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Feeding by the mixotrophic red-tide dinoflagellate *Gonyaulax polygramma*: mechanisms, prey species, effects of prey concentration, and grazing impact

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ABSTRACT: The red-tide dinoflagellate *Gonyaulax polygramma* (GenBank accession number = AJ833631), previously known as an exclusively autotrophic dinoflagellate, has been found to be a mixotrophic species. We investigated feeding mechanisms, types of prey species, and the effects of prey concentration on the growth and ingestion rates of *G. polygramma* when feeding on an unidentified cryptophyte species (equivalent spherical diameter, ESD = 5.6 µm). We also calculated grazing coefficients by combining field data on abundances of *G. polygramma* and co-occurring cryptophytes with laboratory data on ingestion rates obtained in the present study. Among the phytoplankton prey offered, *G. polygramma* ingested small phytoplankton species with ESD ≤17 µm, but did not feed on large phytoplankton species with ESD > 22 µm. *G. polygramma* fed on prey cells by engulfing them through the apical horn, a previously unknown mechanism, as well as through the sulcus. The feeding mechanism of *G. polygramma* on phytoplankton mainly depended on the prey species. Specific growth rates of *G. polygramma* on a cryptophyte increased with increasing mean prey concentration, with saturation occurring at a mean prey concentration of approximately 600 ng C ml^{-1}. The maximum specific (mixotrophic) growth rate of *G. polygramma* on a cryptophyte was 0.278 d^{-1}, under a 14:10 h light:dark cycle of 50 µE m^{-2} s^{-1}, while its (phototrophic) growth rate under the same light conditions without added prey was 0.186 d^{-1}. Its maximum ingestion and clearance rates were 0.18 ng C $grazer^{-1}$ d^{-1} (10.6 cells $grazer^{-1}$ d^{-1}) and 0.18 µl $grazer^{-1}$ h^{-1}, respectively. The grazing coefficients of *G. polygramma* on cryptophytes were up to 0.479 h^{-1}. The results of the present study suggest that *G. polygramma* can have a considerable grazing impact on cryptophyte populations.

KEY WORDS: Feeding process · Harmful algal bloom · Ingestion · Marine · Protist · Red tide

INTRODUCTION

Dense blooms of microalgae or so-called red tides can upset the balance of food webs and cause large-scale mortalities of finfish and shellfish (ECOHAB 1995). They have frequently caused great losses to the aquaculture and tourist industries of many countries. Fully understanding the ecology and physiology of red-tide organisms and thus the mechanisms for the outbreak, persistence, and decline of red tides is a primary concern for scientists in related fields.

Recently, red-tide dinoflagellates, previously known as phototrophic dinoflagellates, have been revealed to be mixotrophic (Bockstahler & Coats 1993, Jacobson & Anderson 1996, Granéli et al. 1997, Stoecker et al. 1997, Smalley et al. 1999, Stoecker 1999, Skovgaard 2000, Jeong et al. 2004). The thecate dinoflagellate *Gonyaulax polygramma* has caused red tides in the

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coastal waters off many countries such as Korea, Japan, China, Hong Kong, USA, Mexico, Belize, South Africa, and Algeria (Grindley & Taylor 1962, Kamykowski 1980, Lam & Yip 1990, Lin et al. 1993, Koizumi et al. 1996, 2001, Faust 2000, Garate-Lizarraga et al. 2001, Ounissi & Retima 2001). Red tides dominated by *G. polygramma* have sometimes caused the mass mortality of finfish and shellfish from anoxia (Grindley & Taylor 1962, Koizumi et al. 1996, Garate-Lizarraga et al. 2001). Recently, we found food vacuoles inside *G. polygramma*, which had previously been known as an exclusively autotrophic dinoflagellate. This observation implies that *G. polygramma* can be mixotrophic. If it is confirmed that *G. polygramma* is mixotrophic, the models for predicting the outbreak, persistence, and decline of red tides dominated by this species and the related management strategies should be adjusted to reflect this fact. However, there have been no reports on the feeding mechanisms, prey species, and growth and grazing rates of *G. polygramma* on its prey yet. To understand the dynamics of red tides dominated by *G. polygramma*, the prey species and the growth and grazing rates of *G. polygramma* should be explored.

We established a monoclonal culture of *Gonyaulax polygramma* and observed its feeding behavior in order to explore feeding mechanisms and determine prey species; feeding frequency was measured when diverse phytoplankton species were provided. Experiments were conducted to determine the effects of prey concentration on the growth and ingestion rates of *G. polygramma* when feeding on an unidentified cryptophyte species (equivalent spherical diameter, ESD = 5.6 µm). We also estimated grazing coefficients attributable to *G. polygramma* on co-occurring cryptophytes using our data for ingestion rates obtained from the laboratory experiments and the abundances of predator and prey in the field. The results of the present study provide a basis for understanding the feeding mechanisms of *G. polygramma*, the interactions between *G. polygramma* and co-occurring phytoplankton, and the dynamics of red tides dominated by *G. polygramma*.

MATERIALS AND METHODS

Preparation of experimental organisms. Phytoplankton species were grown at 20°C in enriched f/2 seawater media (Guillard & Ryther 1962) without silicate, under continuous illumination of 50 µE m^{-2} s^{-1} provided by cool white fluorescent lights (see Table 1). Mean ESD ± standard deviation from the mean was measured by an electronic particle counter (Coulter Multisizer II, Coulter Corporation).

For the isolation and culture of *Gonyaulax polygramma* (GPSMK0209, GenBank accession number = AJ833631), plankton samples collected with a 40 cm diameter, 25 µm mesh plankton net were taken from coastal waters off Saemankeum, Korea, during September 2002, when the water temperature and salinity were 24.4°C and 29.3 psu, respectively. The samples were screened gently through a 154 µm Nitex mesh and placed in 1 l polycarbonate (PC) bottles. Fifty ml of f/2 media were added as food. The bottles were placed on shelves and incubated at 20°C under continuous illumination of 50 µE m^{-2} s^{-1} of cool white fluorescent light. Three days later, aliquots of the enriched water were transferred to 6-well tissue culture plates and a monoclonal culture was established by 2 serial single-cell isolations. Once dense cultures of *G. polygramma* were obtained, they were transferred to 2 l PC bottles containing ca. 500 ml of fresh f/2 seawater media (final culture volume = ca. 1 l) every 2 wk. This strain produces bioluminescence.

Prey species. Expt 1 was designed to investigate whether or not *Gonyaulax polygramma* was able to feed on each target phytoplankton species when unialgal diets of diverse phytoplankton species were provided (Table 1). The initial concentrations of each phytoplankton species offered were similar in terms of carbon biomass. To confirm no ingestion by *G. polygramma* on some phytoplankton species, additional higher prey concentrations were provided.

A dense culture of *Gonyaulax polygramma* maintained in f/2 media and growing photosynthetically in exponential growth phase on shelves and incubated under the continuous illumination of 50 µE m^{-2} s^{-1} 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 *G. polygramma* concentration.

In this experiment, the initial concentrations of *Gonyaulax polygramma* and each target phytoplankton species were established using an autopipette to deliver a predetermined volume of culture with a known cell density to the experimental bottles. Triplicate 80 ml PC bottles (mixtures of *G. polygramma* and phytoplankton) and duplicate predator control bottles (containing *G. polygramma* only) were set up for each target phytoplankton species. The bottles were filled to capacity with freshly filtered seawater, capped, and then placed on a vertically rotating plate at 0.9 rpm and incubated at 20°C under the continuous illumination of 50 µE m^{-2} s^{-1} of cool white fluorescent light. After 6 h incubation, a 5 ml aliquot was removed from each bottle and transferred into a 10 ml bottle. Two 0.1 ml aliquots were placed on slides and then cover-glasses were added. Under these conditions, *G. polygramma* cells were alive, but almost motionless. The protoplasms of >200 *G. polygramma* cells were carefully examined with a com-

pound microscope and/or an epifluorescent microscope at a magnification of 100 to 400 to determine whether or not *G. polygramma* was able to feed on the target prey species.

Feeding frequency. Expt 2 was designed to investigate the effects of prey species on the feeding frequency (FF) of *Gonyaulax polygramma*. FF is the proportion of *G. polygramma* cells that feed, as determined by the presence of ingested prey, and was calculated as the percentage of *G. polygramma* containing 1 or more target prey cells. FFs of *G. polygramma* on *Isochrysis galbana*, a cryptophyte, *Amphidinium carterae*, *Heterosigma akashiwo*, *Heterocapsa triquetra*, *Prorocentrum minimum*, and *Scrippsiella* sp., which had been revealed to be engulfed by *G. polygramma* in Expt 1, were measured. The initial concentrations of predator and prey were the same as in Expt 1 (Table 1).

Expt 2 was set up in the same way as Expt 1. After 10, 30, and 60 min incubation, a 5 ml aliquot was removed from each bottle and transferred into a 10 ml bottle. Two 0.1 ml aliquots were placed on slides and then cover-glasses were added. The protoplasms of >200 *G. polygramma* cells were carefully examined with a compound microscope at a magnification of 100 to 400 and the numbers of *G. polygramma* with and without ingested prey were determined.

Feeding mechanisms. Expts 3 and 4 were designed to investigate the feeding mechanisms of *Gonyaulax polygramma* on a cryptophyte, *Amphidinium carterae*, *Heterosigma akashiwo*, *Heterocapsa triquetra*, *Prorocentrum minimum*, and *Scrippsiella* sp. *Isochrysis galbana* was too small for the feeding process to be observed. The initial concentrations of predator and prey were the same as in Expt 1.

In Expt 3, the initial concentrations of *Gonyaulax polygramma* and the target phytoplankton species were established using an autopipette to deliver a predetermined volume of culture with a known cell density to the experimental bottles. One 80 ml PC bottle (mixtures of *G. polygramma* and phytoplankton) was set up for each target phytoplankton species. The bottle was filled to capacity with freshly filtered seawater, capped, and then well mixed. After 1 min incubation, a 1 ml aliquot was removed from the bottle and transferred into a 1 ml Sedgwick-Rafter counting chamber. By monitoring the behavior of >60 unfed *G. polygramma* cells for each target prey species under a compound microscope at a magnification of 100 to 400, the frequency of engulfment through the apical horn and the sulcus of the predator was measured. All feeding processes between a prey cell being captured and engulfed by each predator were observed. A series of pictures showing the feeding process of a *G. polygramma* cell was taken using a video analyzing system (Toshiba IK-642F) on a compound microscope at a magnification of 100 to 400.

In Expt 4, the time for a prey cell to be completely engulfed through the apical horn of *Gonyaulax polygramma* after the prey cell was contacted by the predator (i.e. handling time) was compared to that through the sulcus of the predator. We selected *Amphidinium carterae* as a test organism because the frequency of engulfment through the apical horn of the predator on this prey was similar to that through the sulcus in Expt 3.

Expt 4 was set up in the same way as Expt 3. After 1 min incubation, a 1 ml aliquot was removed from the bottle and transferred into a 1 ml Sedgwick-Rafter counting chamber. By monitoring the behavior of 10 unfed *Gonyaulax polygramma* cells for each feeding mechanism (i.e. horn and sulcus) under a compound microscope at a magnification of 100 to 400, the time for an *A. carterae* cell to be completely engulfed by *G. polygramma* after the prey cell was contacted by the predator was measured.

Effects of the prey concentration. Expt 5 was designed to investigate the effects of prey concentration on the growth and ingestion rate of *Gonyaulax polygramma*. We measured growth, ingestion, and clearance rates of *G. polygramma* on an unidentified cryptophyte species (ESD = 5.6 µm; carbon content per cell = 0.017 ng C; Strathmann 1967) as a function of prey concentration.

A dense culture of *Gonyaulax polygramma* maintained in f/2 medium and growing photosynthetically under a 14:10 h light:dark cycle of 50 µE m^{-2} s^{-1} for 2 wk was transferred into a 1 l PC bottle. Three 1 ml aliquots from the bottle were counted using a compound microscope to determine the cell concentrations of *G. polygramma*, and the cultures were then used to conduct experiments.

The initial concentrations of *Gonyaulax polygramma* and a cryptophyte were established using an autopipette to deliver predetermined volumes of known cell concentrations to the bottles. Triplicate 80 ml PC experimental bottles (containing mixtures of predator and prey) and triplicate prey control bottles (containing prey only) were set up for each predator-prey combination. Triplicate predator control bottles (containing predator only) were also established at 1 predator concentration. Twenty ml of f/2 medium were added to all bottles, which were then filled to capacity with freshly filtered seawater and capped. To determine the actual predator and prey densities (cells ml^{-1}) at the beginning of the experiment (*G. polygramma*/cryptophyte = 5/18, 13/46, 21/94, 66/189, 116/742, 518/2020, 1057/10259, 1879/58272, 505/0) and after 24, 48, and 72 h incubation, 6 ml aliquots were removed from each bottle and fixed

with 5% Lugol's solution, and all *G. polygramma* cells and all or >300 prey cells in 3 1 ml Sedgwick-Rafter counting chambers were enumerated. Prior to taking subsamples, the condition of *G. polygramma* and its prey was assessed under a dissecting microscope. The bottles were filled again to capacity with f/2 medium, capped, placed on a vertically rotating plate at 0.9 rpm, and incubated at 20°C under a 14:10 h light:dark cycle of 50 µE m^{-2} s^{-1} of cool white fluorescent light. The dilution of the cultures associated with refilling the bottles was taken into consideration in calculating growth and ingestion rates.

The specific growth rate of *Gonyaulax polygramma* (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 concentration of *G. polygramma* at consecutive samplings. The final time (t_2) for the calculation was 48 h, which provided the highest specific growth rate. Mean prey concentrations for 48 h were also calculated by averaging the instantaneous mean prey concentrations at 0 to 24 h and 24 to 48 h. The instantaneous mean prey concentration for each sampling interval was calculated using the equations of Frost (1972).

Data for *Gonyaulax polygramma* growth rate were fitted to a Michaelis-Menten equation:

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

where $\mu_{\max}$ 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 (the prey concentration where $\mu = 0$), and K_{GR} is the prey concentration sustaining ½ $\mu_{\max}$. Data were iteratively fitted to the model using DeltaGraph® (SPSS).

Ingestion and clearance rates for 48 h were also calculated using the equations of Frost (1972) and Heinbokel (1978). Ingestion rate data were fitted to a Michaelis-Menten equation:

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

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 ½ $I_{\max}$.

Grazing impact. We estimated the grazing coefficients attributable to *Gonyaulax polygramma* on cryptophytes by combining field data on abundances of the grazer and the prey with ingestion rates of the grazer on the prey obtained in the present study (see Table 2). Data on the abundances of *G. polygramma* and co-occurring cryptophytes used in this estimation were obtained from the water samples taken off Kwangyang (in 1999–2000), Saemankeum (in 1999–2002), and Tongyoung (in 2004), Korea.

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

$$C_e = \mathrm{PIR} \times 1\ \mathrm{h} = \mathrm{IR} \times G \times 1\ \mathrm{h} \quad (5)$$

where PIR is the population ingestion rate of *G. polygramma* on a cryptophyte in 1 ml of seawater (prey eaten ml^{-1} h^{-1}), IR is the ingestion rate (prey eaten *G. polygramma*$^{-1}$ h^{-1}) of *G. polygramma* on a cryptophyte, and G is the initial abundance (cells ml^{-1}) of *G. polygramma* at the same time as C_i.

RESULTS

Prey species

Among the phytoplankton prey offered, *Gonyaulax polygramma* ingested the small phytoplankton species (the prymnesiophyte *Isochrysis galbana*, an unidentified cryptophyte, the raphidophyte *Heterosigma akashiwo*, the dinoflagellates *Amphidinium carterae*, *Heterocapsa triquetra*, *Prorocentrum minimum*, and *Scrippsiella* sp.; Fig. 1) with ESDs ≤ 17 µm, but it did not feed on the large phytoplankton species (the dinoflagellates *Akashiwo sanguinea*, *Alexandrium tamarense*, *Cochlodinium polykrikoides*, *Gymnodinium catenatum*, *Lingulodinium polyedrum*, and *P. micans*) with ESDs > 22 µm (Table 1).

Feeding frequency

After 10 min incubation, the feeding frequencies (FFs) of *Gonyaulax polygramma* on *Isochrysis galbana*, a cryptophyte, *Amphidinium carterae*, and *Heterosigma akashiwo* (86 to 97%) were much higher than those on *Heterocapsa triquetra*, *Prorocentrum minimum*, and *Scrippsiella* sp. (3 to 34%) (Fig. 2). With increasing elapsed incubation time, FFs of *G. polygramma* on *H. triquetra*, *P. minimum*, and *Scrippsiella* sp. linearly increased. However, after 60 min incubation, FF on *Scrippsiella* sp. was still only 9%, while FF of *G. polygramma* on *H. triquetra* and *P. minimum* had become 87 to 88%.

Table 1. Taxa, sizes, and concentration of *Gonyaulax polygramma* and phytoplankton species offered as food to *G. polygramma* in Expts 1 to 4. To confirm no ingestion by *G. polygramma* on some phytoplankton species, additional higher prey concentrations were provided. Y: *G. polygramma* was observed to contain food cells in the protoplasm; N: *G. polygramma* was observed not to contain food cells. Mean equivalent spherical diameter (ESD, µm) ± standard deviation of the mean was measured by an electronic particle counter (Coulter Multisizer II, Coulter Corporation). n > 2000 for each species. *PRY: Prymnesiophyceae; CRP: Cryptophyceae; RAP: Raphidophyceae; DIN: Dinophyceae

Species	ESD (±SD)	Initial prey concentration (cells ml^{-1})	Feeding
Isochrysis galbana (*PRY)	4.8 (0.2)	100000	Y
Cryptophyte (CRP)	5.6 (1.5)	50000	Y
Amphidinium carterae (DIN)	6.6 (1.5)	33000	Y
Heterosigma akashiwo (RAP)	11.0 (0.4)	10000	Y
Heterocapsa triquetra (DIN)	12.7 (0.6)	6200	Y
Prorocentrum minimum (DIN)	12.9 (3.6)	6000	Y
Scrippsiella sp. (DIN)	17.0 (5.9)	1500	Y
Cochlodinium polykrikoides (DIN)	23.1 (3.2)	1500–4000	N
Alexandrium tamarense (DIN)	24.8 (1.0)	1200–4000	N
Prorocentrum micans (DIN)	26.0 (2.3)	1000–3000	N
Gymnodinium catenatum (DIN)	33.9 (1.6)	500–2000	N
Akashiwo sanguinea (DIN)	36.3 (5.6)	400–1500	N
Lingulodinium polyedrum (DIN)	37.9 (4.5)	400–1500	N
Gonyaulax polygramma (DIN)[a]	32.5 (5.4)		

[a]Densities of *G. polygramma* were 1000 to 1100 cells ml^{-1} for these experiments

Feeding mechanisms

Gonyaulax polygramma fed on phytoplankton cells by engulfing the prey through both the apical horn and the sulcus (Fig. 3). When unialgal diets of phytoplankton species were provided, the ratios of the frequencies of engulfment through the apical horn and the sulcus of *Gonyaulax polygramma* were 83:17 (n = 229) for the cryptophyte, 48:52 (n = 90) for *Amphidinium carterae*, 12:88 (n = 66) for *Heterosigma akashiwo*, 5:95 (n = 77) for *Heterocapsa triquetra*, and 1:99 (n = 75) for *Prorocentrum minimum* prey (Fig. 4). *G. polygramma* fed on *Scrippsiella* sp. cells by engulfing the prey through only the sulcus (n = 126).

The time for an *Amphidinium carterae* cell to be completely engulfed through the apical horn of *Gonyaulax polygramma* after the prey cell was contacted by the predator (mean ± SD, 291 ± 73 s) was not significantly higher than for through the sulcus of the predator (346 ± 79 s) (1-tailed *t*-test, 0.1 > p > 0.05).

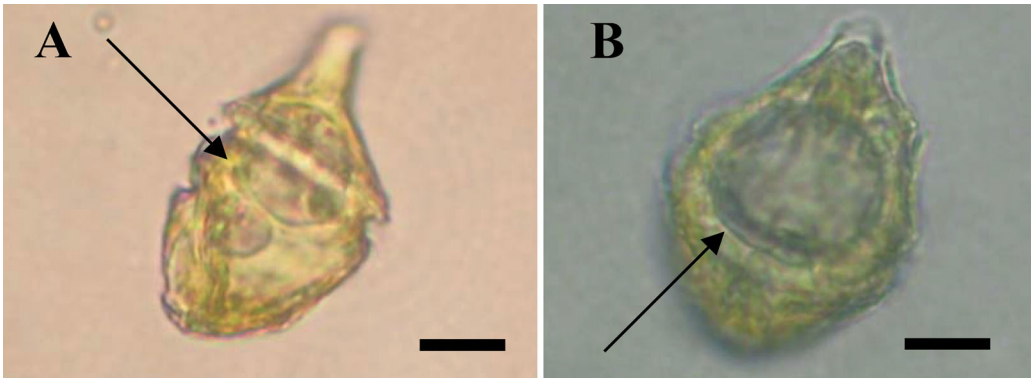

Fig. 1. *Gonyaulax polygramma* with ingested (A) *Heterocapsa triquetra* and (B) *Scrippsiella* sp. cell. Arrows indicate ingested prey cells. Scale bars = 10 µm

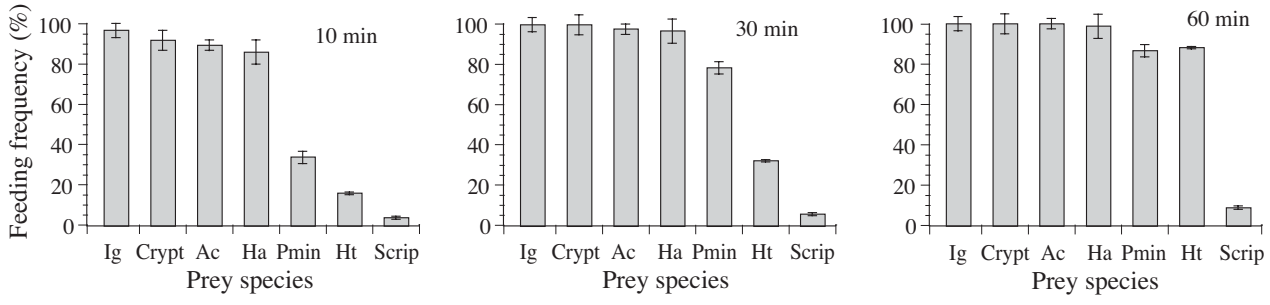

Fig. 2. *Gonyaulax polygramma*. Feeding frequency on *Isochrysis galbana* (Ig), a cryptophyte (Crypt), *Amphidinium carterae* (Ac), *Heterosigma akashiwo* (Ha), *Prorocentrum minimum* (Pmin), *Heterocapsa triquetra* (Ht), and *Scrippsiella* sp. (Scrip) at 3 different elapsed incubation times (10, 30, and 60 min); the proportion of the *G. polygramma* cells observed to contain prey, measured by calculating the percent ratio of *G. polygramma* containing 1 or more target prey cells to total *G. polygramma*. Treatment means ± SD are shown

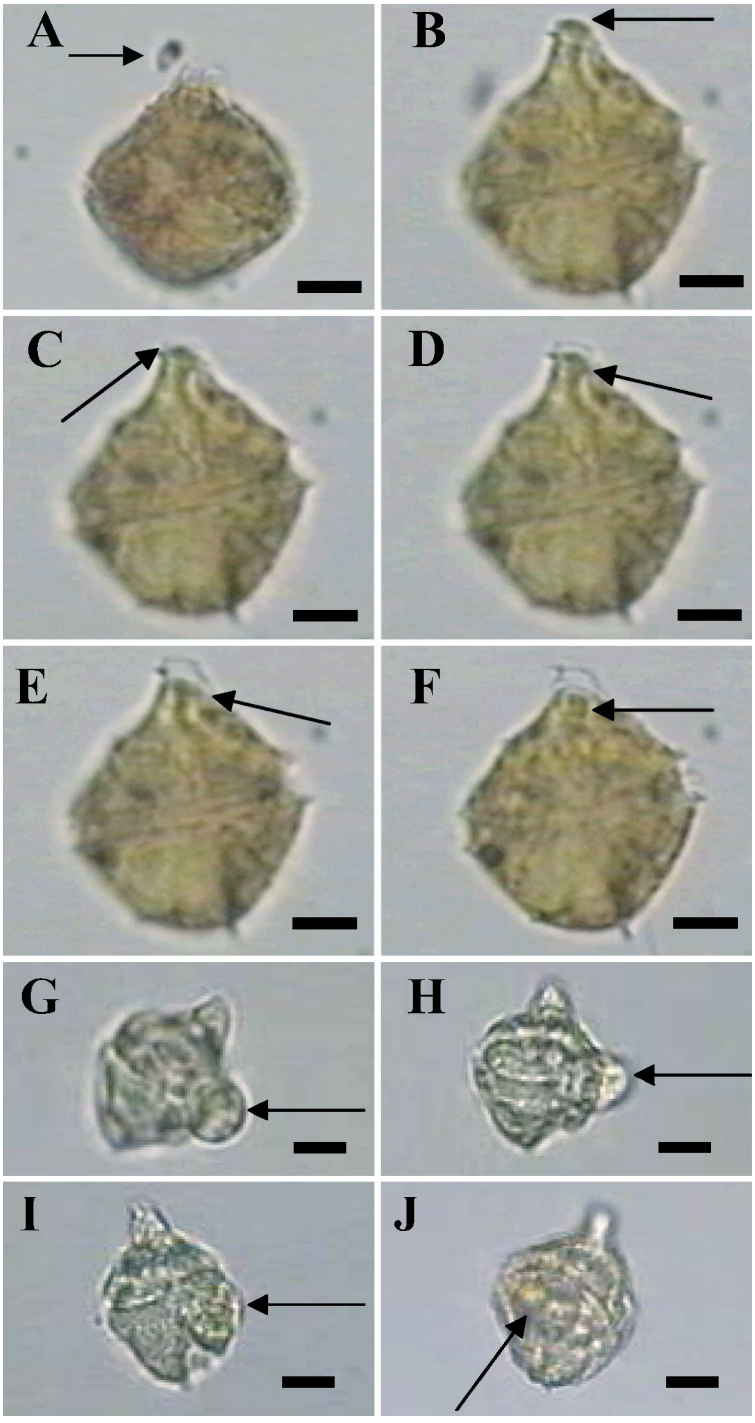

Fig. 3. (A–F) *Gonyaulax polygramma* engulfing a cryptophyte cell through the apical horn. Pictures were taken in sequence using a video analyzing system on a compound microscope. (A) *G. polygramma* and a freely swimming cryptophyte, (B) *G. polygramma* capturing a cryptophyte cell by the apical horn, (C–E) a cryptophyte cell passing through the apical horn of *G. polygramma*, (F) an ingested cryptophyte cell beneath the horn. Predator and prey cells in (B–F) were the same predator and prey cells, respectively. (G–J) *G. polygramma* engulfing a *Prorocentrum minimum* cell through the sulcus. (G,H) a *P. minimum* cell passing through the sulcus of *G. polygramma*, (I,J) an ingested *P. minimum* cell through the sulcus. Predator and prey cells in (G–J) were the same predator and prey cells, respectively. Arrows indicate prey cells. Scale bars = 10 µm

Effects of prey concentration

The specific growth rates of *Gonyaulax polygramma* on a cryptophyte increased with increasing mean prey concentration, with saturation at a mean prey concentration of approximately 600 ng C ml^{-1} (i.e. 35 300 cells ml^{-1}) (Fig. 5). When the data were fitted to Eq. (2), the maximum specific growth rate of *G. polygramma* on a cryptophyte (mixotrophic growth) was 0.278 d^{-1}, under a 14:10 h light:dark cycle of 50 µE m^{-2} s^{-1}, while its growth rate under the same light conditions without added prey (phototrophic growth) was only 0.186 d^{-1}.

Ingestion rates of *Gonyaulax polygramma* feeding on a unialgal diet of a cryptophyte increased continuously with increasing mean prey concentration offered in the present study (Fig. 6). When the data were fitted to Eq. (3), the maximum ingestion rate of *G. polygramma* on a cryptophyte was 0.18 ng C $grazer^{-1}$ d^{-1} (10.6 cells $grazer^{-1}$ d^{-1}). The maximum clearance rate of *G. polygramma* on a cryptophyte was 0.18 µl $grazer^{-1}$ h^{-1}.

Grazing impact

Grazing coefficients (*g*) attributable to *Gonyaulax polygramma* on co-occurring cryptophytes in the coastal waters off Kwangyang (in 1999–2000), Saemankeum (in 1999–2002), and Tongyoung (in 2004), Korea were up to 0.479 h^{-1} (Table 2, Fig. 7). In general, grazing coefficients increased with increasing *G. polygramma* concentration.

DISCUSSION

Prey species

Among the phytoplankton species offered in the present study, *Gonyaulax polygramma* was able to feed on phytoplankton belonging to diverse classes such as Prymnesiophyceae (*Isochrysis galbana*), Cryptophyceae, Raphidophyceae (*Heterosigma akashiwo*), Dinophyceae (*Amphidinium carterae*, *Heterocapsa triquetra* and *Prorocentrum minimum*). All the phytoplankton

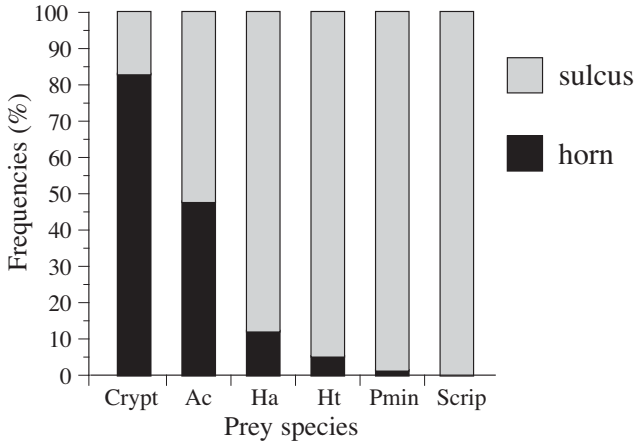

Fig. 4. *Gonyaulax polygramma*. Frequencies of engulfment through the apical horn and the sulcus when feeding on unialgal diets of a cryptophyte (Crypt, n = 229), *Amphidinium carterae* (Ac, n = 90), *Heterosigma akashiwo* (Ha, n = 66), *Heterocapsa triquetra* (Ht, n = 77), *Prorocentrum minimum* (Pmin, n = 75), and *Scrippsiella* sp. (Scrip, n = 126)

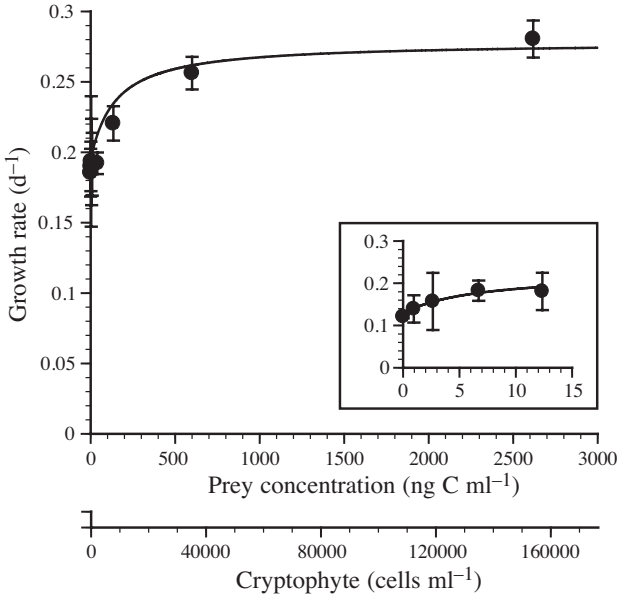

Fig. 5. *Gonyaulax polygramma*. Specific growth rates on an unidentified cryptophyte as a function of mean prey concentration. Symbols represent treatment means ± 1 SE. Curves were fitted by a Michaelis-Menten equation (Eq. 2) using all treatments in the experiment. Growth rate (GR, d^{-1}) = 0.278 $\{(x + 98)/[44 + (x + 98)]\}$, r^2 = 0.47. Inset shows values at low prey concentrations

species ingested by *G. polygramma* had ESDs ≤ 17 µm, while the species not ingested by *G. polygramma* had ESDs > 22 µm. Therefore, whether or not *G. polygramma* is able to ingest a phytoplankton species appears to be mainly affected by the size of the prey species.

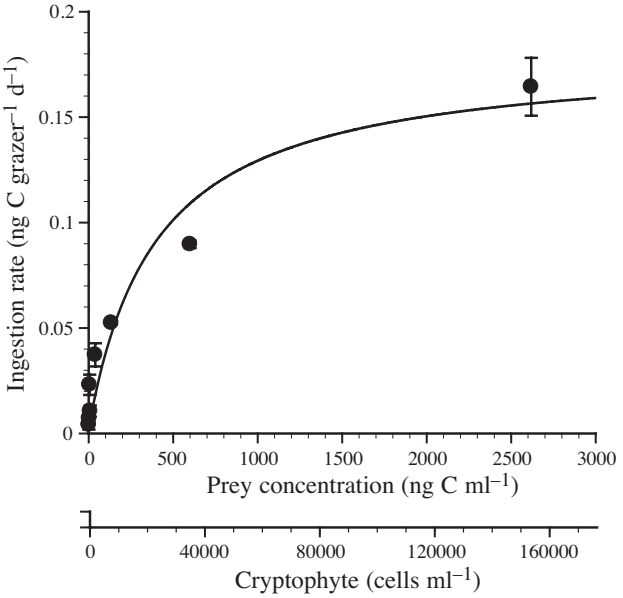

Fig. 6. *Gonyaulax polygramma*. Ingestion rate on an unidentified cryptophyte as a function of mean prey concentration. Symbols represent treatment means ± 1 SE. Curves were fitted by a Michaelis-Menten equation (Eq. 3) using all treatments in the experiment. Ingestion rate (IR, ng C grazer^{-1} d^{-1}) = 0.18 $[x/(393 + x)]$, r^2 = 0.919

Feeding frequency

FFs of *Gonyaulax polygramma* on the smaller prey species such as *Isochrysis galbana*, a cryptophyte, *Amphidinium carterae*, and *Heterosigma akashiwo* after 10 min incubation (86 to 97 %) were much higher than those on the larger prey species such as *Heterocapsa triquetra*, *Prorocentrum minimum*, and *Scrippsiella* sp. (3 to 34 %). The strain of *A. carterae* used in the present study is known to be toxic (Jeong et al. 2001), but the FF of *G. polygramma* on this species was similar to that on the non-toxic algal cryptophyte or *H. akashiwo*. Therefore, the FF appears to be mainly affected by prey size. However, there is still a possibility that the FFs for smaller prey species might have been overestimated compared to larger prey species due to a higher encounter rate for the former species than the latter because the concentrations of the smaller prey species were higher than those for the larger prey species even though their carbon biomasses were similar.

Feeding mechanisms

Gonyaulax polygramma fed on phytoplankton cells by engulfing the prey through both the apical horn and the sulcus. So far, engulfment-feeding mixotrophic and heterotrophic dinoflagellates have only been known

Table 2. Estimation of grazing impact by a *Gonyaulax polygramma* population on cryptophyte populations using the equation for ingestion rate in Fig. 6 legend, and the abundances of co-occurring cryptophytes and *G. polygramma* obtained from the water samples collected off Kwangyang (in 1999–2000), Saemankeum (in 1999–2002), and Tongyoung (in 2004), Korea. GpIR: *G. polygramma*'s population ingestion rate; Gpg: *G. polygramma*'s grazing coefficient (h^{-1})

Cryptophyte concentration (cells ml^{-1})	*G. polygramma* concentration (cells ml^{-1})	GpIR (prey eaten ml^{-1} h^{-1})	Gpg (h^{-1})
75	20000	29	0.479
350	1005	7	0.019
390	3920	29	0.076
440	1620	13	0.031
580	900	10	0.017
710	1810	24	0.034
800	118	2	0.002
1595	105	3	0.002
3550	123	7	0.002

to engulf a prey cell through the sulcus (Schnepf & Elbrächter 1992, Skovgaard 1996, Jeong et al. 1997, 1999, Hansen & Calado 1999). Therefore, this study is the first report of engulfment feeding through the apical horn of dinoflagellates.

The ratios of the frequencies of engulfment through the apical horn and the sulcus of *Gonyaulax polygramma* on a cryptophyte (83:17, ESD = 5.6 µm) and *Amphidinium carterae* (48:52, ESD = 6.6 µm) were much higher than those for *Heterosigma akashiwo* (12:88, ESD = 11.0 µm), *Heterocapsa triquetra* (5:95, 12.6 µm), *Prorocentrum minimum* (1:99, 12.9 µm), or *Scrippsiella* sp. (0:100, 17.0 µm). *G. polygramma* may prefer to engulf a small prey species through the apical horn and a large prey species through the sulcus. Therefore, whether *G. polygramma* uses the apical horn or the sulcus for engulfment may be affected by prey size.

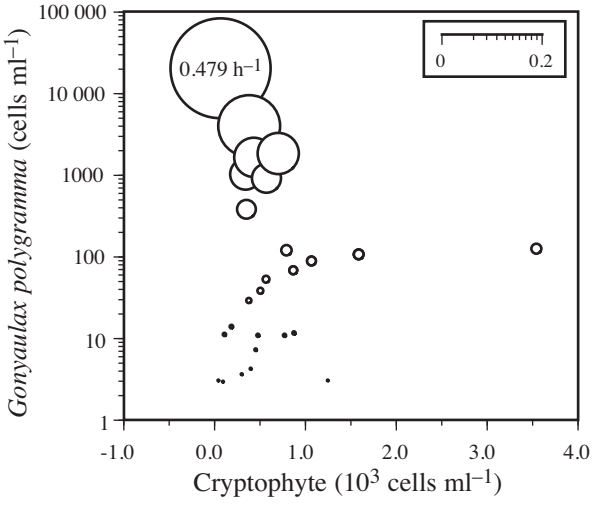

Fig. 7. *Gonyaulax polygramma*. Calculated grazing coefficients (*g*) in relation to the concentration of co-occurring cryptophytes (see text for calculation). N = 37. The scale of the circles in the inset box is *g* (h^{-1}). A value of *g* was 0.479 h^{-1} when the concentrations of cryptophytes and *G. polygramma* were 75 and 20 000 cells ml^{-1}, respectively. The scale for this *g* has been reduced

Effects of prey concentration

Both growth and ingestion rates of *Gonyaulax polygramma* feeding on a unialgal diet of a cryptophyte were affected by the prey concentration. A unialgal diet of a cryptophyte can support a population growth of *G. polygramma* (mixotrophic growth, 0.278 d^{-1}) 49% higher than that without added prey (phototrophic growth, 0.186 d^{-1}) under the conditions provided in the present study. This evidence suggests that *G. polygramma* may be able to increase or maintain its population by feeding on cryptophytes under conditions which are less favorable for phototrophic growth if prey is abundant.

The maximum ingestion and clearance rates of *Gonyaulax polygramma* feeding on a unialgal diet of a cryptophyte under the conditions provided in the present study (10.6 cells $grazer^{-1}$ d^{-1} and 0.18 µl $grazer^{-1}$ h^{-1}, respectively) were comparable to those of *Cochlodinium polykrikoides* on the same prey (9.4 cells $grazer^{-1}$ d^{-1} and 0.33 µl $grazer^{-1}$ h^{-1}, respectively) obtained under a 14:10 h light:dark cycle of 50 µE m^{-2} s^{-1} (Jeong et al. 2004). Therefore, *G. polygramma* may compete with *C. polykrikoides* for a cryptophyte prey if they co-occur.

Grazing impact

Grazing coefficients (*g*) attributable to *Gonyaulax polygramma* on co-occurring cryptophytes obtained in the present study were up to 0.479 h^{-1} (i.e. up to 38% of cryptophyte populations were removed by a *G. polygramma* population in 1 h). The results of the present study suggest that *G. polygramma* may sometimes have a considerable grazing impact on populations of co-occurring cryptophytes. The grazing rates of some mixotrophic dinoflagellates are known to be affected by light and/or nutrient conditions (Hansen & Nielsen 1997, Stoecker et al. 1997, Jeong et al. 1999, Li et al. 2000, Hansen et al. 2000, Jakobsen et al. 2000, Skovgaard et al. 2000). Therefore, the grazing impact of *G. polygramma* on co-occurring cryptophytes may also be affected by light and/or nutrient conditions.

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