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Feeding and grazing impact of the newly described heterotrophic dinoflagellate *Stoeckeria algicida* on the harmful alga *Heterosigma akashiwo*

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ABSTRACT: To investigate the roles of the newly described thecate heterotrophic dinoflagellate *Stoeckeria algicida* (GenBank accession number = AJ841809), which was abundant during the red tides dominated by the harmful raphidophyte *Heterosigma akashiwo*, we measured the abundances of *H. akashiwo* and *S. algicida* in Masan Bay, Korea, from May to July 2004. We also measured growth and ingestion rates of *S. algicida* when feeding on *H. akashiwo* in the laboratory. Grazing coefficients were calculated by combining field data on abundances of *S. algicida* and co-occurring *H. akashiwo* with laboratory data on ingestion rates obtained in the present study. The maximum abundances of *H. akashiwo* during 2 *H. akashiwo* red tides were 58 400 and 99 200 cells ml, while those of *S. algicida* were 1130 and 17 400 cells ml^{-1}, respectively. The time lags between the abundance peaks of *H. akashiwo* and *S. algicida* in these red tide periods were 1 to 2 d. *S. algicida* fed on *H. akashiwo* using a peduncle after anchoring the prey by a tow filament. Specific growth rates of *S. algicida* increased rapidly with increasing mean prey concentration before saturating at *H. akashiwo* concentrations of ca. 350 ng C ml^{-1} (3500 cells ml^{-1}). The maximum specific growth rate of *S. algicida* on *H. akashiwo* was 1.63 d^{-1}. The threshold prey concentration (where net growth = 0) was 1.9 ng C ml^{-1} (19 cells ml^{-1}). Maximum ingestion and clearance rates of *S. algicida* on *H. akashiwo* were 0.75 ng C $grazer^{-1}$ d^{-1} (7.5 cells $grazer^{-1}$ d^{-1}) and 3.7 µl $grazer^{-1}$ h^{-1}, respectively. Calculated grazing coefficients for *S. algicida* on *H. akashiwo* were up to 0.142 min^{-1} (i.e. 13% of *H. akashiwo* populations were removed by a *S. algicida* population in 1 min). The results of the present study suggest that *S. algicida* sometimes has a considerable grazing impact on populations of *H. akashiwo*.

KEY WORDS: Food web · Harmful algal bloom · Ingestion · Peduncle · Protist · Red tide

INTRODUCTION

The red tide phenomenon, discoloration of the surface of the sea due to plankton blooms, can alter the balance of food webs and cause large-scale mortalities of fish and shellfish (ECOHAB 1995). Studies of red tide formation and persistence suggest that grazing pressure may play an important role in bloom dynamics (Watras et al. 1985). In particular, grazing by microzooplankton is believed to contribute to the decline of red tides (Holmes et al. 1967, Eppley & Harrison 1975, Jeong 1995, 1999, Kamiyama et al. 2000, Stoecker & Gustafson 2002, Johnson et al. 2003, Tillmann 2004, Kim & Jeong 2004).

The raphidophyte *Heterosigma akashiwo* can cause large-scale fish mortality rates when abundant during red tides (MacKenzie 1991, Chang et al. 1993, Honjo 1993, Imai et al. 1996). Its density sometimes exceeds 100 000 cells ml^{-1} during red tides (e.g. Nagasaki et al. 1996). The clogging of fishes' gills by *H. akashiwo* may

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be mainly responsible for the mortality rates as there have been no reports on the existence of a *H. akashiwo* toxin. Only a few heterotrophic protists are known to feed on *H. akashiwo*, e.g. the heterotrophic dinoflagellates *Gyrodinium dominans* and *Oxyrrhis marina*, and the prostomatid ciliate *Tiarina fusus* (Nakamura et al. 1995, Jeong et al. 2002, 2003). However, large tintinnid ciliate *Favella* spp. did not ingest this prey (Taniguchi & Takeda 1988) or the ingestion rate was undetectable even though this prey was ingested in the initial incubation (Kamiyama & Arima 2001). We found that the newly described thecate heterotrophic dinoflagellate *Stoeckeria algicida* was often the most dominant heterotrophic protist during red tides dominated by *H. akashiwo* in Masan Bay, Korea, and that the grazer was able to grow well on this red tide prey. However, no study has reported the feeding mechanism, and growth and grazing rates of *S. algicida* on *H. akashiwo* as a function of prey concentration, nor has anyone studied its grazing impact on the prey.

To understand the role of *Stoeckeria algicida* in the dynamics of *Heterosigma akashiwo*, we measured the abundances of *H. akashiwo* and *S. algicida* in Masan Bay, Korea, established a monoclonal culture of *S. algicida*, examined feeding behavior, and conducted experiments to examine its numerical and functional responses when grown on *H. akashiwo*. We also estimated grazing coefficients attributable to *S. algicida* on *H. akashiwo* by combining field data on abundances of *S. algicida* and co-occurring *H. akashiwo* with laboratory data on ingestion rates obtained in the present study.

Maximum growth and grazing rates of *Stoeckeria algicida* on *Heterosigma akashiwo* were compared to those of other heterotrophic protists feeding on the same prey species. The results of the present study provide a basis for understanding the potential of *S. algicida* to influence the population dynamics of *H. akashiwo*.

MATERIALS AND METHODS

Abundances in Masan Bay, Korea. Water samples were taken from the surface during red tides dominated by *Heterosigma akashiwo* from May to July 2004 at a pier in Masan Bay. Samples were taken at 10:00 h using water samplers, and sometimes additional samples were taken at 16:00 h on the same day. Plankton samples for counting were poured into 500 ml polyethylene bottles and preserved with acidic Lugol's solution. After being well mixed, all or >300 *Stoeckeria algicida* and *H. akaskiwo* cells were counted under an inverted microscope with standard transmitted illumination in three 1 ml Sedgwick-Rafter counting chambers (SRCs).

Water temperatures and salinities in the surface waters were measured using a YSI 30 (YSI Incorporated) and pH and dissolved oxygen (DO) concentration were measured using pH-11 (Schott Handy – Lab) and Oxi 197i (WTW), respectively.

Culture of phytoplankton prey. *Heterosigma akashiwo* was grown at 20°C in enriched f/2 seawater media (Guillard & Ryther 1962) without silicate, under continuous illumination of 100 µE m^{-1} s^{-1} provided by cool white fluorescent lights. Only cultures in an exponential growth phase were used for feeding experiments. The carbon content for *H. akashiwo* (0.1 ng C $cell^{-1}$, n > 2000) was estimated from cell volume (700 mm^3) according to Strathmann (1967).

Isolation and culture of *Stoeckeria algicida*. Plankton samples collected with water samplers were taken from a pier in Masan Bay, Korea, during July 2004 when the water temperature and salinity were 24.8°C and 20.6 psu, respectively. The samples were gently screened through a 154 µm Nitex mesh and placed in 6-well tissue culture plates and a monoclonal culture of *S. algicida* was established by 2 serial single cell isolations. As the concentration of *S. algicida* feeding on *Heterosigma akashiwo* increased, the grazers were subsequently transferred to 32, 270, and 500 ml polycarbonate (PC) bottles of fresh *H. akashiwo*. The bottles were again filled to capacity with freshly filtered seawater, capped, and placed on a rotating wheel at 0.9 rpm at 20°C under an illumination of 20 µE m^{-2} s^{-1} of cool white fluorescent light on a 12:12 h light:dark cycle. Once dense cultures of *S. algicida* (8000 to 10 000 cells ml^{-1}) were obtained, they were transferred to 500 ml PC bottles of fresh prey (ca. 30 000 cells ml^{-1}) every day. Experiments were conducted when large volumes of *S. algicida* culture were available. The carbon content for *S. algicida* was estimated from cell volume (see next subsection) according to Menden-Deuer & Lessard (2000).

Stoeckeria algicida is similar to *Pfiesteria piscicida* and some *Pfiesteria*-like species in size and shape, but the plate pattern (Po, cp, X, 4′, 2-3a, 7′′, 6c, 6s, 5′′′, and 2′′′′ in a Kofoidian series) and the sequence of SSU rDNA of *S. algicida* (GenBank accession number = AJ841809) are markedly different from those of *P. piscicida* (Po, cp, X, 4′, 1a, 5′′, 6c, 4s, 5′′′, and 2′′′′ in a Kofoidian series; GenBank accession numbers = AF218805, AF080098) and *Pfiesteria*-like species (Jeong et al. 2005).

Cell volume. Cell length and maximum width of *Stoeckeria algicida* preserved in 5% acidic Lugol's solution (n = 30) were measured using a compound or inverted microscope. The shape of *S. algicida* was estimated as being oval. The cell volume of preserved *S. algicida* was calculated according to the equation: volume = $4/3 \times \pi$ [(cell length + cell width)/4]^3.

Feeding process. A culture of *Stoeckeria algicida* starved for 24 h was transferred to a 6-well-plate chamber containing a dense culture of *Heterosigma akashiwo*. The feeding behavior of >50 unfed *S. algicida* cells was then observed under a dissecting microscope at a magnification of 50×. Some *S. algicida* cells capturing prey cells were placed on slides using a micropipette, cover-glasses were added, and then a series of pictures of *S. algicida* at several different stages of the feeding process were taken using an Olympus digital camera on a compound microscope at a magnification of 100 to 400×.

Growth and ingestion rates. This experiment was designed to measure growth, ingestion, and clearance rates of *Stoeckeria algicida*, as a function of the prey concentration, when feeding on *Heterosigma akashiwo* (Table 1).

Three days before these experiments were conducted, dense cultures (10 000 to 12 000 cells ml^{-1}) of *Stoeckeria algicida* growing on *Heterosigma akashiwo* were transferred into new 500 ml PC bottles of fresh prey (ca. 1000 cells ml^{-1}) every day. This was done to make the growth rate of *S. algicida* almost zero at the end of this pre-incubation. The bottles were filled to capacity with filtered seawater and placed on a rotating wheel to incubate as above. The abundances of *S. algicida* and prey were determined by enumerating cells in three 1 ml SRCs.

The initial concentrations of *Stoeckeria algicida* and *Heterosigma akashiwo* were established using an autopipette to deliver predetermined volumes of known cell concentrations to the bottles. Triplicate 80 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 *S. algicida* were also established at 1 predator concentration. Ten ml of f/2 medium were 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 24, 48, and 72 h of incubation, a 5 ml aliquot for *S. algicida* was removed from each bottle and fixed with 5% Lugol's solution, and all predator cells and all or >200 prey cells were enumerated in three 1 ml SRCs. The ranges of the actual prey and predator concentrations in the experimental bottles at the beginning of the experiment were 23 to 31 263 and 9 to 121 cells ml^{-1}, respectively. The actual predator concentration in the control bottles containing only *S. algicida* was 98 cells ml^{-1}. Prior to taking subsamples, the condition of *S. algicida* and its prey was assessed using a dissecting microscope. The bottles were again filled to capacity with freshly filtered seawater, capped, and placed on a rotating wheel at 0.9 rpm at 20°C under an illumination of 20 µE m^{-2} s^{-1} of cool white fluorescent light on a 12:12 h light:dark cycle. Dilution of the cultures associated with refilling the bottles was considered in calculating growth and ingestion rates.

Table 1. *Stoeckeria algicida* feeding on *Heterosigma akashiwo*. Estimation of grazing impact by a *S. algicida* population on a *H. akashiwo* population using the equations in Fig. 4 derived from the laboratory experiments and the abundances of co-occurring *H. akashiwo* and *S. algicida* obtained from water samples collected off Masan Bay in 2004. HaC: *H. akashiwo* concentration; SaC: *S. algicida* concentration; SaPIR: *S. algicida* population ingestion rate; Sag: *S. algicida* grazing coefficient. Ingestion rates were corrected using Q_{10} = 2.8 (Hansen et al. 1997) because *in situ* water temperatures and the temperature used in the laboratory for this experiment (20°C) were different

HaC (cells ml^{-1})	SaC (cells ml^{-1})	SaPIR (prey ml^{-1} h^{-1})	Sag (h^{-1})
17	69	1	0.070
45	3300	174	4.000
700	17400	5546	8.500
1130	310	82	0.075
4800	8300	3861	1.631
10300	1130	409	0.040
29500	510	178	0.006
41000	505	439	0.011
99200	3700	2015	0.021

The specific growth rate of *Stoeckeria algicida*, µ (d^{-1}), was calculated by averaging the instantaneous growth rates (IGR) for each sampling interval, calculated as:

$$\mathrm{IGR} = \frac{\ln(S_{t_2}/S_{t_1})}{t_2 - t_1} \times 24 \quad (1)$$

where S_{t_1} and S_{t_2} are the concentrations of *S. algicida* at consecutive samplings. The values of t_1 and t_2 used for calculation were 24 and 72 h, respectively, which provided the highest specific growth rate.

Data for *Stoeckeria algicida* growth rate were fitted to a Michaelis-Menten 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 threshold prey concentration (where µ = 0), and K_{GR} is the prey concentration sustaining $\frac{1}{2}\mu_{max}$. Data were iteratively fitted to the model using DeltaGraph® (Delta Point).

Ingestion and clearance rates were calculated using the equations of Frost (1972) and Heinbokel (1978). Incubation times for calculating ingestion and clearance rates were the same as for estimating growth rate. Ingestion rate (IR) data for *Stoeckeria algicida* 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 grazer^{-1} d^{-1} or ng C grazer^{-1} d^{-1}), x is the prey concentration (cells ml^{-1} or ng C ml^{-1}), and K_{IR} is the prey concentration sustaining ½I_{max}.

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

Grazing impact. We estimated grazing coefficients attributable to *Stoeckeria algicida* on *Heterosigma akashiwo* by combining field data on the abundances of *S. algicida* and co-occurring prey with the ingestion rates of the predators on the prey obtained in the present study.

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

$$g = (1/\Delta t)\ \{\ln[C_i/(C_i - C_e)]\} \quad (4)$$

where Δt (h) is a time interval, C_e (cells ml^{-1}) is the number of prey cells eaten by the *Stoeckeria algicida* population in 1 ml of seawater in 1 h, and C_i (cells ml^{-1}) is the initial cell concentration of prey for a given hour. The values of C_e were calculated as:

$$C_e = PIR \times 1\ hour = IR \times G \times 1\ hour \quad (5)$$

where PIR is the population ingestion rate of *S. algicida* on *Heterosigma akashiwo* in 1 ml of seawater (prey eaten ml^{-1} h^{-1}), IR is the ingestion rate (prey eaten grazer^{-1} h^{-1}) of *S. algicida* on *H. akashiwo*, and G is the abundance (grazers ml^{-1}) of *S. algicida* at the same time as C_i. IRs were corrected using Q_{10} = 2.8 (Hansen et al. 1997) because *in situ* water temperatures and the temperature used in the laboratory for this experiment (20°C) were different.

RESULTS

Abundances and hydrography in Masan Bay

Two red tides were dominated by *Heterosigma akashiwo* in Masan Bay between May and July 2004; the first red tide occurred between May 31 and June 8, and the second one occurred between July 10 and July 16 (Fig. 1). The maximum abundances of *H. akashiwo* in the first and second red tide periods were 58 400 and 99 200 cells ml^{-1}, respectively, while those of *Stoeckeria algicida* were 1130 and 17 400 cells ml^{-1}, respectively.

Water temperatures and salinities in the surface waters in the first red tide period were 19.0 to 2.8°C and 24.7 to 29.4 psu, respectively, while those in the second red tide period were 23.7 to 26.0°C and 4.1 to 20.6 psu, respectively (Fig. 1). The pH values and DO concentrations in the surface waters in the first red tide period were 7.90 to 8.33 and 5.2 to 10.3 µM, respectively, while those in the second red tide period were 7.40 to 8.58 and 5.9 to 8.4 µM, respectively.

Feeding process

Stoeckeria algicida fed on *Heterosigma akashiwo* using a peduncle after anchoring the prey by a tow filament (Fig. 2). *S. algicida* deployed a tow filament to anchor a *H. akashiwo* cell. The distance between the predator cell and the prey cell was almost twice the prey cell length when the predator attached the tow filament to the prey cell, but reduced to be similar to the prey cell length before the peduncle deployed. The time lag (mean ± SE) between the deployment of a tow filament and a peduncle was 22 ± 1 s (n = 5). The prey materials (reddish materials in Fig. 2) were moved in inside the predator cell through the peduncle. The time (mean ± SE) for a *H. akashiwo* cell to be completely fed on by a *S. algicida* cell after the predator deployed a peduncle to the prey cell was 129 ± 4 s (n = 5). As the material of the prey cell was moved into the protoplasm of the predator, the size of the prey cell was reduced, while the size of the predator increased. Eventually, round (1 to 2 µm) transparent fecal material remained at the end of this feeding. Up to 5 *S. algicida* cells were observed to deploy their peduncles to a *H. akashiwo* cell simultaneously.

Growth rates

Stoeckeria algicida grew well on *Heterosigma akashiwo*. The specific growth rates of the heterotrophic dinoflagellate increased rapidly with increasing mean prey concentration before saturating at *H. akashiwo* concentrations of ca. 350 ng C ml^{-1} (3500 cells ml^{-1}) (Fig. 3). When the data were fitted to Eq. (2), the maximum specific growth rate (μ_{max}) of *S. algicida* was 1.63 d^{-1}. A threshold prey concentration (where net growth = 0) for *S. algicida* was 1.9 ng C ml^{-1} (19 cells ml^{-1}).

Ingestion and clearance rates

The ingestion rates of *Stoeckeria algicida* on a unialgal diet of *Heterosigma akashiwo* increased with increasing mean prey concentration (Fig. 4). When the

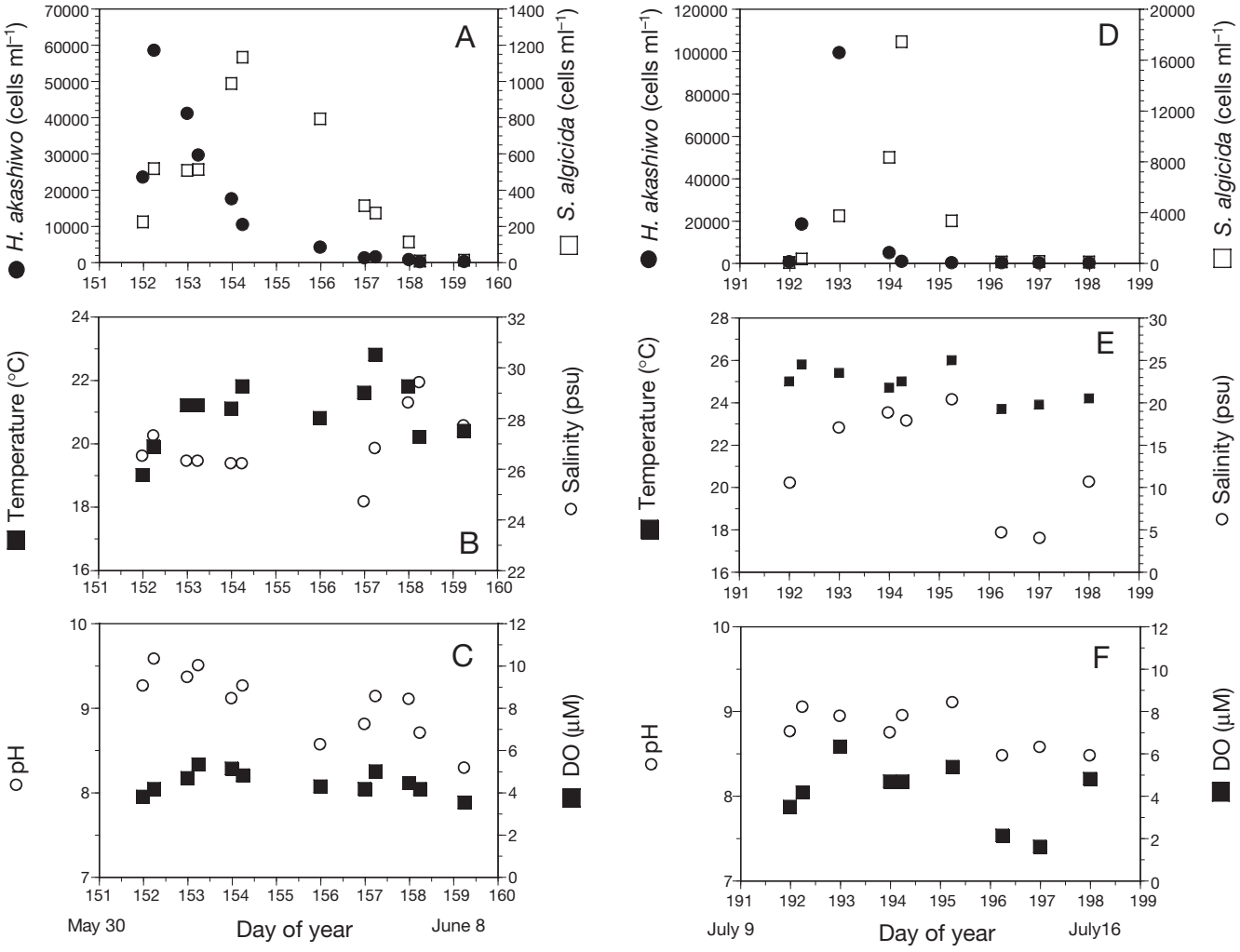

Fig. 1. Abundances of (A,D) *Heterosigma akashiwo* and *Stoeckeria algicida*, (B,E) water temperature and salinity, and (C,F) pH and dissolved oxygen concentration (DO) at a pier in Masan Bay, Korea, in the first *H. akashiwo* red tide period (from May 31 to June 8, 2004) and in the second *H. akashiwo* red tide period (from July 10 to July 16, 2004)

data were fitted to Eq. (3), the maximum ingestion rate of *S. algicida* was 0.75 ng C grazer^{-1} d^{-1} (7.5 prey cells grazer^{-1} d^{-1}).

The maximum clearance rate of *Stoeckeria algicida* on *Heterosigma akashiwo* was 3.7 µl grazer^{-1} h^{-1} at the lowest mean prey concentration of 1 ng C ml^{-1} and the maximum volume-specific clearance rate of *S. algicida* was 9.7×10^6 h^{-1}.

Cell volume

After 72 h incubation, the cell volume of *Stoeckeria algicida*-fed *Heterosigma akashiwo* at the low mean prey concentrations of 1.0 to 1.8 ng C ml^{-1} (320 to 380 µm^3) was slightly larger than that of *S. algicida* without added prey (300 µm^3), but at the higher prey concentration, cell volume increased continuously from 570 to 1350 µm^3 with increasing mean prey concentration (Fig. 5). The largest cell volume of *S. algicida* measured in these experiments (2520 µm^3) was approximately 20 times larger than the smallest one (130 µm^3).

The cell volume of *Stoeckeria algicida* where the maximum volume-specific clearance rate was obtained was 380 µm^3.

Gross growth efficiency

GGE of *Stoeckeria algicida* on *Heterosigma akashiwo* were negative for mean prey concentrations ≤4 ng C ml^{-1}, but efficiencies increased up to 79% with increasing mean prey concentration (Fig. 6).

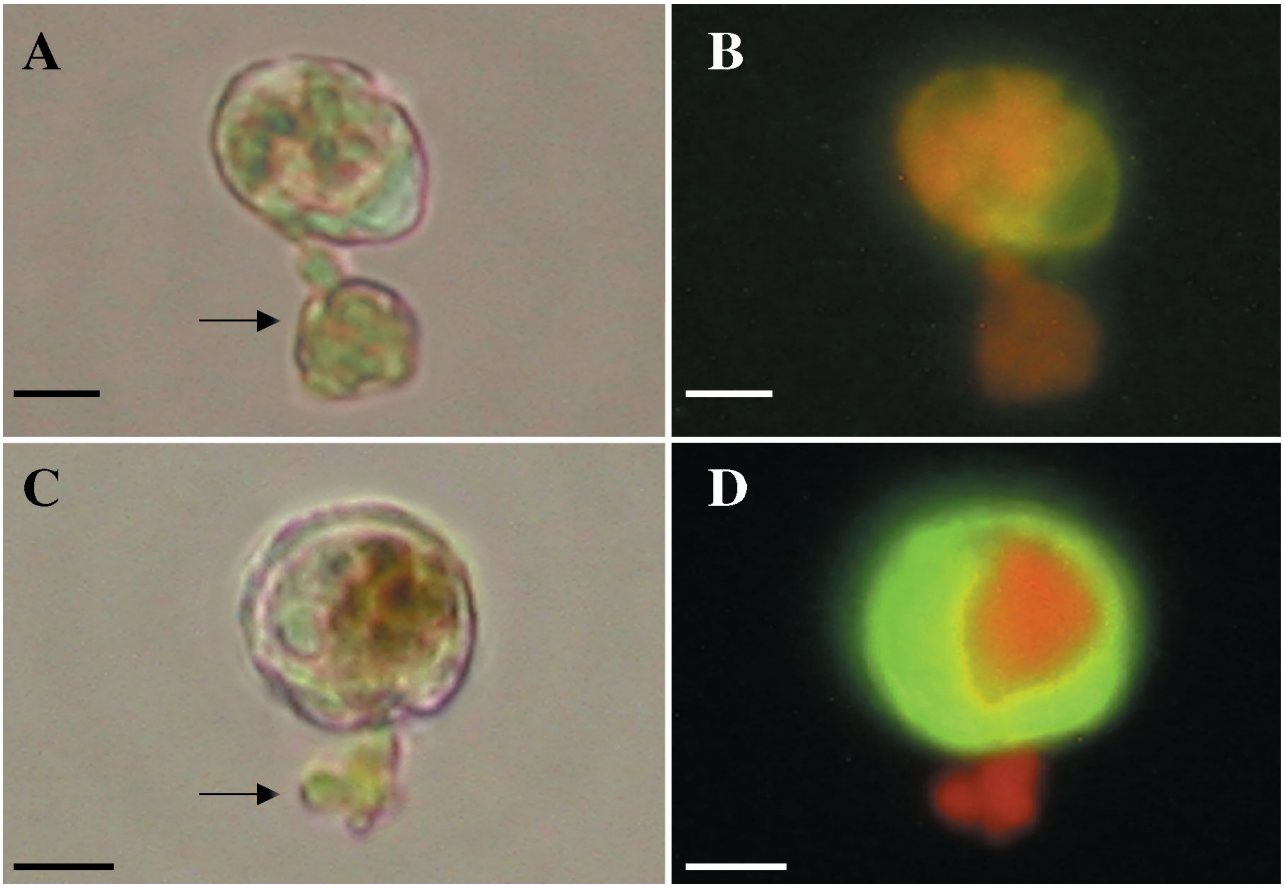

Fig. 2. Feeding by *Stoeckeria algicida* on a *Heterosigma akashiwo* cell using a peduncle. (A,B) Lateral view. (C,D) Anterior view. (A) and (C) are phase photomicrographs and (B) and (D) are photomicrographs taken using epifluorescence. Reddish colors in (B) and (D) are prey materials. Arrows indicate prey cells. All prey and predator cells are the same cells in all panels. Scale bars = 5 µm

Grazing impact

Grazing coefficients attributable to *Stoeckeria algicida* on co-occurring *Heterosigma akashiwo* in Masan Bay, Korea, were up to 1.63 h^{-1} with 2 exceptional values at the decline stages of the red tides dominated by *H. akashiwo*; one value of *g* was 8.5 h^{-1} when the concentrations of *H. akashiwo* and *S. algicida* were 700 and 17 400 cells ml^{-1}, respectively, and another value of *g* was 4.0 h^{-1} when the concentrations of *H. akashiwo* and *S. algicida* were 45 and 3300 cells ml^{-1}, respectively (Fig. 7, Table 1). In general, *g* increased with increasing *S. algicida* concentration.

DISCUSSION

Abundances in Masan Bay

The abundance of *Stoeckeria algicida* in Masan Bay between May and July 2004 peaked 1 to 2 d after that of *Heterosigma akashiwo*. Water masses in the sampling location might be advected over sampling intervals even though the circulation of water masses inside Masan Bay is restricted. If the advection were very small, the time lags between the abundance peaks of *H. akashiwo* and *S. algicida* in these first and second red tide periods could be calculated to be 1 to 2 d.

Feeding mechanism

Stoeckeria algicida feeds on *Heterosigma akashiwo* using a peduncle after anchoring the prey by a tow filament. Peduncle feeders feed on diverse prey species with a wide size range because they are able to feed on prey larger than themselves (Biecheler 1952, cited by Hansen 1991b, Lee 1977, Spero 1982, Hansen 1991a,b, Burkholder & Glasgow 1995). For example, *Pfiesteria* spp. are known to feed on diverse prey items including algae, ciliates, and rotifers (Burkholder & Glasgow 1995). It is, therefore, worthwhile exploring the kinds of prey species used when diverse prey items are provided to *S. algicida*.

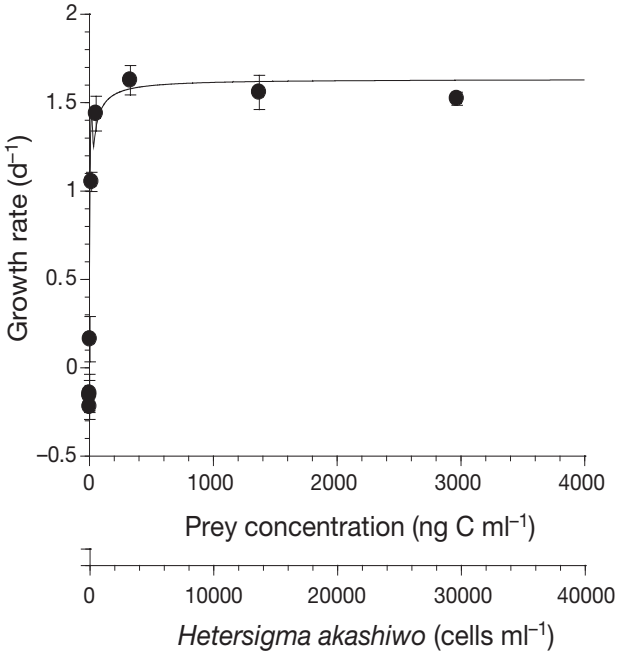

Fig. 3. Specific growth rates of *Stoeckeria algicida* on *Heterosigma akashiwo* as a function of mean prey concentration (x, ng C ml^{-1}). Symbols represent treatment means ± 1 SE. The curve is fitted by a Michaelis-Menten equation (Eq. 2) using all treatments in the experiment. Growth rate (GR, d^{-1}) = 1.63 {(x – 1.9)/[11.6 + (x – 1.9)]}, r^2 = 0.947

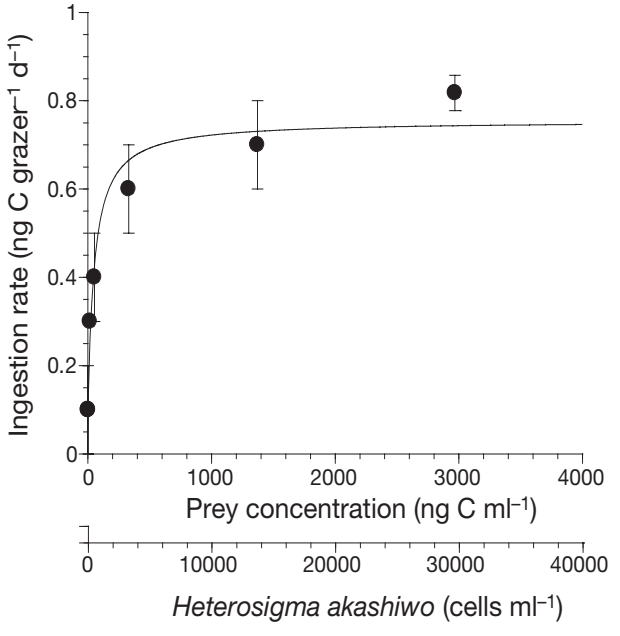

Fig. 4. Ingestion rates of *Stoeckeria algicida* on *Heterosigma akashiwo* as a function of mean prey concentration (x). Symbols represent treatment means ± 1 SE. The curve is fitted by a Michaelis-Menten equation (Eq. 3) using all treatments in the experiment. Ingestion rate (IR, ng C grazer^{-1} d^{-1}) = 0.75 [x/(45.3 + x)], r^2 = 0.915

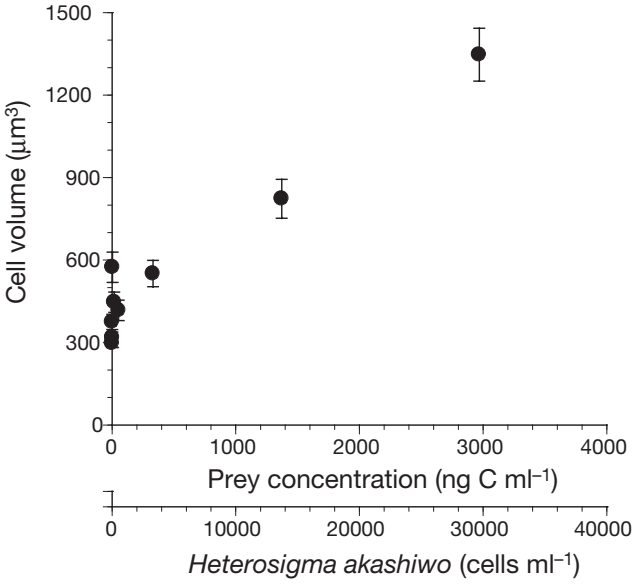

Fig. 5. Cell volume of *Stoeckeria algicida* on *Heterosigma akashiwo* after 72 h incubation as a function of mean prey concentration. Symbols represent treatment means ± 1 SE

Protistan predators on *Heterosigma akashiwo*

Stoeckeria algicida grew well on *Heterosigma akashiwo* in the present study. Only a few heterotrophic protists are known to grow on *H. akashiwo*, e.g. the heterotrophic dinoflagellates *Gyrodinium dominans* and *Oxyrrhis marina*, and the prostomatid ciliate *Tiarina fusus* (Nakamura et al. 1995, Jeong et al. 2002, 2003). However, the large tintinnid ciliate *Favella taraikaensis* did not ingest this prey (Taniguchi & Takeda 1988) or the ingestion rate was undetectable even though this prey was ingested in the initial incubation (Kamiyama & Arima 2001). *S. algicida* is, therefore, one of the few heterotrophic dinoflagellate grazers so far reported to grow on *H. akashiwo*.

Growth and ingestion

The maximum growth rate of *Stoeckeria algicida* on *Heterosigma akashiwo* in the present study (1.63 d^{-1}) was higher than that of any other protistan grazer on the same prey so far reported (Table 2), when corrected to 20°C using Q_{10} = 2.8 (Hansen et al. 1997).

The maximum ingestion rate of *Stoeckeria algicida* on *Heterosigma akashiwo* in the present study (0.75 ng C grazer^{-1} d^{-1}) was lower than that of any other protistan grazer on the same prey so far reported (Table 2). The smaller volume of *S. algicida* might be responsible for its higher growth, despite lower ingestion rates on *H. akashiwo* compared to those of *Oxyrrhis marina* and *Tiarina fusus*.

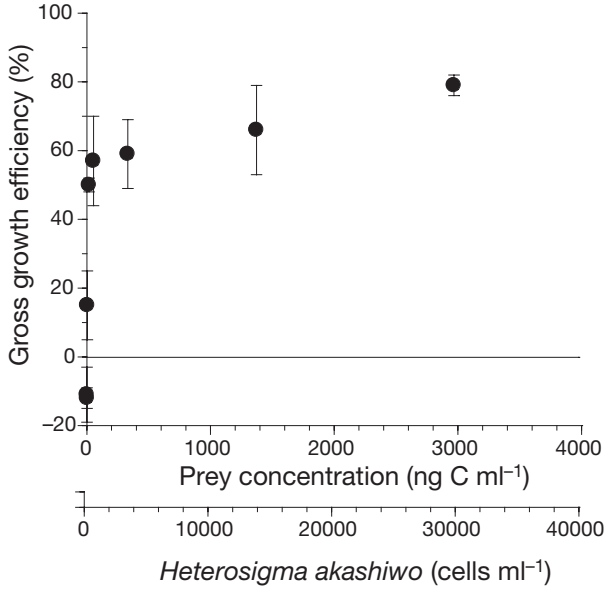

Fig. 6. Gross growth efficiency (GGE), defined as *Stoeckeria algicida* biomass produced (+) or lost (–) per *Heterosigma akashiwo* biomass ingested, as a function of mean prey concentration. Symbols represent treatment means ± 1 SE

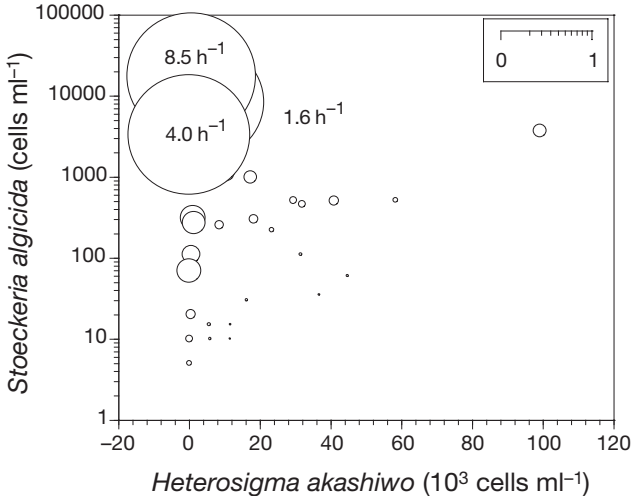

Fig. 7. Calculated grazing coefficients (g, h^{-1}) attributable to *Stoeckeria algicida* on *Heterosigma akashiwo* (see text for calculation), n = 30. The scale of all circles except 2 circles in the inset box is g (h^{-1}). The value of g for one circle was 8.5 h^{-1} when the concentrations of *H. akashiwo* and *S. algicida* were 700 and 17400 cells ml^{-1}, while the value of g for the other circle was 4.0 h^{-1} when the concentrations of *H. akashiwo* and *S. algicida* were 45 and 3300 cells ml^{-1}, respectively. The scales for these 2 values of g were reduced

The maximum clearance rate of *Stoeckeria algicida* on *Heterosigma akashiwo* (3.7 µl $grazer^{-1}$ h^{-1}) was much higher than that of any other protistan grazer on the same prey so far reported (Table 2). *S. algicida*, which feeds on prey cells using a peduncle, might capture and ingest *H. akashiwo* more efficiently at low prey concentration than *Oxyrrhis marina* and *Tiarina fusus*, which feed on prey cells by engulfment.

The maximum volume-specific clearance rate of *Stoeckeria algicida* on *Heterosigma akashiwo* (9.7×10^6 h^{-1}) is higher than that for any other heterotrophic dinoflagellate on phytoplankton. Before the present study, the maximum volume-specific clearance rate of *Protoperidinium bipes* on *Skeletonema costatum* (5.4×10^6 h^{-1}) had been the highest value reported (Jeong et al. 2004). The higher maximum clearance rate of *S. algicida* on *H. akashiwo* (3.7 µl $grazer^{-1}$ h^{-1}) compared with that of *P. bipes* on *S. costatum* (1.0 µl $grazer^{-1}$ h^{-1}) was responsible for its higher maximum volume-specific clearance rate because the cell volume of the former grazer, where the maximum volume-specific clearance rate was obtained (380 μm^3), was larger than that of the latter grazer (184 μm^3). *S. algicida*, might capture and ingest *H. akashiwo* more efficiently at low prey concentration than *P. bipes*, which feeds on prey cells using a feeding veil (pallium).

The maximum GGE of *Stoeckeria algicida* on *Heterosigma akashiwo* (79%) was higher than that for the obligately heterotrophic dinoflagellate *Protoperiidnium huberi* on the diatom *Ditylum brightwelli* (59%), the highest value among the *Protoperiidnium* species so far reported (Buskey et al. 1994). Additional experiments confirmed this high GGE. Like *Pfiesteria* spp., which are also peduncle feeders, mixotrophic growth using kleptoplastids might be partially responsible for this high GGE (Lewitus et al. 1999). Alternatively, the carbon content for a *H. akashiwo* cell might be somewhat underestimated and/or that for a *S. algicida* cell overestimated.

Table 2. Comparison of growth, ingestion and clearance rates of *Stoeckeria algicida* and other protists on *Heterosigma akashiwo*. Rates are corrected to 20°C using Q_{10} = 2.8 (Hansen et al. 1997). PV: predators' volume as $\times 10^3$ μm^3; μ_{max}: maximum growth rate (d^{-1}); I_{max}: maximum ingestion rate (ng C $predator^{-1}$ d^{-1}); C_{max}: maximum clearance rate (µl $predator^{-1}$ h^{-1}); HTD: heterotrophic dinoflagellate; NC: naked ciliate

Predator	PV	μ_{max}	I_{max}	C_{max}	Source
Stoeckeria algicida (HTD)	1.4	1.62	0.75	3.7	this study
Oxyrrhis marina (HTD)	2.6	1.43	1.25	0.3	Jeong et al. (2003)
Gyrodinium dominans (HTD)	4.0	0.15			Nakamura et al. (1995)
Tiarina fusus (NC)	25.4	0.12	7.2	0.7	Jeong et al. (2002)

Grazing impact

Grazing coefficients attributable to *Stoeckeria algicida* on co-occurring *Heterosigma akashiwo* in Masan Bay, Korea, were up to 1.63 h^{-1} (i.e. 80% of *H. akashiwo* populations were removed by a *S. algicida* population in 1 h), with 2 exceptional values at the decline stages of the red tides dominated by *H. akashiwo*. When the *S. algicida* populations are very high and the *H. akashiwo* populations are very low, grazing impact can be so high that grazing has the potential to remove almost all prey cells within hours (Fig. 7, Table 1). Populations of *S. algicida* may, therefore, have considerable grazing impact on populations of *H. akashiwo* and contribute to the decline of red tides dominated by *H. akashiwo*.

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LITERATURE CITED

Biecheler B (1952) Recherches sur les peridiniens. Bull Biol Fr Belg Suppl 36:1–149

Burkholder JM, Glasgow HB Jr (1995) Interactions of a toxic estuarine dinoflagellate with microbial predators and prey. Arch Protistenkd 145:177–188

Buskey EJ, Coulter CJ, Brown SL (1994) Feeding, growth and bioluminescence of the heterotrophic dinoflagellate *Protoperidinium huberi*. Mar Biol 121:373–380

Chang FH, Pridmore R, Boustead N (1993) Occurrence and distribution of *Heterosigma* cf. *akashiwo* (Raphidophyceae) in a 1989 bloom in Big Glory Bay, New Zealand. In: Smayda TJ, Shimizu Y (eds) Toxic phytoplankton blooms in the sea. Elsevier, Amsterdam, p 675–680

ECOHAB (1995) The ecology and oceanography of harmful algal blooms. A national research agenda. Woods Hole Oceanographic Institute, Woods Hole, MA, p 1–66

Eppley RW, Harrison WG (1975) Physiological ecology of *Gonyaulax polyedrum*, a red tide water dinoflagellate of southern California. In: Locicero VR (ed) Proceedings First International Conference on Toxic Dinoflagellate Blooms. Massachusetts Science and Technology Foundation, Wakefield, MA, p 11–22

Frost BW (1972) Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnol Oceanogr 17:805–815

Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Grun. Can J Microbiol 8:229–239

Hansen PJ (1991a) *Dinophysis* — a planktonic dinoflagellate genus which can act both as a prey and a predator of a ciliate. Mar Ecol Prog Ser 69:201–204

Hansen PJ (1991b) Quantitative importance and trophic role of heterotrophic dinoflagellates in a coastal pelagic food web. Mar Ecol Prog Ser 73:253–261

Hansen PJ, Bjornsen PK, Hansen BW (1997) Zooplankton grazing and growth: scaling within the 2–2000-µm body size range. Limnol Oceanogr 42:687–704

Heinbokel JF (1978) Studies on the functional role of tintinnids in the Southern California Bight. I. Grazing and growth rates in laboratory cultures. Mar Biol 47:177–189

Holmes RW, Williams PM, Eppley RW (1967) Red water in La Jolla Bay, 1964–1966. Limnol Oceanogr 12:503–512

Honjo T (1993) Overview on bloom dynamics and physiological ecology of *Heterosigma akashiwo*. In: Smayda T, Shimizu Y (eds) Toxic phytoplankton blooms in the sea. Elsevier, Amsterdam, p 33–41

Imai I, Itakura S, Yamaguchi M, Honjo T (1996) Selective germination of *Heterosigma akashiwo* (raphidophyceae) cysts in bottom sediments under low light conditions: a possible mechanism of red tide initiation. In: Yasumoto T, Oshima Y, Fukuyo Y (eds) Harmful and toxic algal blooms. UNESCO, Paris, p 197–200

Jeong HJ (1995) The interactions between microzooplanktonic grazers and dinoflagellates causing red tides in the open coastal waters off southern California. PhD thesis, University of California, San Diego, CA

Jeong HJ (1999) The ecological roles of heterotrophic dinoflagellates in marine planktonic community. J Eukaryot Microbiol 46:390–396

Jeong HJ, Yoon JY, Kim JS, Yoo YD, Seong KA (2002) Growth and grazing rates of the prostomatid ciliate *Tiarina fusus* on red-tide and toxic algae. Aquat Microb Ecol 28:289–297

Jeong HJ, Kim JS, Yoo YD, Kim ST and 6 others (2003) Feeding by the heterotrophic dinoflagellate *Oxyrrhis marina* on the red-tide raphidophyte *Heterosigma akashiwo*: a potential biological method to control red tides using mass-cultured grazers. J Eukaryot Microbiol 50:274–282

Jeong HJ, Yoo YD, Kim ST, Kang NS (2004) Feeding by the heterotrophic dinoflagellate *Protoperidinium bipes* on the diatom *Skeletonema costatum*. Aquat Microb Ecol 36:171–179

Jeong HJ, Kim JS, Park JY, Kim JH and 5 others (2005) *Stoeckeria algicida* n. gen., n. sp. (Dinophayceae) from the coastal waters off southern Korea: morphology and small subunit ribosomal DNA gene sequence. J Eukaryot Microbiol (in press)

Johnson MD, Rome M, Stoecker DK (2003) Microzooplankton grazing on *Prorocentrum minimum* and *Karlodinium micrum* in Chesapeake Bay. Limnol Oceanogr 48:238–248

Kamiyama T, Arima S (2001) Feeding characteristics of two tintinnid ciliate species on plankton including harmful species: effects of prey size on ingestion rates and selectivity. J Exp Mar Biol Ecol 257:281–296

Kamiyama T, Itakura S, Nagasaki K (2000) Changes in microbial loop components: effects of a harmful algal bloom formation and its decay. Aquat Microb Ecol 21:21–30

Kim JS, Jeong HJ (2004) Feeding by the heterotrophic dinoflagellates *Gyrodinium dominans* and *G. spirale* on the red-tide dinoflagellate *Prorocentrum minimum*. Mar Ecol Prog Ser 280:85–94

Lee RE (1977) Saprophytic and phagotrophic isolates of the colorless heterotrophic dinoflagellate *Gyrodinium lebouriae* Herdman. J Mar Biol Assoc UK 57:303–315

Lewitus AJ, Glasgow HB Jr, Burkholder JM (1999) Kleptoplastidy in the toxic dinoflagellate *Pfiesteria piscicida* (Dinophyceae). J Phycol 35:303–312

MacKenzie L (1991) Toxic and noxious phytoplankton in Big Glory Bay, Stewart Island, New Zealand. J Appl Phycol 3: 19–34

Menden-Deuer S, Lessard E (2000) Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnol Oceanogr 45:569–579

Nagasaki K, Itakura S, Imai I, Nakagiri S, Yamaguchi M (1996) The disintegration process of a *Heterosigma akashiwo* (raphidophyceae) red tide in northern Hirosima Bay, Japan, during the summer of 1994. In: Yasumoto T, Oshima Y, Fukuyo Y (eds) Harmful and toxic algal blooms. UNESCO, Paris, p 251–254

Nakamura Y, Suzuki SY, Hiromi J (1995) Growth and grazing of a naked heterotrophic dinoflagellate, *Gyrodinium dominans*. Aquat Microb Ecol 9:157–164

Spero HJ (1982) Phagotrophy in *Gymnodinium fungiforme* (Pyrrhophyta): the peduncle as an organelle of ingestion. J Phycol 18:356–360

Stoecker DK, Gustafson DE (2002) Predicting grazing mortality of an estuarine dinoflagellate, *Pfiesteria piscicida*. Mar Ecol Prog Ser 233:31–38

Strathmann RR (1967) Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnol Oceanogr 12:411–418

Taniguchi A, Takeda Y (1988) Feeding rate and behavior of the tintinnid ciliate *Favella taraikaensis* observed with a high speed VTR system. Mar Microb Food Webs 3:21–34

Tillmann U (2004) Interactions between planktonic microalgae and protozoan grazers. J Eukaryot Microbiol 51: 156–168

Watras CJ, Garcon VC, Olson RJ, Chishom SW, Anderson DM (1985) The effect of zooplankton grazing on estuarine blooms of the toxic dinoflagellate *Gonyaulax tamarensis*. J Plankton Res 7:891–908

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