; n = 30) was similar to that of cells satiated with *H. akashiwo* (mean ± SE = 1.2 ± 0.01; range = 1.1 to 1.4; n = 30).

The SSU, ITS 1, 5.8 S, ITS 2, and partial LSU rDNA sequences of *Pfiesteria piscicida* obtained from 5 independent PCRs for each isolate were identical (2540 nucleotides; Table 1). The DNA sequence of strain PPMS0507 was identical to that of USA isolates, but the DNA sequences of strains PPBS0507,
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PPIC0507, PPKS0507, PPKY0507, and PPYS0507 differed by 1 bp (A instead of C at the 63rd nucleotide in SSU) (Table 1).

**Prey species**

Among the prey offered in the present study, *Pfiesteria piscicida* was able to feed on the diatom *Skeletonema costatum*, the cryptophytes *Rhodomonas salina* and an unidentified cryptophyte species, the naked mixotrophic dinoflagellates *Amphidinium carterae*, *Akashiwo sanguinea*, *Cochlodinium polykrikoides*, *Gymnodinium catenatum*, the small thecate mixotrophic dinoflagellate *Heterocapsa rotundata*, the raphidophytes *Chattonella ovata* and *Heterosigma akashiwo*, the blood cells of perch and flounder, and human red blood cells, whereas it did not feed on living cells of large thecate mixotrophic dinoflagellates *Prorocentrum minimum*, *Heterocapsa triquetra*, *Scrippsiella trochoidea*, *Alexandrium catenella*, *Gonyaulax polygramma*, and *Lingulodinium polyedrum* that were of ESD > 12 μm (Table 2). However, *P. piscicida* was able to feed on dead cells of these large thecate mixotrophic dinoflagellates.

**Comparison of growth and ingestion rates at single prey concentrations**

We measured the growth and ingestion rates of *Pfiesteria piscicida* on 9 edible prey species at single prey concentrations (Table 3). When mean prey concentrations were ca. 800 to 1700 ng C ml⁻¹, the specific growth rates of *P. piscicida* on *Heterocapsa rotundata* and *Chattonella ovata* (0.32 to 0.37 d⁻¹, n = 6) and *Cochlodinium polykrikoides*, *Akashiwo sanguinea*, and *Gymnodinium catenatum* (0.15 to 0.17 d⁻¹, n = 6) were significantly lower than those of *P. piscicida* on perch blood cells, *Rhodomonas salina*, the unidentified cryptophyte, *Amphidinium carterae*, and *Heterosigma akashiwo* (0.74 to 1.57 d⁻¹, n = 12; p < 0.01, 1-tailed t-test) (Table 3).

The ingestion rates of *Pfiesteria piscicida* on *Heterocapsa rotundata*, *Chattonella ovata*, *Cochlodinium polykrikoides*, *Akashiwo sanguinea*, and *Gymnodinium catenatum* (0.03 to 0.19 ng C predator⁻¹ d⁻¹, n = 15) were also significantly lower than those of *P. piscicida* on perch blood cells, *Rhodomonas salina*, the unidentified cryptophyte, *Amphidinium carterae*, and *Heterosigma akashiwo* (0.48 to 3.59 ng C predator⁻¹ d⁻¹, n = 12; p < 0.01, 1-tailed t-test) (Table 3).

Data from this study show that specific growth rates of *Pfiesteria piscicida* on 8 naked microalgae were negatively correlated with prey size (p < 0.05, linear regression ANOVA), whereas ingestion rates showed no clear correlation with prey size (p > 0.1) (Fig. 2).

**Effects of prey concentration**

We investigated the numerical and functional responses of *Pfiesteria piscicida* when fed prey species that supported a relatively high rate of growth of this predator. With increasing mean prey concentration, the specific growth rate of *P. piscicida* on each diet of perch blood cells, *Rhodomonas salina*, *Amphidinium carterae*, and *Heterosigma akashiwo* increased rapidly with increasing mean prey concentration before reaching saturation at mean prey concentrations of ca. 100 to 600 ng C ml⁻¹ (Figs. 3A–D). In contrast, that on the unidentified cryptophyte increased rapidly at prey concentrations less than ca. 1100 ng C ml⁻¹, but increased slowly at higher prey concentrations (Fig. 3E). When data were fitted to Eq. (2), the maxi-
Table 2. Expt 1. Prey species, size (equivalent spherical diameter, ESD, μm), and concentration (cells ml⁻¹) of prey offered as food to *Pfiesteria piscicida*. To confirm no ingestion of some prey species, additional higher prey concentrations were provided. T: thecate; NT: non-thecate; Y: *P. piscicida* was observed to feed on living cells; N: *P. piscicida* was not observed to feed on living cells.

**Table 3.** *Pfiesteria piscicida*. Comparisons of growth (μR, d⁻¹) and ingestion rates (IR, ng C predator⁻¹ d⁻¹) (means ±SE, n = 3) on dinoflagellate (DN), raphidophyte (RA), and cryptophyte (CR) species at single mean prey concentrations (MPC, ng C ml⁻¹)
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(FIG. 5A). After 48 h incubation, the mean cell volume of P. piscicida fed Rhodomonas salina, Amphidinium carterae, or Heterosigma akashiwo increased from 250–350 to 910–1230 μm³ with increasing mean prey concentration before becoming saturated, whereas those of starved P. piscicida were 110 to 250 μm³ (Fig. 5B–D). However, mean cell volume of P. piscicida fed the unidentified cryptophyte did not change markedly (290 to 350 μm³) with increasing mean prey concentration, and that of starved P. piscicida was 240 μm³ (Fig. 5E).

Gross growth efficiency

GGE of Pfiesteria piscicida fed perch blood cells, Rhodomonas salina, Amphidinium carterae, and Heterosigma akashiwo were negative at mean prey concentrations of ≤4 to 67 ng C ml⁻¹. However, GGE of P. piscicida at higher mean prey concentrations were 3 to 14% for perch blood cells, 7 to 27% for A. carterae, 23 to 62% for H. akashiwo, and 30 to 103% for R. salina.

Potential grazing effect

Grazing coefficients attributable to Pfiesteria piscicida on co-occurring Amphidinium spp. in coastal waters off Masan, Kwangyang, and Yeosu in 2004–05, when abundances of Amphidinium spp. and P. piscicida + PLDs were 1 to 16 000 cells ml⁻¹ (mean ± SE = 2180 ± 740 cells ml⁻¹, n = 35) and 4 to 2560 cells ml⁻¹ (240 ± 80 cells ml⁻¹), respectively, were 0.01 to 1.07 h⁻¹ (0.20 ± 0.05 h⁻¹) (Fig. 6A).

Grazing coefficients attributable to Pfiesteria piscicida on co-occurring Heterosigma akashiwo in coastal waters off Masan in 2004–05, when abundances of H. akashiwo and P. piscicida + PLDs were 20 to 87 680 cells ml–¹ (mean ± SE = 12 890 ± 3740 cells ml⁻¹, n = 29) and 40 to 10 200 cells ml⁻¹ (1530 ± 440 cells ml⁻¹), respectively, were 0.01 to 0.45 h⁻¹ (0.06 ± 0.02 h⁻¹) (Fig. 6B).

Grazing coefficients attributable to Pfiesteria piscicida on co-occurring cryptophytes in coastal waters off Masan, Busan, Kwangyang, and Yeosu in 2004–05, when abundances of cryptophytes and P. piscicida + PLDs were 40 to 392 440 cells ml–¹ (mean ± SE = 54 460 ± 10 240 cells ml⁻¹, n = 57) and 10 to 10 200 cells ml–¹ (860 ± 240 cells ml–¹), respectively, were 0.00 to 0.22 h⁻¹ (0.03 ± 0.01 h⁻¹) (Fig. 6C).

DISCUSSION

Morphology and DNA sequences of Korean isolates of Pfiesteria piscicida

This study showed that Pfiesteria piscicida was present in several locations along the coasts of southern and western Korea. Pfiesteria piscicida collected from Masan (strain PPMS0507) and several USA isolates may have the same origin, because their DNA sequence and morphology were identical (Table 1). However, the DNA sequences of P. piscicida isolated from 5 other Korean locations (strains PPBS0507, PPIC0507, PPKS0507, PPKY0507, PPYS0507) differed by 1 bp (A instead of C at the 63rd nucleotide in SSU) from USA isolates (Table 1; Tengs et al. 2003). The 63rd nucleotide of these 5 Korean isolates was the same as that of the Norway isolate (Jakobsen et al. 2002), but the 75th and 78th nucleotides of these isolates (G and A, respectively) differed from those of the Norway isolate (C and G). We analyzed the sequences of >5 DNA copies for each isolate, and thus there is no possibility that these differences are just variants.
within the many copies of the ribosomal gene. Therefore, the DNA sequence of these 5 Korean isolates is unique and reported for the first time in the present study. However, the morphology of strain PPMS0507 and other Korean isolates was identical (Table 1). Therefore, a 1 bp difference in the DNA sequence may not be enough to affect the morphology of *P. piscicida*; nevertheless, it may be useful to understand the similarity among isolates from waters of different continents or countries, and thus the origins of each isolate.

The means of cell length and width of live *Pfiesteria piscicida* cells satiated with *Heterosigma akashiwo* (13.5 and 11.0 μm, respectively) were less than those of

![Fig. 3. *Pfiesteria piscicida* (strain PPIC0507). Specific growth rates (μ) on (A) perch blood cells, (B) *Rhodomonas salina*, (C) *Amphidinium carterae*, (D) *Heterosigma akashiwo*, and (E) an unidentified cryptophyte (ESD 5.6 μm) as a function of mean prey concentration (x, ng C ml⁻¹). Data are mean ± 1 SE. Curves fitted by Eq. (2) using all treatments in Expt 3.](image-url)
the PLD Stoeckeria algicida satiated with the same prey (16.8 and 12.9 μm, respectively) (Jeong et al. 2005a). However, the ranges of cell length and width of live P. piscicida cells satiated with H. akashiwo (9.1 to 17.0 and 6.4 to 14.2 μm, respectively) overlapped with the lower boundaries of the ranges of cell length and width of S. algicida satiated with H. akashiwo (14.4 to 20.8 and 10.0 to 17.4 μm, respectively) (Jeong et al. 2005a). The ratios of cell length to cell width of P. piscicida satiated with H. akashiwo (mean = 1.2; range = 1.0 to 1.4) were similar to those of S. algicida (mean = 1.3; range = 1.1 to 1.6). Therefore, based only on morphology, it may be very difficult to completely distinguish P. piscicida from S. algicida in natural water samples collected during H. akashiwo blooms. Analyses of DNA sequences, DNA probes, and/or SEM or calcoflour-stained images are needed to distinguish one species from the other.

Fig. 4. Pfiesteria piscicida (strain PPIC0507). Ingestion rates (IR) of (A) perch blood cells, (B) Rhodomonas salina, (C) Amphidinium carterae, (D) Heterosigma akashiwo, and (E) an unidentified cryptophyte (ESD 5.6 μm) as a function of mean prey concentration (x, ng C ml $^{-1}$). Data are mean ± 1 SE. Curves fitted by Eq. (3) (A–D) or by linear equation (E) using all treatments in Expt 3.
Prey species

Before the present study, mixotrophic dinoflagellates and raphidophytes had not been tested as prey for *Pfiesteria piscicida*, even though there is a high probability that *P. piscicida* does consume them; many mixotrophic dinoflagellate species and/or some raphidophyte species usually co-occur with *P. piscicida* (authors’ pers. obs.). This study demonstrated that *P. piscicida* was able to feed on all naked mixotrophic dinoflagellates, the smallest thecate dinoflagellate *Heterocapsa rotundata*, and all raphidophytes offered, but that it did not feed on large thecate dinoflagellates of ESD > 12 μm. The presence/absence of dinoflagellate theca may be a critical factor that affects the predation of *P. piscicida* on target prey. *P. piscicida* may have difficulties in capturing a large swimming thecate dinoflagellate cell and/or extracting materials from the prey using a peduncle. Immobility of prey cells may also be important—*P. piscicida* was able to feed on diatoms that have hard SiO₂ valves and dead cells of large thecate mixotrophic dinoflagellates. These findings suggest that *P. piscicida* may grow favorably under conditions when naked dinoflagellates, raphidophyte, and/or cryptophytes are abundant, or during the declining stage of blooms of large thecate dinoflagellates.

Growth and ingestion

The results of the present study show that the growth of *Pfiesteria piscicida* was affected by the kind of prey and prey concentration, with highest growth rates on
small naked algae *Amphidinium carterae* and *Heterosigma akashiwo*, relatively low growth on large naked algae *Akashiwo sanguinea*, *Chattonella ovata*, *Cocchlidium polykrikoides*, and/or *Gymnodinium catenatum*, and no growth on large thecate mixotrophic dinoflagellates *Prorocentrum minimum*, *Heterocapsa triqueta*, *Scrippsiella trochoidea*, *Gonyaulax polygramma*, *Alexandrium catanella*, and *Lingulodinium polyedrum*. Therefore, blooms dominated by small naked algae may be indicative of conditions that favor the presence of *P. piscicida*.

The growth rates of *Pfiesteria piscicida* on 8 naked algae were significantly negatively correlated with the prey size, whereas ingestion rates showed no clear correlation with prey size. *Pfiesteria piscicida* might spend more energy when capturing, handling, and extracting materials from larger prey species using a peduncle than from smaller prey species, because the energy obtained from larger and smaller prey were not significantly different.

The observed maximum growth rate ($\mu_{\text{max}}$) of Korean isolate PPIC0507 fed *Rhodomonas salina* (mean $\pm$ SE = 1.47 $\pm$ 0.01 d$^{-1}$, observed $\mu_{\text{max}}$: 1.41 d$^{-1}$, $\mu_{\text{max}}$ obtained from Eq. 2) determined in the present study was very similar to that of USA isolate NCSU 113-3 fed *Rhodomonas* sp. (1.5 d$^{-1}$, observed $\mu_{\text{max}}$: 1.76 d$^{-1}$, $\mu_{\text{max}}$ obtained using Monad functions; Lin et al. 2004), previously known as the optimal prey for *P. piscicida*. The $\mu_{\text{max}}$ of 2 other USA isolates (Parrow et al. 2001) fed *Rhodomonas* sp. were also similar to that of the Korean isolate PPIC0507. Therefore, the Korean and USA isolates have similar growth rates when fed *Rhodomonas* spp., in addition to similar DNA sequences and morphology.

In the present study, the $\mu_{\text{max}}$ of *Pfiesteria piscicida* fed perch blood cells (mean $\pm$ SE = 1.69 $\pm$ 0.03 d$^{-1}$, observed $\mu_{\text{max}}$: 1.74 d$^{-1}$, $\mu_{\text{max}}$ obtained from Eq. 2) was greater than that when fed *Rhodomonas salina*. Therefore, it appears that, among the prey tested in the present study, perch blood cells were optimal for *P. piscicida*. Flounder blood cells also supported high growth of *P. piscicida* in our preliminary test. Due to the dense population of fish maintained in a cage, the fish make a lot of gashes on each other; thus, *P. piscicida* may enter these gashes and suck blood cells of the fishes, which may cause illness or death of the fish (Burkholder et al. 2001a, Glasgow et al. 2001).

The $\mu_{\text{max}}$ of *Pfiesteria piscicida* on *Amphidinium carterae* (1.21 d$^{-1}$) was slightly higher than that of the heterotrophic dinoflagellate *Oxyrrhis marina* on the same prey (1.17 d$^{-1}$, Jeong et al. 2001a), but much higher than that of another heterotrophic dinoflagellate, *Polykrikos kofoidii* (0.10 d$^{-1}$) or the ciliates *Tiarina fusus* (~0.04 d$^{-1}$) and *Strombidinopsis* sp. (<0 d$^{-1}$; Jeong et al. 1999, 2001b, 2002). Therefore, *P. piscicida* is expected to be one of the dominant heterotrophic protists with *O. marina* during the red tides dominated by *A. carterae*.

The $\mu_{\text{max}}$ of *Pfiesteria piscicida* when fed *Heterosigma akashiwo* (1.10 d$^{-1}$) was lower than that of heterotrophic dinoflagellates *Stoeckeria algicida* (1.63 d$^{-1}$, Jeong et al. 2005b) or *Oxyrrhis marina* when fed the same prey (1.43 d$^{-1}$; Jeong et al. 2003). Nevertheless, a $\mu_{\text{max}}$ of 1.10 d$^{-1}$ is still high. Therefore, we expect high abundances of mixed populations of *P. piscicida*, *S. algicida*, and *O. marina* during blooms dominated by *H. akashiwo*, whereas species with lower growth rates such as the heterotrophic dinoflagellate *Gyrodinium dominans* or ciliate *Tiarina fusus* ($\mu_{\text{max}}$: 0.12 to 0.15 d$^{-1}$) may not be able to compete and thus will be less abundant (Nakamura et al. 1995, Jeong et al. 2002).

The highest maximum ingestion rate ($I_{\text{max}}$) of *Pfiesteria piscicida* observed in the present study (4.3 ng C predator$^{-1}$ d$^{-1}$), obtained when perch blood cells were provided as food, was much higher than $I_{\text{max}}$ when algal prey was provided (0.7 to 1.1 ng C predator$^{-1}$ d$^{-1}$). The liquid materials of perch blood cells were observed to be easily transferred into the body of *P. piscicida* through a peduncle. The $I_{\text{max}}$ of *P. piscicida* fed perch blood cells (ca. 480 cells predator$^{-1}$ d$^{-1}$) was much higher than that when fed tilapia blood cells (220 cells predator$^{-1}$ d$^{-1}$, no available carbon-base unit; Glasgow et al. 1998). Therefore, ingestion rates of *P. piscicida* when fed blood cells of different fish species may differ; thus, it would be worthwhile to measure ingestion rates when blood cells of other fish species are provided, so that such comparisons can be made in the future. The highest $I_{\text{max}}$ of *P. piscicida* obtained in the present study is also much higher than that of PLD *Stoeckeria algicida* (0.8 ng C predator$^{-1}$ d$^{-1}$; Jeong et al. 2005b). In our preliminary test, the $I_{\max}$ of *S. algicida* was low when fed perch blood cells (ca. 0.4 ng C predator$^{-1}$ d$^{-1}$). Therefore, the ability of *P. piscicida* to feed on blood cells accounts for its high $I_{\text{max}}$ in comparison with that of *S. algicida*.

The $I_{\text{max}}$ of *Pfiesteria piscicida* when fed the unidentified cryptophyte (1.1 ng C predator$^{-1}$ d$^{-1}$) was much higher than those of the mixotrophic dinoflagellates *Cocchlidium polykrikoides*, *Gonyaulax polygramma*, *Heterocapsa triqueta*, *Prorocentrum donghaiense*, or *Prorocentrum micans* when fed the same prey (0.03 to 0.18 ng C predator$^{-1}$ d$^{-1}$; Jeong et al. 2004a, 2005c,d). According to our field data, the maximum abundances of *P. piscicida* (combined with PLDs) in Korean waters were ca. 10 000 to 20 000 cells ml$^{-1}$, while those of *C. polykrikoides*, *G. polygramma*, or *H. triqueta* were 20 000 to 30 000 cells ml$^{-1}$. If the abundances of *P. piscicida* and these mixotrophic dinoflagellates are similar at a given time, the unidentified cryptophyte will be removed by *P. piscicida* at a much greater rate than by the mixotrophic dinoflagellates.
The maximum GGE of *P. piscicida* fed *Amphidinium carterae* (27\%) was observed to be similar to those of heterotrophic dinoflagellates fed algae (Jeong & Latz 1994, Naustvoll 1998, Kim & Jeong 2004). However, GGE of *P. piscicida* fed perch blood cells were distinctly low (3 to 14\%). Perch blood cells might not be nutritious prey for *P. piscicida*. GGEs of *P. piscicida* fed *Heterosigma akashiwo and Rhodomonas salina* were high (23 to 62 and 30 to 103\%, respectively). The kleptoplastids from *R. salina* and *H. akashiwo* may support mixotrophic growth of *P. piscicida*, as reported by Lewitus et al. (1999).

**Potential grazing effect**

In our estimation of grazing coefficients attributable to *P. piscicida* (+PLDs) on co-occurring algal prey, *P. piscicida* (+PLDs) removed 0.6 to 66\% (mean ± SE = 15 ± 3\%) of populations of co-occurring *Amphidinium* spp. in 1 h. In addition, *P. piscicida* (+PLDs) removed 0.1 to 36\% (mean ± SE = 5 ± 2\%) of populations of co-occurring *Heterosigma akashiwo* and 0.02 to 20\% (mean ± SE = 2 ± 1\%) of cryptophytes in 1 h. Therefore, grazing by *P. piscicida* (+PLDs) may sometimes have a considerable effect on populations of *Amphidinium* spp., *H. akashiwo*, or cryptophytes. However, in the present study, our estimation of grazing coefficients attributable to *P. piscicida* on co-occurring algal prey was limited by our inability to distinguish this dinoflagellate from PLDs. For better estimation of the effect of grazing of *P. piscicida* and PLDs on algal prey, it would be worthwhile to measure the ingestion rates of other PLDs as a function of prey concentration.

The maximum abundances of *P. piscicida* and PLDs in natural environments are ca. 10,000 to 25,000 cells ml\(^{-1}\) (e.g. Glasgow et al. 2001, Jeong et al. 2005b), whereas those of other heterotrophic dinoflagellates such as *Oxyrrhis marina*, *Polykrikos* spp., *Gyrodinium* spp., and *Protoperidinium* spp. are 100 to 2000 cells ml\(^{-1}\) (Lessard 1984, Jacobson 1987, Jeong 1995, 1999, Jeong et al. 2001b, Sherr & Sherr 2002, Jeong et al. 2004b). The effects of *P. piscicida* and PLD grazing on blooms of the small naked algae tested in the present study may be greater than those of other heterotrophic dinoflagellates, because rates of ingestion by *P. piscicida* on some of these algae were comparable to those by other heterotrophic dinoflagellates on the same prey. Widespread distribution of *P. piscicida* in Korean waters and the ability of *P. piscicida* to graze on mixotrophic dinoflagellates, raphidophytes, and cryptophytes implies that *P. piscicida* may play important roles in dynamics of blooms dominated by these algal prey.

Acknowledgements. We thank Seong Taek Kim, Jae Yoon Song, Kyeong Ah Seong, Dong Hyun Kim, Seung Hyun Lee, Myung Ok Park, Jeong Eun Song, and Soo Kyeeom Kim for sampling and technical support. This study was funded by a Sooteuk grant from MOMAF & KMI and a NRL grant from MOST & KOSEF (M1-0500-00-0174).

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**Editorial responsibility:** Urania Christaki, Wimereux, France

**Submitted:** March 23, 2006; **Accepted:** June 29, 2006
**Proofs received from author(s):** September 19, 2006