

# Feeding by the Heterotrophic Dinoflagellate *Oxyrrhis marina* on the Red-Tide Raphidophyte *Heterosigma akashiwo*: a Potential Biological Method to Control Red Tides Using Mass-Cultured Grazers

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**ABSTRACT.** As part of the development of a method to control the outbreak and persistence of red tides using mass-cultured heterotrophic protist grazers, we measured the growth and ingestion rates of cultured *Oxyrrhis marina* (a heterotrophic dinoflagellate) on cultured *Heterosigma akashiwo* (a raphidophyte) in bottles in the laboratory and in mesocosms (ca. 60 liter) in nature, and those of the cultured grazer on natural populations of the red-tide organism in mesocosms set up in nature. In the bottle incubation, specific growth rates of *O. marina* increased rapidly with increasing concentration of cultured prey up to ca. 950 ng C ml<sup>-1</sup> (equivalent to 9,500 cells ml<sup>-1</sup>), but were saturated at higher concentrations. Maximum specific growth rate ( $\mu_{max}$ ),  $K_{GR}$  (prey concentration sustaining 0.5  $\mu_{max}$ ) and threshold prey concentration of *O. marina* on *H. akashiwo* were 1.43 d<sup>-1</sup>, 104 ng C ml<sup>-1</sup>, and 8.0 ng C ml<sup>-1</sup>, respectively. Maximum ingestion and clearance rates of *O. marina* were 1.27 ng C grazer<sup>-1</sup> d<sup>-1</sup> and 0.3  $\mu$ l grazer<sup>-1</sup> h<sup>-1</sup>, respectively. Cultured *O. marina* grew well effectively reducing cultured and natural populations of *H. akashiwo* down to a very low concentration within 3 d in the mesocosms. The growth and ingestion rates of cultured *O. marina* on natural populations of *H. akashiwo* in the mesocosms were 39% and 40%, respectively, of those calculated based on the results from the bottle incubation in the laboratory, while growth and ingestion rates of cultured *O. marina* on cultured *H. akashiwo* in the mesocosms were 55% and 36%, respectively. Calculated grazing impact by *O. marina* on natural populations of *H. akashiwo* suggests that *O. marina* cultured on a large scale could be used for controlling red tides by *H. akashiwo* near aquaculture farms that are located in small ponds, lagoons, semi-enclosed bays, and large land-aqua tanks to which fresh seawater should be frequently supplied.

**Key Words.** Biological control, growth, grazing, ingestion, harmful algal bloom, protist.

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 often caused great loss to the aquaculture and tourist industries in many countries. For example, a huge red tide dominated by *Cochlodinium polykrikoides* and *Gymnodinium impudicum* caused a loss of US \$60 million in Korean aquaculture industries in the fall of 1995 (NFRDA 1998). Developing methods of controlling the outbreak and persistence of red tides is a primary concern to the aquaculture and tourist industries and scientists in related fields.

Until now, several methods have been used or suggested for controlling the outbreak and persistence of red tides. Examples include the use of clay (NFRDA 1998), NaOCl produced by the electrolysis of natural seawater (Jeong et al. 2002b), a centrifugal device (KORDI 1998), protistan grazers (Jeong 2001), bacteria (Doucette, McGovern, and Babinchak 1999; Imai, Ishida, and Hata 1993), viruses (Nagasaki, Tarutani, and Yamaguchi 1999; Tarutani, Nagasaki, and Yamaguchi 2000), and parasitic dinoflagellates (Coats 1999; Taylor 1968). The clay dispersal method has been intensively used for controlling the persistence of red tides in Korea since 1996. This method helps in drastically reducing the losses caused by red tide dominated by *C. polykrikoides* and/or *G. impudicum*. However, the clay dispersal method may have some difficulties: (1) getting enough clay every year is difficult because of limited resources; (2) secondary effects on benthic communities have not been extensively tested; and (3) to load, carry, and spray clay, a lot of labor, time, and ships are necessary. Therefore, alternative effective methods should be developed.

High grazing pressure by zooplankton is known to sometimes prevent natural populations of red-tide organisms forming red-tide patches (e.g. Watras et al. 1985). Grazing by heterotrophic

protists is also believed to contribute to the decline of red tides (Eppley and Harrison 1975; Holmes, Williams, and Eppley 1967; Jeong 1995; Matsuyama, Miyamoto, and Kotani 1999). Heterotrophic protistan grazers cultured on a large scale may reduce the natural populations of red-tide organisms down to a very low concentration after being introduced into water parcels containing the red-tide organisms. This Biological method of Controlling the outbreak and persistence of Red tides using mass-cultured heterotrophic protistan Grazers (BCRG method), if developed, may have significant merits: (1) Heterotrophic protistan grazers are originally isolated from natural seawater, and are easily eaten by metazooplankton after ingesting red-tide organisms (Stoecker and Egloff 1987; Stoecker and Sanders 1985). Therefore, introduction of the grazer is much safer than that of clay materials, NaOCl, or other chemicals. (2) Heterotrophic protist grazers grow fast (i.e. 2 divisions per day) by ingesting red-tide organisms (Hansen 1992; Jeong and Latz 1994; Jeong et al. 1999a, b, 2001a, b; Stoecker, Guillard, and Kavee 1981). Therefore, the grazers can rapidly increase their populations in a short period by ingesting red-tide organisms after being introduced into red-tide waters. This will enable the grazers to dissipate large-scale red-tide patches even when a small number of grazers are initially introduced.

The raphidophyte *Heterosigma akashiwo* can cause large-scale fish mortality rates when forming red tides (Chang, Pridmore, and Boustead 1993; Honjo 1993; Imai et al. 1996; MacKenzie 1991). Its density sometimes exceeds 100,000 cells ml<sup>-1</sup> during red tides (Nagasaki et al. 1996; Jeong et al., unpubl. data). Clogging gills of finfishes may be mainly responsible for the mortality rates because there have been no reports on its toxin. Only a few heterotrophic protists are known to feed on *H. akashiwo*. For example, the heterotrophic dinoflagellate *Gyrodinium dominans* and the prostomatid ciliate *Tiarina fusus* can grow on *H. akashiwo* (Jeong et al. 2002a; Nakamura, Suzuki, and Hiromi 1995). However, the large tintinnid ciliate *Favella* spp. did not ingest this prey (Taniguchi and Takeda 1988) or the ingestion rate was undetectable even though this

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prey was ingested in the initial incubation (Kamiyama and Arima 2001). We found that the heterotrophic dinoflagellate *Oxyrrhis marina* was the most dominant heterotrophic protist (concentration = 138–209 *Oxyrrhis ml*<sup>-1</sup>) in red-tide patches dominated by *H. akashiwo* (concentration = 9,800–15,000 *Heterosigma ml*<sup>-1</sup>) in Kunsan Port in May, 2000 and the grazer was able to grow well on the red-tide prey (Jeong et al. unpubl. data).

As part of the development of the BCRG method, we measured the growth and grazing rates of cultured *O. marina* on *H. akashiwo* in bottles in the laboratory and in mesocosms in nature, and those of the cultured grazer on natural populations of the red-tide organism in mesocosms set up in nature. In addition, we compared planktonic assemblages in the experimental (added *O. marina* + natural red-tide water) and control (natural red-tide water) mesocosms to investigate whether introduction of cultured grazers would alter the natural populations of other protists. The results of the present study provide a basis for potential use of heterotrophic protist grazers cultured in large scale to control populations of red-tide organisms in natural environments or large land-aqua tanks along the coastal areas.

#### MATERIALS AND METHODS

**Preparation of experimental organisms.** *Heterosigma akashiwo* (HAKS01) was grown at 20 °C in enriched *f/2* seawater media (Guillard and Ryther 1962; salinity = 29.5–31.0 psu) without silicate and with continuous illumination of 50  $\mu\text{E m}^{-2}\text{s}^{-1}$  provided by cool-white fluorescent lights. The carbon content for *H. akashiwo* (0.1 ng C per cell) was estimated from the cell vol. according to Strathmann (1967).

For the isolation and culture of *Oxyrrhis marina*, plankton samples collected with a 25-cm diam., 25- $\mu\text{m}$  mesh plankton net were taken from the mouth of Keum Estuary, Kunsan, Korea, during May, 2001, when the water temperature was 16 °C. The samples were screened gently through 154- $\mu\text{m}$  Nitex mesh and placed in 1-L polycarbonate (PC) bottles. *Amphidinium carterae* (ca. 8,000 cells  $\text{ml}^{-1}$ ) and 50 ml of *f/2* media were added as food. Bottles were placed on plankton wheels rotating at 0.9 rpm and incubated at 20 °C under continuous illumination of 20  $\mu\text{E m}^{-2}\text{s}^{-1}$  of cool-white fluorescent light. Two 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 *O. marina* were obtained, they were transferred to 500- or 1,000-ml PC bottles of fresh *H. akashiwo* prey (ca. 15,000 cells  $\text{ml}^{-1}$ ) every two or three days. Experiments for the bottle incubation were conducted when 2 L of *O. marina* culture was available. A total of 200 L of *O. marina* (concentration = ca. 8,000 cells  $\text{ml}^{-1}$ ) were used for mesocosm experiments by using an automatic system of growing daily 300 L of *O. marina* (concentration = ca. 8,000–10,000 cells  $\text{ml}^{-1}$ ). The automatic system of growing the predator consists of two major parts (Fig. 1): one part (Part A) grows phytoplankton as prey and the other part (Part B) grows the predator. Part A consists of 4 different-sized transparent chambers (4, 20, 80, 320 L). Each smaller chamber is located at a higher level than the next largest chamber. Adjacent chambers are connected with a flexible pipe in which a solenoid valve (M-06-2PV-5 CKD Co., Japan) controls flow. Each chamber contains cultures of  $\frac{1}{4}$  of the chamber volume at  $t = 0$  d. Cells in each chamber grow rapidly under a continuous illumination of 100–150  $\mu\text{E m}^{-2}\text{s}^{-1}$  provided by cool-white fluorescent lights. At  $t = 1$  d, *f/2* seawater media of  $\frac{1}{4}$  of each chamber vol. is supplied by a pump to the original culture containing increased prey cells. One-quarter of the total vol. of the culture in the chamber is supplied to a chamber containing

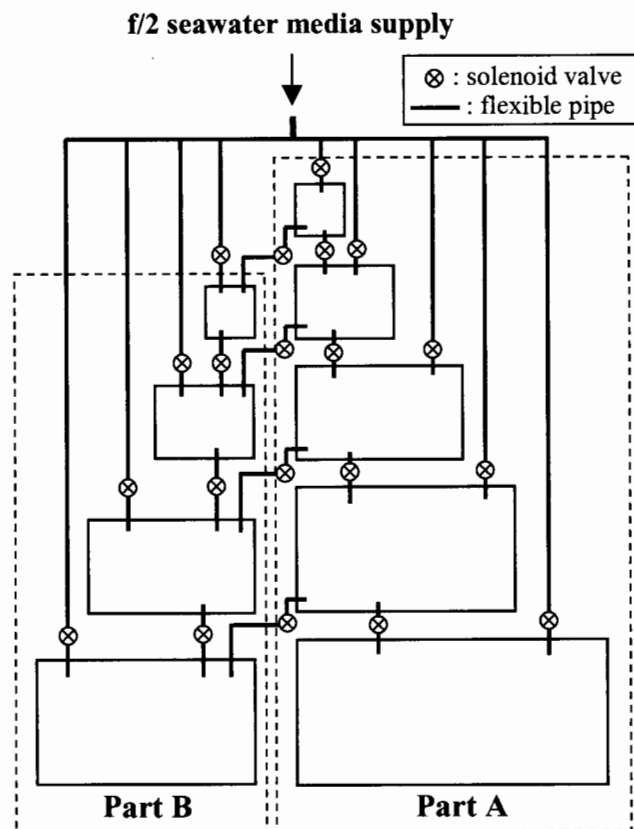


Fig. 1. Diagram of an automatic system of growing daily 300 L of *Oxyrrhis marina* (see text).

predators in Part B, another two-quarters to the next largest chamber in Part A, and the other one-quarter remains in the original chamber. A chamber containing prey in Part A is connected with a pipe to the same-sized chamber at a lower level and containing the predator in Part B. Therefore, by gravity, a culture of prey enters the chamber containing the predator if a solenoid valve is open. The timing and duration of opening and closing the valves are controlled by a computer system (MarineBio Co., Kunsan, Korea).

**Growth and ingestion rates of *Oxyrrhis marina* on cultured *Heterosigma akashiwo* in bottles.** Experiment 1 was designed to measure the growth, ingestion, and clearance rates of *O. marina* on *H. akashiwo* as a function of the prey concentration.

Two days before these experiments were conducted, dense cultures of *O. marina* growing on *H. akashiwo* were transferred into 1-L PC bottles containing low concentrations of the prey (ca. 50 cells  $\text{ml}^{-1}$ ). This was done to minimize possible residual growth resulting from the ingestion of prey during batch culture. The bottles were filled to capacity with filtered seawater and placed on rotating wheels to incubate as above, except that illumination was provided on a 14 h:10 h light-dark cycle. To monitor the condition of, and interaction between, predator and prey species, cultures were periodically removed from the rotating wheels, examined by looking through the surface of capped bottles using a dissecting microscope, and then returned to the rotating wheels. Once *H. akashiwo* cells were no longer detectable, three 1-ml aliquots from each bottle were counted using a compound microscope to determine cell concentrations of *O. marina*, and the cultures were then used to conduct experiments.

For each experiment, initial concentrations of *O. marina* and *H. akashiwo* were established using an autopipette to deliver predetermined volumes of known cell concentrations to the bottles. Triplicate 270-ml PC experimental bottles (mixtures of predator and prey) and triplicate control bottles (prey only) were set up at each predator-prey combination. Triplicate control bottles containing only *O. marina* were also established at one predator concentration. Thirty milliliters of f/2 medium were added to all bottles, which were then filled to capacity with seawater filtered by 0.2- $\mu\text{m}$  CP filter (Chisso Filter Co. Ltd., Osaka, Japan) and capped. To determine actual initial predator and prey densities (cells  $\text{ml}^{-1}$ ) at the beginning of the experiment (*O. marina*/*H. akashiwo* = 3/95, 9/266, 12/1086, 22/2654, 36/8102, 84/16049, 92/31574, 69/0), a 10-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 enumerating cells in three 1-ml Sedgwick-Rafter counting chambers (SRCs). Cells in five more 1-ml SRCs were additionally counted at the 4 low predator concentrations. The bottles were filled again to capacity with freshly filtered seawater, capped, and placed on rotating wheels under the environmental conditions described above. Dilution of the cultures associated with refilling the bottles was considered in calculating growth and ingestion rates.

Ten-ml aliquots were taken from each bottle at 24, 48, and 72 h and fixed with 5% Lugol's solution and the abundances of *O. marina* and *H. akashiwo* were determined by counting all or > 300 cells in three 1-ml SRCs. Cells in five more 1-ml SRCs were additionally counted at the 4 low predator concentrations. Prior to taking subsamples, the condition of *O. marina* and its prey were assessed using a dissecting microscope as described above. After subsampling, bottles were again filled to capacity with freshly filtered seawater and placed back on the rotating wheels.

The specific growth rate  $\mu$  ( $\text{d}^{-1}$ ) of *O. marina* was calculated by averaging the instantaneous growth rates (IGR) for each sampling interval, calculated as:

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

where  $S_{t_1}$  and  $S_{t_2}$  = the concentration of *O. marina* at consecutive samplings. The final  $t_2$  for calculation was 48 h because after 72 h prey concentrations had been already largely reduced. Mean prey concentrations (cells  $\text{ml}^{-1}$ ) for 48 h were calculated by averaging the instantaneous mean prey concentration at 0–24 h and 24–48 h. An instantaneous mean prey concentration for each sampling interval was calculated using the equations of Frost (1972).

Data for *O. marina* growth rates were fitted to a Michaelis-Menten equation:

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

where  $\mu_{\text{max}}$  = the maximum growth rate ( $\text{d}^{-1}$ ),  $x$  = prey concentration (cells  $\text{ml}^{-1}$  or ng C  $\text{ml}^{-1}$ ),  $x'$  = threshold prey concentration (the prey concentration where  $\mu = 0$ ), and  $K_{\text{GR}}$  = the prey concentration sustaining  $\frac{1}{2} \mu_{\text{max}}$ . Data were iteratively fitted into the model using DeltaGraph<sup>®</sup> (SPSS Inc., Chicago, IL, USA).

Ingestion and clearance rates were calculated using the equations of Frost (1972) and Heinbokel (1978). The incubation time for calculating ingestion and clearance rates was the same as for estimating growth rate. Ingestion rate data were fitted to a Michaelis-Menten equation:

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

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

**Growth and ingestion rates of *Oxyrrhis marina* on cultured *Heterosigma akashiwo* in mesocosms.** Experiment 2 was designed to measure the growth and ingestion rates of cultured *O. marina* on cultured *H. akashiwo* in mesocosms set up in Masan Bay, southeastern Korea, on June 6, 2002 when a huge red tide dominated by *H. akashiwo* occurred.

The mesocosms used in these experiments were 50 cm in diam. and 50 cm in height. They were surrounded by waterproof cloth on the top and bottom and 5- $\mu\text{m}$  Nitex mesh in the middle. A circle of 40-cm in diam. at the top of the mesocosms was open. In the laboratory, seawater filtered by 0.2- $\mu\text{m}$  CP filter (Chisso Filter Co. Ltd., Osaka, Japan) penetrated the mesh at a speed of ca. 6 L  $\text{min}^{-1}$ , but *O. marina* and *H. akashiwo* did not pass through the mesh. Each 60-L mesocosm was supported by a stainless frame (ca. 8 kg in weight) and kept afloat by 4 buoys of 30 cm in diam. and 40 cm in length. Four mesocosms (MC1, MC2, MC3, MC4; see followings for details) were established simultaneously at the same location.

For Expt. 2, two mesocosms were filled by ca. 20 L of natural water through the mesh for approximately 5 min. A mixture of cultured predator (density = ca. 6,000 cells  $\text{ml}^{-1}$ , 25 L) and prey (density = ca. 45,000–50,000 cells  $\text{ml}^{-1}$ , 30 L) was added into one experiment mesocosm (MC1) and only cultured prey (density = ca. 45,000–50,000 cells  $\text{ml}^{-1}$ , 30 L) was added to one control mesocosm (MC2). This experiment started 2 h later after the predator and prey had been added into the mesocosm ( $t = 0$  h) when the apparently stable conditions (water volume, abundance of plankton) inside the mesocosms were established.

Five-hundred-ml aliquots were taken from each mesocosm after the first 4 h and then approximately every 6–8 h for 73 h after the contents inside the mesocosms were gently but well mixed by a wide rod. Three 100-ml aliquots were fixed with 5% Lugol's solution, 5% Bouin's solution, and 4% glutaldehyde. The abundances of *H. akashiwo* and *O. marina* in aliquots fixed with Lugol's solution were determined by counting > 300 cells in three 1-ml SRCs. Dilution of the abundances associated with water penetrating into a mesocosm through the mesh due to the balance of water levels was considered in calculating growth and ingestion rates. Water temperature and salinity were also measured simultaneously using YSI 600 XLM (YSI Inc., Yellow Springs, Ohio, USA).

Net growth and ingestion rates of *O. marina* on *H. akashiwo* for the first 50 h for which continuous positive growth of the predator occurred were calculated by averaging the instantaneous net growth and ingestion rates for each sampling interval as described above. However, due to different sampling intervals (i.e. one 4 h and one 6 h, and the other 8 h), mean prey concentration (MPC, cells  $\text{ml}^{-1}$ ) for the first 50 h was calculated as:

$$\text{MPC} = \sum \text{IMPC} \times (t_2 - t_1) / T \quad (4)$$

where IMPC = instantaneous mean prey concentration for each sampling interval ( $t_2 - t_1$ );  $T = 50$  h.

**Growth and ingestion rates of *Oxyrrhis marina* on natural populations of *Heterosigma akashiwo* in mesocosms.** Experiment 3 was designed to measure the growth and ingestion rates of cultured *O. marina* on the natural populations of *H. akashiwo* in mesocosms set up at the same location and time as in Expt. 2.

For the experiment, red-tide waters containing mostly *H. akashiwo* (ca. 30,000 cells  $\text{ml}^{-1}$ , > 90% of phytoplankton as-

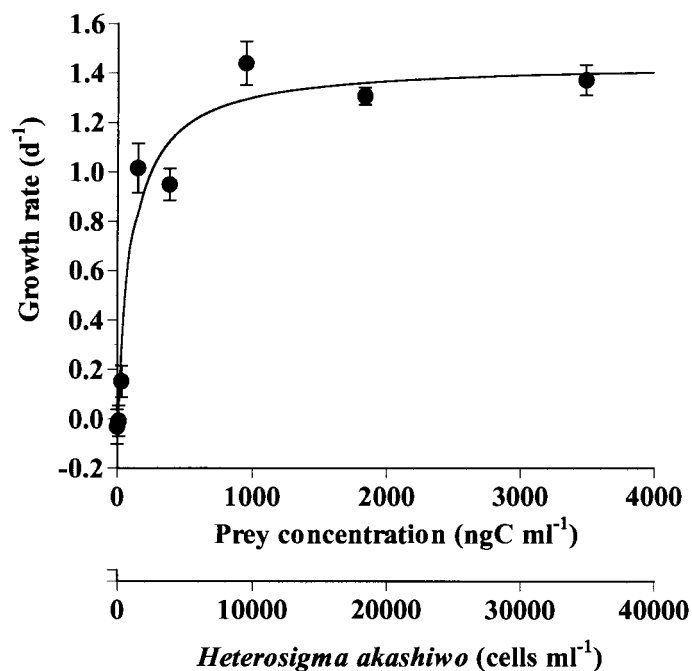


Fig. 2. Numerical response of *Oxyrrhis marina* on *Heterosigma akashiwo* as a function of mean prey concentration ( $x$ ). Symbols represent treatment means  $\pm$  Standard Error. The curves were fitted by a Michaelis-Menten equation [Eq. (2)] using all treatments in the experiment. Growth rate ( $\text{GR}, \text{d}^{-1}$ ) =  $1.43 [(x - 8.0)/(104 + (x - 8.0))]$ ,  $r^2 = 0.93$ .

semblage) were poured into two mesocosms: one experimental mesocosm [MC3; mixtures of added *O. marina* (density = ca. 7,000 cells  $\text{ml}^{-1}$ , 25 L) and natural plankton assemblage] and one control mesocosm (MC4; natural plankton assemblage only) were set up adjacent to MC1 and MC2 in Expt 2.

Five-hundred-ml aliquots were taken from each mesocosm and then fixed as in Expt. 2. The abundances of *H. akashiwo* and *O. marina* were determined as in Expt. 2. Co-occurring diatoms, autotrophic dinoflagellates, and heterotrophic dinoflagellates in the aliquots fixed with Lugol's solution and glutaldehyde were also counted under a compound microscope and an epifluorescent microscope. Naked ciliates and tintinnids in the aliquots fixed with Bouin's solution were enumerated using a quantitative protargol stain method (Montagnes and Lynn 1993). When the mesocosm experiments were terminated, all the contents of the mesocosms were filtered through a 100- $\mu\text{m}$  mesh net and any retained organisms were fixed with final concentration 4% formalin. Metazooplankton were enumerated under a dissecting microscope. Water temperature and salinity were also measured as in Expt. 2.

Net growth and ingestion rates of *O. marina* on *H. akashiwo* for the first 50 h were calculated using the abundances of the predator and prey as in Expt 2.

## RESULTS

**Growth and ingestion rates of *Oxyrrhis marina* on cultured *Heterosigma akashiwo* in bottle incubation (Expt. 1).** *O. marina* grew well on unialgal diets of *H. akashiwo*. The specific growth rates increased with increasing mean prey concentration up to ca. 950  $\text{ng C ml}^{-1}$  (9,500 cells  $\text{ml}^{-1}$ ), but were saturated at higher prey concentrations (Fig. 2). When the data were fitted to Eq. (2), the maximum specific growth rates ( $\mu_{\text{max}}$ ) and  $K_{\text{GR}}$  (prey concentration sustaining 0.5  $\mu_{\text{max}}$ ) were 1.43  $\text{d}^{-1}$

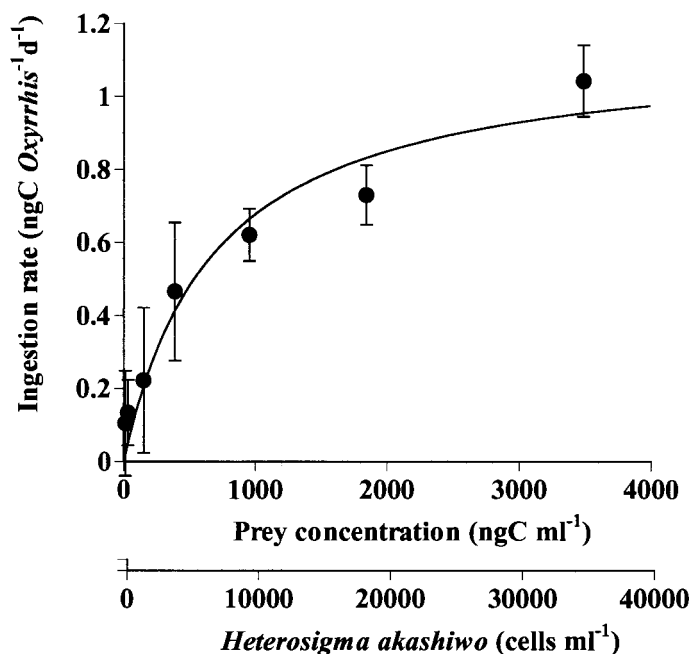


Fig. 3. Functional response of *Oxyrrhis marina* on *Heterosigma akashiwo* as a function of mean prey concentration ( $x$ ). Symbols represent treatment means  $\pm$  Standard Error. The curves are fitted by a Michaelis-Menten equation [Eq. (3)] using all treatments. Ingestion rate ( $\text{IR}, \text{ng C predator}^{-1} \text{d}^{-1}$ ) =  $1.25 [x/(704 + x)]$ ,  $r^2 = 0.77$ .

and 104  $\text{ng C ml}^{-1}$  (1,040 cells  $\text{ml}^{-1}$ ), respectively. The threshold prey concentration (where net growth = 0) was 8.0  $\text{ng C ml}^{-1}$  (80 cells  $\text{ml}^{-1}$ ).

The ingestion rate of *O. marina* on unialgal diets of *H. akashiwo* increased rapidly with increasing mean prey concentration up to ca. 1,800  $\text{ng C ml}^{-1}$  (18,000 cells  $\text{ml}^{-1}$ ), and slowly, but continuously, increased at higher prey concentrations (Fig. 3). When the data were fitted to Eq. (3), the maximum ingestion rate and  $K_{\text{IR}}$  (prey concentration sustaining 0.5  $\mu_{\text{max}}$ ) of *O. marina* on *H. akashiwo* were 1.25  $\text{ng C grazer}^{-1} \text{d}^{-1}$  (12.5 cells  $\text{grazer}^{-1} \text{d}^{-1}$ ) and 704  $\text{ng C ml}^{-1}$  (7,040 cells  $\text{ml}^{-1}$ ), respectively. The maximum clearance rate of *O. marina* was 0.3  $\mu\text{l grazer}^{-1} \text{h}^{-1}$  at the mean prey concentration of 11  $\text{ng C ml}^{-1}$ .

**Growth and ingestion rates of *Oxyrrhis marina* on cultured *Heterosigma akashiwo* in mesocosms (Expt. 2).** With increasing elapsed incubation time, the concentrations of *H. akashiwo* in the experimental mesocosm (MC1) in Expt. 2, 13,800 – 17,600 cells  $\text{ml}^{-1}$ , did not markedly change over the first 18 h, but continuously decreased between 18 and 58 h and down to 10 cells  $\text{ml}^{-1}$  at 73 h (Fig. 4A). With increasing elapsed incubation time, the concentration of *H. akashiwo* in control mesocosm (MC2) increased from 19,000 to 50,400 cells  $\text{ml}^{-1}$  with a depression between 50 and 66 h. The concentration of *O. marina* in MC1 increased from 2,100 to 9,025 cells  $\text{ml}^{-1}$  between 0 and 50 h, but decreased to 4,150 cells  $\text{ml}^{-1}$  by 73 h, probably due to food limitation (Fig. 4B).

Net growth and ingestion rates of *O. marina* on *H. akashiwo*, calculated by using the equations (1)–(4) for the first 50 h, were 0.724  $\text{d}^{-1}$  and 0.28  $\text{ng C grazer}^{-1} \text{d}^{-1}$  (2.8 cells  $\text{grazer}^{-1} \text{d}^{-1}$ ), respectively. The mean prey concentration at this interval was 1,259  $\text{ng C ml}^{-1}$  (12,590 cells  $\text{ml}^{-1}$ ).

**Growth and ingestion rates of *Oxyrrhis marina* on natural populations of *Heterosigma akashiwo* in mesocosms (Expt. 3).** With increasing elapsed incubation time, the concentration of *H. akashiwo* in the experimental mesocosm (MC3) in Expt.

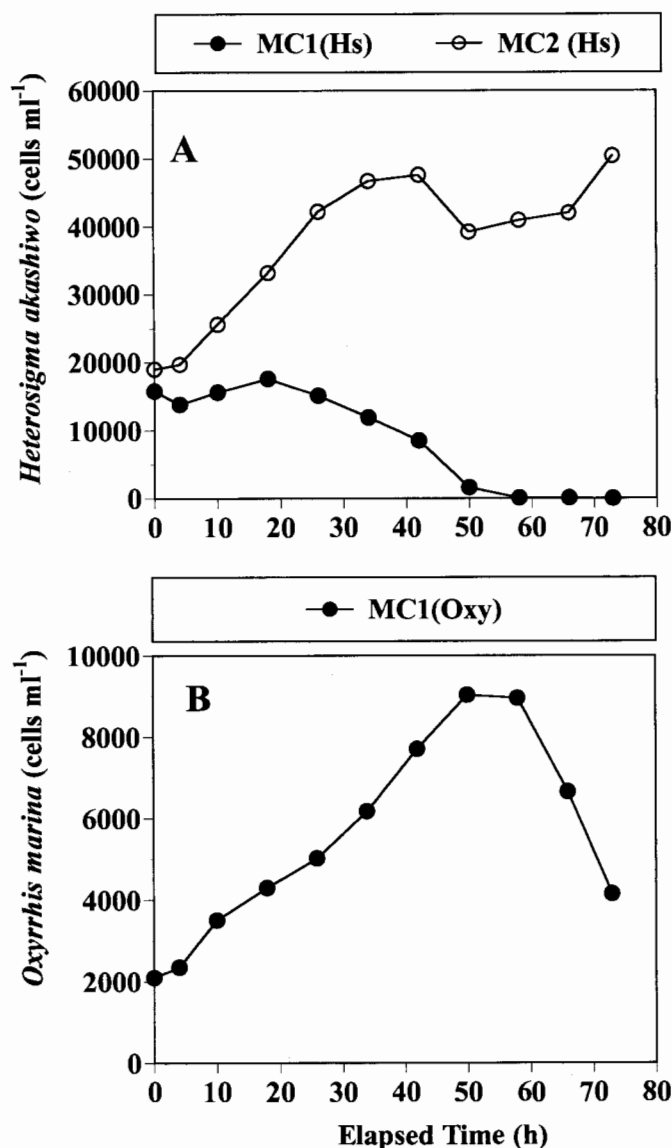


Fig. 4. Cell concentrations of *Heterosigma akashiwo* (A) and *Oxyrrhis marina* (B) as a function of elapsed incubation time in 100-L mesocosms set up in Masan Bay, Korea between 12:00 pm, June 6 ( $t = 0$  h) and 13:00 pm, June 9 ( $t = 73$  h), 2002 (Expt 2). One mesocosm (MC1) contained *H. akashiwo* and *O. marina*, while the other mesocosm (MC2) contained only *H. akashiwo* as control. *H. akashiwo* and *O. marina* were all originally cultured in the laboratory.

3, 19,400–22,800 cells ml<sup>-1</sup>, did not markedly change over the first 10 h, but continuously decreased between 10 and 66 h and down to 52 cell ml<sup>-1</sup> at 73 h (Fig. 5A). With increasing elapsed incubation time, the concentration of *H. akashiwo* in the control mesocosm (MC4) increased from 21,800 to 71,000 cells ml<sup>-1</sup>. The concentration of *O. marina* in MC3 increased from 2,915 to 8,250 cells ml<sup>-1</sup> between 0 and 50 h, but did not change between 50 and 66 h (Fig. 5B). There were no *O. marina* cells in the natural plankton assemblage in MC4.

Net growth and ingestion rates of *O. marina* on *H. akashiwo*, calculated with using Eq. (1)–(4) for the first 50 h, were 0.523 d<sup>-1</sup> and 0.33 ng C grazer<sup>-1</sup>d<sup>-1</sup> (3.3 cells grazer<sup>-1</sup>d<sup>-1</sup>), respectively. The mean prey concentration at this interval was 1,437 ng C ml<sup>-1</sup> (14,370 cells ml<sup>-1</sup>).

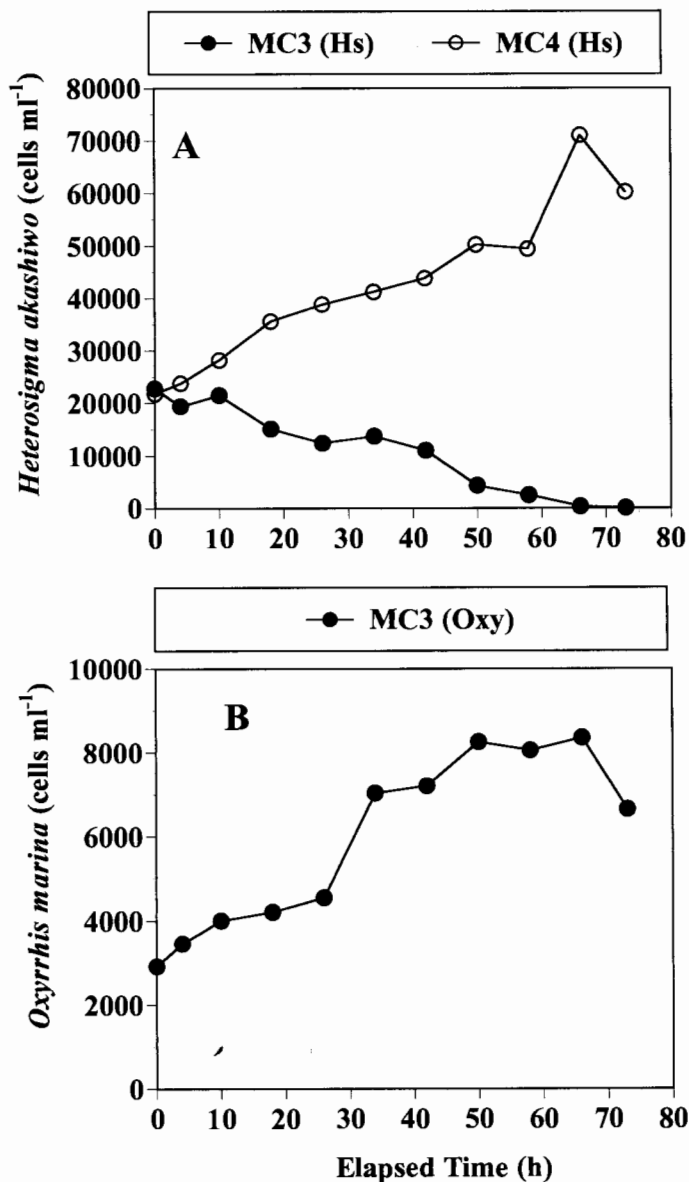


Fig. 5. Cell concentrations of *Heterosigma akashiwo* (A) and *Oxyrrhis marina* (B) as a function of elapsed incubation time in 100-L mesocosms set up in Masan Bay, Korea between 12:00 pm, June 6 ( $t = 0$  h) and 13:00 pm, June 9 ( $t = 73$  h), 2002 (Expt 3). One mesocosm (MC3) contained a natural population of *H. akashiwo* and *O. marina*, while the other mesocosm (MC4) contained a natural population of *H. akashiwo* only as control. *O. marina* was originally cultured in the laboratory.

**Difference in the concentrations of other protists and metazooplankton between the experimental and control mesocosms in Expt. 3.** With increasing elapsed incubation time, the concentrations of total diatoms in the experimental (MC3) and control (MC4) mesocosms in Expt. 3 showed a different pattern: the diatom concentration in the experimental mesocosm increased with fluctuation between 1,302 and 2,100 cells ml<sup>-1</sup>, whereas that in the control mesocosm (MC4) decreased from 867 to 459 cells ml<sup>-1</sup> (Fig. 6A). *Skeletonema costatum* was the dominant diatom species (> 90% of total diatoms).

With increasing elapsed incubation time, the concentrations of total phototrophic dinoflagellates (PTD) in MC3 and MC4

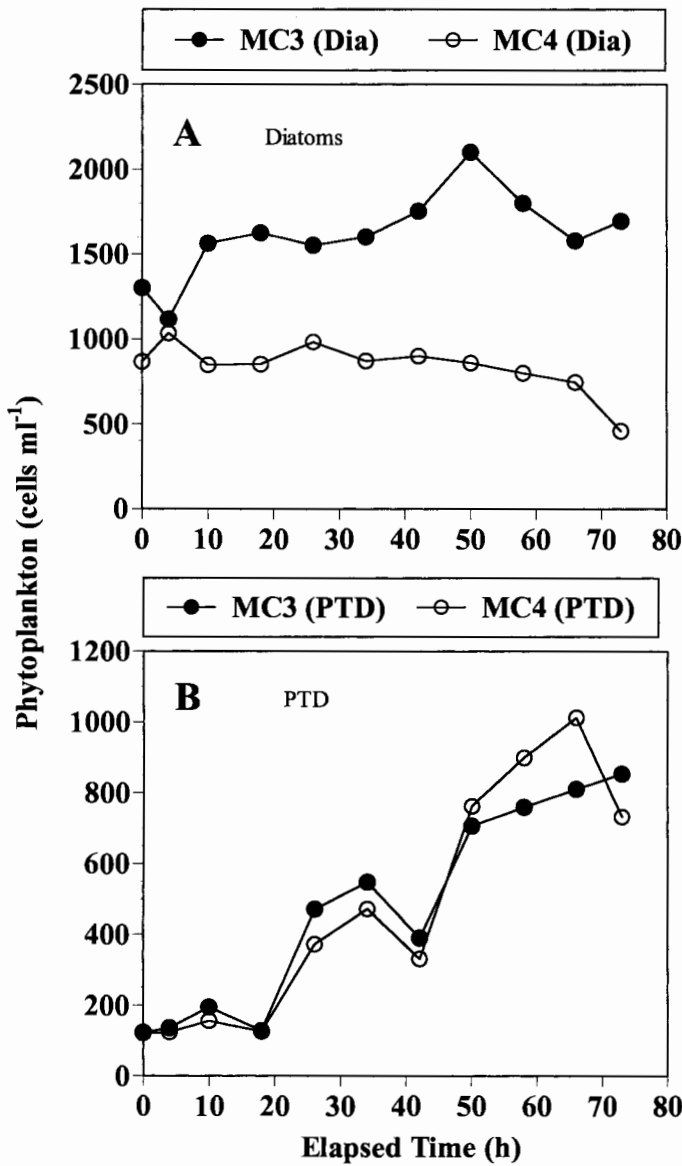


Fig. 6. Cell concentrations of total diatoms (A) and phototrophic dinoflagellates (PTD) (B) as a function of elapsed incubation time as in Fig. 5.

showed a similar pattern: the concentrations in MC3 and MC4 increased from 123 to 853 cells ml<sup>-1</sup> and from 121 to 1,012 cells ml<sup>-1</sup>, respectively (Fig. 6B). *Prorocentrum minimum* was the dominant dinoflagellate species (> 90% of total phototrophic dinoflagellates).

With increasing elapsed incubation time, the concentration of total heterotrophic dinoflagellates (HTD) excluding *O. marina* in MC3 fluctuated between 17 and 46 cells ml<sup>-1</sup>, and did not have a consistent pattern, while that in MC4 fluctuated between 15 and 67 cells ml<sup>-1</sup>, and generally increased (Fig. 7A). *Protoperidinium bipes* and *Oxyphysis oxytoxoides* were the most and second dominant heterotrophic dinoflagellate species.

With increasing elapsed incubation time, the concentrations of total ciliates in the MC3 and MC4 showed a similar pattern: the ciliate concentrations in MC3 and MC4 continuously increased from 45 to 119 cells ml<sup>-1</sup> and from 16 to 121 cells ml<sup>-1</sup>, respectively. The naked ciliate *Strombidium* sp. (40 × 20

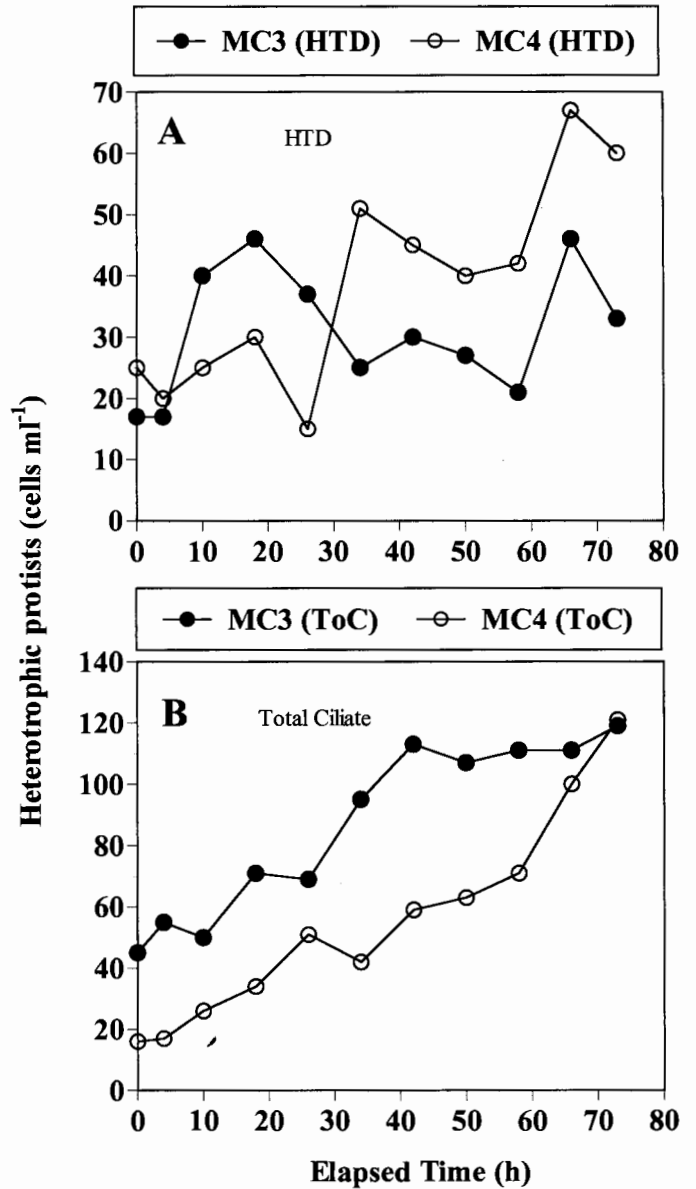


Fig. 7. Cell concentrations of total heterotrophic dinoflagellates (HTD) excluding *Oxyrrhis marina* (A) and ciliates (B) as a function of elapsed incubation time as in Fig. 5.

µm in size) and the tintinnid ciliate *Stenosemella nivalis* were the dominant species.

The abundances of total metazooplankton in MC3 and MC4 at the end of this experiment were 12.6 and 2.3 inds. l<sup>-1</sup>, respectively. Only the cladocera *Podon intermedius* and barnacle larvae were present.

**Physical properties in mesocosms in Experiments 2 & 3.** The water temperature and salinity inside the mesocosms prior to addition of cultured organisms were 23.5 °C and 28.7 psu. Water temperature in the experimental and control mesocosms in Expt. 2 (MC1 and MC2) and Expt. 3 (MC3 and MC4) fluctuated between 21.4 and 26.9 °C over a diurnal cycle. Salinity in the experimental and control mesocosms in Expt. 2 (MC1 and MC2) and Expt. 3 (MC3 and MC4) gradually decreased from 28.9 to 25.9 psu.

Table 1. Comparison of growth, ingestion, and clearance rates of *Oxyrrhis marina* on the different phytoplankton species at 20 °C.

Prey species	$\mu_{\max}^a$	$I_{\max}^b$	$C_{\max}^c$	Reference
<i>Heterosigma akashiwo</i>	1.43	1.25	0.3	This study
<i>Phaeodactylum tricornutum</i>	1.30	2.6	0.05	Goldman et al. (1989)
<i>Dunaliella tertiolecta</i>	0.79		0.34	Goldman et al. (1989)
<i>Isochrysis galbana</i>	0.79	7	0.36	Goldman et al. (1989)
<i>Amphidinium carterae</i>	1.17	2.8	2.4	Jeong et al. (2001b)
<i>Fibrocapsa japonica</i>	0.72			Tillmann & Reckermann (2002)

<sup>a</sup>  $\mu_{\max}$  (maximum growth rate as d<sup>-1</sup>).

<sup>b</sup>  $I_{\max}$  (maximum ingestion rate in ng C grazer<sup>-1</sup> d<sup>-1</sup>).

<sup>c</sup>  $C_{\max}$  (maximum clearance rate as  $\mu$ l grazer<sup>-1</sup> h<sup>-1</sup>).

## DISCUSSION

The present study shows that: (1) *Oxyrrhis marina* grew well on *Heterosigma akashiwo*, (2) *O. marina* was able within 3 d to reduce both natural and cultured populations of *H. akashiwo* down to a very low prey concentration in mesocosms set up in nature, and (3) introduction of cultured *O. marina* could cause a large change in the concentrations of diatoms and a small change in the concentrations of heterotrophic dinoflagellates excluding *O. marina* between the experimental and control mesocosms containing red-tide waters dominated by *H. akashiwo*. Phototrophic dinoflagellates and ciliates continuously increased in numbers.

**Growth, ingestion, and clearance rates of *Oxyrrhis marina* on cultured *Heterosigma akashiwo* in bottle incubation.** So far, only a few heterotrophic protists have been known to feed on *H. akashiwo* (Jeong et al. 2002a; Nakamura, Suzuki, and Hiromi 1995). *O. marina* readily ingests this prey and grows well when feeding on a strain of *H. akashiwo* tested in the present study. However, an ingestion rate of *O. marina* on a toxic clone of the prymnesiophyte *Chrysochromulina polylepis* was lower by 27% than that on a non-toxic clone (John, Tillmann, and Medlin 2002). Verity and Stoecker (1982) suggested harmful effects by *H. akashiwo* (*Olisthodiscus luteus*) on the growth of tintinnids. Therefore, it is worthwhile to test whether the growth and/or ingestion of *O. marina* on a toxic strain of *H. akashiwo* are reduced compared to those on a non-toxic strain.

The maximum growth rate of *O. marina* on *H. akashiwo* (1.43 d<sup>-1</sup>) is much higher than that for the prostomatid ciliate *Tiarina fusus* (0.12 d<sup>-1</sup>, Jeong et al. 2002a) or the heterotrophic dinoflagellate *Gyrodinium dominans* (0.15 d<sup>-1</sup>, Nakamura, Suzuki, and Hiromi 1995) feeding on *H. akashiwo* when corrected to 20 °C using a  $Q_{10}$  of 2.8 (Hansen, Bjornsen, and Hansen 1997). Therefore, *O. marina* could dominate the abundance of heterotrophic protists during red tides dominated by *H. akashiwo* if *O. marina* is originally present.

The maximum growth rate ( $\mu$ ) of *O. marina* on *H. akashiwo* (1.43 d<sup>-1</sup>) is higher than that of *O. marina* on the diatom *Phaeodactylum tricornutum* (1.30 d<sup>-1</sup>), *Amphidinium carterae* (1.17), *Dunaliella tertiolecta* (0.79), *Isochrysis galbana* (0.79), or *Fibrocapsa japonica* (0.72) (Goldman, Dennett, and Gordin 1989; Jeong et al. 2001b; Tillmann and Reckermann 2002) (Table 1). However, the maximum ingestion rate (1.25 ng C grazer<sup>-1</sup>d<sup>-1</sup>) of *O. marina* on *H. akashiwo* obtained in this study is lower than that of *O. marina* on *P. tricornutum* (2.6 ng C grazer<sup>-1</sup>d<sup>-1</sup>). Since the growth efficiency of *O. marina* on *H. akashiwo* is higher than that for *P. tricornutum*, *H. akashiwo* may be nutritionally better prey for *O. marina* than *P. tricornutum*. The C:N ratio of *H. akashiwo* in a log phase (5.1–7.5) is lower than that of *P. tricornutum* (8.6) (Goldman, Dennett, and Gordin 1989). Otherwise, the carbon content for *H. akashiwo* used in

the present study (0.1 ng C per cell) might be underestimated; the carbon contents for this species in literature were 0.12–0.43 ng C per cell (Smayda 1998).

**Growth, ingestion, and clearance rates of *Oxyrrhis marina* on *Heterosigma akashiwo* in mesocosms.** *O. marina* eliminated most prey cells in a short period and grew well in mesocosms when either cultured or natural populations of *H. akashiwo* were provided as prey. The net growth (0.523 d<sup>-1</sup>) rates of *O. marina* on a natural population of *H. akashiwo* in mesocosms (MC3) were lower than that on a culture population of the prey in mesocosms (MC1) (0.724 d<sup>-1</sup>), while the ingestion rates (0.33 ng C grazer<sup>-1</sup> d<sup>-1</sup>) of *O. marina* on a natural population of *H. akashiwo* were higher than that on a culture population of the prey (0.28 ng C grazer<sup>-1</sup> d<sup>-1</sup>) when the mean prey concentrations in MC3 and MC1 were 1,437 and 1,259 ng C ml<sup>-1</sup>, respectively. Predation by other zooplankters on *O. marina* in MC3 might lower this net growth rate of *O. marina* on the natural population of *H. akashiwo*. There have been no studies on the feeding by cladocerans on *O. marina*, but there is a possibility that the predators feed on the prey species because cladocerans have been known to feed on diverse diatoms and phototrophic and heterotrophic dinoflagellates of 4–115  $\mu$ m in size (Kim, Onbé, and Yoon 1989; Kim, Yoon, and Onbé 1993). However, the growth and ingestion rates of *O. marina* on a natural population of *H. akashiwo* in the mesocosms were 39 and 40%, respectively, of the values (1.33 d<sup>-1</sup> and 0.84 ng C grazer<sup>-1</sup> d<sup>-1</sup>, respectively), calculated by interpolating the rates obtained from the bottle incubation using Eq. (2) & (3) at the same mean prey concentrations. Fluctuations of temperature, salinity, or light intensity in the mesocosms might lower the growth and ingestion rates in the mesocosms compared to bottle incubation in which the environmental variables were kept constant in the laboratory. Biological factors such as bacteria and viruses and/or chemical factors such as possible heavy metals and pesticide compounds in Masan Bay might also reduce these rates.

**Difference in the concentrations of other protists between the experimental and control mesocosms in Experiment 3.** Introduction of cultured *Oxyrrhis marina* caused a large change in the concentrations of diatoms and a small change in the concentration of heterotrophic dinoflagellates excluding *O. marina* between the experimental and control mesocosms containing red tide waters dominated by *Heterosigma akashiwo*. Phototrophic dinoflagellates and ciliates continued to increase in numbers. A drastic increase in the concentration of *H. akashiwo* in the control mesocosm (MC4) might be partially responsible for the decrease in the concentration of total diatoms, but obviously did not affect the concentration of phototrophic dinoflagellates. In the natural environment, replacement of the harmful alga *H. akashiwo* by the non-harmful diatom *Skeleto-*

*nema costatum* after the introduction of cultured *O. marina* may be useful for some copepods: *H. akashiwo* has been known to be rejected by the copepod *Acartia omorii* (Uye and Takamatsu 1990) whereas *S. costatum* is easily ingested by several copepods, such as *Acartia tonsa*, *Eucalanus pileatus*, and *Temora stylifera* (Paffenhöfer and Knowles 1978; Reeve and Walter 1977). The concentrations of the dominant and second dominant heterotrophic dinoflagellates *Protoperidinium bipes* and *Oxyphysis oxytoxoides* in MC4 generally increased with fluctuations, while those in MC3 just fluctuated without a consistent pattern. Whether *P. bipes* or *O. oxytoxoides* is able to feed and grow on *H. akashiwo* has not been reported yet.

**Application of the Biological method of Controlling the outbreak and persistence of Red tides using mass-cultured heterotrophic protistan Grazers (BCRG method).** Developing methods of controlling the outbreak and persistence of red tides is an imperative task in many countries that suffer from great losses in the aquaculture and tourism industries every year. Only a few countries (e.g. Korea) use control methods (e.g. clay dispersal) for red-tide outbreaks (NFRDA 1998). It has been suggested that non-biological methods should be used in urgent situations (i.e. when red-tide patches almost reach aquaculture cages) due to possible secondary effects; in most non-biological methods some heterotrophic protists are killed at the concentrations of geo-chemical materials that kills red-tide organisms (Jeong et al. 2002b).

We built an automatic system that produces 300-L *Oxyrrhis marina* (concentration = 8,000–10,000 cells ml<sup>-1</sup>) per day using *Amphidinium carterae* or *Heterosigma akashiwo* as prey. MarineBio Co., (Kunsan, Korea) is building an automatic system for producing 10<sup>4</sup>-L *O. marina* per day. By using the results presented here and by making some assumptions, the time for 10<sup>4</sup>-L *O. marina* (concentration = 8,000 cells ml<sup>-1</sup>) to eliminate *H. akashiwo* cells in a red-tide patch of 100 m long, 10 m wide, and 1 m deep containing homogeneously distributed prey of 20,000 cells ml<sup>-1</sup> can be estimated. When *O. marina* is introduced into a part of a red-tide patch dominated by *H. akashiwo*, the grazer is expected to move to and attack other prey cells in adjacent parts of the patch after eliminating most prey cells in the original part. Therefore, unlike the mesocosms in which *O. marina* could not move to adjacent waters containing dense populations of *H. akashiwo* and thus the growth and ingestion rates of the grazer on the prey were reduced due to food limitation, the rates in the red-tide patch in the open system may not markedly change if the concentration of the prey in the adjacent parts increases or remains similar. The growth and ingestion rates of cultured *O. marina* on a natural population of *H. akashiwo* in MC3 were 39–40% of the calculated rates obtained from the bottle incubation at the same mean prey concentrations. Therefore, the growth and ingestion rates of cultured *O. marina* on natural populations of *H. akashiwo* with concentrations  $\geq 20,000$  cells ml<sup>-1</sup> could maintain approximately 40% of the maximum growth and ingestion rates obtained from the bottle incubation (0.57 d<sup>-1</sup> and 50 cells grazer<sup>-1</sup> d<sup>-1</sup>, respectively). If the growth rate of the prey is 0.23 d<sup>-1</sup> as obtained from the mesocosm experiment (Expt. 3), *O. marina* should be able to dissipate the red-tide patch within 9 d.

The results of the present study provide a possibility of controlling red tides using mass cultured grazers. However, open natural environments can be quite different from mesocosms in hydrographic, geochemical, and biological conditions, such as current, suspended material concentration, and the ratios of target prey and alternative prey. If higher trophic level predators do not consume the grazers of red-tide organisms, then decomposition of these starving grazers (i.e. accumulation of organic materials) may cause anoxic conditions. To use the BCRG

method effectively in open natural environments, small-scale trials should be attempted and contingent problems solved before mass-cultured grazers are used widely for control of large-scale red tides.

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