

Mixotrophy in the Phototrophic Harmful Alga *Cochlodinium polykrikoides* (Dinophyceae): Prey Species, the Effects of Prey Concentration, and Grazing Impact

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ABSTRACT. We first reported here that the harmful alga *Cochlodinium polykrikoides*, which had been previously known as an autotrophic dinoflagellate, was a mixotrophic species. We investigated the kinds of prey species and the effects of the prey concentration on the growth and ingestion rates of *C. polykrikoides* 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 *C. polykrikoides* and co-occurring cryptophytes with laboratory data on ingestion rates obtained in the present study. *Cochlodinium polykrikoides* fed on prey cells by engulfing the prey through the sulcus. Among the phytoplankton prey offered, *C. polykrikoides* ingested small phytoplankton species that had ESD's $\leq 11 \mu\text{m}$ (e.g. the prymnesiophyte *Isochrysis galbana*, an unidentified cryptophyte, the cryptophyte *Rhodomonas salina*, the raphidophyte *Heterosigma akashiwo*, and the dinoflagellate *Amphidinium carterae*). It did not feed on larger phytoplankton species that had ESD's $\geq 12 \mu\text{m}$ (e.g. the dinoflagellates *Heterocapsa triquetra*, *Prorocentrum minimum*, *Scrippsiella* sp., *Alexandrium tamarense*, *Prorocentrum micans*, *Gymnodinium catenatum*, *Akashiwo sanguinea*, and *Lingulodinium polyedrum*). Specific growth rates of *C. polykrikoides* on a cryptophyte increased with increasing mean prey concentration, with saturation at a mean prey concentration of approximately 270 ng C ml⁻¹ (i.e. 15,900 cells ml⁻¹). The maximum specific growth rate (mixotrophic growth) of *C. polykrikoides* on a cryptophyte was 0.324 d⁻¹, under a 14:10 h light-dark cycle of 50 $\mu\text{E m}^{-2} \text{s}^{-1}$, while its growth rate (phototrophic growth) under the same light conditions without added prey was 0.166 d⁻¹. Maximum ingestion and clearance rates of *C. polykrikoides* on a cryptophyte were 0.16 ng C grazer⁻¹d⁻¹ (9.4 cells grazer⁻¹d⁻¹) and 0.33 $\mu\text{l grazer}^{-1}\text{h}^{-1}$, respectively. Calculated grazing coefficients by *C. polykrikoides* on cryptophytes were 0.001–0.745 h⁻¹ (i.e. 0.1–53% of cryptophyte populations were removed by a *C. polykrikoides* population in 1 h). The results of the present study suggest that *C. polykrikoides* sometimes has a considerable grazing impact on populations of cryptophytes.

Key Words. Growth, harmful algal bloom, ingestion, marine, protist, red tide.

DENSE blooms of microalgae or so-called red tides can upset the balance of food webs and cause large-scale mortalities of fin-fish and shellfish (ECOHAB 1995). They have frequently caused great losses to the aquaculture and tourist industries of many countries. Red tides dominated by the harmful alga *Cochlodinium polykrikoides* (reported maximum concentrations = 48,000 cells ml⁻¹, NFRDI 2003) caused losses of US \$60 million in the Korean aquaculture industries in 1995 (NFRDI 1998) and US \$10–20 million per year in 2000–2003. They also caused losses of CAN \$2 million in Canada in 1999 (Whyte et al. 2001) and US \$36 million in Japan in 2000 (Kim et al. 2004). *Cochlodinium polykrikoides* has been known to kill fin-fish and shellfish by producing radical oxygen (Kim et al. 1999). Large-scale mortality of abalone in large land-aquaculture tanks in Wando, Korea, due to *C. polykrikoides* being carried in fresh seawater supplied on a daily basis to the tanks, occurred first in the fall of 2003, which entailed losses of US \$15 million. Red tides dominated by *C. polykrikoides* have recently been observed in several other countries, such as China (Huang and Dong 2000) and Japan (summarized by Kim et al. 2004), and have also caused economic losses to their aquacultures. To predict and control the outbreak of red tides dominated by *C. polykrikoides* in Korea, the Korean government and the union of aquaculture industries have spent more than US \$10 million annually. Therefore, fully understanding the ecology and physiology of *C. polykrikoides* and thus the mechanisms for the outbreak, persistence, and decline of red tides dominated by *C. polykrikoides* is a primary concern for scientists in the field of red tide research.

Recently, we found food vacuoles inside *C. polykrikoides*, which had been known previously as an autotrophic dinoflagellate. This observation implies that *C. polykrikoides* can be a mixotroph. If it is confirmed that *C. polykrikoides* can be a

mixotroph, the models for predicting the outbreak, persistence, and decline of red tides dominated by *C. polykrikoides* and related management strategies should be adjusted to reflect this fact. Recently, several red-tide dinoflagellates, which had previously been known as phototrophic, are now considered to be mixotrophic (Bockstahler and Coats 1993; Jacobson and Anderson 1994, 1996; Li, Stoecker, and Coats 1996; Stoecker et al. 1997; Jeong et al. 1997; Stoecker 1999; Granéli et al. 1997; Smalley, Coats, and Adam 1999; Skovgaard 1996, 2000). To understand the ecology and physiology of *C. polykrikoides* and the dynamics of red tides dominated by *C. polykrikoides*, the prey preferences, feeding behavior, and growth and grazing of *C. polykrikoides* should be explored.

We established a monoclonal culture of *C. polykrikoides* and observed the feeding behavior to explore its feeding mechanisms. We conducted experiments to investigate its preferences for prey species and to determine the effects of the prey concentration on the growth and ingestion rates of *C. polykrikoides* when feeding on an unidentified cryptophyte species (Equivalent Spherical Diameter, ESD = 5.6 μm). We also estimated grazing coefficients attributable to *C. polykrikoides* on 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 *C. polykrikoides*, the interactions between *C. polykrikoides* and co-occurring phytoplankton, and the dynamics of red tides dominated by *C. polykrikoides*.

MATERIALS AND METHODS

Cultivation of experimental organisms. Phytoplankton species (Table 1) were grown at 20 °C in enriched f/2 seawater medium (Guillard and Ryther 1962) without silicate and with continuous illumination of 50 $\mu\text{E m}^{-2}\text{s}^{-1}$ provided by cool-white fluorescent lights. Mean equivalent spherical diameter (ESD) \pm standard deviation of the mean was measured by an

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Table 1. Taxa, sizes, and concentration of *Cochlodinium polykrikoides* and phytoplankton species offered as food to *C. polykrikoides*. To confirm no ingestion by *C. polykrikoides* on some phytoplankton species, additional higher prey concentrations were provided. Y—*C. polykrikoides* was observed to engulf a target prey cell; N—*C. polykrikoides* was observed not to engulf a target prey cell. Mean equivalent spherical diameter (ESD, μm) \pm standard deviation of the mean was measured by an electronic particle counter (Coulter Multisizer II, Coulter Corporation, Miami, Florida, USA). $n > 2000$ for each species.

Species	ESD (\pm SD)	Initial prey concentration (cells ml ⁻¹)	Feeding
<i>Isochrysis galbana</i> (*PRY)	4.8 (0.2)	100,000	Y
Unidentified cryptophyte (CRP)	5.6 (1.5)	50,000	Y
<i>Amphidinium carterae</i> (DIN)	6.6 (1.5)	33,000	Y
<i>Rhodomonas salina</i> (CRP)	7.0 (2.0)	12,000	Y
<i>Heterosigma akashiwo</i> (RAP)	11.0 (0.4)	10,000	Y
<i>Heterocapsa triquetra</i> (DIN)	12.7 (0.6)	5,000	N
<i>Prorocentrum minimum</i> (DIN)	12.9 (3.6)	6,200	N
<i>Scrippsiella</i> sp. (DIN)	17.0 (5.9)	1,500–3,000	N
<i>Alexandrium tamarensense</i> (DIN)	24.8 (1.0)	1,200–4,000	N
<i>Prorocentrum micans</i> (DIN)	26.0 (2.3)	1,000–3,000	N
<i>Gymnodinium catenatum</i> (DIN)	33.9 (1.6)	500–2,000	N
<i>Akashiwo sanguinea</i> (DIN)	36.3 (5.6)	500–1,500	N
<i>Lingulodinium polyedrum</i> (DIN)	37.9 (4.5)	500–1,500	N
# <i>Cochlodinium polykrikoides</i> (DIN)	23.1 (3.2)		

* PRY: Prymnesiophyceae; CRP: Cryptophyceae; RAP: Raphidophyceae; DIN: Dinophyceae.

The densities of *C. polykrikoides* were 1,000 cells ml⁻¹ for these experiments.

electronic particle counter (Coulter Multisizer II, Coulter Corporation, Miami, Florida, USA).

For the isolation and culture of *C. polykrikoides*, plankton samples collected with a clean bucket were taken from the coastal waters off Tongyoung, Korea, during August, 2002, when the water temperature and salinity were 22.4 °C and 27.0 psu, respectively. The samples were screened gently through a 154- μm Nitex mesh and placed in 1-L polycarbonate (PC) bottles to which 50 ml of f/2 nutrient medium were added. The bottles were placed on shelves and incubated at 20 °C under the continuous illumination of 50 $\mu\text{E m}^{-2} \text{s}^{-1}$ 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 two serial single-cell isolations. Once dense cultures of *C. polykrikoides* were obtained, they were transferred every two weeks to 2-L PC bottles of fresh f/2 seawater medium.

Feeding process and prey species. These experiments were designed to investigate the feeding process of *C. polykrikoides* and whether or not the predator was able to feed on target phytoplankton species when unialgal diets of various phytoplankton species were provided (Table 1). The initial concentration of each phytoplankton species offered was similar to other species in terms of carbon biomass. To confirm that *C. polykrikoides* did not ingest some phytoplankton species, additional higher prey concentrations were also provided.

A dense culture of *C. polykrikoides* maintained in f/2 medium and growing photosynthetically on shelves and incubated under the continuous illumination of 50 $\mu\text{E m}^{-2} \text{s}^{-1}$ was transferred to a 1-L polycarbonate (PC) bottle containing freshly filtered seawater. Three 1-ml aliquots were then removed from the bottle and examined using a compound microscope to determine *C. polykrikoides* concentration.

In this experiment, the initial concentrations of *C. polykrikoides* and target phytoplankton species were established using an autopipette to deliver a predetermined volume of culture with known cell density to the experimental bottles. Triplicate 32-ml PC bottles with mixtures of *C. polykrikoides* and phytoplankton were set up for each target phytoplankton species. The bottles were filled to capacity with freshly filtered seawater,

capped, and then well mixed. One minute later, a 1-ml aliquot was removed from the bottle and transferred into a 1-ml Sedgwick-Rafter chamber. The feeding behavior of >50 unfed *C. polykrikoides* cells for each target prey species was observed under a compound microscope at a magnification of 100–400 \times . Pictures of *C. polykrikoides* at several different stages of the feeding process were taken using an Olympus camera on a compound microscope at a magnification of 100–400 \times . The bottles were filled again to capacity with fresh seawater filtered by a 0.2- μm CP filter (Chisso Filter Co. LTD., Osaka, Japan), capped, and placed on shelves and incubated at 20 °C under the continuous illumination of 50 $\mu\text{E m}^{-2} \text{s}^{-1}$ 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, *C. polykrikoides* cells were alive, but almost motionless. The protoplasm of >200 *C. polykrikoides* cells was carefully examined with a compound microscope and/or an epifluorescence microscope at a magnification of 100–400 \times to determine whether or not *C. polykrikoides* was able to feed on the target prey species.

Effects of the prey concentration. These experiments were designed to investigate the effects of prey concentration on the growth and ingestion rate of *C. polykrikoides*. We measured growth, ingestion, and clearance rates of *C. polykrikoides* on an unidentified cryptophyte species (Equivalent Spherical Diameter, ESD = 5.6 μm) as a function of prey concentration.

A dense culture of *C. polykrikoides* maintained in f/2 medium and growing photosynthetically under a 14:10 h light-dark cycle of 50 $\mu\text{E m}^{-2} \text{s}^{-1}$ 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 *C. polykrikoides*, and the cultures were then used to conduct experiments.

The initial concentrations of *C. polykrikoides* and a cryptophyte were established using an autopipette to deliver predetermined volumes of known cell concentrations to the bottles. Triplicate 32-ml PC experimental bottles (mixtures of predator and prey) and triplicate control bottles (prey only) were set up

for each predator-prey combination. Triplicate control bottles containing only *C. polykrikoides* were also established at one predator concentration. Five milliliters 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 initial predator and prey densities (cells ml⁻¹) at the beginning of the experiment (*C. polykrikoides*/cryptophyte = 4/29, 5/60, 14/98, 30/187, 66/818, 331/3952, 831/8930, 335/0), a 5-ml aliquot was removed from each bottle, fixed with 5% Lugol's solution, and examined with a compound microscope to determine predator and prey abundance, by counting cells in three 1-ml Sedgwick-Rafter counting chambers (SRCs). The bottles were filled again to capacity with freshly filtered seawater, capped, laid on shelves, 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. We did not use plankton wheels in these experiments because cryptophytes were distributed almost homogeneously even when bottles were not rotated and *C. polykrikoides* often had negative growth if bottles were rotated. The dilution of the cultures associated with refilling the bottles was taken into consideration in calculating growth and ingestion rates.

Ten-milliliter aliquots were taken from each bottle after 48 h incubation, fixed with 5% Lugol's solution, and the abundances of *C. polykrikoides* and the cryptophyte were determined by counting all or >300 cells in five 1-ml SRCs. Prior to taking subsamples, the condition of *C. polykrikoides* and its prey were assessed using a dissecting microscope as described above.

Specific growth rates for 48 h were calculated using the equations of Frost (1972) and Heinbokel (1978). Data for *C. polykrikoides* growth rate were fitted to a Michaelis-Menten equation:

$$\mu = \frac{\mu_{\max}(x - x')}{K_{GR} + (x - x')} \quad (1)$$

where μ_{\max} = the maximum growth rate (d⁻¹); x = prey concentration (cells ml⁻¹ or ng C ml⁻¹), x' = threshold prey concentration (the prey concentration where $\mu = 0$), K_{GR} = the prey concentration sustaining $\frac{1}{2} \mu_{\max}$. Data were iteratively fitted to the model using DeltaGraph® (SPSS Inc., Chicago, IL, USA).

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:

$$IR = \frac{I_{\max}}{K_{IR} + (x)} \quad (2)$$

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

Grazing impact. We estimated grazing coefficients attributable to *C. polykrikoides* 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 *C. polykrikoides* and co-occurring cryptophytes used in this estimation were obtained from the water samples off Kohung (in 1998–1999) and Saemankeum (in 1999), Korea.

Grazing coefficients (g, h⁻¹) were calculated as:

$$g = (1/\Delta t)\{\ln[C_i/(C_i - C_e)]\} \quad (3)$$

where Δt (h) is a time interval, C_e (cells ml⁻¹) is the number of prey cells eaten by the *C. polykrikoides* population in 1 ml of seawater in an hour, and C_i (cells ml⁻¹) is the initial prey cell concentration on a given hour. The values of C_e were calculated as:

Table 2. Estimation of grazing impact by a *Cochlodinium polykrikoides* population on cryptophyte populations using the equation in Fig. 3 in this study, and the abundances of co-occurring cryptophytes and *C. polykrikoides* obtained from the water samples collected off Kohung (in 1998–1999) and Saemankeum (in 1999), Korea. C_{PIR} = *C. polykrikoides*'s population ingestion rate; C_g = *C. polykrikoides*'s grazing coefficient (h⁻¹).

Cryptophyte concentration (cells ml ⁻¹)	<i>C. polykrikoides</i> concentration (cells ml ⁻¹)	C _{PIR} (prey eaten ml ⁻¹ h ⁻¹)	C _g (h ⁻¹)
10	80	0.2	0.021
24	2,065	12.8	0.745
41	1,062	11.1	0.311
49	433	5.3	0.115
118	68	1.9	0.016
186	82	3.5	0.019
243	53	2.9	0.012
311	25	1.7	0.005
684	25	3.1	0.005
853	32	4.5	0.005
1,615	23	4.7	0.003

$$C_e = \text{PIR} \times 1 \text{ h} = \text{IR} \times G \times 1 \text{ h} \quad (4)$$

where PIR is the population ingestion rate of *C. polykrikoides* on a cryptophyte in 1 ml of seawater (prey eaten ml⁻¹h⁻¹), IR is the ingestion rate (prey eaten *C. polykrikoides*⁻¹h⁻¹) of *C. polykrikoides* on a cryptophyte, and G is the initial abundance (cells ml⁻¹) of *C. polykrikoides* at the same time as C_i.

RESULTS

Feeding process and prey species. *Cochlodinium polykrikoides* fed on phytoplankton cells by engulfing the prey through the sulcus (Fig. 1). Prey cells were engulfed along the sulcus in the region where the sulcus joined the girdle.

The time (mean ± standard error) for an unidentified cryptophyte cell (ESD = 5.6 μm) to be completely engulfed by *C. polykrikoides* after the prey cell was contacted by the predator was 245 ± 29 sec (N = 5).

Among the phytoplankton prey offered, *C. polykrikoides* ingested the small phytoplankton species [the prymnesiophyte *Isochrysis galbana*, an unidentified cryptophyte (ESD = 5.6 μm), the cryptophyte *Rhodomonas salina*, the raphidophyte *Heterosigma akashiwo*, and the dinoflagellate *Amphidinium carterae*] which had ESD's ≤ 11 μm, but it did not feed on the large phytoplankton species [the dinoflagellates *Heterocapsa triquetra*, *Prorocentrum minimum*, *Scrippsiella* sp., *Alexandrium tamarense*, *P. micans*, *Gymnodinium catenatum*, *Akashiwo sanguinea*, and *Lingulodinium polyedrum*] which had ESD's ≥ 12 μm (Table 1). *Cochlodinium polykrikoides* was observed to attempt to engulf *H. triquetra* and *P. minimum*, but it failed to engulf them due to the large size of the prey. However, the predator did not even attempt to engulf the other large phytoplankton species.

Effects of prey concentration. The specific growth rates of *C. polykrikoides* feeding on a unialgal diet of a cryptophyte were significantly affected by the prey concentration (1-way ANOVA, $p < 0.025$). With increasing mean prey concentration the specific growth rates of *C. polykrikoides* on a cryptophyte increased, with saturation at a mean prey concentration of approximately 270 ng C ml⁻¹ (i.e. 15,900 cells ml⁻¹) (Fig. 2). When the data were fitted to Eq. (1), the maximum specific growth rate of *C. polykrikoides* on a cryptophyte (mixotrophic growth) was 0.324 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 condi-

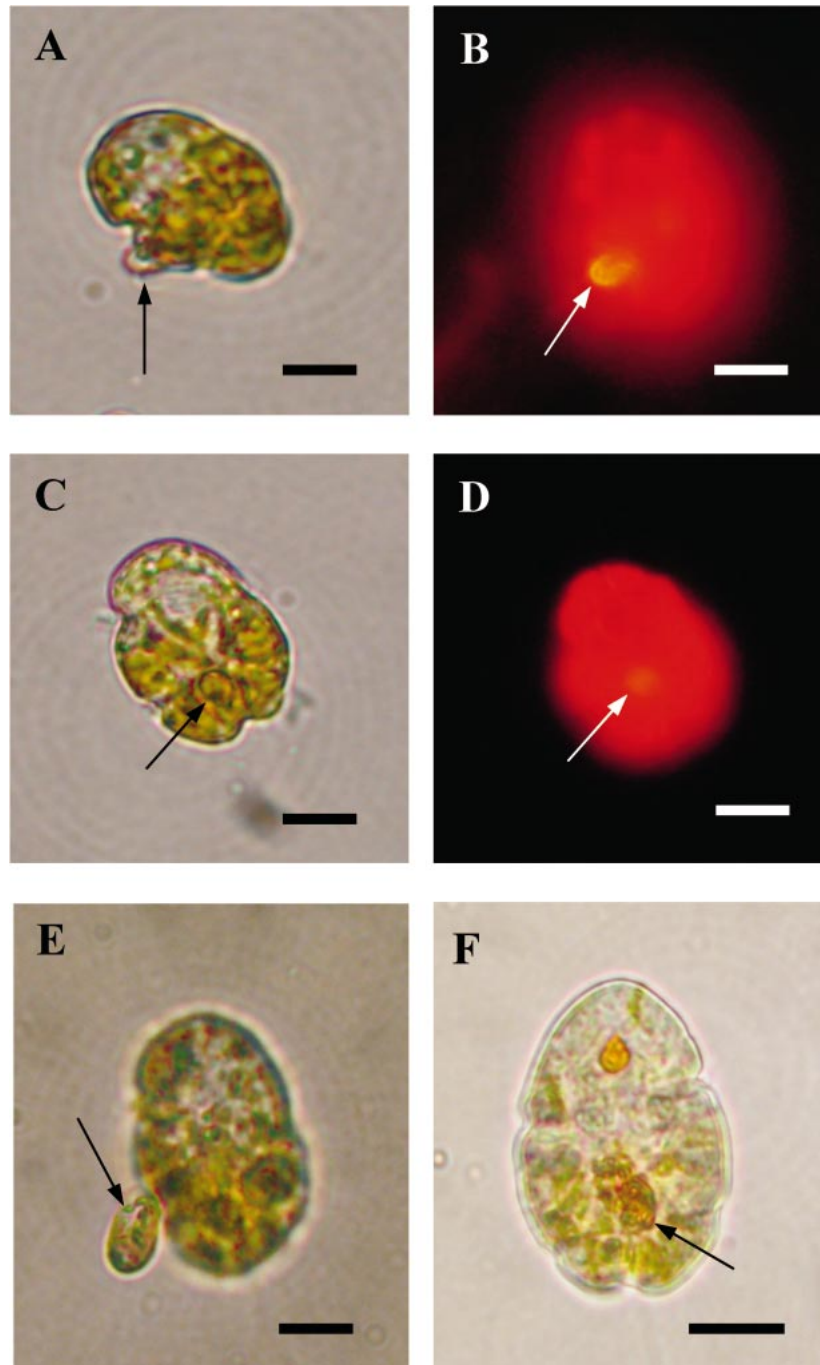


Fig. 1. The feeding process of *Cochlodinium polykrikoides*. **A, B.** *Cochlodinium polykrikoides* has engulfed approximately half of the body of an unidentified cryptophyte cell. **C, D.** An ingested cryptophyte cell inside the same predator cell as in (A). **E.** A *C. polykrikoides* has captured a *Rhodomonas* cell by the sulcus. **F.** Another *C. polykrikoides* has ingested a *Rhodomonas* cell. Arrows indicate ingested prey cells. A, C, E, F are phase photomicrographs and B and D are photomicrographs taken using epifluorescence. All scale bars = 10 μm .

tions without added prey (phototrophic growth) was only 0.166 d^{-1} .

The ingestion rates of *C. polykrikoides* feeding on a unialgal diet of a cryptophyte were also significantly affected by the prey concentration (1-way ANOVA, $p < 0.001$). The ingestion rates of *C. polykrikoides* on a cryptophyte increased continuously with increasing mean prey concentration offered in the present study (Fig. 3). When the data were fitted to Eq. (2), the maximum ingestion rate of *C. polykrikoides* on a cryptophyte

was 0.16 $\text{ng C grazer}^{-1}\text{d}^{-1}$ (9.4 $\text{cells grazer}^{-1}\text{d}^{-1}$). The maximum clearance rate of *C. polykrikoides* on a cryptophyte was 0.33 $\mu\text{l grazer}^{-1}\text{h}^{-1}$.

Grazing impact. Grazing coefficients (g) attributable to *C. polykrikoides* on co-occurring cryptophytes in the coastal waters off Kohung (1998–1999) and Saemankeum (1999), Korea were 0.001–0.745 h^{-1} (Table 2 and Fig. 4). In general grazing coefficients increased with increasing *C. polykrikoides* concentration.

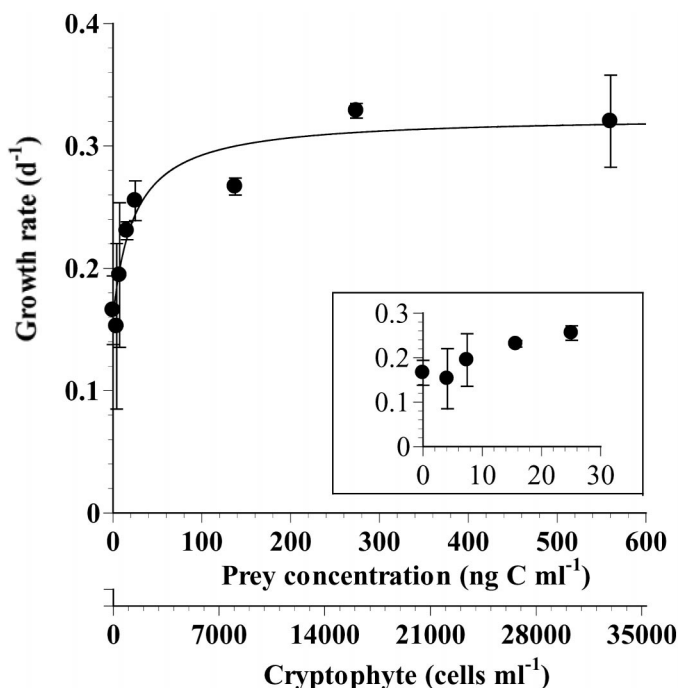


Fig. 2. Specific growth rates (d⁻¹) of *Cochlodinium polykrikoides* on an unidentified cryptophyte as a function of mean prey concentration (ng C ml⁻¹ and cells ml⁻¹). Symbols represent treatment means ± 1 S.E (N = 3 for each prey concentration). The curves were fitted by a Michaelis-Menten equation [Eq. (1)] using all treatments in the experiment. Growth rate (GR, d⁻¹) = 0.324 [(x + 11)/(12 + (x + 11))], r² = 0.54. Inset shows values at low prey concentrations.

DISCUSSION

Feeding process and prey species. The present study is the first to report that *C. polykrikoides*, which had been previously known as an autotrophic dinoflagellate, can be mixotrophic. Among the phytoplankton prey offered, *C. polykrikoides* ingested the smaller phytoplankton species such as *I. galbana*, an unidentified cryptophyte (ESD = 5.6 μm), *R. salina*, *H. akashiwo*, and *A. carterae*, which had ESD's ≤ 11 μm, but it did not feed on the larger phytoplankton species, such as *H. triquetra*, *P. minimum*, *Scrippsiella* sp., *A. tamarense*, *P. micans*, *G. catenatum*, *A. sanguinea*, and *L. polyedrum*, which had ESD's ≥ 12 μm. The ESD of *H. akashiwo* was slightly smaller than that of *H. triquetra* or *P. minimum*, but *C. polykrikoides* was able to engulf the former prey species, whereas it failed to engulf the latter prey species. When *C. polykrikoides* attempted to engulf *H. akashiwo*, *H. triquetra*, and *P. minimum*, the naked alga *H. akashiwo* was engulfed while being squeezed somewhat, but the thecate algae *H. triquetra* and *P. minimum* could not be squeezed and thus were not engulfed. Therefore, whether or not *C. polykrikoides* is able to ingest a phytoplankton species might be mainly affected by the size of the prey species and partially by its body flexibility. The results of the present study suggest that to predict the outbreak of red tides dominated by *C. polykrikoides*, the phytoplankton species that co-occur should be taken into consideration.

Effects of prey concentration. Both growth and ingestion rates of *C. polykrikoides* feeding on a unialgal diet of a cryptophyte were significantly affected by the prey concentration. A unialgal diet of a cryptophyte can support a population growth of *C. polykrikoides* (mixotrophic growth, 0.324 d⁻¹) 95% higher than that without added prey (phototrophic growth,

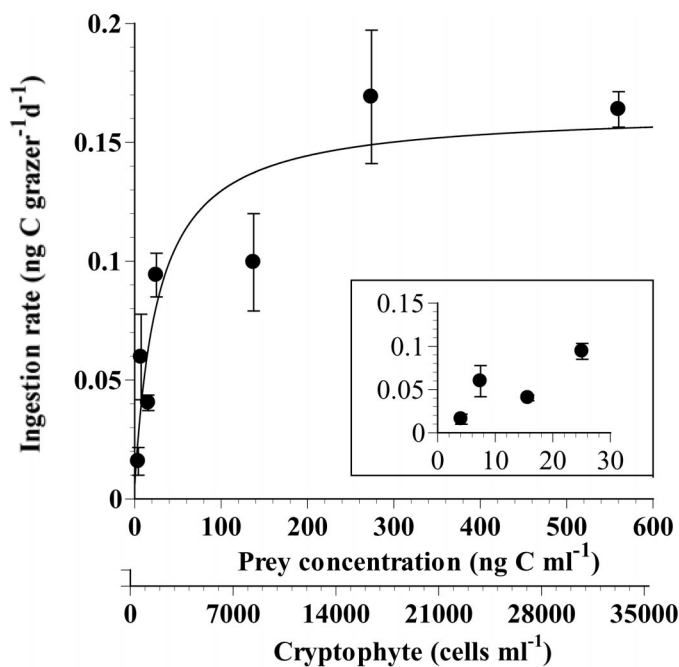


Fig. 3. Ingestion rate (ng C grazer⁻¹d⁻¹) of *Cochlodinium polykrikoides* on an unidentified cryptophyte as a function of mean prey concentration (ng C ml⁻¹ and cells ml⁻¹). Symbols represent treatment means ± 1 S.E (N = 3 for each prey concentration). The curves were fitted by a Michaelis-Menten equation [Eq. (2)] using all treatments in the experiment. Ingestion rate (IR, ng C grazer⁻¹d⁻¹) = 0.16 [x/(26.3 + x)], r² = 0.73. Inset shows values at low prey concentrations.

0.166 d⁻¹) under the conditions provided in the present study. This evidence suggests that *C. polykrikoides* may be able to increase or maintain its population by feeding on cryptophytes under conditions less favorable for phototrophic growth and if prey is abundant.

At the initial stage of red tides dominated by *C. polykrikoides*

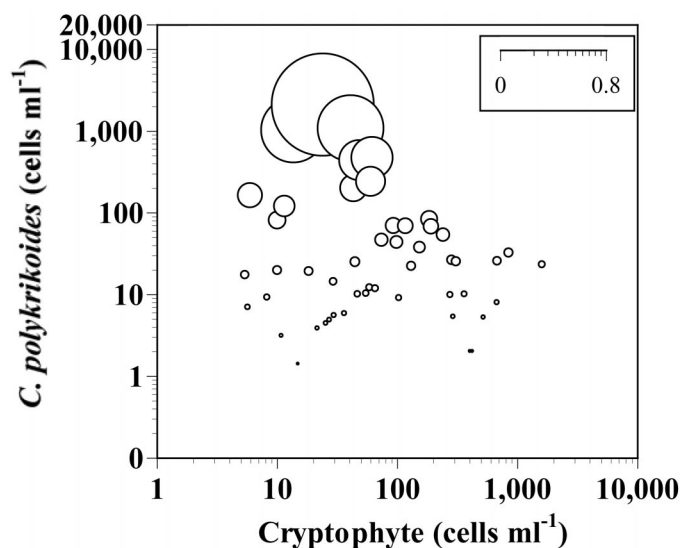


Fig. 4. Calculated grazing coefficients of *Cochlodinium polykrikoides* in relation to the concentration of co-occurring cryptophytes (see text for calculation). N = 55. The scale of the circles in the inset box is g (h⁻¹).

in Korean waters, red tide patches dominated by *C. polykrikoides* have usually been observed in the offshore waters where nutrient concentrations in the surface waters are relatively low. On the other hand, the patches dominated by diatoms have been observed in the nearshore waters where nutrient concentrations in the surface waters are relatively high (Jeong et al. 2000; Yang et al. 2000). Jeong et al. (2000) suggested that *C. polykrikoides* might increase its population during diurnal vertical migration in the offshore water, while much faster growing *Skeletonema costatum* (the most dominant diatom) outgrow slower growing *C. polykrikoides* in nearshore waters. The results of the present study suggest that *C. polykrikoides* may obtain additional nutrients for growth by ingesting small photosynthetic prey. Thus, the presence of co-occurring small prey might be partially responsible for the outbreaks of red tides dominated by *C. polykrikoides* in the offshore waters.

The maximum ingestion rate of *C. polykrikoides* feeding on a unialgal diet of a cryptophyte under the conditions provided in the present study (9.4 cells grazer⁻¹d⁻¹) was similar to that of *Mesodinium rubrum* on the same prey (8.9 cells grazer⁻¹d⁻¹) obtained at 15 °C under continuous illumination of 60 μE m⁻²s⁻¹ (Yih et al. 2004). *Cochlodinium polykrikoides* and *M. rubrum* often co-occur in western Korean waters (Jeong et al., unpubl. data) and thus they may compete with each other for cryptophyte prey.

Grazing impact. Grazing coefficients (g) attributable to *C. polykrikoides* on co-occurring cryptophytes obtained in the present study were 0.001–0.745 h⁻¹; 0.1–53% of cryptophyte populations were removed by a *C. polykrikoides* population in 1 h (Fig. 4). The maximum concentration of *C. polykrikoides* so far reported was 48,000 cells ml⁻¹ (NFRDI 2003). The results of the present study suggest that *C. polykrikoides* may sometimes have 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 and Nielsen 1997; Hansen et al. 2000; Jakobsen, Hansen, and Larsen 2000; Jeong et al. 1999; Li, Stoecker, and Coats 2000; Skovgaard, Hansen, and Stoecker 2000; Stoecker et al. 1997). Therefore, the grazing impact by *C. polykrikoides* on co-occurring cryptophytes may also be affected by light and/or nutrient conditions.

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