

Ecology of *Gymnodinium aureolum*. II. Predation by common heterotrophic dinoflagellates and a ciliate

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ABSTRACT: We investigated whether the common heterotrophic dinoflagellates *Gyrodinium dominans*, *Oxyrrhis marina*, *Pfiesteria piscicida*, *Polykrikos kofoidii*, *Proto-peridinium bipes* and *Stoeckeria algicida*, and the naked ciliate *Strombidinopsis* sp., were able to feed on the mixotrophic red-tide dinoflagellate *Gymnodinium aureolum* (GenBank accession no. FN392226). We also measured the growth and ingestion rates of *G. dominans*, *O. marina*, *P. kofoidii*, and *Strombidinopsis* sp. on *G. aureolum* as a function of prey concentration. We calculated grazing coefficients by combining field data on abundance of small *Gyrodinium* spp. (25 to 35 μm in cell length) and *Strombidinopsis* spp. (>70 μm) and co-occurring *G. aureolum* with laboratory data on ingestion rates obtained in this study. *G. dominans*, *O. marina*, *P. kofoidii*, and *Strombidinopsis* sp. were able to feed on *G. aureolum*, whereas *P. piscicida*, *P. bipes*, and *S. algicida* were not. The maximum growth rates of *G. dominans*, *O. marina*, *Strombidinopsis* sp. and *P. kofoidii* on *G. aureolum* were 0.92, 0.71, 0.44 and 0.11 d^{-1} , respectively. However, the maximum ingestion rates of *G. dominans* on *G. aureolum* (2.0 $\text{ng C predator}^{-1} \text{d}^{-1}$) were comparable with that of *P. kofoidii* (2.3 $\text{ng C predator}^{-1} \text{d}^{-1}$), but much lower than that of *Strombidinopsis* sp. (69.7 $\text{ng C predator}^{-1} \text{d}^{-1}$). Calculated grazing coefficients for small heterotrophic *Gyrodinium* spp. and large *Strombidinopsis* spp. on *G. aureolum* were up to 0.40 d^{-1} and 0.25 d^{-1} , respectively (i.e. up to 33 and 22% of *G. aureolum* populations were removed by small *Gyrodinium* and *Strombidinopsis* populations in 1 d, respectively). The results of the present study suggest that small *Gyrodinium* spp. and *Strombidinopsis* sp. sometimes have considerable grazing effect on populations of *G. aureolum*.

KEY WORDS: Graze · Growth · Harmful algal bloom · Ingestion · Protist · Red tide

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INTRODUCTION

The dinoflagellate *Gyrodinium aureolum* was first described by Hulburt (1957) and had been considered a common bloom-forming species in temperate waters (Tangen 1977, Potts & Edwards 1987, Nielsen & Tønseth 1991, Blasco et al. 1996). However, considerable confusion arose regarding identity of this species. An isolate from the Pettaquamscutt River, USA, which was similar to the original description of *Gyrodinium aure-*

olum by Hulburt (1957), was designated as *Gymnodinium aureolum*, while the European isolates that were formerly identified as *Gyrodinium aureolum*, *Gymnodinium nagasakiense* and *Gymnodinium mikimotoi* were finally designated as *Karenia mikimotoi* (Daugbjerg et al. 2000, Hansen et al. 2000). *Gymnodinium aureolum* has a horseshoe-shaped apical groove, nuclear chambers and a nuclear fibrous connective (NFC), while *K. mikimotoi* has a linear apical groove and does not have a nuclear chamber or a NFC.

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At present, it is difficult to determine whether *Gyrodinium aureolum* isolates reported by researchers before Daugbjerg et al. (2000) and Hansen et al. (2000) were *Gymnodinium aureolum* or *K. mikimotoi*. Thus, there have been only a few studies on the ecophysiology of the actual *Gymnodinium aureolum* species since settlement of the confusion over taxonomy (Tang et al. 2008, Jeong et al. 2010, this issue).

Jeong et al. (2010) revealed that *Gymnodinium aureolum*, originally thought to be an exclusively autotrophic dinoflagellate, is a mixotrophic dinoflagellate. The population dynamics of a mixotrophic dinoflagellate are affected mainly by growth and mortality. However, to date, no studies exist on mortality of *G. aureolum* caused by predation. Even for *Karenia mikimotoi*, only a few studies have been done to determine its mortality resulting from predation (Lee & Hirayama 1992, Nakamura et al. 1995, Kamiyama & Arima 2001); Nakamura et al. (1995) reported that the heterotrophic dinoflagellate *Gyrodinium dominans* had a growth rate of 0.4 to 0.7 d⁻¹ when they fed on *K. mikimotoi* (previously *Gymnodinium mikimotoi*). Another heterotrophic dinoflagellate *Noctiluca scintillans* was able to feed on *K. mikimotoi* (previously *G. nagasakiense*) (Lee & Hirayama 1992). Also, a Japanese strain of *Favella ehrenbergii* was able to feed on a strain of *K. mikimotoi* although its ingestion rate (based on carbon biomass) was lower than that when it fed on other mixotrophic dinoflagellate prey, with the exception of *Prorocentrum triestinum* (Kamiyama & Arima 2001). The heterotrophic dinoflagellate *Diplopsalis lenticula* was shown to feed on *Gyrodinium aureolum*, although it remains unclear whether this prey was *Gymnodinium aureolum* or *K. mikimotoi* (Naustvoll 1998). To understand the ecology of *Gymnodinium aureolum* in natural environments, it is necessary to explore mortality of *G. aureolum* due to predation by using a clearly identified culture of *G. aureolum*.

Karenia mikimotoi is known to produce undefined toxic compounds and is harmful to the ciliate *Favella ehrenbergii*, copepods, larvae of benthos and fish (Gill & Harris 1987, Uye & Takamatsu 1990, Hansen 1995, Smolowitz & Shumway 1997, Yamasaki et al. 2004). However, the toxins or toxic compounds of *Gymnodinium aureolum* have not been detected. Tang et al. (2008) reported that the juvenile fish *Cyprinodon variegata* was not killed when exposed to 3 strains of *G. aureolum* at concentrations of 10 900 to 14 500 cells ml⁻¹. Jeong et al. (2010) also reported that the Korean strain of *G. aureolum* used in the present study did not kill the brine shrimp *Artemia salina* at the concentrations of 4940 to 15 180 cells ml⁻¹. Therefore, unlike *K. mikimotoi*, *G. aureolum* is likely to be a non-toxic dinoflagellate, and the interactions between *G. aureolum* and predators may be quite different from those

between *K. mikimotoi* and predators. To understand the population dynamics of *G. aureolum* and its potential predators in natural environments, it is necessary to explore the interactions between *G. aureolum* and potential predators.

In general, the grazing effects of heterotrophic dinoflagellates or ciliates on red tide dinoflagellates are much higher than that of copepods, as the abundances of heterotrophic dinoflagellates or ciliates are much greater than that of copepods (i.e. usually 100 to 10 000 times greater) even though the ingestion rates of heterotrophic dinoflagellates or ciliates are considerably lower (i.e. usually 10 to 100 times lower) (Calbet et al. 2003, Calbet & Landry 2004, Kim & Jeong 2004, Turner & Borkman 2005). Therefore, to investigate grazing pressure by potential predators on *Gymnodinium aureolum*, growth, ingestion and mortality of *G. aureolum* due to predation by heterotrophic dinoflagellates and ciliates need to be measured.

Recently *Gymnodinium aureolum* has formed red tides off the western coasts of Korea. We isolated and established a clonal culture of the dinoflagellate during a red tide event dominated by *G. aureolum* in 2008. During this red tide, we found several ciliates and heterotrophic dinoflagellates; therefore, it is highly possible that some of these ciliates and heterotrophic dinoflagellates feed on *G. aureolum*. By using this culture, we observed feeding behavior, examined the kind of prey consumed and measured growth and grazing rates of this species (Jeong et al. 2010). The present study also investigated feeding by 6 common heterotrophic dinoflagellates and one ciliate on this dinoflagellate. We (1) tested whether the heterotrophic dinoflagellates *Gyrodinium dominans*, *Oxyrrhis marina*, *Pfiesteria piscicida*, *Polykrikos kofoidii*, *Protoperdinium bipes* and *Stoeckeria algicida* and the ciliate *Strombidinopsis* sp. were able to feed on *G. aureolum*; (2) measured the growth and/or ingestion rates of *G. dominans*, *O. marina*, *P. kofoidii* and *Strombidinopsis* sp. on *G. aureolum* as a function of prey concentration; and (3) calculated grazing coefficients by combining field data on abundance of *G. dominans* (or *Strombidinopsis* sp.) and co-occurring *G. aureolum* with laboratory data on ingestion rates obtained in this study.

The results of the present study provide a basis for understanding the interactions between *Gymnodinium aureolum* and heterotrophic protists and their population dynamics in marine planktonic food webs.

MATERIALS AND METHODS

Preparation of experimental organisms. For isolation and culture of *Gymnodinium aureolum* (GenBank accession no. FN392226), plankton samples were col-

lected with water samplers from the waters off Saemankeum, Korea, during March 2008 when the water temperature and salinity were 10.0°C and 30.5, respectively (Table 1). These samples were gently passed through a 154 µm Nitex mesh screen and placed in 6-well tissue culture plates. A clonal culture of *G. aureolum* was established by 2 serial single cell isolations. As the concentration of *G. aureolum* increased, *G. aureolum* was subsequently transferred to 32, 120, 270 and 500 ml polycarbonate (PC) bottles (Nalge Nunc International) containing fresh f/2 seawater media. The bottles were filled to capacity with freshly filtered seawater, capped and placed on a shelf at 20°C under an illumination of 20 µE m⁻² s⁻¹ of cool white fluorescent light on a 14 h light: 10 h dark cycle.

For the isolation and culture of the heterotrophic dinoflagellate predators *Gyrodinium dominans*, *Oxyrrhis marina*, *Polykrikos kofoidii*, *Pfiesteria piscicida*, *Stoeckeria algicida* and *Protoberidinium bipes*, plankton samples were collected with water samplers from the coastal waters off Masan, Incheon or Shiwha, Korea, from 2001 to 2008, and a clonal culture of each species was established by 2 serial single-cell isolations (Table 1).

For the isolation and culture of *Strombidinopsis* sp., plankton samples were collected with water samplers from a pier in Masan Bay, Korea, during May 2009 when the water temperature and salinity were 20.2°C and 30.1, respectively (Table 1). A clonal culture of *Strombidinopsis* sp. (70 to 160 µm in cell length) was established by 2 serial single cell isolations as described by Jeong et al. (2008a).

The carbon contents for *Gymnodinium aureolum* (0.44 ng C per cell, n = 50), the heterotrophic dinoflagellates and the ciliate were estimated from cell volume according to Menden-Deuer & Lessard (2000). The cell volume of the predators was estimated by means of the following methods: Kim & Jeong (2004) for *Gyrodinium dominans*; Jeong et al. (2008b) for *Oxyrrhis marina*; Jeong et al. (2003a) for *Polykrikos kofoidii*; Jeong et al. (2007) for *Pfiesteria piscicida* and

Stoeckeria algicida; Jeong et al. (2004) for *Protoberidinium bipes*; and Jeong et al. (2008a) for *Strombidinopsis* sp.

Feeding occurrence. Expt 1 was designed to test whether each of *Gyrodinium dominans*, *Oxyrrhis marina*, *Pfiesteria piscicida*, *Polykrikos kofoidii*, *Protoberidinium bipes*, *Stoeckeria algicida* and *Strombidinopsis* sp. was able to feed on *Gymnodinium aureolum* (Table 2).

Approximately 8 × 10⁵ *Gymnodinium aureolum* cells were added to each of two 80 ml PC bottles containing *Polykrikos kofoidii* (100 cells ml⁻¹), each of the other heterotrophic dinoflagellates (1000 to 5000 cells ml⁻¹), or the ciliate (20 cells ml⁻¹) (final prey concentration ca. 10 000 cells ml⁻¹). One control bottle (without prey) was set up for each experiment. The bottles were placed on a plankton wheel rotating at 0.9 rpm and incubated at 20°C under an illumination of 20 µE m⁻² s⁻¹ on a 14 h light:10 h dark cycle.

Aliquots of 5 ml were removed from each bottle after 1, 2, 6 and 24 h incubation and then transferred into 6-well plate chambers (or glass microscope slides). Approximately 200 cells of each predator in the plate chamber (or glass microscope slide) were observed under a dissecting microscope (or inverted microscope) at a magnifications ranging from 20 to 90× (or 100 to 630×) to determine whether the predator was able to feed on the *Gymnodinium aureolum*. Cells of the predator containing ingested *G. aureolum* cells were photographed at a magnification of 400 to 630× with a digital camera (Zeiss AxioCam HRC5, Carl Zeiss) mounted on the microscope.

Growth and ingestion rates. Expts 2 to 5 were designed to measure the growth and ingestion rates of *Gyrodinium dominans*, *Oxyrrhis marina*, *Polykrikos kofoidii* and *Strombidinopsis* sp. as a function of the prey concentration when they fed on *Gymnodinium aureolum* (Table 2). Only these 3 heterotrophic dinoflagellates and the ciliate were shown to feed on *G. aureolum* among the heterotrophic protists tested.

Table 1. Isolation and maintenance conditions of the experimental organisms. Sampling location and time, water temperature (*T*, °C) and salinity for isolation, and prey species and concentrations (cells ml⁻¹) for maintenance. MTD: mixotrophic dinoflagellate; HTD: heterotrophic dinoflagellate; CIL: ciliate

Organism	Location	Date	<i>T</i>	Salinity	Prey species	Concentration
<i>Gyrodinium dominans</i> (HTD)	Masan Bay	Apr 2007	15.1	33.4	<i>Amphidinium carterea</i>	30 000–40 000
<i>Oxyrrhis marina</i> (HTD)	Keum Estuary	May 2001	16.0	27.7	<i>Amphidinium carterea</i>	8000
<i>Polykrikos kofoidii</i> (HTD)	Masan Bay	Jun 2007	20.2	32.2	<i>Lingulodinium polyedrum</i>	4000
<i>Strombidinopsis</i> sp. (CIL)	Masan Bay	May 2009	20.2	30.1	<i>Prorocentrum minimum</i>	20 000–30 000
<i>Pfiesteria piscicida</i> (HTD)	Off Incheon	Jul 2005	24.0	25.4	<i>Amphidinium carterea</i>	20 000–30 000
<i>Stoeckeria algicida</i> (HTD)	Masan Bay	May 2007	20.9	30.1	<i>Heterosigma akashiwo</i>	30 000
<i>Protoberidinium bipes</i> (HTD)	Shiwha Bay	Nov 2008	13.0	28.5	<i>Skeletonema costatum</i>	50 000
<i>Gymnodinium aureolum</i> (MTD)	Off Saemankeum	Mar 2008	10.0	30.5		

Table 2. Experimental design to assess predation on *Gymnodinium aureolum* by various planktonic predator species. The numbers in prey and predator columns are the actual initial concentrations (cells ml⁻¹) of prey and predator. In each experiment, the lowest *G. aureolum* concentration was tested against the lowest predator concentration, the next lowest against the next lowest, etc. Values within parentheses in the prey and predator columns are the corresponding prey and predator concentrations in the predator-only control bottles. Feeding occurrence of each predator fed *Gymnodinium aureolum* in Expt 1 is represented by Y (feeding observed) or N (no feeding observed)

Expt	<i>G. aureolum</i> concentration	Species	Predator concentration	Feeding
1	10 000	<i>Gyrodinium dominans</i>	1000	Y
		<i>Oxyrrhis marina</i>	3000	Y
		<i>Polykrikos kofoidii</i>	100	Y
		<i>Protoperidinium bipes</i>	1000	N
		<i>Pfiesteria piscicida</i>	5000	N
		<i>Stoeckeria algicida</i>	5000	N
		<i>Strombidinopsis</i> sp.	20	Y
2	20, 50, 110, 210, 490, 1080, 2120, 5480, 11 300 (0)	<i>Gyrodinium dominans</i>	4, 8, 20, 24, 28, 38, 69, 159, 409 (90)	
3	20, 50, 90, 240, 520, 1160, 2370, 5590, 10 800 (0)	<i>Oxyrrhis marina</i>	5, 8, 13, 39, 85, 149, 394, 638, 966 (960)	
4	20, 50, 90, 180, 430, 950, 1980, 4640, 8360 (0)	<i>Polykrikos kofoidii</i>	5, 10, 20, 77, 62, 94, 135, 136, 129 (60)	
5	40, 90, 220, 590, 1130, 2680, 6180, 8260 (0)	<i>Strombidinopsis</i> sp.	5, 5, 6, 8, 8, 12, 21, 21 (20)	

The day before these experiments were conducted, dense cultures of predator species growing on algal prey were transferred into 500 ml PC bottles containing 2 different concentrations of the target prey as follows: (1) ca. 100 *Gymnodinium aureolum* cells ml⁻¹ for 5 lower prey concentrations and ca. 500 *G. aureolum* cells ml⁻¹ for 4 upper prey concentrations for *G. dominans*; (2) ca. 200 *G. aureolum* cells ml⁻¹ for 4 lower prey concentrations and ca. 1000 *G. aureolum* cells ml⁻¹ for 5 upper prey concentrations for *Oxyrrhis marina*; (3) ca. 100 *G. aureolum* cells ml⁻¹ for 5 lower prey concentrations and ca. 500 *G. aureolum* cells ml⁻¹ for 4 upper prey concentrations for *Polykrikos kofoidii*; (4) ca. 300 *G. aureolum* cells ml⁻¹ for 4 lower prey concentrations and ca. 1500 *G. aureolum* cells ml⁻¹ for 4 upper prey concentrations for *Strombidinopsis* sp. The use of two different concentrations was meant to minimize possible residual growth resulting from the ingestion of prey during batch culture. The bottles were filled to capacity with freshly filtered seawater, capped and placed on plankton wheels rotating at 0.9 rpm and incubated at 20°C under the illumination of 20 µE m⁻² s⁻¹ on a 14 h light: 10 h dark cycle. To monitor the conditions and interaction between predator and prey species, the cultures were periodically removed from the rotating wheels, examined by observation through the surface of the capped bottles by means of a dissecting microscope (Olympus SZX12, Olympus) and returned to the rotating wheels. Once the target prey cells were no longer detectable, three 1 ml aliquots from each bottle were viewed under a light microscope (Olympus BX51, Olympus) and counted to determine cell concentrations of predator species, and the cultures were then used to conduct these experiments.

For each experiment, the initial concentrations of predator species and *Gymnodinium aureolum* were established by means of an autopipette that delivered predetermined volumes of known cell concentrations to the bottles. In preliminary tests, ingestion rates, and in turn growth rates, of a predator on prey at low prey concentrations were lower than those at higher prey concentrations. After incubation, ratios of predator relative to prey at low prey concentrations usually decreased (i.e. predator concentration slowly increased, remained unchanged or decreased, whereas prey concentration slowly decreased or remained unchanged), while those at high prey concentrations increased (i.e. predator concentration rapidly increased, whereas prey concentration rapidly decreased). Thus, we established higher initial ratios of predator relative to prey at low prey concentrations than for those at high prey concentrations. Triplicate 42 ml PC experiment bottles (80 ml bottles for the ciliate) containing mixtures of predator and prey and triplicate control bottles (prey only) were set up at each predator–prey combination. Triplicate control bottles containing only predator species were also established at one predator concentration. To obtain similar water conditions, the water of a predator culture was filtered through a 0.7 µm GF/F filter and then added to the prey control bottles in the same volume that was added to the experiment bottles for each predator–prey combination. All bottles were then filled to capacity with freshly filtered seawater and capped. To determine the actual predator and prey concentrations at the beginning of the experiment, a 5 ml aliquot was removed from each bottle, fixed with 5% Lugol's solution and examined with a

light microscope to determine predator and prey abundance by enumerating the cells in three 1 ml Sedgwick-Rafter chambers (SRCs). The bottles were refilled to capacity with freshly filtered seawater, capped and placed on rotating wheels under the conditions described above. Dilution of the cultures associated with refilling the bottles was considered when calculating growth and ingestion rates. A 10 ml aliquot was taken from each bottle after 48 h incubation (24 h incubation for the ciliate) and fixed with 5% Lugol's solution. The abundance of predator species and the prey were determined by counting all or >300 cells in three 1 ml SRCs. Before taking the subsamples, the condition of predator species and the prey was assessed by means of a dissecting microscope as described above.

The specific growth rate of predator species, μ (d^{-1}), was calculated as:

$$\mu = \ln(P_t/P_0)/t \quad (1)$$

where P_0 and P_t are the concentrations of predator species at 0 and 48 h (or 24 h for the ciliate), respectively.

We tested >100 curve fittings for the data on the growth rate of each predator on *Gymnodinium aureolum* using TableCurve® 2D v.5.01 (SYSTAT Software) and found best curve fits for each predator. Best curve fits for the data on growth and ingestion rates of *Gyrodinium dominans*, *Oxyrrhis marina* and *Strombidinopsis* sp. on *G. aureolum* were a Michaelis-Menten equation or Ivlev equation. The coefficients of determination (r^2) of the Michaelis-Menten equation and Ivlev equation for growth and ingestion rates of each predator were very similar, and thus, we provided both curve fits. The Michaelis-Menten equation for growth rate data was:

$$\mu = \mu_{\max} (X - X')/[K_{\text{GR}} + (X - X')] \quad (2)$$

where μ_{\max} = maximum growth rate (d^{-1}), X = prey concentration (cells ml^{-1} or ng C ml^{-1}), X' = threshold prey concentration (the prey concentration where $\mu = 0$) and K_{GR} = prey concentration sustaining $\frac{1}{2} \mu_{\max}$. Data were iteratively fitted to the model using DeltaGraph® (Delta Point). The Ivlev equation for growth rate (μ , d^{-1}) data was:

$$\mu = \mu_{\max} [1 - e^{-k(X - X')}] \quad (3)$$

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 $\mu = 0$) and k = constant. Data were iteratively fitted to the model using DeltaGraph.

Ingestion and clearance rates were calculated using the equations of Frost (1972) and Heinbokel (1978). The incubation times for calculating ingestion and clearance rates were the same as those for estimating

growth rate. The Michaelis-Menten equation for ingestion rate (IR, cells predator $^{-1}$ d^{-1} or ng C predator $^{-1}$ d^{-1}) data was:

$$\text{IR} = I_{\max} X/(K_{\text{IR}} + X) \quad (4)$$

where I_{\max} = maximum ingestion rate (cells predator $^{-1}$ d^{-1} or ng C predator $^{-1}$ d^{-1}), X = prey concentration (cells ml^{-1} or ng C ml^{-1}) and K_{IR} = prey concentration sustaining $\frac{1}{2} I_{\max}$. The Ivlev equation for ingestion rate data was:

$$\text{IR} = I_{\max} (1 - e^{-kX}) \quad (5)$$

where I_{\max} = maximum ingestion rate (cells predator $^{-1}$ d^{-1} or ng C predator $^{-1}$ d^{-1}), X = prey concentration (cells ml^{-1} or ng C ml^{-1}) and k = constant. Data were iteratively fitted to the model using DeltaGraph®.

Growth rate data of *Polykrikos kofoidii* were not well fitted to any equation. The best curve fit for ingestion rate data of *P. kofoidii* was a logistic curve fit as follows:

$$\text{IR} = I_{\max} \ln(X + A) + B \quad (6)$$

where I_{\max} = maximum ingestion rate (cells predator $^{-1}$ d^{-1} or ng C predator $^{-1}$ d^{-1}), X = prey concentration (cells ml^{-1} or ng C ml^{-1}) and A and B are constants. Data were iteratively fitted to the model using DeltaGraph. However, the value extrapolated using this curve when prey concentration was zero (i.e. no prey) was positive (i.e. feeding occurred). Therefore, this curve is valid only when the prey concentration is larger than a certain prey concentration.

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

Grazing impact. We estimated grazing coefficients attributable to small heterotrophic *Gyrodinium* spp. (25 to 35 μm in cell length) or large *Strombidinopsis* spp., (>70 μm in cell length) on *Gymnodinium aureolum* by combining field data on abundances of small *Gyrodinium* spp. (or *Strombidinopsis* sp.) and prey with ingestion rates of the predators on the prey obtained in the present study. We assumed that the ingestion rates of the other small heterotrophic *Gyrodinium* spp. (or *Strombidinopsis* spp.) on *G. aureolum* are the same as that of *Gyrodinium dominans* (or *Strombidinopsis* sp.). The data on the abundances of *G. aureolum* and co-occurring small heterotrophic *Gyrodinium* spp. (or *Strombidinopsis* spp.) used in this estimation were obtained from water samples collected from 2006 to 2008 from the coastal waters off Saemankeum, at fixed stations. The grazing coefficients (g , d^{-1}) were calculated as:

$$g = CR \times P \times 24 \quad (7)$$

where CR is the clearance rate ($\text{ml predator}^{-1} \text{h}^{-1}$) of a predator on *Gymnodinium aureolum* at a given prey concentration and P is the predator concentration (cells ml^{-1}). CRs were calculated as:

$$CR = IR(h)/X \quad (8)$$

where $IR(h)$ is the ingestion rate ($\text{cells eaten predator}^{-1} \text{h}^{-1}$) of the predator on the prey and X is the prey concentration (cells ml^{-1}). CRs 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 sometimes different.

RESULTS

Feeding occurrence and growth rate

Gyrodinium dominans, *Oxyrrhis marina*, *Polykrikos kofoidii* and *Strombidinopsis* sp. were able to feed on *Gymnodinium aureolum*, while *Pfiesteria piscicida*, *Proto-peridinium bipes* and *Stoeckeria algicida* were not (Fig. 1). *P. piscicida*, *P. bipes* and *S. algicida* were observed not to deploy a tow filament to capture *G. aureolum* cells.

The specific growth rates of *Gyrodinium dominans* feeding on *Gymnodinium aureolum* increased rapidly with increasing mean prey concentration up to ca. 445 ng C ml^{-1} ($1010 \text{ cells ml}^{-1}$), but became saturated at higher concentrations (Fig. 2A). When the data were fitted to Eq. (2), the maximum specific growth rate (μ_{max}) of *G. dominans* feeding on *G. aureolum* was 0.92 d^{-1} . The feeding threshold prey concentration for the growth of the predator (no growth) in Eq. (2) was 76 ng C ml^{-1} ($173 \text{ cells ml}^{-1}$). However, when the data were fitted to Eq. (3), the μ_{max} of *G. dominans* feeding on *G. aureolum* was 0.79 d^{-1} . The feeding threshold prey concentration in Eq. (3) was 86 ng C ml^{-1} ($195 \text{ cells ml}^{-1}$).

The specific growth rates of *Oxyrrhis marina* feeding on *Gymnodinium aureolum* increased rapidly with increasing mean prey concentration up to ca. 80 ng C ml^{-1} ($180 \text{ cells ml}^{-1}$), but became saturated at higher concentrations (Fig. 2B). When the data were fit-

ted to Eq. (2), the μ_{max} of *O. marina* feeding on *G. aureolum* was 0.71 d^{-1} . The feeding threshold prey concentration for the growth of the predator (no growth) in Eq. (2) was 8 ng C ml^{-1} (18 cells ml^{-1}). However, when the data were fitted to Eq. (3), the μ_{max} of *O. marina* feeding on *G. aureolum* was 0.65 d^{-1} . The feeding threshold prey concentration in Eq. (3) was 9 ng C ml^{-1} (20 cells ml^{-1}).

The specific growth rates of *Polykrikos kofoidii* feeding on *Gymnodinium aureolum* increased with increasing mean prey concentration (Fig. 2C). The growth rates were negative at mean prey concentrations $< 830 \text{ ng C ml}^{-1}$ ($< 1900 \text{ cells ml}^{-1}$), but positive at mean prey concentrations $> 2090 \text{ ng C ml}^{-1}$ ($> 4750 \text{ cells ml}^{-1}$). At the given prey concentrations,

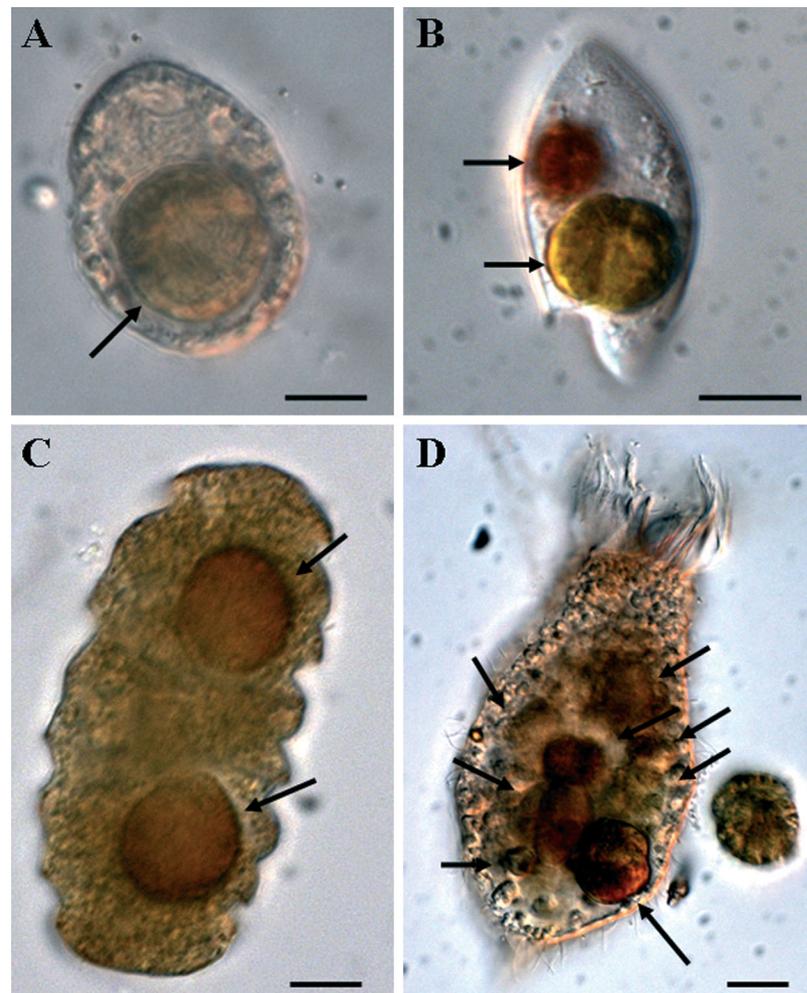


Fig. 1. Feeding by (A–C) heterotrophic dinoflagellates and (D) a ciliate on the mixotrophic dinoflagellate *Gymnodinium aureolum*. (A) *Gyrodinium dominans* with an ingested *G. aureolum* cell, (B) *Oxyrrhis marina* with 2 ingested *G. aureolum* cells, (C) *Polykrikos kofoidii* with 2 ingested *G. aureolum* cells, (D) *Strombidinopsis* sp. with several ingested *G. aureolum* cells. Arrows indicate ingested prey cells. All photographs were taken by means of an inverted microscope.

Scale bars = $10 \mu\text{m}$

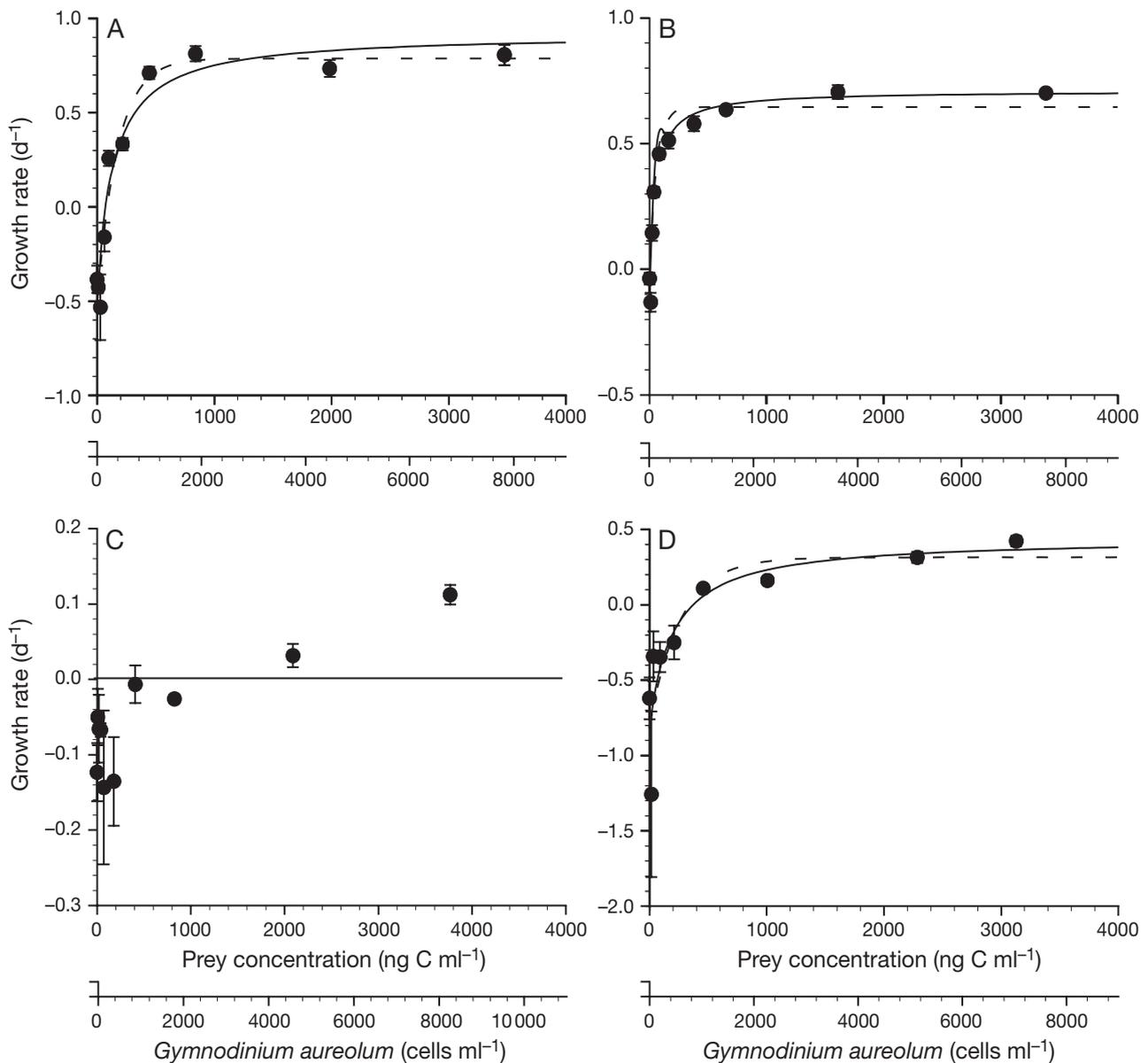


Fig. 2. Specific growth rates (μ , d^{-1}) of the heterotrophic dinoflagellates and a ciliate feeding on the mixotrophic dinoflagellate *Gymnodinium aureolum* as a function of mean prey concentration (X). (A) *Gyrodinium dominans* predator, (B) *Oxyrrhis marina*, (C) *Polykrikos kofoidii*, (D) *Strombidinopsis* sp. Symbols represent treatment means \pm 1 SE. A Michaelis-Menten equation (Eq. 2, solid line) and an Ivlev equation (Eq. 3, dashed line) were used to produce curves for (A), (B) and (D) for all treatments in the experiments. (A) $\mu = 0.92 \cdot (X - 76.2) / [207 + (X - 76.2)]$, $r^2 = 0.905$ for Eq. (2) and $\mu = 0.79 \cdot [1 - e^{-0.00573(X - 86.1)}]$, $r^2 = 0.920$ for Eq. (3); (B) $\mu = 0.71 \cdot (X - 7.8) / [55.2 + (X - 7.8)]$, $r^2 = 0.939$ for Eq. (2) and $\mu = 0.65 \cdot [1 - e^{-0.01612(X - 8.9)}]$, $r^2 = 0.930$ for Eq. (3); (D) $\mu = 0.44 \cdot (X - 370) / [570 + (X - 370)]$, $r^2 = 0.601$ for Eq. (2) and $\mu = 0.31 \cdot [1 - e^{-0.00372(X - 337)}]$, $r^2 = 0.593$ for Eq. (3)

the highest growth rate of *P. kofoidii* feeding on *G. aureolum* was 0.11 d^{-1} .

The specific growth rates of *Strombidinopsis* sp. feeding on *Gymnodinium aureolum* increased continuously with increasing mean prey concentration up to ca. 460 ng C ml^{-1} ($1050 \text{ cells ml}^{-1}$), but slowly increased at higher concentrations (Fig. 2D). When the data were fitted to Eq. (2), the μ_{max} of *Strombidinop-*

sis sp. feeding on *G. aureolum* was 0.44 d^{-1} . The feeding threshold prey concentration for the growth of the predator (no growth) in Eq. (2) was 370 ng C ml^{-1} ($840 \text{ cells ml}^{-1}$). However, when the data were fitted to Eq. (3), the μ_{max} of *Strombidinopsis* sp. feeding on *G. aureolum* was 0.31 d^{-1} . The feeding threshold prey concentration in Eq. (3) was 340 ng C ml^{-1} ($770 \text{ cells ml}^{-1}$).

Ingestion and clearance rates

The ingestion rates of *Gyrodinium dominans* feeding on *Gymnodinium aureolum* increased rapidly with increasing mean prey concentration up to ca. 1980 ng C ml⁻¹ (4500 cells ml⁻¹), but became saturated at higher concentrations (Fig. 3A). When the data were fitted to Eq. (4), the maximum ingestion rate (I_{\max}) of *G. dominans* feeding on *G. aureolum* was 2.0 ng C predator⁻¹ d⁻¹ (4.5 cells predator⁻¹ d⁻¹). However, when the data were fitted to Eq. (5), the I_{\max} of *G. dominans* feeding on *G. aureolum* was 1.6 ng C predator⁻¹ d⁻¹ (3.6 cells predator⁻¹ d⁻¹). The maximum clearance rate of *G. dominans* feeding on *G. aureolum* was 0.1 µl predator⁻¹ h⁻¹. Gross growth efficiencies (GGEs) of *G. dominans* feeding on *G. aureolum* at the prey concentrations where the ingestion rates were saturated were 29 to 43%.

The ingestion rates of *Oxyrrhis marina* feeding on *Gymnodinium aureolum* increased rapidly with increasing mean prey concentration up to ca. 80 ng C ml⁻¹ (180 cells ml⁻¹), but became saturated at higher concentrations (Fig. 3B). When the data were fitted to Eq. (4), the I_{\max} of *O. marina* feeding on *G. aureolum* was 0.51 ng C predator⁻¹ d⁻¹ (1.2 cells predator⁻¹ d⁻¹). However, when the data were fitted to Eq. (5), the I_{\max} of *O. marina* feeding on *G. aureolum* was 0.46 ng C predator⁻¹ d⁻¹ (1.0 cells predator⁻¹ d⁻¹). The maximum clearance rate of *O. marina* feeding on *G. aureolum* was 0.2 µl predator⁻¹ h⁻¹. GGEs of *O. marina* feeding on *G. aureolum* at the prey concentrations where the ingestion rates were saturated were 29 to 62%.

The ingestion rates of *Polykrikos kofoidii* feeding on *Gymnodinium aureolum* continuously increased with increasing mean prey concentration (Fig. 3C). At the given prey concentrations, the highest ingestion rate of *P. kofoidii* feeding on *G. aureolum* was 2.3 ng C predator⁻¹ d⁻¹ (5.3 cells predator⁻¹ d⁻¹). However, when the data were fitted to both Eq. (5) and Eq. (6) at prey concentrations of <3770 ng C predator⁻¹ d⁻¹ (<8560 cells predator⁻¹ d⁻¹), the I_{\max} of *P. kofoidii* feeding on *G. aureolum* was 2.2 ng C predator⁻¹ d⁻¹ (5.0 cells predator⁻¹ d⁻¹). The maximum clearance rate of *P. kofoidii* feeding on *G. aureolum* was 2.5 µl predator⁻¹ h⁻¹. GGEs of *P. kofoidii* feeding on *G. aureolum* at the prey concentrations where the 2 highest ingestion rates were achieved were 29 to 40%.

The ingestion rates of *Strombidinopsis* sp. feeding on *Gymnodinium aureolum* increased rapidly with increasing mean prey concentrations <2280 ng C ml⁻¹ (<5190 cells ml⁻¹), but became saturated at higher concentrations (Fig. 3D). When the data were fitted to Eq. (4), the I_{\max} of *Strombidinopsis* sp. feeding on *G. aureolum* was 69.7 ng C predator⁻¹ d⁻¹ (158 predator⁻¹

d⁻¹). However, when the data were fitted to Eq. (5), the I_{\max} of *Strombidinopsis* sp. feeding on *G. aureolum* was 51.0 ng C predator⁻¹ d⁻¹ (116 cells predator⁻¹ d⁻¹). The maximum clearance rate of *Strombidinopsis* sp. feeding on *G. aureolum* was 5.9 µl predator⁻¹ h⁻¹. GGEs of *O. marina* feeding on *G. aureolum* at the prey concentrations where the ingestion rates were saturated were 9 to 18%.

Relationships among maximum growth and ingestion rates of the predators

Both μ_{\max} and I_{\max} of *Gyrodinium dominans* feeding on algal prey were not significantly correlated with prey size (linear regression ANOVA, $p > 0.1$; Fig. 4A,B). The μ_{\max} and I_{\max} of *G. dominans* feeding on *Gymnodinium aureolum* obtained with Eqs. (2) & (4) were the second and third highest, respectively, among the algal prey (Fig. 4A,B). The ratio of μ_{\max} relative to I_{\max} of *G. dominans* feeding on *G. aureolum* was higher than that of the mixotrophic dinoflagellate *Heterocapsa triquetra* and the raphidophyte *Chattonella antiqua* (Fig. 4C).

Both μ_{\max} and I_{\max} of *Oxyrrhis marina* feeding on algal prey were not significantly correlated with prey size (linear regression ANOVA, $p > 0.1$; Fig. 5A,B). The I_{\max} of *O. marina* feeding on *Gymnodinium aureolum* was lower than that for any other algal prey (Fig. 5A). However, the μ_{\max} of *O. marina* feeding on *G. aureolum* was comparable with that for the haptophyte *Isochrysis galbana*, the diatom *Dunaliella tertiolecta* and the raphidophyte *Fibrocapsa japonica*, while it was lower than that for other algal prey (Fig. 5B). Also, the μ_{\max} of *O. marina* feeding on the algal prey was not significantly correlated with their I_{\max} ($p > 0.1$; Fig. 5C).

Both μ_{\max} and I_{\max} of *Polykrikos kofoidii* feeding on the mixotrophic dinoflagellates showed significant positive correlations with prey size (linear regression ANOVA, $p < 0.05$ for both rates; Fig. 6A,B). The I_{\max} of *P. kofoidii* feeding on *Gymnodinium aureolum* was the lowest amongst the prey species tested, while the μ_{\max} of *P. kofoidii* feeding on *G. aureolum* was also almost the lowest (Fig. 6A,B). The μ_{\max} of *P. kofoidii* feeding on the mixotrophic dinoflagellates also showed significant positive correlations with their I_{\max} ($p < 0.01$; Fig. 6C).

Both μ_{\max} and I_{\max} of *Strombidinopsis* sp. on algal prey did not correlate significantly with prey size (linear regression ANOVA, $p > 0.1$; Fig. 7A,B). The I_{\max} of *Strombidinopsis* sp. feeding on *Gymnodinium aureolum* (70 ng C ciliate⁻¹d⁻¹) are considerably lower than those of *Strombidinopsis* sp. on the mixotrophic dinoflagellates *Lingulodinium polyedrum*, *Akashiwo sanguinea* (previously *Gymnodinium*

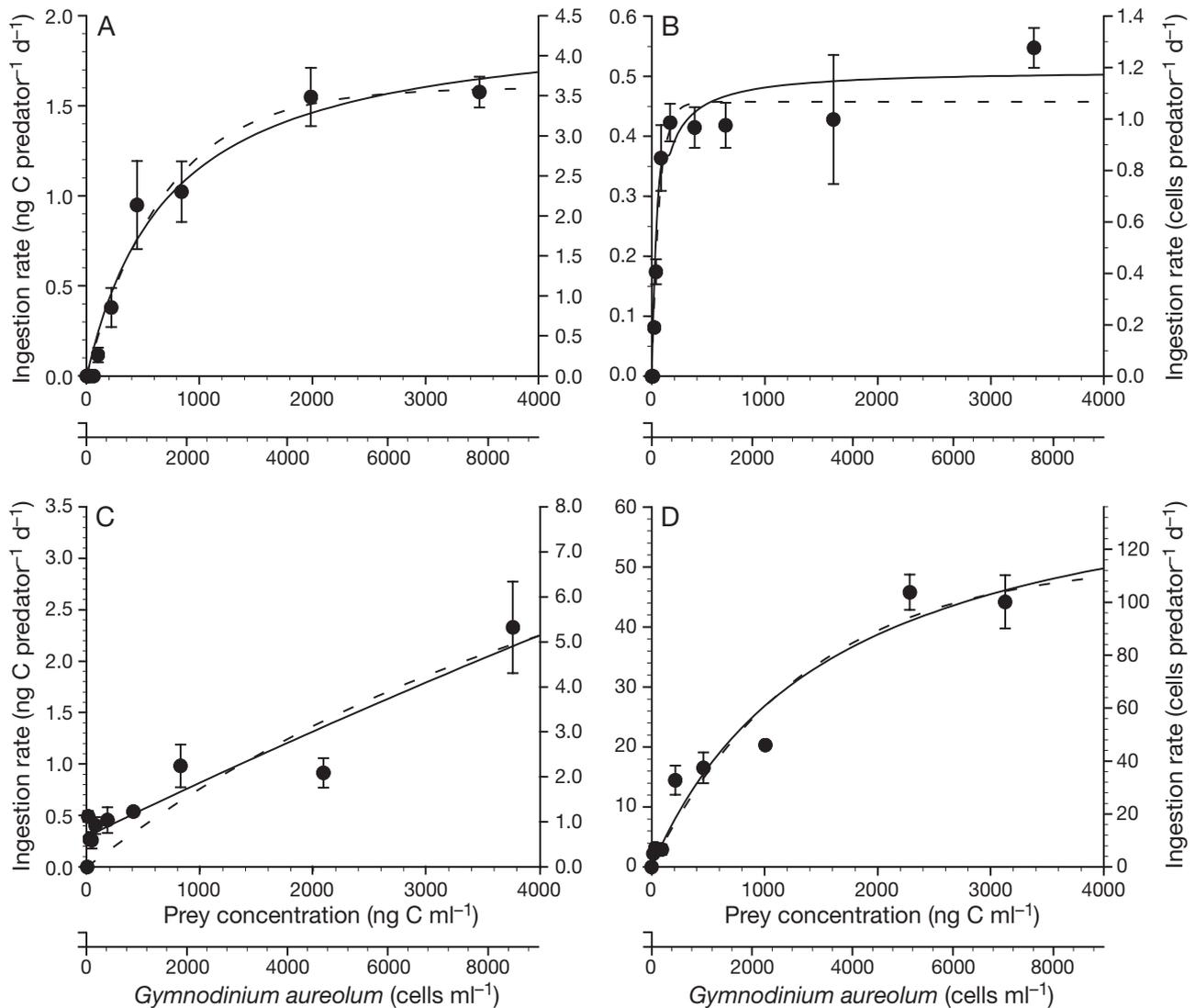


Fig. 3. Ingestion rates (IR, $\text{ng C predator}^{-1} \text{d}^{-1}$) of heterotrophic dinoflagellates and a ciliate feeding on the mixotrophic dinoflagellate *Gymnodinium aureolum* as a function of mean prey concentration (X). (A) *Gyrodinium dominans* predator, (B) *Oxyrrhis marina*, (C) *Polykrikos kofoidii*, (D) *Strombidinopsis* sp. Symbols represent treatment means ± 1 SE. A Michaelis-Menten equation (Eq. 4, solid line) and an Ivlev equation (Eq. 5, dashed line) were used to produce the curves for (A), (B) and (D) for all treatments in the experiments. (A) $\text{IR} = 2.0 \cdot X / (727 + X)$, $r^2 = 0.905$ for Eq. (4), and $\text{IR} = 1.6 \cdot (1 - e^{-0.00144 X})$, $r^2 = 0.908$ for Eq. (5); (B) $\text{IR} = 0.51 \cdot X / (60.6 + X)$, $r^2 = 0.844$ for Eq. (4) and $\text{IR} = 0.46 \cdot (1 - e^{-0.0142 X})$, $r^2 = 0.857$ for Eq. (5); (D) $\text{IR} = 69.7 \cdot X / (1599 + X)$, $r^2 = 0.925$ for Eq. (4) and $\text{IR} = 51.0 \cdot (1 - e^{-0.00074 X})$, $r^2 = 0.924$ for Eq. (5). A logistic equation (Eq. 6, solid line) and an Ivlev equation (Eq. 5, dashed line) were used to produce the curves for (C) at prey concentrations of $< 3770 \text{ ng C predator}^{-1} \text{d}^{-1}$ (< 8560 cells $\text{predator}^{-1} \text{d}^{-1}$) for all treatments in the experiment. $\text{IR} = 11 \cdot \ln(X + 20610) - 109$, $r^2 = 0.770$ for Eq. (6) and $\text{IR} = 5.7 \cdot (1 - e^{-0.00013 X})$, $r^2 = 0.701$ for Eq. (5)

sanguineum), *Scrippsiella trochoidea*, *Cochlodinium polykrikoides* and *Prorocentrum minimum* (207 to 353 $\text{ng C ciliate}^{-1} \text{d}^{-1}$) (Fig. 7A). The μ_{max} of *Strombidinopsis* sp. feeding on *G. aureolum* (0.44 d^{-1}) was lower than for any other mixotrophic dinoflagellate (0.54 to 1.77 d^{-1}). The μ_{max} of *Strombidinopsis* sp. on the mixotrophic dinoflagellate was significantly and positively correlated with their I_{max} ($p < 0.05$; Fig. 7C).

Grazing impact

When the abundances of *Gymnodinium aureolum* and small heterotrophic *Gyrodinium* spp. (25 to 35 μm in cell length) in the coastal waters off Saemankeum, Korea, in 2006 to 2007 (sample size $n = 27$) were 13 to 4033 cells ml^{-1} and 2 to 78 cells ml^{-1} , respectively, grazing coefficients attributable to small heterotrophic *Gyrodinium* spp. on co-occurring *G. aureolum* were

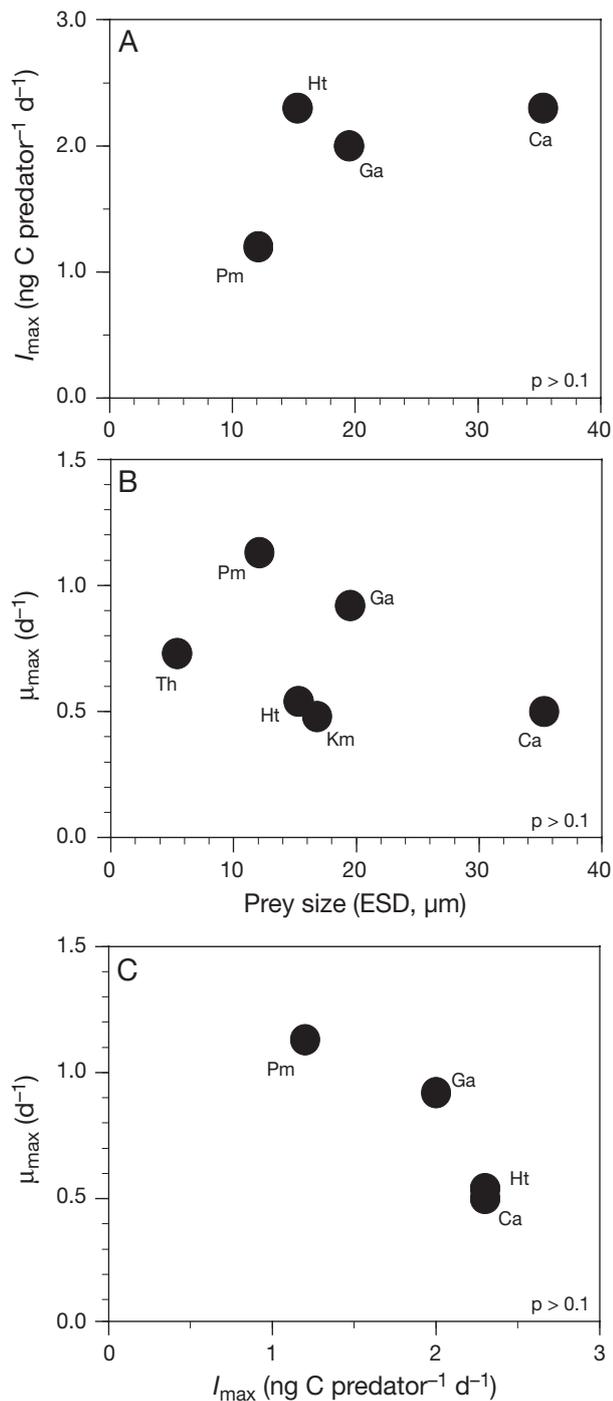


Fig. 4. *Gyrodinium dominans*. (A) Maximum ingestion (I_{\max}) and (B) growth rates (μ_{\max}) of *G. dominans* feeding on autotrophic/mixotrophic algal prey (●) as a function of prey size (equivalent spherical diameter [ESD], μm) and (C) μ_{\max} as a function of the I_{\max} . The p-values in (A), (B) and (C) were all $p > 0.1$ (linear regression ANOVA). Ca: *Chattonella antiqua*; Ga: *Gymnodinium aureolum*; Ht: *Heterocapsa triquetra*; Km: *Karenia mikimotoi*; Pm: *Prorocentrum minimum*; Th: *Thalassiosira* sp. I_{\max} and/or μ_{\max} on algal prey were obtained from Nakamura et al. (1992, 1995) and Kim & Jeong (2004)

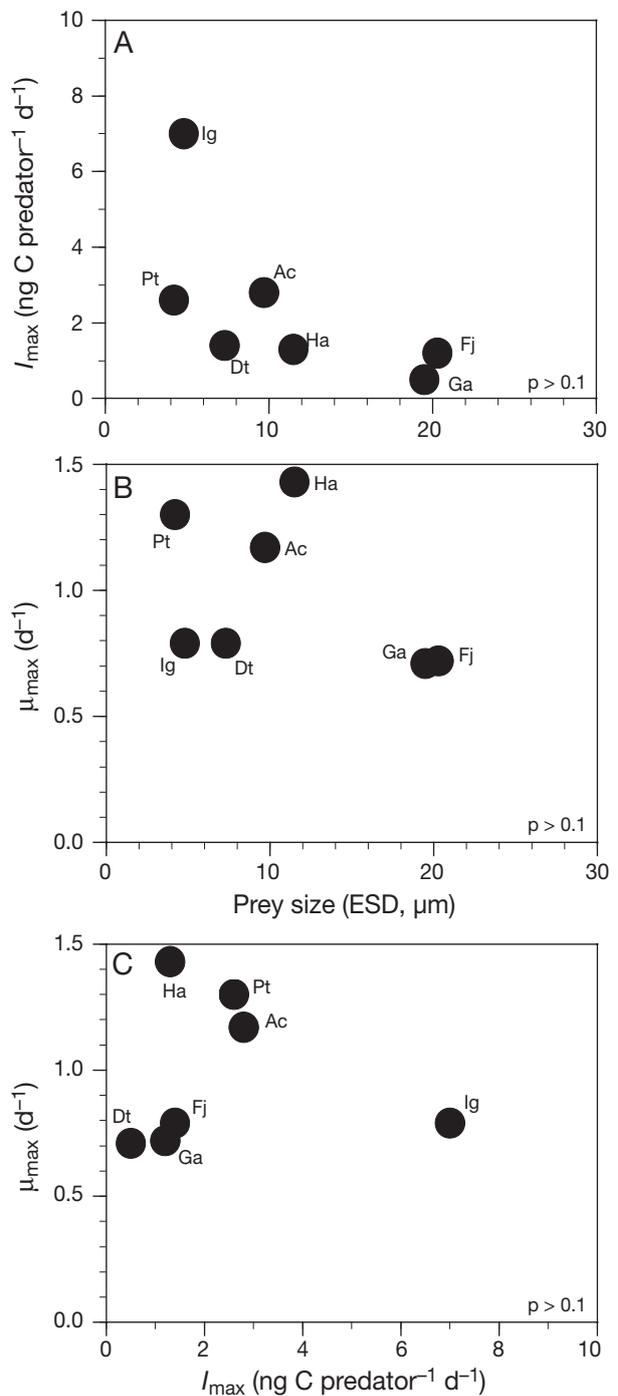


Fig. 5. *Oxyrrhis marina*. (A) Maximum ingestion (I_{\max}) and (B) growth rates (μ_{\max}) of *O. marina* feeding on autotrophic/mixotrophic algal prey (●) as a function of prey size (equivalent spherical diameter [ESD], μm) and (C) μ_{\max} as a function of I_{\max} . The p-values in (A), (B) and (C) were all $p > 0.1$ (linear regression ANOVA). Ac: *Amphidinium carterae*; Dt: *Dunaliella tertiolecta*; Fj: *Fibrocapsa japonica*; Ga: *Gymnodinium aureolum*; Ha: *Heterosigma akashiwo*; Ig: *Isochrysis galbana*; Pt: *Phaeodactylum tricorutum*. I_{\max} and/or μ_{\max} on algal prey were obtained from Goldman et al. (1989), Jeong et al. (2001a, 2003b), and Tillmann & Reckermann (2002)

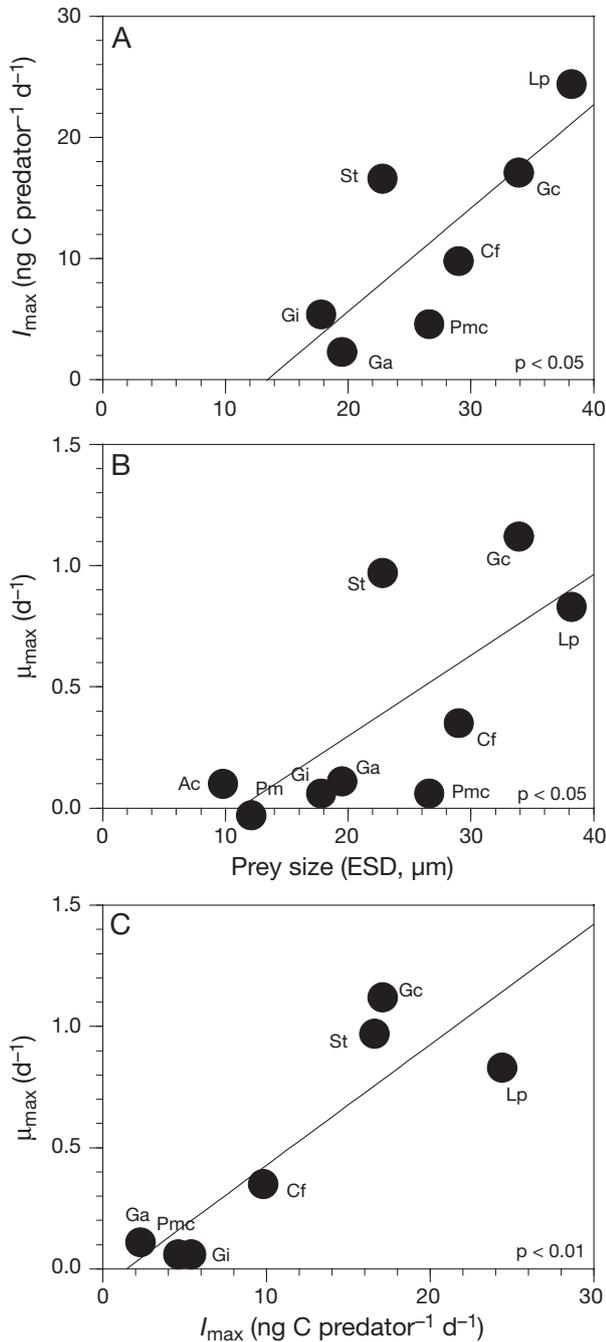


Fig. 6. *Polykrikos kofoidii*. Maximum (A) ingestion (I_{\max} , ng C predator⁻¹ d⁻¹) and (B) growth rates (μ_{\max} , d⁻¹) of *P. kofoidii* on autotrophic/mixotrophic algal prey (●) as a function of prey size (equivalent spherical diameter [ESD], μm) and (C) μ_{\max} as a function of I_{\max} . The p-values in (A) and (B) were $p < 0.05$, but the p-value in (C) was $p < 0.01$ (linear regression ANOVA). The equations of the linear regression were: (A) $I_{\max} = 0.853 \cdot \text{ESD} - 11.4$, $r^2 = 0.616$; (B) $\mu_{\max} = 0.0331 \cdot \text{ESD} - 0.375$, $r^2 = 0.494$; (C) $\mu_{\max} = 0.0497 \cdot I_{\max} - 0.0695$, $r^2 = 0.767$. Ac: *Amphidinium carterae*; Cf: *Ceratium furca*; Ga: *Gymnodinium aureolum*; Gc: *Gymnodinium catenatum*; Gi: *Gymnodinium impudicum*; Lp: *Lingulodinium polyedrum*; Pmc: *Prorocentrum micans*; Pm: *P. minimum*; St: *Scrippsiella trochoidea*. I_{\max} and/or μ_{\max} on algal prey were obtained from Jeong et al. (2001b)

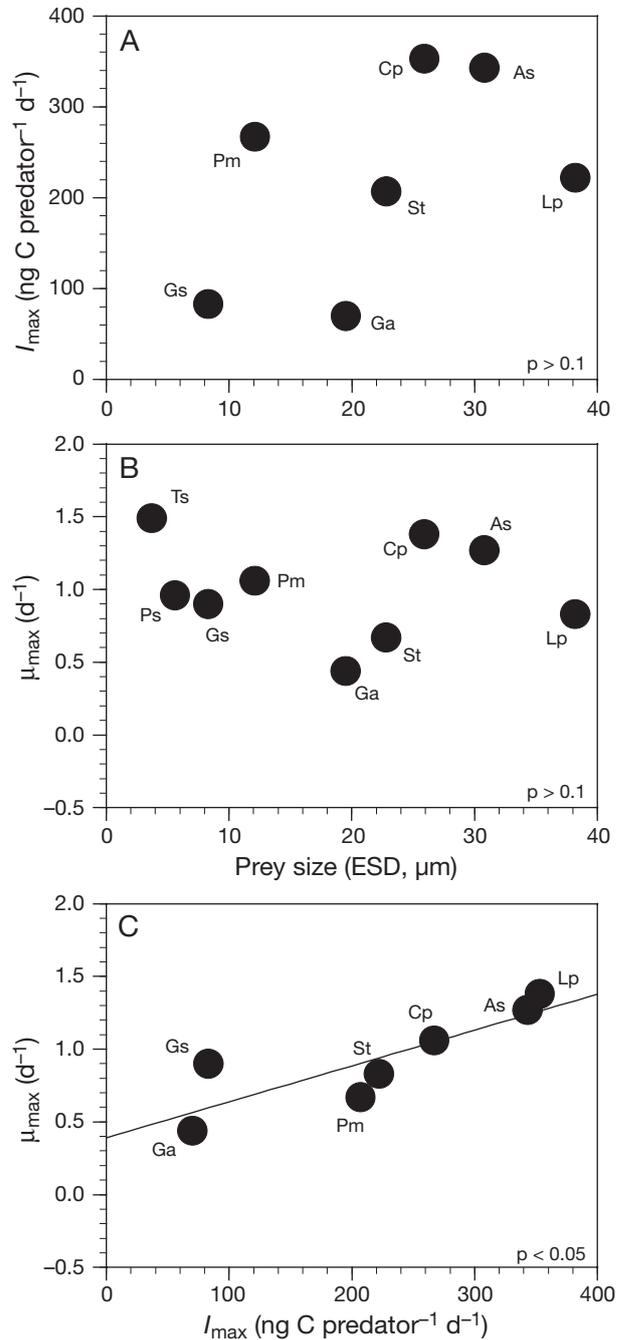


Fig. 7. *Strombidinopsis* sp. Maximum (A) ingestion (I_{\max} , ng C predator⁻¹ d⁻¹) and (B) growth rates (μ_{\max} , d⁻¹) of *Strombidinopsis* sp. on autotrophic/mixotrophic algal prey (●) as a function of prey size (equivalent spherical diameter [ESD], μm) and (C) μ_{\max} as a function of the I_{\max} . The p-values in (A) and (B) were $p > 0.1$, but the p-value in (C) was $p < 0.05$ (linear regression ANOVA). The equations of the linear regression for autotrophic/mixotrophic algal prey only was (C) $\mu_{\max} = 0.003 \cdot I_{\max} + 0.391$, $r^2 = 0.712$. As: *Akashiwo sanguinea*; Cp: *Cochlodinium polykrikoides*; Ga: *Gymnodinium aureolum*; Gs: *G. simplex*; Lp: *Lingulodinium polyedrum*; Pm: *Prorocentrum minimum*; Ps: *Prynomonas salina*; St: *Scrippsiella trochoidea*; Ts: *Thalassiosira pseudonana*. I_{\max} and/or μ_{\max} on algal prey were obtained from Buskey & Hyatt (1995), Montagnes et al. (1996), Jeong et al. (1999), and Montagnes & Lessard (1999)

0.002 to 0.40 d⁻¹ (Fig. 8A). In addition, when the abundances of *Gymnodinium aureolum* and large *Strombidinopsis* spp. (>70 µm in cell length) in the coastal waters off Saemankeum in 2006 to 2008 (sample size n = 20) were 10 to 4425 cells ml⁻¹ and 0.1 to 3.8 cells ml⁻¹, respectively, grazing coefficients attributable to large *Strombidinopsis* spp. on co-occurring *Gymnodinium aureolum* were 0.007 to 0.25 d⁻¹ (Fig. 8B).

DISCUSSION

Feeding occurrence

This study is the first report on feeding by heterotrophic dinoflagellates and ciliates on actual *Gymnodinium aureolum* since the confusion over taxonomy was settled (Daugbjerg et al. 2000, Hansen et al. 2000).

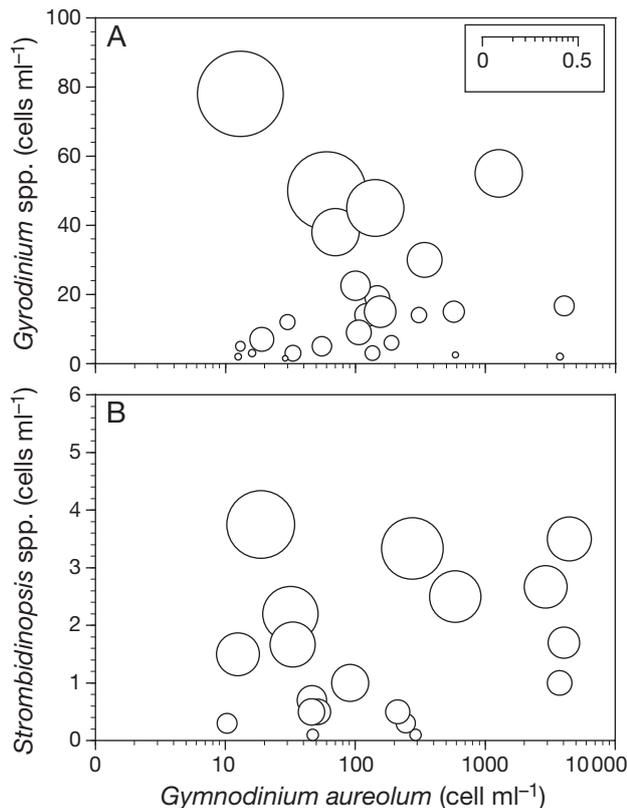


Fig. 8. *Gyrodinium* spp. and *Strombidinopsis* spp. Calculated grazing coefficients of (A) small heterotrophic *Gyrodinium* spp. (n = 27) and (B) *Strombidinopsis* spp. (n = 20) in relation to the concentration of co-occurring *Gymnodinium aureolum* see Eq. (7) for calculation). Clearance rates, measured under the conditions provided in the present study, were corrected with a $Q_{10} = 2.8$ (Hansen et al. 1997) because *in situ* water temperatures and the temperature used in the laboratory for this experiment (20°C) were sometimes different. Inset shows the scale of the circles that represent grazing coefficients (g, d^{-1})

Gyrodinium dominans, *Oxyrrhis marina*, *Polykrikos kofoidii* and *Strombidinopsis* spp. are known to feed on prey by engulfing them (Kim & Jeong 2004, Nakamura et al. 1995, Jeong et al. 2001a, 2003a, 2008a), while *Pfiesteria piscicida*, *Protoperidinium bipes* and *Stoeceria algicida* feed by means of the peduncle or pallium (Burkholder & Glasgow 2001, Jeong et al. 2004, 2005). Therefore, the heterotrophic protists that feed by engulfing their prey and were tested in the present study were able to feed on *G. aureolum*, whereas those that feed using their peduncle or pallium did not feed on *G. aureolum*. *P. piscicida*, *P. bipes*, and *S. algicida* did not deploy a tow filament to capture *G. aureolum* cells. *P. piscicida* is known to feed on the naked mixotrophic dinoflagellates *Cochlodinium polykrikoides*, *Akashiwo sanguinea* and *Gymnodinium catenatum*, which are larger than *G. aureolum* (Jeong et al. 2006). Furthermore, the mean swimming speeds of *C. polykrikoides* and *G. catenatum* (450 to 1060 µm s⁻¹) are greater than that of *G. aureolum* (390 µm s⁻¹), while the speed achieved by *A. sanguinea* (190 µm s⁻¹) is slower than that of *G. aureolum* (Jeong et al. 1999, 2010, authors' unpubl. data). Therefore, size and/or swimming speed of *G. aureolum* may not be primarily responsible for nonfeeding of *P. piscicida* on *G. aureolum*. On the other hand, *G. dominans*, *O. marina*, *P. kofoidii* and *Strombidinopsis* spp. are comparable in size or larger than *G. aureolum*, while *P. piscicida*, *P. bipes* and *S. algicida* are smaller. Therefore, the size of the predators may affect their ability to feed on *G. aureolum*. On the basis of the results of the present study, a high abundance of large heterotrophic dinoflagellates and/or ciliates that engulf their prey, but no or low abundance of heterotrophic dinoflagellates that feed using their small peduncle or pallium is expected to occur during red tides dominated by *G. aureolum*.

Oxyrrhis marina and *Polykrikos kofoidii* are known to be killed by the toxic mixotrophic dinoflagellate *Alexandrium tamarense* at concentrations of ca. 3000 to 4000 cells ml⁻¹ (Cho & Matsuoka 2000, Tillmann & John 2002). The present study showed that *O. marina* and *P. kofoidii* grew well while feeding on *Gymnodinium aureolum*. This suggests that *G. aureolum* is not toxic or has much weaker toxins than *A. tamarense*. Furthermore, the large ciliate *Strombidinopsis* sp. used in this study fed and grew well on *G. aureolum*, while *K. mikimotoi* (previously *Gyrodinium aureolum*) is known to be harmful to the large ciliate *Favella ehrenbergii* (Hansen 1995). This evidence confirmed that, unlike *K. mikimotoi*, *Gymnodinium aureolum* does not have toxic compounds strong enough to kill larval fish and/or brine shrimp as reported by Tang et al. (2008) and Jeong et al. (2010). There have been some studies reporting harmful effects by *K. mikimotoi* on gut tissues as determined by

histological examination of some juvenile bivalve mollusks, such as the bay scallop *Argopecten irradians* and the oyster *Crassostrea virginica* (Smolowitz & Shumway 1997). The copepods *Pseudocalanus elongatus* and *Temora longicornis* are known to select the nontoxic dinoflagellate *Gyrodinium instriatum* in preference to *K. mikimotoi* (Schultz & Kioerboe 2009). However, on the basis of the results of the present study, there is a high probability that *G. aureolum* does not have harmful effects on these larvae at the histological level and copepods may not select nontoxic dinoflagellate prey in preference to *G. aureolum*.

Growth and ingestion rates

The μ_{\max} and I_{\max} of *Gyrodinium dominans*, *Oxyrrhis marina* and *Strombidinopsis* sp. feeding on *Gymnodinium aureolum* obtained by means of Michaelis-Menten equations (Eqs. 2 & 4) were slightly higher than those obtained by means of Ivlev equations (Eqs. 3 & 5), even though r^2 -values of both equations for each data set were similar. There were 2 characteristic differences between these 2 equations. The rate of increase in the growth or ingestion rates at low and medium prey concentrations in the Ivlev equations were higher than those determined from Michaelis-Menten equations; however, μ_{\max} and I_{\max} obtained by means of Michaelis-Menten equations were higher than those obtained by means of Ivlev equation because the rates obtained from the Michaelis-Menten equations continuously and detectably increased, while those obtained from the Ivlev equations continuously, but undetectably, increased. Thus, an Ivlev equation may produce the best fit curve for both growth and ingestion rates of *G. dominans*, while a Michaelis-Menten equation may produce the best fit curve for growth and ingestion rates of *O. marina* and *Strombidinopsis* spp.

The μ_{\max} and I_{\max} of *Polykrikos kofoidii*, *Oxyrrhis marina*, and *Strombidinopsis* sp. when feeding on *Gymnodinium aureolum* obtained using Eqs. (2) & (4) were lower than that when they fed on any other algal prey (Figs. 4 to 7). The μ_{\max} and I_{\max} of *P. kofoidii* when feeding on the algal prey showed significant positive correlations with prey size. Thus, the almost smallest size of *G. aureolum* may be mainly responsible for these lower μ_{\max} and I_{\max} of *P. kofoidii* compared with the other prey. Interestingly, *G. aureolum* is almost the largest of the prey species on which *O. marina* is able to feed. *O. marina* may have difficulty in capturing and ingesting this large edible prey, and this may be mainly responsible for the lower μ_{\max} and I_{\max} of *G. aureolum*. The size of *G. aureolum* was middle among the sizes of the edible prey species for *Strombidinopsis*

sp. Large ciliates such as *Strombidinopsis* spp. and *Favella ehrenbergii* have been reported to be negatively affected by the presence of toxic algal prey; the growth rates of *Strombidinopsis* spp. and *F. ehrenbergii* decreased when the toxic dinoflagellates *Amphidinium carterae* and *Alexandrium tamarense*, respectively, were provided as prey (Hansen 1995, Jeong et al. 1999). Thus, *G. aureolum* may have weak toxins that negatively affect the growth and ingestion of *Strombidinopsis* spp., but do not negatively affect its survival. The size of *G. aureolum* was intermediate among the sizes of the edible prey species for *Gyrodinium dominans* as well. However, unlike *Strombidinopsis* sp., the μ_{\max} and I_{\max} of *G. dominans* feeding on *G. aureolum* were comparable with or higher than those when feeding on the algal prey. Several heterotrophic dinoflagellates have been known to detoxify phytotoxins (Jeong et al. 2001a, 2003a). *G. dominans* grew well on the toxic dinoflagellate *Karenia mikimotoi* (previously *Gymnodinium mikimotoi*) (0.48 d^{-1} when corrected to 20°C using a Q_{10} of 2.8, Hansen et al. 1997), even though the μ_{\max} of *G. dominans* on *K. mikimotoi* was half the μ_{\max} on *G. aureolum* (0.9 d^{-1}). Therefore, *G. aureolum* may affect the growth and ingestion of diverse predators in several different ways (e.g. lower and upper prey size limits, presence and toxicity of toxins).

The μ_{\max} of *Polykrikos kofoidii* and *Strombidinopsis* sp. on the mixotrophic dinoflagellates showed significant positive correlations with I_{\max} , while that of *Gyrodinium dominans* and *Oxyrrhis marina* did not. The I_{\max} of *P. kofoidii* and *Strombidinopsis* spp. on the mixotrophic dinoflagellates were 2.3 to 24.4 ng C predator⁻¹ d⁻¹ and 70 to 353 ng C predator⁻¹ d⁻¹, respectively, while that of *G. dominans* and *O. marina* were 1.2 to 2.3 ng C predator⁻¹ d⁻¹ and 0.3 to 7.0 ng C predator⁻¹ d⁻¹, respectively. This evidence suggests that *P. kofoidii* and *Strombidinopsis* sp. may not have difficulty in capturing and ingesting these algal preys and are able to convert ingested carbon to body carbon at similar rates, regardless of the prey species (i.e. they may effectively digest any prey). In contrast, *G. dominans* and *O. marina* may have difficulty in capturing and ingesting these algal prey, in particular large prey species (i.e. differential energy spent for feeding) and/or may convert ingested carbon to body carbon at different rates for different prey species (i.e. small predators may digest some prey easily and others with more difficulty).

Grazing impact

Grazing coefficients attributable to small heterotrophic *Gyrodinium* spp. (25 to 35 μm in cell length) on

co-occurring *Gymnodinium aureolum* in the coastal waters off Saemankeum in 2006 to 2007 ranged between 0.002 and 0.40 d⁻¹ (i.e. up to 33% of *G. aureolum* populations could be removed by small *Gyrodinium* populations in 1 d). Grazing coefficients attributable to *Gyrodinium dominans* were high (2 to 78 cells ml⁻¹) in a wide range of *G. aureolum* abundances (13 to 4033 cells ml⁻¹). We assumed that the ingestion rates of the other small heterotrophic *Gyrodinium* spp. feeding on *G. aureolum* are the same as that of *G. dominans*. *Gyrodinium guttula* co-occurs in the sampling waters, but it was not distinguishable from *G. dominans* in fixed samples under light microscope. We did not measure the growth and ingestion rates of *G. guttula* feeding on *G. aureolum* because we do not have a culture of this species. Thus, our estimation may be under- or overestimated if the growth and ingestion rates of *G. guttula* feeding on *G. aureolum* are significantly different from those of *G. dominans* and the abundance of *G. guttula* is not negligible.

In addition, grazing coefficients attributable to *Strombidinopsis* spp. (>70 µm in cell length) on co-occurring *Gymnodinium aureolum* in the coastal waters off Saemankeum in 2006 to 2008 were 0.007 to 0.25 d⁻¹ (i.e. up to 22% of *G. aureolum* populations could be removed by large *Strombidinopsis* populations in 1 d). Grazing coefficients attributable to *Strombidinopsis* spp. were also high (0.1 to 3.8 cells ml⁻¹) in a wide range of *G. aureolum* abundances (20 to 4030 cells ml⁻¹). Therefore, the populations of *Strombidinopsis* spp. or *Gyrodinium dominans* may have a considerable grazing impact on *G. aureolum* not only during the development or declining stages of red tides dominated by *G. aureolum*, but also at the fully developed stage of these red tides.

Studies on the formation, persistence and decline of red tide suggest that grazing pressure may sometimes play an important role in bloom dynamics (Watras et al. 1985, Turner et al. 1998, Turner 2006). In particular, grazing by heterotrophic protists is believed to contribute to the decline of red tides or cause changes in the abundance of the causative species (Jeong 1995, Kamiyama & Matsuyama 2005, Turner 2006, Buskey 2008, Stoecker et al. 2008). This study is an important example showing that grazing by heterotrophic dinoflagellates and ciliates may contribute to the decline of red tides.

Some studies have suggested a biological method for controlling red tides using mass cultured grazers (Jeong et al. 2003b, 2008a). *Strombidinopsis* spp. or *Gyrodinium dominans* are strong candidates for developing a biological method for controlling *Gymnodinium aureolum* red tides by increasing the abundance of these predators and introducing them to aqua cages in natural environments or aqua tanks on land.

Acknowledgements. We thank Dr. K. A. Seong for technical support and E. Potvin for improving the English of the manuscript. This paper was funded by grants from the National Research Foundation (2009-0058298) and an award to H.J.J. from the Ecological Disturbance Research Program, Korea Institute of Marine Science and Technology Promotion, Ministry of Land, Transportation and Marine Affairs (KMLTM), and an award to J.S.K. from the Korean Research Foundation (KRF-2007-359-C00042).

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*Editorial responsibility: Robert Sanders,
Philadelphia, Pennsylvania, USA*

*Submitted: August 17, 2009; Accepted: January 13, 2010
Proofs received from author(s): April 12, 2010*