Research Article

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Feeding by the newly described heterotrophic dinoflagellate *Aduncodinium glandula*: having the most diverse prey species in the family Pfiesteriaceae

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To explore the feeding ecology of the newly described heterotrophic dinoflagellate *Aduncodinium glandula* in the family Pfiesteriaceae, its feeding behavior and prey species were investigated. Additionally, the growth and ingestion rates of *A. glandula* on the mixotrophic dinoflagellates *Heterocapsa triquetra* and *Akashiwo sanguinea*, its optimal and suboptimal prey, respectively were measured. *A. glandula* fed on prey through a peduncle after anchoring to the prey using a tow filament. *A. glandula* ate all algal prey and perch blood cells tested and had the most diverse prey species in the family Pfiesteriaceae. Unlike for other pfiesteriacean species, *H. triquetra* and *A. sanguinea* support the positive growth of *A. glandula*. However, the cryptophytes *Rhodomonas salina* and *Teleaulax* sp. and the phototrophic dinoflagellate *Amphidinium carterae* did not support the positive growth of *A. glandula*. Thus, *A. glandula* may have a unique kind of prey and its optimal prey differs from that of the other pfiesteriacean dinoflagellates. With increasing mean prey concentration, the growth rates of *A. glandula* on *H. triquetra* and *A. sanguinea* increased rapidly and then slowed or became saturated. The maximum growth rates when feeding on *H. triquetra* and *A. sanguinea* were 1.004 and 0.567 d^{-1}, respectively. Further, the maximum ingestion rates of *A. glandula* on *H. triquetra* and *A. sanguinea* were 0.75 and 1.38 ng C $predator^{-1}$ d^{-1}, respectively. There is no other pfiesteriacean species having *H. triquetra* and *A. sanguinea* as optimal and suboptimal prey. Thus, *A. glandula* may be abundant during blooms dominated by these species not preferred by the other pfiesteriacean dinoflagellates.

Key Words: food web; harmful algal bloom; ingestion; peduncle; protist; red tide

INTRODUCTION

Heterotrophic dinoflagellates are ubiquitous protists in marine environments and major components in marine ecosystems (Lessard 1984, 1991, Jacobson and Anderson 1986, Jeong et al. 2010*b*, Yoo et al. 2013*a*). They play diverse ecological roles in the marine ecosystems as effective grazers on diverse prey items, including bacteria, phytoplankton, heterotrophic nanoflagellates, other heterotrophic dinoflagellates, ciliates, and eggs and early naupliar stages of metazoans (Jeong 1999, Jeong et al. 2010*b*, 2015, Yoo et al. 2013*a*, 2013*b*), and are important prey items for large ciliates, metazooplankton, and larval fish (Klein Breteler 1980, Gifford and Dagg 1991, Jeong et al. 2007*b*). They also sometimes control populations of red tide species (Jeong et al. 2005*a*, 2015, Yoo et al. 2013*a*).

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Therefore, to understand the cycling of materials in marine ecosystems and red tide dynamics, the interactions between heterotrophic dinoflagellates and their prey or predators and the impact of grazing by heterotrophic dinoflagellates on populations of red tide species must be elucidated.

Before 2015, 7 genera, *Amyloodinium*, *Cryptoperidiniopsis*, *Luciella*, *Paulsenella*, *Pfiesteria*, *Stoeckeria*, and *Tyrannodinium*, had been established in the family Pfiesteriaceae (Landsberg et al. 1994, Steidinger et al. 1996, 2006, Jeong et al. 2005*b*, Litaker et al. 2005, Marshall et al. 2006, Mason et al. 2007, Calado et al. 2009). The kind of prey that can be consumed and the maximum growth and ingestion rates for a given prey species differ among pfiesteriacean species, even though they have the same feeding mechanism, peduncle feeding (e.g., Lim et al. 2014). Thus, the ecological niche and roles in marine ecosystems differ among pfiesteriacean species. The kind of prey and maximum growth and ingestion rates have been suggested to be related to the evolution of the species in this family (Lim et al. 2014). Therefore, when a new genus or species in the family Pfiesteriaceae is discovered, its feeding behavior, kind of prey, and maximum growth and ingestion rates should be explored to understand its ecological roles in marine ecosystems and evolution in the family.

Recently, a new heterotrophic dinoflagellate from the waters of Masan Bay, Korea, was isolated. This dinoflagellate had thin plates arranged in a Kofoidian series of Po, X, 4′, 2a, 6″, 6c, PC, 3 + s, 5‴, 0p, and 2⁗, similar to species in the family Pfiesteriaceae (Kang et al. 2015). Furthermore, this dinoflagellate genetically belonged to the family Pfiesteriaceae, although it had a conspicuous apical hook (finger-like projection) on the epitheca, unlike the other species in the family. Therefore, based on its morphology and genetics, it was assigned to be a new species in a new genus, *Aduncodinium glandula*, in the family Pfiesteriaceae. It is worthwhile to explore its feeding capability and then compare the results to those for other pfiesteriacean species.

In the present study, the feeding behavior of *A. glandula* was investigated. Several feeding experiments were conducted to determine: 1) the kind of prey that *A. glandula* can feed on, and whether it is similar to that of other species in this family; 2) whether *A. glandula* feeds on prey in the same way as species in other genera; and 3) the growth and ingestion rates of *A. glandula* on the optimal and suboptimal prey species, and whether the maximum growth and ingestion rates of *A. glandula* are comparable with those of species in other genera on the same prey species. The results of this study provide a basis for understanding the interactions and population dynamics occurring between *A. glandula* and its prey species; and the role of *A. glandula* in marine communities.

MATERIALS AND METHODS

Collection and culture of *Aduncodinium glandula*

A. glandula (GenBank accession No. LK934662) isolated from plankton samples collected from Masan Bay, Korea, in March 2013 was used in this study (Kang et al. 2015). Dense cultures of *A. glandula* were transferred to 250-mL polystyrene cell culture Petri flasks (BD Falcon, Bedford, MA, USA) containing fresh prey, *Akashiwo sanguinea* (ca. 1,000-2,000 cells mL^{-1}) or *Heterocapsa triquetra* (ca. 2,000-5,000 cells mL^{-1}), each week. These culture flasks were capped and placed lying on a shelf at 20°C under an illumination of 20 μE m^{-2} s^{-1} of cool white fluorescent light on a 14 : 10 h light-dark cycle.

To ensure that none of the established cultures were contaminated, DNA extracts of each culture were analyzed using species-specific primers for *A. glandula*, *Stoeckeria algicida*, *Stoeckeria changwonensis*, *Pfiesteria piscicida*, and *Luciella masanensis* as in Kang et al. (2015). This screening confirmed that each culture comprised only the target species.

Preparation of prey

Phototrophic cells were grown in enriched f/2 seawater media without silicate in 2-L polycarbonate (PC) bottles (Guillard and Ryther 1962). The bottles were placed on shelves and incubated at 20°C in a controlled-temperature chamber under a 14 : 10 h light-dark cycle at 20 μE m^{-2} s^{-1}.

The strain of *H. triquetra* used in the present study was isolated from Shiwha Bay, Korea in 2012. The strain of *A. sanguinea* was originated from Scripps Institution of Oceanography, University of California, San Diego, CA, USA. Perch blood cells, obtained by cutting the tailfin of live adults (ca. 50 cm), were maintained at 4°C. The experiments on the perch blood cells were started within 2-3 h of the cells being obtained.

The carbon contents of *H. triquetra* (0.22 ng C $cell^{-1}$), *A. sanguinea* (2.23 ng C $cell^{-1}$), and the perch blood cells (0.009 ng C $cell^{-1}$, n > 2,000) were used according to Jeong et al. (2006, 2007*a*).

Feeding occurrence

Experiment 1 was designed to investigate the ability of *A. glandula* to feed on individual target species when unialgal diets of diverse algal species were provided (Table 1). The initial concentrations of each algal species had a similar carbon biomass.

A culture of approximately 2,000 cells mL^{-1} of *A. glandula* growing on *A. sanguinea* was transferred to a single 50-mL culture flask containing freshly filtered seawater after *A. sanguinea* became undetectable. This culture was maintained for 1 d. Three 1-mL aliquots were then removed from the flask and examined with a light microscope to determine the concentration of *A. glandula*.

The initial concentrations of *A. glandula* (ca. 500 cells mL^{-1}) and each of the target algal species were established using an autopipette to deliver a predetermined volume of culture with a known cell density into the experimental flasks. For each algal species, triplicate 50-mL culture flasks containing 5-mL mixtures of *A. glandula* and the prey, and duplicate predator control flasks containing a 5-mL culture of *A. glandula* only were set up. The flasks were placed lying on a shelf and incubated.

After 6, 12, 24, and 48 h, >30 *A. glandula* cells in the flask were monitored under a dissecting microscope with a magnification of ×40-63 to determine the ability of *A. glandula* to feed on the target prey species. *A. glandula* cells feeding on the target prey cell using their peduncle were transferred to a slide, and photographs of the feeding were taken using digital cameras on an epifluorescence microscope (Zeiss-Axiovert 200M; Carl Zeiss Ltd., Göttingen, Germany) at a magnification of ×100-400.

Feeding behaviors

Experiment 2 was designed to investigate the feeding behavior of *A. glandula* when provided with unialgal diets of *H. triquetra* and *A. sanguinea* as prey. The initial concentrations of predator and prey used in Experiment 2 were the same as those described above for Experiment 1.

Table 1. Taxa, sizes, and initial prey concentration of prey species offered as food to *Aduncodinium glandula* in experiment 1

Species	ESD (± SD)	Initial prey concentration (cells mL^{-1})	Feeding by *A. glandula*
Diatoms			
Skeletonema costatum	5.9 (1.1)	100,000	Y
Prymnesiophytes			
Isochrysis galbana	4.8 (0.2)	150,000	Y
Cryptophytes			
Teleaulax sp.	5.6 (1.5)	100,000	Y
Rhodomonas salina	8.8 (1.5)	50,000	Y
Rhaphidophytes			
Heterosigma akashiwo	11.5 (1.9)	30,000	Y
Chattonella ovata	40.0 (1.6)	1,000	Y
Dinoflagellates			
Heterocapsa rotundata (T)	5.8 (0.4)	100,000	Y
Amphidinium carterae (NT)	9.7 (1.6)	30,000	Y
Prorocentrum minimum (T)	12.1 (2.5)	10,000	Y
Heterocapsa triquetra (T)	15.0 (4.3)	1,000	Y
Scrippsiella trochoidea (T)	22.8 (2.7)	3,000	Y
Cochlodinium polykrikoides (NT)	25.9 (2.9)	2,000	Y
Prorocentrum micans (T)	26.6 (2.8)	3,000	Y
Akashiwo sanguinea (NT)	30.8 (3.5)	100	Y
Alexandrium tamarense (T)	32.6 (2.7)	3,000	Y
Gymnodinium catenatum (NT)	33.9 (1.6)	3,000	Y
Lingulodinium polyedrum (T)	38.2 (3.6)	2,000	Y
Blood cells			
Perch	6.1 (0.5)	200,000	Y

To confirm the absence of ingestion by the predator on certain prey species, additional higher prey concentrations were provided. The mean equivalent spherical diameter (ESD, μm ± standard deviation [SD]) was measured using an electronic particle counter (Coulter Multisizer II; Coulter Corporation, Miami, FL, USA) (n > 2,000 for each species).
Y, the predator was observed to feed on a food cell; T, thecate; NT, non-thecated.

For each target species, a single 50-mL culture flask containing a mixture of *A. glandula* and the prey was set up. After 1 h of incubation, all of the feeding processes from the time a prey cell was captured to the time that the prey was completely ingested by the predator were observed by monitoring the behavior of >60 unfed *A. glandula* cells for each prey species under a light microscope at a magnification of ×100-630.

The feeding process of an *A. glandula* cell was photographed using a video analyzing system (Sony DXC-C33; Sony Co., Tokyo, Japan) mounted on a compound microscope at a magnification of ×100-630. The time lag (n = 5) between the deployment of a peduncle of *A. glandula* and the time for a prey cell to be completely ingested by an *A. glandula* cell after the predator had deployed its peduncle to the prey cell were measured when *H. triquetra* was provided as prey. In addition, the feeding process of *A. glandula* on *A. sanguinea* (n = 5) was recorded.

Growth and ingestion rates as a function of prey concentration

Experiment 3 was designed to measure the growth and ingestion rates of *A. glandula* on *H. triquetra* and *A. sanguinea* as a function of prey concentration. In the preliminary test, *A. glandula* grew well on *H. triquetra* and *A. sanguinea*, but did not grow on *Amphidinium carterae*, *Heterosigma akashiwo*, *Rhodomonas salina*, or *Teleaulax* sp.

For the experiment on *H. triquetra* prey, cultures of 1,000-2,000 cells mL^{-1} of *A. glandula* growing on *H. triquetra* were transferred to a single 250-mL culture flask containing freshly filtered seawater after *H. triquetra* became undetectable. This culture was maintained for 1 day. Similarly, for the experiment on *A. sanguinea* prey, cultures of 2,000-3,000 cells mL^{-1} of *A. glandula* growing on *A. sanguinea* were transferred to a single 250-mL culture flask containing freshly filtered seawater after *A. sanguinea* became undetectable. This culture was maintained for 1 day. Three 1-mL aliquots were then collected from the flask and examined using a light microscope to determine the concentration of *A. glandula*.

The initial concentrations of *A. glandula* and each of the target prey species were established by using an autopipette to deliver predetermined volumes of known cell concentrations to the bottles (Table 2). For each predator-prey combination, triplicate experimental 50-mL culture flasks (containing a 10-mL mixture of predator and prey) and triplicate control flasks (containing a 10-mL culture of prey only) were set up. Triplicate control flasks containing a 10-mL culture of only *A. glandula* were also established at a single predator concentration. To ensure similar water conditions, the water of the predator culture was filtered through a 0.7-μm GF/F filter, and this was added to the prey control flasks at the same volume as the predator culture added into the experimental flasks for each predator-prey combination. To determine the actual predator and algal prey concentrations at the start of the experiment and after 2 days, a 5-mL aliquot was removed from each bottle and fixed with 5% Lugol's solution; all or >200 predator and prey cells in triplicate 1-mL Sedgwick-Rafter chambers were then counted. Only 5 mL of water in each 50-mL flask was incubated to increase encounter rates between predators and prey because *A. glandula* swam near the bottom of the flask (i.e., to make a shallow depth in the flasks).

The specific growth rate of *A. glandula* was calculated as follows:

$$\mu = \frac{\mathrm{Ln}\,(C_t / C_0)}{t} \quad (1)$$

, where C_0 is the initial concentration of *A. glandula* and C_t is the final concentration after time t. The period was 2 days. The mean prey concentration was calculated using the equation of Frost (1972). The ingestion and clearance rates were calculated using the equations of Frost (1972) and Heinbokel (1978).

The carbon content of *A. glandula* was estimated from the cell volume according to the method of Menden-Deuer and Lessard (2000).

Table 2. Experimental design

Species	Prey	Species	Predator
	Density		Density
Heterocapsa triquetra	89, 164, 381, 928, 1,994, 3,353, 9,589	*Aduncodinium glandula*	23, 31, 71, 152, 106, 96, 231 (234)
Akashiwo sanguinea	45, 108, 420, 889, 1,323, 2,331, 3,340, 5,267	*Aduncodinium glandula*	23, 29, 80, 108, 141, 182, 271, 283 (280)
Perch blood cell	102,333, 204,222	*Aduncodinium glandula*	736, 1,494 (731)

Values shown in parentheses in the predator column are the predator densities in the control bottles. The numbers in the prey and predator columns are the actual initial densities (cells mL^{-1}) of prey and predators.

Data for *A. glandula* growth rate were fitted to the following equation:

$$\mu = \frac{\mu_{max} (x - x')}{K_{GR} + (x - x')} \quad (2)$$

, where μ_{max} is the maximum growth rate (d^{-1}), x is the prey concentration (cells mL^{-1} or ng C mL^{-1}), x' is the threshold prey concentration (i.e., the prey concentration where $\mu = 0$), and K_{GR} is the prey concentration sustaining 1/2 μ_{max}. Data were iteratively fitted to the model using DeltaGraph (SPSS Inc., Chicago, IL, USA).

Ingestion rate (IR) data were fitted to a Michaelis-Menten equation:

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

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

Swimming speed

A culture of *A. glandula* of approximately 2,000 cells mL^{-1} growing on *A. sanguinea* was transferred into a 500-mL PC bottle. When prey was undetectable, an aliquot from the bottle was added to a 50-mL cell culture flask and allowed to acclimate for 30 min. The video camera focused on an individual field viewed as a single circle in a cell culture flask under a dissecting microscope at 20°C. The swimming of *A. glandula* cells was then recorded at a magnification of ×40 by using a video analyzing system (SV-C660; Samsung, Seoul, Korea) and a charge-coupled device camera (KP-D20BU; Hitachi, Tokyo, Japan). The mean and maximum swimming velocities for all swimming cells viewed during the first 10 min were analyzed. The average swimming speed (n = 20) was calculated based on the linear displacement of cells in 1 s during single-frame playback.

RESULTS

Prey species and feeding behaviors

A. glandula ingested all of the algal prey species offered as food (i.e., the diatoms *Skeletonema costatum*; the cryptophytes *Teleaulax* sp. and *R. salina*; the raphidophytes *H. akashiwo* and *Chattonella ovata*; and the dinoflagellates *Heterocapsa rotundata*, *A. carterae*, *Prorocentrum minimum*, *H. triquetra*, *Cochlodinium polykrikoides*, *Scrippsiella trochoidea*, *P. micans*, *A. sanguinea*, *Gymnodinium catenatum*, and *Lingulodinium polyedrum*) and perch blood cells (Table 1, Figs 1 & 2, Supplementary Videos 1 & 2). *A. glandula* fed on its prey through a peduncle after anchoring to the prey cell using a tow filament. Typically, several *A. glandula* cells attacked a prey cell together (Fig. 2). In particular, it took a long time for *A. glandula* cells to ingest a large *A. sanguinea* cell, so several predator cells would approach and collectively attack the prey cell after one *A. glandula* cell deployed its peduncle (Fig. 2).

The time (mean ± standard error [SE], n = 5) for a larger naked prey *A. sanguinea* cell to be completely ingested by two *A. glandula* cells, after the first attacking predator had deployed its peduncle to the prey cell (1,040 ± 184 s), was longer than that for the thecate but smaller prey *H. triquetra* (538 ± 57 s, n = 4). However, the time (mean ± SE, n = 5) for an *A. sanguinea* cell to be completely ingested by five *A. glandula* cells after the first attacking predator had deployed its peduncle to the prey cell (566 ± 147 s, n = 5) was shorter than the time taken by two *A. glandula* cells.

Growth and ingestion rates

A unialgal diet of *H. triquetra* or *A. sanguinea* supported the positive growth of *A. glandula*. By contrast, a unialgal diet of *A. carterae*, *R. salina*, or *Teleaulax* sp., or perch blood cells did not support growth (Table 3).

With increasing mean prey concentration, the specific growth rates of *A. glandula* increased rapidly at *H. trique-*

Table 3. Growth and ingestion data for the heterotrophic dinoflagellate *Aduncodinium glandula* on algal prey species

Prey	μ_{max}	K_{GR}	x'	r^2	I_{max}	K_{IR}	r^2	C_{max}
Heterocapsa triquetra	1.004	694	67.8	0.916	0.75	195	0.704	0.08
Akashiowo sanguinea	0.567	127	6.0	0.954	1.38	191	0.433	0.29
Perch blood cells	0.088[a]	-	-	-	0.43[a]	-	-	0.02[a]

Parameters are for numerical and / or functional responses from Eqs. (2) and (3) as presented in Figs 3 and 4.
μ_{max}, maximum growth rate (d^{-1}); K_{GR}, prey concentration sustaining 0.5 μ_{max} (ng C mL^{-1}); x', threshold prey concentration (ng C mL^{-1}); I_{max}, maximum ingestion rate (ng C predator^{-1} d^{-1}); K_{IR}, prey concentration sustaining (0.5 I_{max}, ng C mL^{-1}); C_{max}, maximum clearance rate (μL predator^{-1} h^{-1}).
[a]Highest value among the growth or ingestion rates measured at the given prey concentrations.

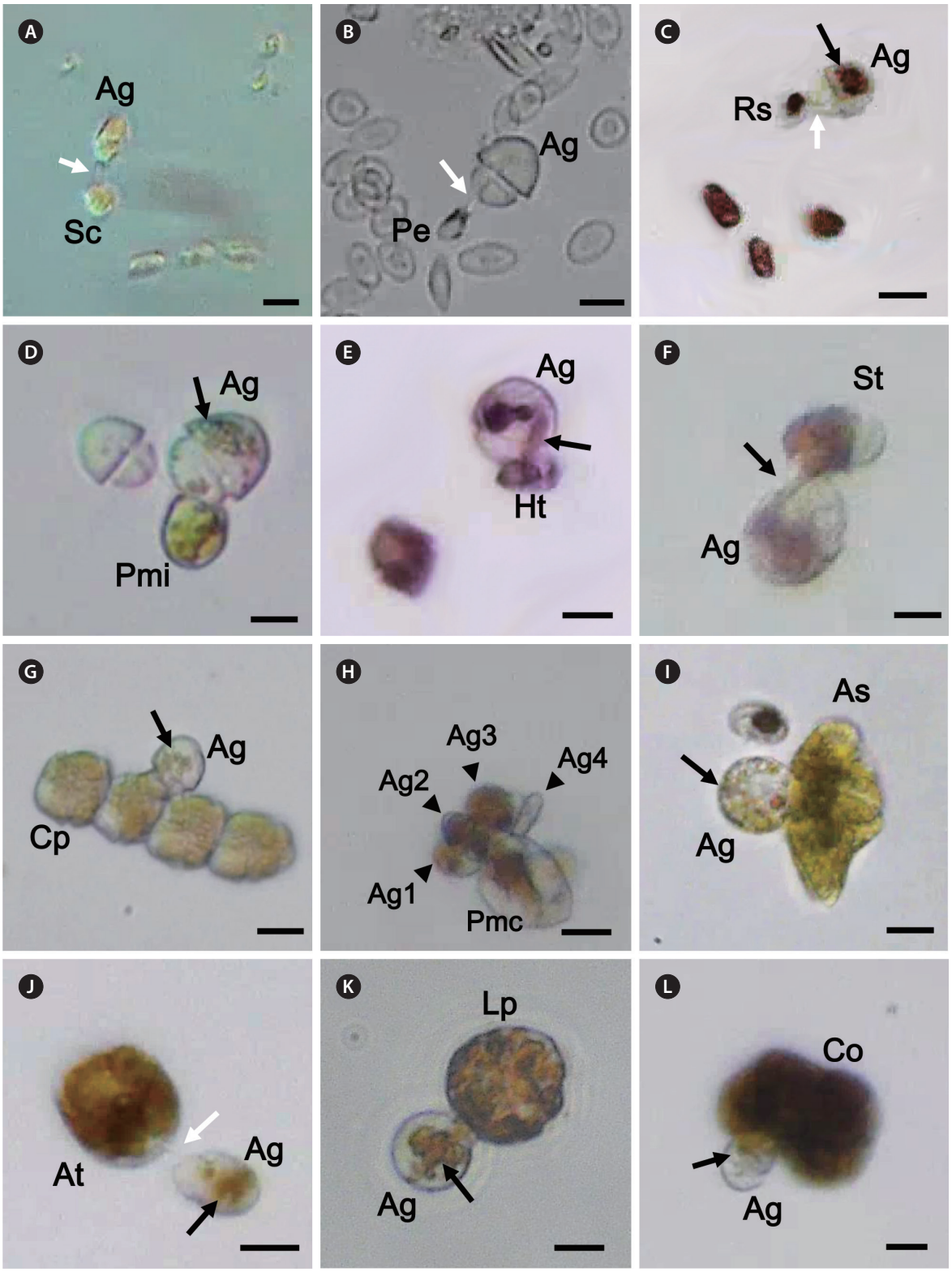

Fig. 1. Feeding of *Aduncodinium glandula* (Ag) on diverse prey species. Ag cells feeding on *Skeletonema costatum* (Sc) (A), perch blood cell (Pe) (B), *Rhodomonas salina* (Rs) (C), *Prorocentrum minimum* (Pmi) (D), *Heterocapsa triquetra* (Ht) (E), *Scrippsiella trochoidea* (St) (F), *Cochlodinium polykrikoides* (Cp) (G), *Prorocentrum micans* (Pmc) (H), *Akashiwo sanguinea* (As) (I), *Alexandrium tamarense* (At) (J), *Lingulodinium polyedrum* (Lp) (K), and *Chattonella ovata* (Co) (L). An *A. glandula* cell sucking materials (black arrows) from a prey cell through a peduncle (white arrows). Arrowheads in panel H indicate Ag cells. Scale bars represent: A-L, 10 μm.

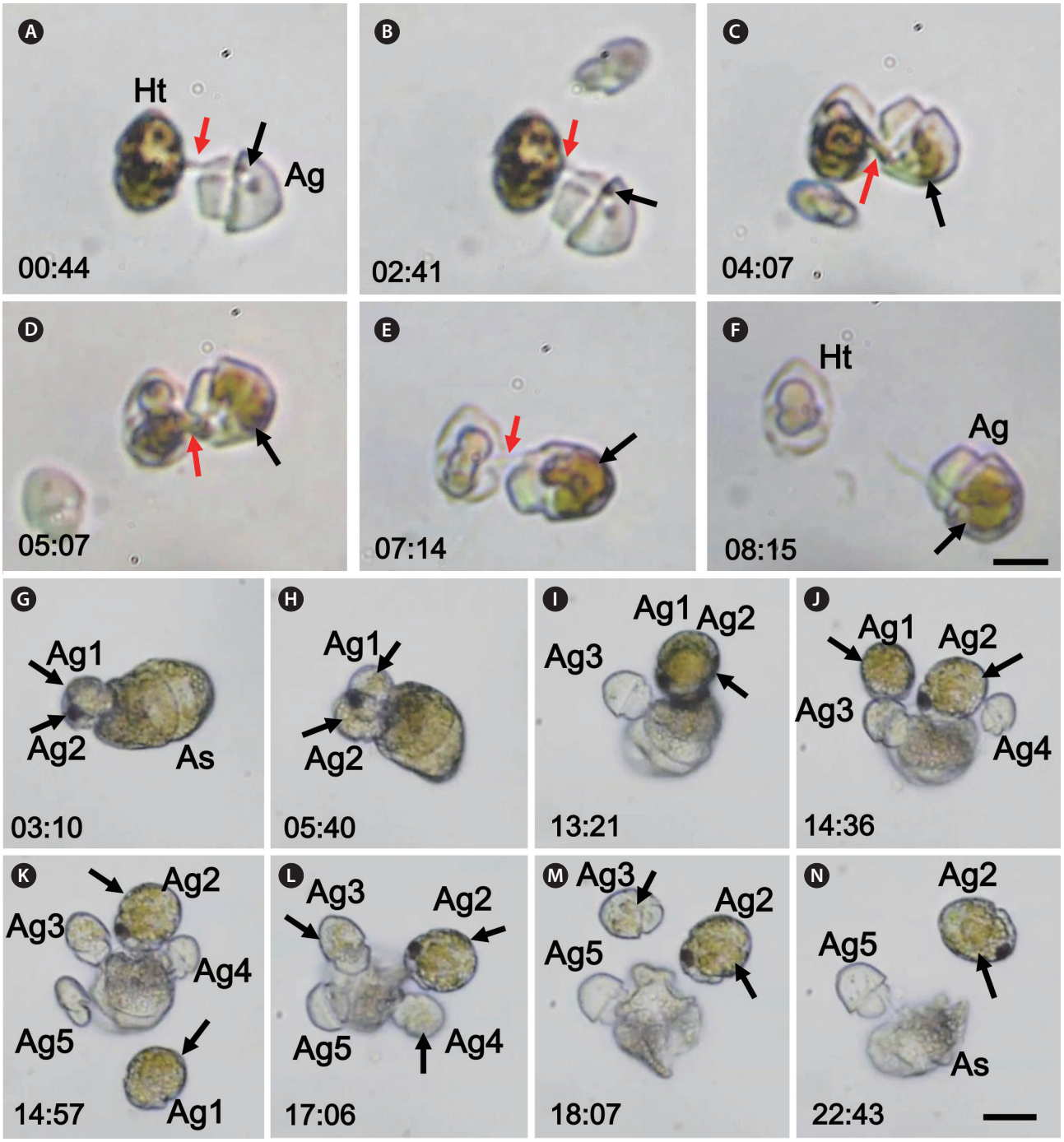

Fig. 2. Feeding process of *Aduncodinium glandula* (Ag) on *Heterocapsa triquetra* (Ht) (Supplementary Video 1) (A-F) and *Akashiwo sanguinea* (As) (Supplementary Video 2) (G-N). (A & B) *A. glandula* cell deploying a peduncle (red arrows) to the surface of a *H. triquetra* cell. (C-E) The amount of materials (arrows) from the prey cell inside the protoplasm of the predator cell increases. (F) *A. glandula* cell swimming away after feeding on *H. triquetra* cell. (G & H) Two *A. glandula* cells (Ag1 and Ag2) sucking materials (arrows) from an *A. sanguinea* cell through peduncles. Third (Ag3) (I), fourth (Ag4) (J), and fifth *A. glandula* (Ag5) (K & L) cells subsequently start to attack the *A. sanguinea* cell. The first *A. glandula* cell (Ag1) (K & L) and the fourth (Ag4) cell (M) swimming away after feeding on *A. sanguinea* cell. (N) All *A. glandula* cells except Ag5 left or are leaving the *A. sanguinea* cell, which is almost empty. The numbers indicate the elapsed time after a peduncle of a predator cell was deployed (min : sec). The same *H. triquetra* and *A. glandula* cells are shown in A-F and the same *A. sanguinea* cell is shown in panel G-N. Scale bars represent: A-N, 10 μm.

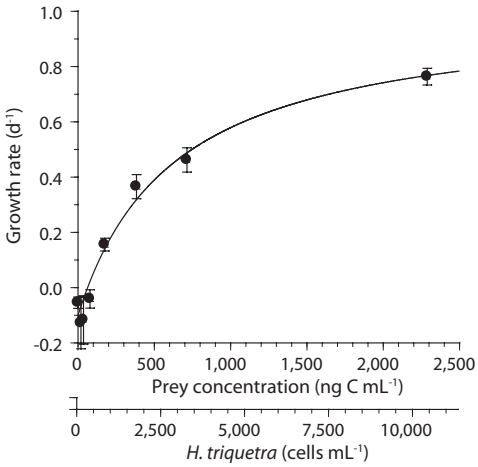

Fig. 3. Specific growth rates of *Aduncodinium glandula* on *Heterocapsa triquetra* as a function of the mean prey concentration (x, ng C mL^{-1}). Symbols represent treatment means ± 1 standard error. The curve was fitted by a Michaelis-Menten equation [Eq. (2)] using all treatments in the experiment. Growth rate (d^{-1}) = 1.004 {(x - 67.8) / [694 + (x - 67.8)]}, r^2 = 0.916.

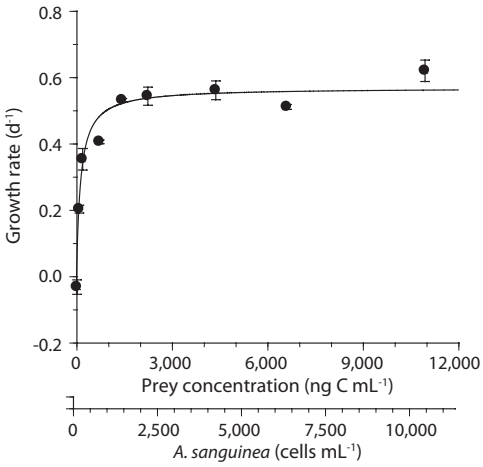

Fig. 4. Specific growth rates of *Aduncodinium glandula* on *Akashiwo sanguinea* as a function of the mean prey concentration (x, ng C mL^{-1}). Symbols represent treatment means ± 1 standard error. The curve was fitted by a Michaelis-Menten equation [Eq. (2)] using all treatments in the experiment. Growth rate (d^{-1}) = 0.567 {(x - 6.0) / [127 + (x - 6.0)]}, r^2 = 0.954.

tra concentrations less than 714 ng C mL^{-1} or 3,245 cells mL^{-1} and then more slowly at higher prey concentrations (Fig. 3). When the data were fitted to Eq. (2), the maximum specific growth rate of *A. glandula* on *H. triquetra* was 1.004 d^{-1}; the K_{GR} was 694 ng C mL^{-1} or 3,154 cells mL^{-1}; and the threshold prey concentration was 67.8 ng C mL^{-1} or 315 cells mL^{-1}.

With increasing mean prey concentration, the specific growth rates of *A. glandula* increased rapidly at *A. sanguinea* concentrations less than 638 ng C mL^{-1} or 286 cells mL^{-1} (Fig. 4). When the data were fitted to Eq. (2), the maximum specific growth rate of *A. glandula* on *A. sanguinea* was 0.567 d^{-1}; the K_{GR} was 127 ng C mL^{-1} or 57 cells mL^{-1}; and the threshold prey concentration was 6.0 ng C mL^{-1} or 2.7 cells mL^{-1}.

With increasing mean prey concentration, the ingestion rates of *A. glandula* increased rapidly at *H. triquetra* concentrations less than 383 ng C mL^{-1} or 1,740 cells mL^{-1}, but became saturated at higher prey concentrations (Fig. 5). When the data were fitted to Eq. (3), the maximum ingestion rate of *A. glandula* on *H. triquetra* was 0.75 ng C $predator^{-1}$ d^{-1} or 3.4 cells $predator^{-1}$ d^{-1}; and K_{IR} was 195 ng C mL^{-1} or 886 cells mL^{-1}. The maximum clearance rate of *A. glandula* on *H. triquetra* was 0.08 µL $predator^{-1}$ h^{-1}.

With increasing mean prey concentration, the ingestion rates of *A. glandula* increased rapidly at *A. sanguinea* concentrations less than 1,424 ng C mL^{-1} or 638 cells mL^{-1}, but became saturated at higher prey concentrations (Fig. 6). When the data were fitted to Eq. (3), the maximum ingestion rate of *A. glandula* on *A. sanguinea* was 1.38 ng C $predator^{-1}$ d^{-1} or 0.6 cells $predator^{-1}$ d^{-1}; and K_{IR} was 191 ng C mL^{-1} or 86 cells mL^{-1}. The maximum clearance rate of *A. glandula* on *A. sanguinea* was 0.29 µL $predator^{-1}$ h^{-1}.

Swimming speed

The average (±SE, n = 20) and maximum swimming speeds of *A. glandula* under the experimental conditions were 439 (±12) and 546 µm s^{-1}, respectively.

DISCUSSION

Prey species and feeding behaviors

A. glandula fed on all algal prey and perch blood cells tested. Prior to this study, in the family Pfiesteriaceae, *S. changwonensis* was the species that could feed on the most diverse prey species (Table 4). However, *A. glandula* feeds on *S. trochoidea* and *G. catenatum*, while *S. changwonensis* does not. Therefore, now *A. glandula* is the species can feed on the most diverse prey species among the pfiesteriacean species. The ranking of the pfiesteriacean species tested in terms of the number of prey species consumed was *A. glandula* (18/18 species eaten) > *S. changwonensis* (15/17 species eaten) > *L. masanensis* (12/19 species eaten) > *P. piscicida* (12/19 species eaten) > *Cryptoperidiniopsis brodyi* (4/8 species eaten) > *Pfiesteria*

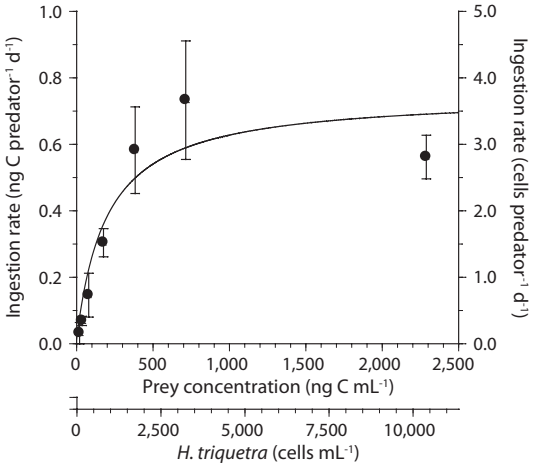

Fig. 5. Ingestion rates of *Aduncodinium glandula* on *Heterocapsa triquetra* as a function of the mean prey concentration (x). Symbols represent treatment means ± 1 standard error. The curve was fitted by a Michaelis-Menten equation [Eq. (3)] using all treatments in the experiment. Ingestion rate (ng C grazer^{-1} d^{-1}) = 0.75 [x / (195 + x)], r^2 = 0.708.

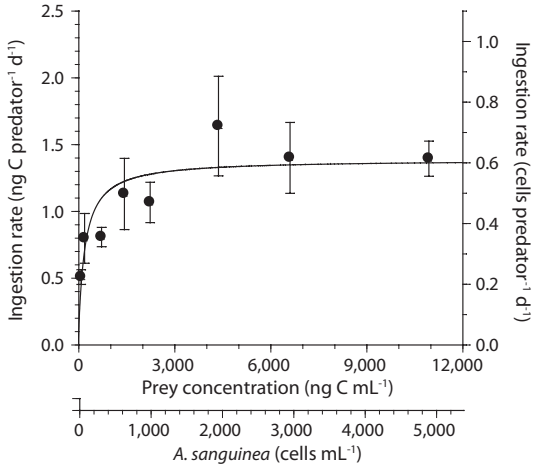

Fig. 6. Ingestion rates of *Aduncodinium glandula* on *Akashiwo sanguinea* as a function of the mean prey concentration (x). Symbols represent treatment means ± 1 standard error. The curve was fitted by a Michaelis-Menten equation [Eq. (3)] using all treatments in the experiment. Ingestion rate (ng C grazer^{-1} d^{-1}) = 1.38 [x / (191 + x)], r^2 = 0.433.

shumwayae (3/8 species eaten) > *S. algicida* (1/19 species eaten) (Table 4). Furthermore, *A. glandula* could eat more species than could the similar-sized heterotrophic dinoflagellate *Gyrodiniellum shiwhaense* (7/16 species eaten) and the mixotrophic dinoflagellates *Biecheleria cincta* (previously *Woloszynskia cincta*; 6/16 species eaten), *Symbiodinium voratum* (6/16 species eaten), *Gymnodinium aureolum* (6/18 species eaten), *Paragymnodinium shiwhaense* (6/16 species eaten), and *Gymnodinium smaydae* (4/16 species eaten) in other families, but the same as *Karlodinium armiger* could eat (10/10 species eaten) (Table 4). The maximum swimming speed of *A. glandula* is lowest among the pfiesteriacean dinoflagellates, so factors other than swimming speed (and in turn the rate of encounter between predator and prey cells) may be responsible for its having the most diverse prey species (Lim et al. 2014, our unpublished data) (Table 4). *A. glandula* may have enzymes that are more diverse than those of other species in the Pfiesteriaceae. In the phylogenetic trees based on small subunit and large subunit ribosomal DNA, *A. glandula* is divergent from the clade consisting of *Pfiesteria* spp., *Luciella* spp., and *Cryptoperidiniopsis* spp. and another clade consisting of *Stoeckeria* spp. (Kang et al. 2015). Therefore, *A. glandula* may have prey-related genes that differ from those of the other species in the family Pfiesteriaceae. Based on the diversity of its prey, *A. glandula* is likely to have more diverse prey-related genes than the other pfiesteriacean dinoflagellates. The high diversity of prey species for *A. glandula* may mean that it is present in more diverse marine environments than are other species.

A. glandula can feed on *S. trochoidea* and *Alexandrium tamarense*, which are not eaten by the other pfiesteriacean species. Furthermore, besides *S. changwonensis*, *A. glandula* is the only pfiesteriacean dinoflagellate that can feed on the common red-tide dinoflagellates *P. minimum*, *H. triquetra*, *Prorocentrum micans*, and *L. polyedrum* (Table 4). Moreover, besides *P. piscicida*, *A. glandula* is the only pfiesteriacean species that can feed on the toxic dinoflagellate *G. catenatum*. Therefore, *A. glandula* may be the only pfiesteriacean species present during *A. tamarense* blooms, while it is expected to be one of only two pfiesteriacean species present during *P. minimum*, *H. triquetra*, *P. micans*, *L. polyedrum*, and *G. catenatum* blooms.

A. glandula feeds on algal prey and fish blood cells through a peduncle after anchoring to a prey cell using a tow filament. This feeding mechanism is similar to that of species in the other genera in the family Pfiesteriaceae (Burkholder et al. 1992, Burkholder and Glasgow 1997, Jeong et al. 2005*a*, 2006, 2007*a*, Baek et al. 2010). Because the morphology of *A. glandula* is considerably different from those of the other genera in the family, it was originally allocated to another genus and family (*Katodinium* in the family Gymnodiniaceae). However, *A. glandula* has the same feeding mechanism as all pfiesteriacean species.

It took longer for an *A. sanguinea* cell to be completely

Table 4. Comparison of prey species, and the maximum growth and ingestion rates of the peduncle-feeding MD and HD

Prey species / Predator	ESD	HD								MD					
Trophic mode		Ag	Sc	Sa	Lm	Pfp	Pfs	Cb	Gsh	Sv	Bc	Ga	Ps	Ka	Gsm
Prymnesiophytes															
Isochrysis galbana	4.8	Y	Y	-	-	-	-	-	Y	Y	Y	Y	Y	Y	Y
Diatoms															
Skeletonema costatum	5.9	**Y**	**Y**	N	**Y**	**Y**	-	-	N	N	N	N	N	-	N
Thalassiosira weissflogii	11.8	-	-	-	-	-	-	-	-	-	-	-	-	Y	-
Thalassiosira rotula	29.3	-	-	N	Y	Y	-	-	-	-	-	N	-	-	-
Cryptophytes															
Teleaulax sp.	5.6	Y	Y	**N**	Y	Y	-	-	Y	Y	Y	Y	Y	-	**N**
Teleaulax amphioxeia	7.3	-	-	**-**	-	-	-	-	-	-	-	-	-	Y	**-**
Rhodomonas salina	8.8	Y	Y	**N**	Y	Y	-	-	Y	Y	Y	Y	Y	Y	**N**
Rhodomonas sp.	-	-	-	-	-	-	Y	Y	-	-	-	-	-	-	-
Rhaphidophytes															
Heterosigma akashiwo	11.5	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	**N**
Chattonella ovata	40.0	Y	Y	N	Y	Y	N	Y	-	-	-	-	-	-	N
Dinoflagellates															
Heterocapsa rotundata	5.8	Y	Y	**N**	Y	Y	-	-	Y	Y	Y	Y	Y	Y	Y
Amphidinium carterae	9.7	Y	Y	**N**	Y	Y	-	-	Y	Y	Y	Y	Y	-	**N**
Prorocentrum minimum	12.1	**Y**	**Y**	N	N	N	N	N	**Y**	N	N	N	N	**Y**	N
Heterocapsa triquetra	15.0	**Y**	**Y**	N	N	N	N	N	N	N	N	N	N	**Y**	**Y**
Scrippsiella trochoidea	22.8	**Y**	N	N	N	N	-	-	N	N	N	N	N	-	**Y**
Cochlodinium polykirkoides	25.9	**Y**	**Y**	N	**Y**	**Y**	-	-	N	N	N	N	N	-	N
Prorocentrum micans	26.6	**Y**	**Y**	N	N	N	N	N	N	N	N	N	N	**Y**	N
Akashiwo sanguinea	30.8	Y	Y	N	Y	Y	Y	Y	N	N	N	N	N	Y	N
Gonyaulax polygramma	32.5	-	-	N	N	N	-	-	N	N	N	N	N	-	-
Alexandrium tamarense	32.6	**Y**	-	N	N	N	N	N	-	N	N	N	N	-	N
Gymnodinium catenatum	33.9	**Y**	N	N	Y	**Y**	-	-	-	-	-	N	-	-	-
Lingulodinium polyedrum	38.2	**Y**	**Y**	N	N	N	-	-	N	N	N	N	N	-	N
Blood cells															
Perch blood cell	-	Y	Y	**N**	Y	Y	-	-	-	-	-	-	-	-	-
MGR	-	1.00	0.38	1.63	1.46	1.74	0.72	0.61	1.05	0.47	0.50	0.17	1.10	0.65	2.23
MIR	-	1.4	0.4	0.8	2.6	4.3	-	-	0.5	0.1	0.5	0.1	0.4	1.0	1.6
MSS	-	546	617	549	680	670	-	-	782	180	378	576	863	-	707
Reference	-	a	b	c, d, e	d, f	d, e, f, g, h	i	i	j	f, k	l	m	e	n	o

Bold indicates different results from other predators.

MD, mixotrophic dinoflagellates; HD, heterotrophic dinoflagellates; ESD, equivalent spherical diameter (μm); Ag, *Aduncodinium glandula*; Sc, *Stoeckeria changwonensis*; Sa, *Stoeckeria algicida*; Lm, *Luciella masanensis*; Pfp, *Pfiesteria piscicida*; Pfs, *Pfiesteria shumwayae*; Cb, *Cryptoperidiniopsis brodyi*; Gsh, *Gyrodiniellum shiwhaense*; Sv, *Symbiodinium voratum*; Bc, *Biecheleria cincta*; Ga, *Gymnodinium aureolum*; Ps, *Paragymnodinium shiwhaense*; Ka, *Karlodinium armiger*; Gsm, *Gymnodinium smaydae*; -, not tested; Y, predator was observed to feed on a living food cell; N, predator was not observed to feed on a living food cell; MGR, maximum growth rate (d^{-1}); MIR, maximum ingestion rate (ng C $predator^{-1}$ d^{-1}); MSS, maximum swimming speed (m s^{-1}).

a, This study; b, Lim et al. (2014); c, Jeong et al. (2005*a*); d, Jeong et al. (2007*a*); e, Yoo et al. (2010); f, our unpublished data; g, Burkholder and Glasgow (1997); h, Jeong et al. (2006); i, Baek et al. (2010); j, Jeong et al. (2011); k, Jeong et al. (2012); l, Kang et al. (2011); m, Jeong et al. (2010*a*); n, Berge et al. (2008); o, Lee et al. (2014).

ingested by two *A. glandula* cells after the first attacking predator had deployed its peduncle to the prey cell than it took to ingest an *H. triquetra* cell. The size of *A. sanguinea* (equivalent spherical diameter [ESD] = 30.8 μm) is ca. twice the size of *H. triquetra* (ESD = 15.0 μm). Therefore, the time for a prey cell to be completely ingested by *A. glandula* cells is likely to be proportional to the cell size of the prey. Furthermore, the time for an *A. sanguinea* cell to be completely ingested by five *A. glandula* cells was shorter than that by two predators. Thus, the time for a prey cell to be completely ingested by *A. glandula* cells is likely to be affected by the number of predator cells.

Growth and ingestion rates

H. triquetra and *A. sanguinea* supported relatively high positive growth of *A. glandula*, but *A. carterae*, *H. akashiwo*, *R. salina* (or *Rhodomonas* sp.), *Teleaulax* sp., and perch blood cells, which are known to support relatively high positive growth of *Pfiesteria* spp., *L. masanensis*, *C. brodyi*, and *Stoeckeria* spp., did not support growth or only supported very low positive growth for *A. glandula* (Table 5). Furthermore, the optimal and suboptimal prey species for *A. glandula* are different from those of the other pfiesteriacean species. Therefore, the feeding of *A. glandula* is clearly different from those of the other pfiesteriacean species, so it has an ecological niche different from those of the other pfiesteriacean species in marine food webs. In addition, *A. glandula* is expected to be abundant during or after *H. triquetra* and *A. sanguinea* red tides, while the other pfiesteriacean species are abundant during or after *A. carterae*, *H. akashiwo*, and cryptophyte red tides.

The maximum ingestion rate of *A. glandula* (1.4 ng C predator^{-1} d^{-1}) is intermediate among those of pfiesteriacean species (Tables 4 & 5); the maximum ingestion rate of *A. glandula* is lower than those of *L. masanensis* and *P. piscicida*, but greater than those of *S. changwonensis* and *S. algicida*. The prey species supporting the maximum ingestion rate of *A. glandula* is *A. sanguinea*, while that for *L. masanensis* and *P. piscicida* is perch blood cells. *A. glandula* may have difficulty in capturing, handling, ingesting, and digesting large and actively swimming *A. sanguinea* cells, while *L. masanensis* and *P. piscicida* may not have difficulty in capturing, handling, ingesting, and digesting motionless perch blood cells. Furthermore, the maximum growth rate of *A. glandula* (1.00 d^{-1}) is also intermediate among those of pfiesteriacean species (Tables 4 & 5); the maximum growth rate of *A. glandula* is lower than that of *L. masanensis*, *P. piscicida*, and *S. algicida*, but greater than *C. brodyi*, *P. shumwayae*, and *S. changwonensis*. The maximum ingestion rate of *A. glandula* is much

Table 5. Comparison of the maximum growth and ingestion rates of each pfiesteriacean species on the algal and fish blood cell prey that support positive growth of the predator

Predator	Ag	Cb	Lm	Pfp	Pfs	Sa	Sc
Prey species							
Cryptophytes							
Teleaulax sp.	-	-	0.23 (0.44)	1.15 (1.11)	-	-	-
Rhodomonas salina	-	-	-	1.41 (0.72)	-	-	-
Rhodomonas sp.	-	**0.49**	0.64	-	**0.52**	-	-
Rhaphidophytes							
Heterosigma akashiwo	-	-	0.20 (0.16)	1.10 (0.75)	-	**1.63 (0.75)**	**0.38 (0.35)**
Dinoflagellates							
Amphidinium carterae	-	-	0.59 (0.32)	1.22 (1.08)	-	-	-
Heterocapsa triquetra	**1.00 (0.75)**	-	-	-	-	-	-
Akashiwo sanguinea	0.57 (1.38)	-	-	-	-	-	-
Blood cells							
Perch	0.09 (0.43)[a]	-	**1.46 (2.61)**	**1.74 (4.3)**	-	-	0.35 (0.27)
Reference	a	b	b, c	d	b	e	f

Bold indicates the optimal prey for each predator; maximum growth rate (d^{-1}) and maximum ingestion rate (ng C predator^{-1} d^{-1}) in parenthesis.
Ag, *Aduncodinium glandula*; Cb, *Cryptoperidiniopsis brodyi*; Lm, *Luciella masanensis*; Pfp, *Pfiesteria piscicida*; Pfs, *Pfiesteria shumwayae*; Sa, *Stoeckeria algicida*; Sc, *Stoeckeria changwonensis*.
[a]Highest value among the growth or ingestion rates measured at the given prey concentrations.
a, This study; b, Baek et al. (2010); c, Jeong et al. (2007*a*); d, Jeong et al. (2006); e, Jeong et al. (2005*a*); f, Lim et al. (2014).

lower than those of *L. masanensis* and *P. piscicida*, but greater than those of *S. changwonensis* and *S. algicida*, and these differences may be responsible for the differences in the maximum growth rates. Moreover, the ratio of the maximum growth rate relative to the maximum ingestion rate of *A. glandula* on the thecate dinoflagellate *H. triquetra* (1.3) is lower than that of *S. algicida* on the naked raphidophyte *H. akashiwo* (2.2). Therefore, the conversion of *H. triquetra* prey cells to *A. glandula* predator cells may be smaller than that of *H. akashiwo* prey cells to *S. algicida* predator cells.

The maximum ingestion rate of *A. glandula* on *H. triquetra* is 2 to 3 times greater than that of the mixotrophic dinoflagellates *G. smaydae* and *K. armiger* on the same prey (Table 6). *A. glandula* is considerably larger than *G. smaydae* and *K. armiger*. Therefore, *A. glandula* is likely to have a higher maximum ingestion rate than those of *G. smaydae* and *K. armiger*. In addition, the maximum ingestion rates of heterotrophic dinoflagellates are generally higher than those of mixotrophic dinoflagellates (Jeong et al. 2010*b*). Therefore, the heterotrophy of *A. glandula* may be partially responsible for its having maximum ingestion rates higher than the mixotrophic *G. smaydae* and *K. armiger*. However, the maximum growth rate of *A. glandula* on *H. triquetra* is comparable to that of *G. smaydae* on the same prey (Table 6). *G. smaydae* feeding on *H. triquetra* may have a growth rate slightly higher than *A. glandula* on the same prey because possible phototrophic growth during feeding may elevate the maximum growth rate (i.e., mixotrophic growth). Moreover, the maximum ingestion rate of *A. glandula* is considerably lower than that of *Gyrodinium dominans*, even though the sizes of *A. glandula* and *G. dominans* are similar. *A. glandula* is a peduncle feeder, while *G. dominans* is an engulfment feeder. Therefore, the peduncle of *A. glandula* may be a less effective tool for feeding on *H. triquetra* than the engulfment of *G. dominans*. However, the maximum growth rate of *G. dominans* on *H. triquetra* is considerably lower than that of *A. glandula*. *G. dominans* ingests whole *H. triquetra* cells including the theca, while *A. glandula* sucks prey materials excluding the theca. Therefore, the conversion of ingested *H. triquetra* cells to *G. dominans* mass may be less effective than that for *A. glandula*. Alternatively, engulfment feeding may require more energy than peduncle feeding. During *H. triquetra* red tides, *A. glandula* is likely to be as abundant as *G. smaydae* and *Gyrodinium spirale* and more abundant than *G. dominans*.

The maximum ingestion rate of *A. glandula* on *A. sanguinea* is much greater than that of *P. piscicida* on the same prey (Table 6). *A. glandula* is much larger than *P. piscicida*, which may be the reason for the difference in the maximum ingestion rate; the larger *A. glandula* may have less difficulty in handling large *A. sanguinea* cells than do the smaller *P. piscicida*. In turn, the much greater

Table 6. Comparison of the growth and ingestion rates of *Aduncodinium glandula* and dinoflagellate predators on *Heterocapsa triquetra*, *Akashiwo sanguinea*, and perch blood cells

Prey	Predator	ESD	μ_{max}	I_{max}	Reference
Heterocapsa triquetra	*Gymnodinium smaydae* (MD)	10.6	1.05	0.24	Lee et al. (2014)
	Karlodinium armiger (MD)	16.7	0.80	0.37	Berge et al. (2008)
	Gyrodinium dominans (HD)	20.0	0.54	2.3	Nakamura et al. (1995)
	Aduncodinium glandula (HD)	21.0	1.00	0.75	This study
	Protoperidinium steinii (HD)	25.8	0.30	-	Naustvoll (2000)
	Gyrodinium spirale (HD)	31.8	1.08	7.5	Hansen (1992)
Akashiwo sanguinea	*Pfiesteria piscicida* (HD)	13.5	0.15[a]	0.06[a]	Jeong et al. (2006)
	Aduncodinium glandula (HD)	21.0	0.57	1.38	This study
	Protoperidinium cf. *divergens* (HD)	61.0	0.27	-	Jeong and Latz (1994)
	Protoperidinium crassipes (HD)	73.0	0.12	-	Jeong and Latz (1994)
Perch blood cell	*Luciella masanensis* (HD)	13.5	1.46	2.61	Jeong et al. (2007*a*)
	Pfiesteria piscicida (HD)	13.5	1.74	4.30	Jeong et al. (2006)
	Stoeckeria changwonensis (HD)	13.9	0.35	0.58	Lim et al. (2014)
	Aduncodinium glandula (HD)	21.0	0.09	0.43	This study

Rates were corrected to 20°C using Q_{10} = 2.8 (Hansen et al. 1997).

ESD, equivalent spherical diameter (μm); μ_{max}, maximum growth rate (d^{-1}); I_{max}, maximum ingestion rate (ng C predator^{-1} d^{-1}); MD, mixotrophic dinoflagellate; HD, heterotrophic dinoflagellate.

[a]Value at a single mean prey concentration at which the growth or ingestion rate of the predator on the optimal prey was saturated.

maximum ingestion rate of *A. glandula* is likely to cause its higher maximum growth rate than that of *P. piscicida*. The much larger sizes of the heterotrophic dinoflagellates *Protoperidinium crassipes* and *P.* cf. *divergens* may be partially responsible for their having growth rates lower than that of *A. glandula*. During *A. sanguinea* red tides, *A. glandula* is likely to be more abundant than *P. piscicida*, *P. crassipes*, and *P.* cf. *divergens*.

The maximum ingestion rate of *A. glandula* on perch blood cells was slightly greater than that of *S. changwonensis*, but much lower than those of *P. piscicida* and *L. masanensis*. In the phylogenetic tree based on large subunit ribosomal DNA, *A. glandula*, the clade consisting of *Stoeckeria* spp., and the clade consisting of *Pfiesteria* spp. and *Luciella* spp. are clearly divergent from each other (Kang et al. 2015). *A. glandula* and *Stoeckeria* spp. may not have genes for enzymes related to detecting, ingesting, and digesting blood cells, whereas *Pfiesteria* spp. and *Luciella* spp. have them.

In conclusion, the results of this study show that 1) *A. glandula* has the most diverse prey species among the pfiesteriacean dinoflagellates; 2) *H. triquetra* and *A. sanguinea* are the optimal and suboptimal prey species for *A. glandula*, and these are different from those for the other pfiesteriacean dinoflagellates; 3) the maximum growth and ingestion rates of *A. glandula* are intermediate among those of pfiesteriacean species; and, therefore, 4) the ecological niche of *A. glandula* is likely to be different from that of other pfiesteriacean dinoflagellates in marine food webs.

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SUPPLEMENTARY VIDEOS

1. Ag on Ht (Jang) - *Aduncodinium glandula* feeding on *Heterocapsa triquetra* (www.e-algae.kr).

2. Ag on As (Jang) - *Aduncodinium glandula* feeding on *Akashiwo sanguinea* (www.e-algae.kr).

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