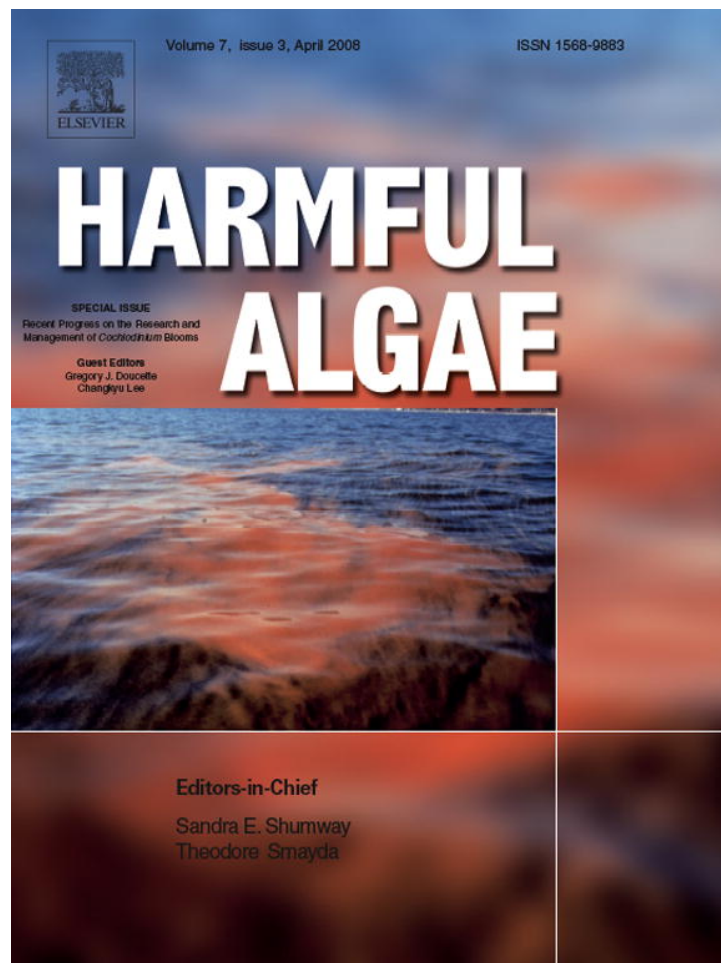


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## Control of the harmful alga *Cochlodinium polykrikoides* by the naked ciliate *Strombidinopsis jeokjo* in mesocosm enclosures

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### Abstract

Red tides dominated by the harmful dinoflagellate *Cochlodinium polykrikoides* have caused annual losses of USD \$5–60 million to the Korean aquaculture industry annually since 1995 and a loss of USD \$3 million during a 1999 net-pen fish mortality event in Canada. In order to evaluate the potential to control *C. polykrikoides* red tides dominated by using mass-cultured heterotrophic protistan grazers, we monitored the abundance of *Strombidinopsis jeokjo* (a naked ciliate) and *C. polykrikoides* after mass-cultured *S. jeokjo* was introduced into mesocosms (ca. 60 l) deployed in situ and containing natural red tide waters dominated by *C. polykrikoides*. Water temperature, salinity, and pH, as well as the abundance of co-occurring other protists and metazooplankton were measured concurrently. To compare the growth and ingestion rates of *S. jeokjo* feeding on cultured versus natural populations of *C. polykrikoides*, we also monitored the abundance of cultured *C. polykrikoides* and *S. jeokjo* in bottles during laboratory grazing experiments. *S. jeokjo* introduced into the mesocosms grew well, effectively reducing natural populations of *C. polykrikoides* from approximately 1000 cells ml<sup>-1</sup> to below 10 cells ml<sup>-1</sup> within 2 days. The growth and ingestion rates of cultured *S. jeokjo* on natural populations of *C. polykrikoides* in the mesocosms for the first 30 h (0.72 day<sup>-1</sup> and 51 ng C grazer<sup>-1</sup> day<sup>-1</sup>) were 84% and 44%, respectively, of those measured in the laboratory during bottle incubations with similar initial prey concentrations. The calculated grazing impact of *S. jeokjo* on natural populations of *C. polykrikoides* suggests that large-scale cultures of this ciliate could be used for controlling red tides by *C. polykrikoides* in small areas.

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**Keywords:** *Cochlodinium polykrikoides*; Growth; Harmful algal bloom; Ingestion; Management; Mitigation; Red tide

### 1. Introduction

Dense algal blooms, frequently referred to as red tides, have occurred in the waters off many countries (Jeong, 1995; Turner et al., 2000; Sordo et al., 2001; Alonso-Rodriguez and Ochoa, 2004; Seong et al., 2006). These events can upset the balance of food webs, cause large-scale mortalities of fin-fish and shellfish in natural environments (ECO HAB, 1995), and result in great losses to the aquaculture and tourist industries of many countries. Phototrophic dinoflagellates, heterotrophic dinoflagellates, phototrophic nanoflagellates, and diatoms have been

identified as causative organisms of red tides (Smayda, 1997; Parrow and Burkholder, 2003; Jeong et al., 2005b,c; Mason et al., 2007). In particular, phototrophic dinoflagellates are important red tide organisms in marine ecosystems due to their adverse impacts on other organisms (e.g., Tillmann and John, 2002).

Red tides dominated by the phototrophic dinoflagellate *Cochlodinium polykrikoides* caused losses of USD \$60 million in the Korean aquaculture industry in 1995 (NFRDI, 1998) and US \$10–20 million per year in 2000–2003. The death of net-pen fish in British Columbia, Canada during 1999 resulted in losses of USD \$3 million (Whyte et al., 2001). A large-scale abalone mortality in land-based, flow-through tanks in Wando, Korea during fall 2003 was traced to the supply of fresh seawater containing *C. polykrikoides*, with a cost to the industry of USD

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\$15 million. *C. polykrikoides* has been reported to kill fin-fish and shellfish by producing radical oxygen after clogging their gills (Kim et al., 1999). Blooms of *C. polykrikoides* have recently been observed in several other countries, including China (Huang and Dong, 2000), Russia (Vershinin et al., 2005), and Japan (summarized by Kim et al., 2004), and have also led to considerable losses for their aquaculture industries. Developing methods of controlling the outbreak and persistence of red tides dominated by *C. polykrikoides* and thereby reducing their economic impacts is thus an urgent task.

Methods of controlling the outbreak and persistence of red tides should not cause serious secondary effects on other marine organisms, yet be easily used in natural environments at a relatively low cost. Many potential control methods have been used or suggested, including the application of clay (NFRDI, 1998; Sengco and Anderson, 2004), NaOCl produced by the electrolysis of natural seawater (Jeong et al., 2002), protistan grazers (Jeong et al., 2003; Tillmann, 2004), algicidal bacteria (Imai et al., 1993; Doucette et al., 1999; Mayali and Azam, 2004), viruses (Nagasaki et al., 1999; Tarutani et al., 2000; Brussaard, 2004), and parasitic dinoflagellates (Coats, 1999; Park et al., 2004). Non-biological methods, such as the clay dispersal and NaOCl dispersal, have been used since 1996 for effectively controlling the persistence of red tides in Korea. However, these methods kill certain heterotrophic protists at the concentrations similar to those causing the death of red tide organisms (Jeong et al., 2002), which may, in turn, alter marine planktonic food webs and eventually impact natural fish communities. Therefore, methods that minimize secondary effects on other marine organisms need to be developed.

One of the factors contributing to the development of red tides is the inability of grazing pressure to suppress the growth of causative organisms (e.g., Watras et al., 1985). The introduction of mass-cultured effective grazers may increase grazing pressure and thus control the red tides. Heterotrophic protistan grazers cultured on a large-scale and introduced into water parcels containing red tide organisms may increase grazing pressure and effectively reduce natural population of these harmful species down to very low concentrations. Recently, *C. polykrikoides*, which had previously been known as exclusively autotrophic, was revealed to be a mixotrophic dinoflagellate (Jeong et al., 2004c) capable of feeding on diverse prey items, including the cyanobacterium *Synechococcus* (Jeong et al., 2005d), heterotrophic bacteria (Seong et al., 2006), haptophytes, raphidophytes, and other mixotrophic dinoflagellates (Jeong et al., 2004c). In Korean waters, red tides dominated by *C. polykrikoides* have occurred offshore where nitrate and phosphate concentrations were low (Jeong et al., 2000a; Yang et al., 2000). *C. polykrikoides* likely obtains carbon and nutrients by feeding on various prey species (i.e., mixotrophy) in these waters. However, among the heterotrophic protists tested thus far, only a few species have been reported to ingest *C. polykrikoides* (Jeong et al., 1999a,b, 2000b, 2005c, 2006). Therefore, *C. polykrikoides* may have a low mortality rate due to predation by heterotrophic protists in natural environments, leading to the formation of red tides if growth conditions are favorable.

In order to evaluate the potential to control *C. polykrikoides* red tides using mass-cultured heterotrophic protistan grazers, we (1) monitored the abundances of mass-cultured *Strombidinopsis jeokjo* (Ciliophora: Choreotrichida) and natural populations of *C. polykrikoides* in mesocosms set up in nature, following introduction of the grazers, and (2) simultaneously measured the abundances of diatoms, phototrophic dinoflagellates excluding *C. polykrikoides*, heterotrophic dinoflagellates, and ciliates excluding *S. jeokjo* in experimental (added *S. jeokjo* + natural red tide water) and control (natural red tide water only) mesocosms to investigate whether introduction of cultured grazers alters the natural populations of other protists. In addition, to comparing the growth and ingestion rates of *S. jeokjo* on cultured and natural populations of *C. polykrikoides*, we (3) measured the growth and grazing rates of cultured *S. jeokjo* on *C. polykrikoides* in bottles in the laboratory. Results of the present study provide a basis for potential use of mass-cultured *S. jeokjo* to control *C. polykrikoides* in natural environments or large land-based flow-through tanks along coastal areas and for assessing of the effects of introduced grazers on populations of other protists.

## 2. Materials and methods

### 2.1. Preparation of experimental organisms

*Cochlodinium polykrikoides* (CPTY02) was grown at 20 °C in enriched *f/2* seawater medium (Guillard and Ryther, 1962; salinity = 29.5–31.0 psu) without silicate under a continuous illumination of 50  $\mu\text{E m}^{-2} \text{s}^{-1}$  provided by cool white fluorescent lights. The carbon content for *C. polykrikoides* (0.7 ng C per cell) was estimated from the cell volume according to Strathmann (1967).

For the isolation and culture of *Strombidinopsis jeokjo* (Jeong et al., 2004a), plankton samples collected with a 40-cm diameter, 25- $\mu\text{m}$  mesh plankton net were taken from the mouth of the Mankyong Estuary, Kunsan, Korea, during July, 2003, when water temperature and salinity were 23.5 °C and 26 psu, respectively. Samples were screened gently through a 202- $\mu\text{m}$  Nitex mesh and placed in 1-l polycarbonate (PC) bottles. A mixture of *Prorocentrum micans* (ca. 1000 cells  $\text{ml}^{-1}$ ) and *Lingulodinium polyedrum* (ca. 500 cells  $\text{ml}^{-1}$ ) in 50 ml of *f/2* medium were added as food. The bottles were placed on plankton wheels rotating at 0.9 rpm and incubated at 20 °C under a 14 h:10 h light–dark cycle at 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 of *S. jeokjo* was established by two serial single-cell isolations. Once dense cultures of *S. jeokjo* were obtained, they were transferred to 500 or 1000-ml PC bottles (10- or 20-l bottles for mesocosm experiments) of fresh *P. micans* (ca. 1500 cells  $\text{ml}^{-1}$ ) or *L. polyedrum* prey (ca. 500 cells  $\text{ml}^{-1}$ ) every 2 or 3 days. Experiments for the bottle incubations were conducted when 2-l *S. jeokjo* culture was available. A total of 50 l of *S. jeokjo* (ca. 30 cells  $\text{ml}^{-1}$ ) were used for the mesocosm experiments. The mean ( $\pm$ standard error,  $n = 100$ ) length, width, and oral diameter of *S. jeokjo* used

in the present study were 130 ( $\pm 3$ ), 88 ( $\pm 1$ ), and 68 ( $\pm 1$ )  $\mu\text{m}$ , respectively.

### 2.2. Growth and ingestion rates of *Strombidinopsis jeokjo* on natural populations of *Cochlodinium polykrikoides* in mesocosms

Experiment (Expt) 1 was designed to measure the growth and ingestion rates of cultured *Strombidinopsis jeokjo* feeding on natural populations of *Cochlodinium polykrikoides* in mesocosms set up in coastal waters off Tongyoung, south-eastern Korea, in October 2003, coinciding with intense red tides dominated by *C. polykrikoides*.

The mesocosms used in these experiments were 50 cm in diameter and 50 cm in height. They were surrounded by waterproof cloth on the top and bottom and 15- $\mu\text{m}$  Nitex mesh in the middle. A circle of 40-cm diameter at the top of the mesocosms was open. Each 60-l mesocosm was supported by a stainless steel frame weighting ca. 8 kg and kept afloat by 4 buoys of 30 cm in diameter and 40 cm in length. In laboratory tests, filtered seawater (0.2- $\mu\text{m}$  CP filter; Chisso filter Co. Ltd., Japan) penetrated the mesh at a speed of ca. 60 l  $\text{min}^{-1}$  when the mesocosm was empty, yet *S. jeokjo* and *C. polykrikoides* did not pass through the mesh. After introducing cultures of *S. jeokjo* or subsampling (500 ml), seawater inside or outside the mesocosm set up in natural environments penetrated the mesh based on pressure differential. However, when the level of seawater inside and outside the mesocosms was equal, the exchange rate of seawater through the mesh was low. Six mesocosms (MC1, MC2, MC3, MC4, MC5, MC6; see below for details) were established simultaneously at the same location.

For Expt 1, red tide water containing *C. polykrikoides* (ca. 1500 cells  $\text{ml}^{-1}$ ) was poured into five mesocosms: three experimental mesocosms [MC1, MC2, MC3; mixtures of added *S. jeokjo* (density = ca. 30 cells  $\text{ml}^{-1}$ , 10 l) and natural plankton assemblage] and two prey control mesocosms (MC4, MC5; natural plankton assemblage only); one predator control mesocosm (MC6; cultured *S. jeokjo* only) was set up. The experiment started 2 h after adding the predator and prey to the mesocosm ( $t = 0$  h), when apparently stable conditions (water volume, abundance of plankton) had been established inside the mesocosms.

Five hundred-millilitre aliquots were taken from each mesocosm at 0, 6, 17, 24, 30, 41, 48, and 54 h, after the contents inside the mesocosms were gently but well mixed with a wide rod. Three 100-ml aliquots were fixed with 5% Lugol's solution, 5% Bouin's solution, and 4% glutaldehyde. The abundances of *C. polykrikoides* and *S. jeokjo* in the samples fixed with Lugol's solution were determined by counting all or  $>300$  cells in three 1-ml Sedgwick-Rafter counting chambers (SRCs). Dilution of the abundances associated with water penetrating into a mesocosm through the mesh during equilibration of water levels was considered in calculating growth and ingestion rates. Water temperature and salinity were also measured simultaneously (YSI model 600 XLM; YSI Inc., Yellow Springs, OH, USA).

The specific growth rate,  $\mu$  ( $\text{d}^{-1}$ ), of *S. jeokjo* was calculated by averaging the instantaneous growth rates (IGR) for each sampling interval ( $t_2 - t_1$ ) with weight for time due to different intervals, calculated as:

$$\mu = \sum \text{IGR} \times \frac{(t_2 - t_1)}{T} \quad (1)$$

where  $T = 30$  h during which rapid growth of the grazer occurred. IGR for each sampling interval ( $t_2 - t_1$ ) was calculated as:

$$\text{IGR} = \frac{\ln(S_{t_2}/S_{t_1})}{t_2 - t_1} \times 24 \quad (2)$$

where  $S_{t_1}$  and  $S_{t_2}$  = the concentration of *S. jeokjo* at consecutive samplings. Mean prey concentrations (cells  $\text{ml}^{-1}$ ) for 30 h were calculated by averaging the instantaneous mean prey concentrations for each sampling interval ( $t_2 - t_1$ ) with weight. An instantaneous mean prey concentration for each sampling interval was calculated using the equations of Frost (1972).

Ingestion rates were calculated by averaging the instantaneous ingestion rates for each sampling interval ( $t_2 - t_1$ ) with weight. Instantaneous ingestion rates were calculated using the equations of Frost (1972) and Heinbokel (1978). The incubation time for calculating ingestion rates was the same as for estimating growth rate.

Co-occurring diatoms, phototrophic dinoflagellates, and heterotrophic dinoflagellates in the aliquots fixed with Lugol's solution and glutaldehyde were counted under a compound microscope and an epifluorescent microscope. Naked ciliates and tintinnids in the samples fixed with Bouin's solution were counted using a quantitative protargol stain method (Montagnes and Lynn, 1993). When the mesocosm experiments were terminated, their entire contents of the mesocosms were filtered through a 100- $\mu\text{m}$  mesh net and any retained organisms were fixed with a final concentration 4% formalin. Metazooplankton were counted under a dissecting microscope.

### 2.3. Growth and ingestion rates of *Strombidinopsis jeokjo* on cultured *Cochlodinium polykrikoides* in bottles

Expt 2 was designed to measure the growth and ingestion rates of *Strombidinopsis jeokjo* on *Cochlodinium polykrikoides* at an initial prey concentration similar to that used in Expt 1 above (natural prey population experiment).

One day before this experiment was conducted dense cultures of *S. jeokjo* growing on *Prorocentrum micans* were transferred into 1-l PC bottles containing low concentrations of *C. polykrikoides* (c.a. 100 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 a rotating wheel to incubate, as above. To monitor the condition of, and interaction between, predator and prey species, bottles were periodically removed from the rotating wheel, examined under a dissecting microscope, and then replaced. Once *C. polykrikoides* cells were no longer detectable, three 1-ml aliquots from each bottle were counted using a compound microscope to determine cell

concentrations of *S. jeokjo* and the cultures were then used to conduct experiments.

The initial concentrations of *S. jeokjo* and *C. polykrikoides* 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. Triplicate control bottles containing only *S. jeokjo* were also established at one predator concentration. Thirty millilitres of *f/2* medium were added to all bottles, which were then filled to capacity with filtered seawater (0.2- $\mu\text{m}$  CP filter; Chisso Filter Co. Ltd., Japan) and capped. To establish the actual initial predator and prey densities (cells  $\text{ml}^{-1}$ ) at the beginning of the experiment (*S. jeokjo*/*C. polykrikoides* = 4.0/1250), a 10-ml aliquot was removed from each bottle, fixed with 5% Lugol's solution, and predator and prey abundance, determined by counting cells in five 1-ml SRCs under a compound microscope. 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 caused by refilling the bottles was taken into consideration in calculating growth and ingestion rates.

Ten-millilitre aliquots were taken from each bottle at 6, 18, 24, 30 h and fixed with 5% Lugol's solution, and the abundances of *S. jeokjo* and *C. polykrikoides* were determined by counting all or  $>300$  cells in seven 1-ml SRCs. Prior to taking subsamples, the condition of *S. jeokjo* and its prey were assessed using a dissecting microscope as described above. After subsampling, the bottles were again filled to capacity with freshly filtered seawater and placed back on the rotating wheels.

The specific growth and ingestion rates and mean prey concentration of *S. jeokjo* on *C. polykrikoides* were calculated as described above.

### 3. Results

#### 3.1. Physical properties in mesocosms

Mean water temperature and salinity inside the mesocosms prior to addition of cultured grazers were 22.1 °C and 29.8 psu, respectively. Water temperature in the experimental and control mesocosms fluctuated between 20.5 and 21.8 °C over a diurnal cycle. Salinity in the experimental and control mesocosms ranged from 29.8 to 31.7 psu. The pH in the experimental and control mesocosms did not change markedly (7.5–7.8). Water temperature and salinity outside the mesocosms were similar to those inside the mesocosms.

#### 3.2. Growth and ingestion rates of *Strombidinopsis jeokjo* on natural populations of *Cochlodinium polykrikoides* in the mesocosms

The mean concentration of *Cochlodinium polykrikoides* in the experimental mesocosms (MC1, MC2, MC3) in Expt 1, decreased relatively slowly from 1030 to 770 cells  $\text{ml}^{-1}$  between 0 and 24 h, but rapidly from 770 to 32 cells  $\text{ml}^{-1}$  between 24 and 41 h, and down to 8 cells  $\text{ml}^{-1}$  at 48 h

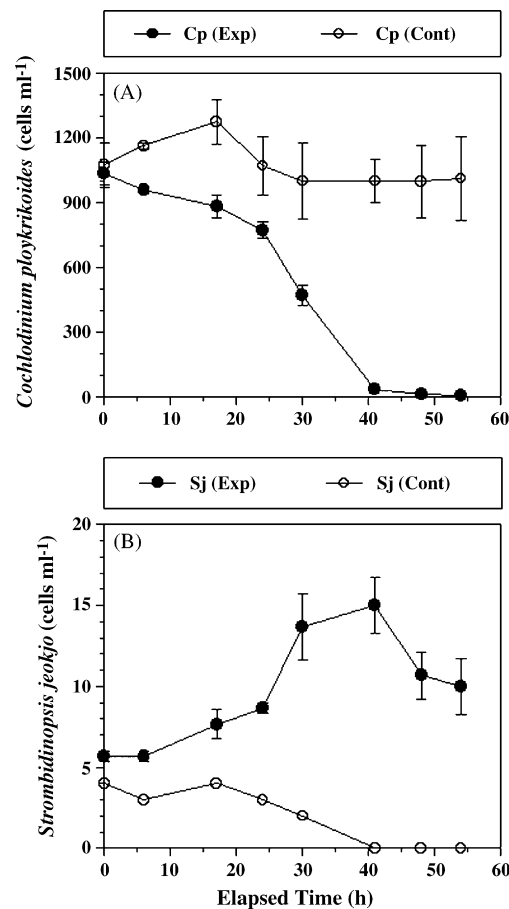


Fig. 1. Cell concentrations of *Cochlodinium polykrikoides* (Cp) (A) and *Strombidinopsis jeokjo* (Sj) (B) as a function of elapsed incubation time in 60-l mesocosms set up in coastal water off Tongyoung, Korea between 14:00 pm, October 1 ( $t = 0$  h) and 20:00 pm, October 3 ( $t = 54$  h), 2003 (Expt 1). Symbols represent treatment means  $\pm$  standard error. Three experimental (Exp) mesocosms (MC1, 2, 3 – closed circles) contained natural populations of *C. polykrikoides* and *S. jeokjo*, while two other mesocosms (MC4, 5 – open circles) contained only *C. polykrikoides* and another mesocosm (MC6 – open circles) only *S. jeokjo* as controls (Cont). *S. jeokjo* was originally cultured in the laboratory. Prey and grazer concentrations immediately after subsampling (500 ml aliquots) were slightly lower than before removal of the sample due to the dilution by ambient seawater penetrating into the mesocosms during volume re-equilibration.

(Fig. 1A). By comparison, the mean concentration of *C. polykrikoides* in control mesocosms (MC4, MC5; *C. polykrikoides* only) increased slightly from 1070 to 1270 cells  $\text{ml}^{-1}$  between 0 and 17 h, but declined slightly from 1270 to 1010 cells  $\text{ml}^{-1}$  between 17 and 54 h. The mean concentration of *Strombidinopsis jeokjo* in the experimental mesocosms increased from 5.7 to 15.0 cells  $\text{ml}^{-1}$  between 0 and 41 h, but decreased to 10.0 cells  $\text{ml}^{-1}$  by 54 h, probably due to food limitation (Fig. 1B). In contrast, the mean concentration of *S. jeokjo* in the control mesocosm (*S. jeokjo* only) decreased from 4 to 2 cells  $\text{ml}^{-1}$  between 0 and 30 h, whereas no *S. jeokjo* cells were detected at 41 h.

Net growth and ingestion rates of *S. jeokjo* on *C. polykrikoides* calculated by using Eqs. (1) and (2) for the first 30 h, were 0.715  $\text{day}^{-1}$  and 51 ng C grazer $^{-1}$   $\text{day}^{-1}$  (72 cells grazer $^{-1}$   $\text{day}^{-1}$ ), respectively. The mean *C. polykri-*

*koides* concentration over this interval was 844 ng C ml<sup>-1</sup> (591 cells ml<sup>-1</sup>). However, net growth and ingestion rates of *S. jeokjo* on *C. polykrikoides*, calculated between 6 and 30 h in which exponential growth of the grazer occurred, were 0.886 day<sup>-1</sup> and 43 ng C grazer<sup>-1</sup> day<sup>-1</sup> (62 cells grazer<sup>-1</sup> day<sup>-1</sup>), respectively. The mean *C. polykrikoides* concentration at this interval was 808 ng C ml<sup>-1</sup> (565 cells ml<sup>-1</sup>).

### 3.3. Difference in the concentrations of other protists and metazooplankton between the experimental and control mesocosms

The mean concentrations of total diatoms in the experimental (MC1, MC2, MC3) and control (MC4, MC5) mesocosms showed a similar growth pattern for the first 17 h, but quite different growth patterns later during the incubations: the mean diatom concentrations in the experimental and control mesocosms increased from 6240 to 13,530 cells ml<sup>-1</sup> (net growth rate = 1.10 day<sup>-1</sup>) and from 7630 to 15,070 cells ml<sup>-1</sup> (0.96 day<sup>-1</sup>) between 0 and 17 h, respectively; however, for the last 37 h, mean diatom concentrations in the experimental mesocosms increased from 13,550 to 50,220 cells ml<sup>-1</sup> (net growth rate = 0.85 day<sup>-1</sup>), whereas those for control mesocosms increased from 15,070 to

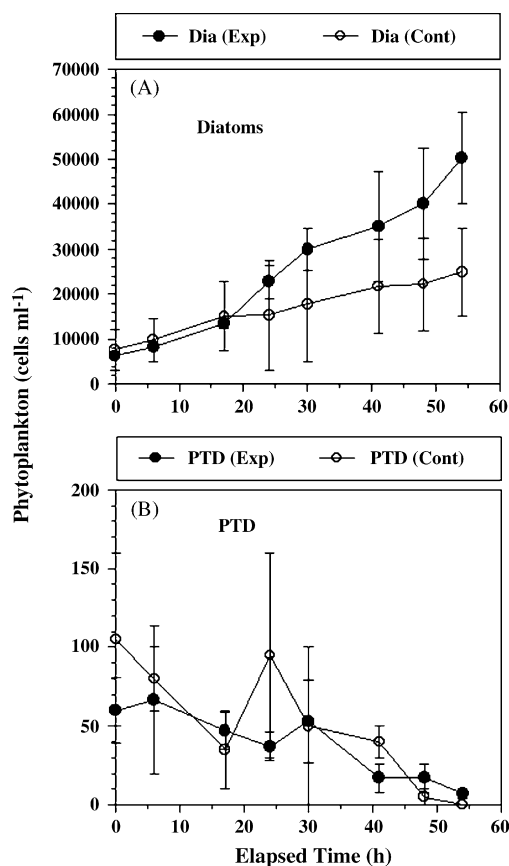


Fig. 2. Cell concentrations of total diatoms (Dia) (A) and phototrophic dinoflagellates excluding *C. polykrikoides* (PTD) (B) as a function of elapsed incubation time as in Fig. 1.

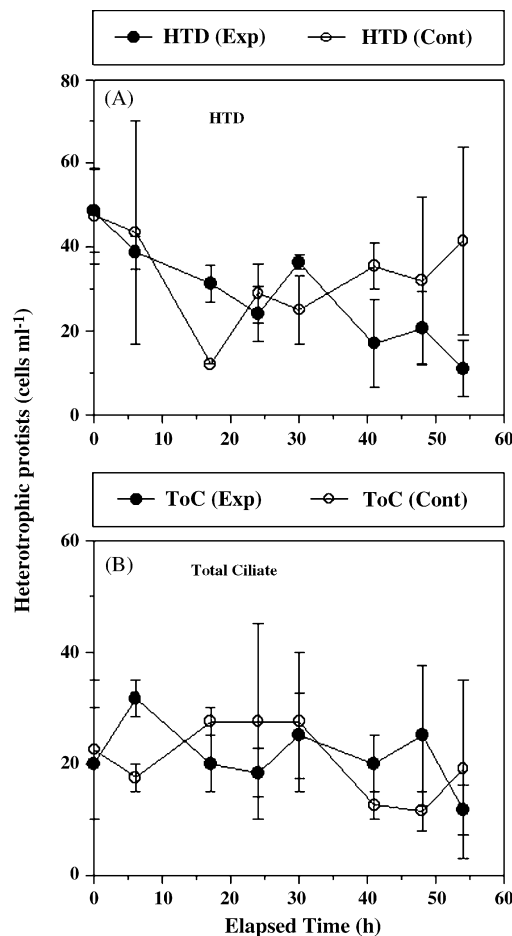


Fig. 3. Cell concentrations of total heterotrophic dinoflagellates (HTD) (A) and the ciliates excluding *Strombidinopsis* (B) as a function of elapsed incubation time as in Fig. 1.

24,985 cells ml<sup>-1</sup> (0.33 day<sup>-1</sup>; Fig. 2A). *Skeletonema costatum* was the dominant diatom species (>80% of total diatoms).

The mean concentrations of total phototrophic dinoflagellates (PTD) excluding *Cochlodinium polykrikoides* in the experimental (MC 1, MC2, MC3) and control (MC4, MC5) mesocosms showed a similar pattern throughout the incubations: concentrations in experimental and control mesocosms decreased from 60 to 7 cells ml<sup>-1</sup> and from 105 to 1 cells ml<sup>-1</sup>, respectively (Fig. 2B). *Prorocentrum minimum* was the dominant dinoflagellate species (>80% of total phototrophic dinoflagellates).

The mean concentrations of total heterotrophic dinoflagellates (HTD) in the experimental (MC1, MC2, MC3) and control (MC4, MC5) mesocosms showed a different pattern: mean concentration in experimental mesocosms generally decreased from 49 to 11 cells ml<sup>-1</sup>, while that in the control mesocosms declined from 48 to 12 cells ml<sup>-1</sup>, between 0 and 17 h, but increased from 12 to 42 cells ml<sup>-1</sup> for last 37 h (Fig. 3A). *Gyrodinium* spp. were the most dominant heterotrophic dinoflagellate species.

The mean concentrations of total ciliates in the experimental (MC1, MC2, MC3) and control (MC4, MC5) mesocosms, excluding *Strombidinopsis jeokjo*, fluctuated between 32 and

12 cells ml<sup>-1</sup> and between 28 and 12 cells ml<sup>-1</sup>, respectively (Fig. 3B). The tintinnid ciliate *Tintinnopsis beroidea* was the dominant species.

The mean abundances of total metazooplankton in the experimental, *Cochlodinium* only control, and *Strombidinopsis* only control mesocosms at the end of Expt 1 were 0.53, 0.33, and 0.43 indiv. l<sup>-1</sup>, respectively. The copepod *Acartia omorii*, the cladoceran *Evadne nordmanii*, and barnacle larvae were the most dominant metazooplankton.

#### 3.4. Growth and ingestion rates of *Strombidinopsis jeokjo* on cultured *Cochlodinium polykrikoides* in bottles

The mean concentration of *Cochlodinium polykrikoides* in the experimental bottles in Expt 2, decreased continuously from 1250 down to 9 cells ml<sup>-1</sup> at 30 h (Fig. 4A) and mean concentrations in control bottles also declined continuously from 1210 to 850 cells ml<sup>-1</sup> over the same interval (Fig. 4A). Half of the decrease in cell levels was due to dilution after subsampling. The mean concentration of *Strombidinopsis*

*jeokjo* in the experimental bottles increased from 4.0 to 10.8 cells ml<sup>-1</sup> between 0 and 24 h, but decreased to 10.0 cells ml<sup>-1</sup> at 30 h (Fig. 4B). In the control bottles (*S. jeokjo* only), mean concentrations of *S. jeokjo* decreased from 7.0 to 0.1 cells ml<sup>-1</sup> between 0 and 25 h; however, no *S. jeokjo* cells were detected at 30 h.

Net growth and ingestion rates of *S. jeokjo* on *C. polykrikoides*, calculated by using Eqs. (1) and (2) for the first 30 h, were 0.855 day<sup>-1</sup> and 116 ng C grazer<sup>-1</sup> day<sup>-1</sup> (166 cells grazer<sup>-1</sup> day<sup>-1</sup>), respectively. The mean *C. polykrikoides* concentration during this interval was 302 ng C ml<sup>-1</sup> (432 cells ml<sup>-1</sup>). However, net growth and ingestion rates of *S. jeokjo* on *C. polykrikoides*, calculated between 6 and 24 h (i.e., period of exponential grazer growth), were 1.782 day<sup>-1</sup> and 127 ng C grazer<sup>-1</sup> day<sup>-1</sup> (181 cells grazer<sup>-1</sup> day<sup>-1</sup>), respectively. The mean *C. polykrikoides* concentration over this interval was 255 ng C ml<sup>-1</sup> (365 cells ml<sup>-1</sup>).

## 4. Discussion

#### 4.1. Growth and ingestion rates of *Strombidinopsis jeokjo* on *Cochlodinium polykrikoides* in laboratory bottle incubations and mesocosms in nature

*S. jeokjo* eliminated most *C. polykrikoides* cells in a short period and grew well both in laboratory bottle incubations and in mesocosms containing red tide water where *C. polykrikoides* dominated the protistan assemblages. Besides large *Strombidinopsis* spp., the heterotrophic dinoflagellates *Noctiluca scintillans* and *Pfiesteria piscicida* are known to feed on *C. polykrikoides* (Jeong et al., 2000b, 2006). However, *N. scintillans* may be able to capture and ingest only unhealthy prey cells during the declining stages of red tides dominated by *C. polykrikoides* because its swimming speed is much lower than that of *C. polykrikoides* (Jeong et al., 1999a). The maximum ingestion rate of *P. piscicida* on *C. polykrikoides* is very low (0.03 ng C predator<sup>-1</sup> day<sup>-1</sup>; Jeong et al., 2006). Also, many mixotrophic dinoflagellates, heterotrophic dinoflagellates, and ciliates tested thus far were not able to feed on *C. polykrikoides* (Jeong et al., 2005c). Therefore, the large *Strombidinopsis* spp. is a strong candidate to control red tides dominated by *C. polykrikoides* by the approach of introducing mass-cultured grazers to the red tide waters in natural environments or large land-based flow-through tanks.

The specific growth rate of *S. jeokjo* on a diet of cultured *C. polykrikoides* for the first 30 h herein (0.855 day<sup>-1</sup>) was similar to that (0.850 day<sup>-1</sup>) of *Strombidinopsis* sp., calculated by interpolating the rates obtained by Jeong et al. (1999b) at the same mean prey concentrations; whereas, the ingestion rate of *S. jeokjo* used in the present study (116 ng C grazer<sup>-1</sup> day<sup>-1</sup>) was lower by 50% (230 ng C grazer<sup>-1</sup> day<sup>-1</sup>) than that employed by Jeong et al. (1999b). The mean length, width, and oral diameter of *S. jeokjo* (130, 88, and 68 μm, respectively) were much smaller than for *Strombidinopsis* sp. (198, 100, and 74 μm, respectively), which may be responsible for this lower ingestion rate compared to the latter species, even though they belong to the same genus.

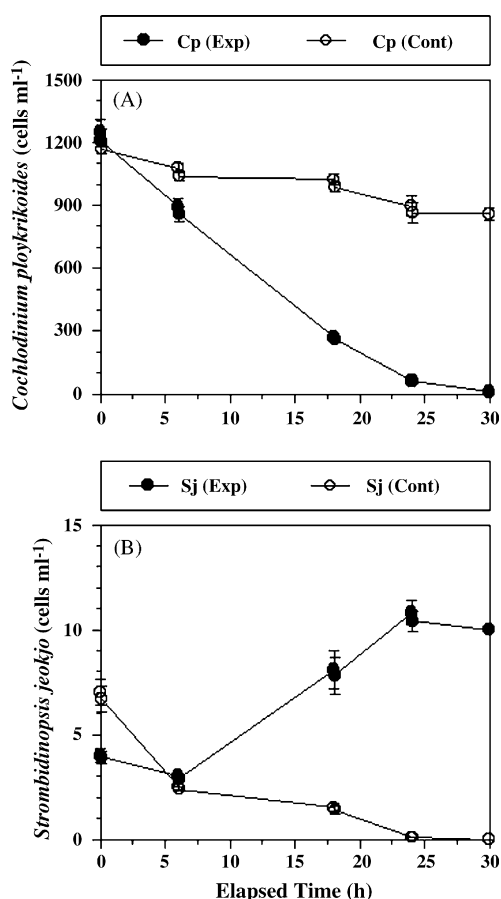


Fig. 4. Cell concentrations of *Cochlodinium polykrikoides* (Cp) (A) and *Strombidinopsis jeokjo* (Sj) (B) as a function of elapsed incubation time in bottle incubations (Expt 2). Symbols represent treatment means  $\pm$  standard error. *C. polykrikoides* and *S. jeokjo* were originally cultured in the laboratory. The prey and grazer concentrations immediately after subsampling (10 ml aliquots) were slightly lower than before removal of the sample due to the dilution of the cultures by refilling the bottles. Direct adjacent symbols at each time point representing pre- and post-subsampling values.

Net growth and ingestion rates of *S. jeokjo* on a natural *C. polykrikoides* population in the mesocosms calculated for the first 30 h ( $0.715 \text{ day}^{-1}$  and  $51 \text{ ng C grazer}^{-1} \text{ day}^{-1}$ , respectively) were 84% and 44%, respectively, of the bottle incubation values ( $0.855 \text{ day}^{-1}$  and  $116 \text{ ng C grazer}^{-1} \text{ day}^{-1}$ , respectively) at a similar initial prey concentration. Temperature or salinity fluctuations and/or irregularly disturbed water in the mesocosms might have lowered the growth and ingestion rates in the mesocosms compared to bottle incubations under constant laboratory conditions. Biological components of the natural red tide water (e.g., bacteria, viruses) might also have reduced these rates. Moreover, growth of *S. jeokjo* in the mesocosms might have benefited from other prey in addition to *C. polykrikoides* compared to bottle incubations containing a single prey species (i.e., *C. polykrikoides*). The higher ratio for net growth rate of *S. jeokjo* feeding on a natural *C. polykrikoides* population in the mesocosms versus bottle incubations (84%), compared to the ratio for ingestion rate (44%), is consistent with this idea. In addition, the mean concentration of heterotrophic dinoflagellates in the experimental mesocosms declined considerably more than in the control mesocosms (from 49 to 11 cells  $\text{ml}^{-1}$  versus from 48 to 42 cells  $\text{ml}^{-1}$ , respectively; Fig. 4A). *S. jeokjo* is known to feed on heterotrophic dinoflagellates such as *Gyrodinium dominans*, *Oxyrrhis marina*, *Pfiesteria piscicida*, and *Stoeckeria algicida* in the laboratory (Jeong et al., 2004b, 2007b). The ratio of net growth rate of *S. jeokjo* in mesocosms containing a natural *C. polykrikoides* population to the values for bottle incubations (84%) was much higher than that of *O. marina* feeding on *Heterosigma akashiwo* (39%), whereas the ratios of ingestion rates for *S. jeokjo* and *O. marina* in these same experiments were similar (44% and 40%, respectively; Jeong et al., 2003). These results suggest that the much larger *S. jeokjo* (ca.  $250,000 \mu\text{m}^3$ ) might have had more alternative prey available in mesocosms than *O. marina* ( $2000 \mu\text{m}^3$ ). When introducing mass-cultured *S. jeokjo* into red tide waters for biological control, the presence of alternative prey species will be an important consideration.

#### 4.2. Difference in the concentrations of other protists between the experimental and control mesocosms

Introduction of cultured *S. jeokjo* caused a large increase in diatom concentrations after only 17 h incubation compared with smaller fluctuations in heterotrophic dinoflagellates over 41 h and essentially no change in either phototrophic dinoflagellates (excluding *Cochlodinium polykrikoides*) or ciliates (excluding *S. jeokjo*) in experimental versus control mesocosms containing *C. polykrikoides* red tide waters. Net growth rate of total diatoms in experimental mesocosms ( $0.85 \text{ day}^{-1}$ ) greatly exceeded that in the control mesocosms ( $0.33 \text{ day}^{-1}$ ) between 17 and 54 h, coinciding with a rapid decline in *C. polykrikoides* concentration due to predation by *S. jeokjo*. There are several possible explanations for this disparity diatom growth (mostly *Skeletonema costatum*) between experimental (MC1, 2, 3) and control mesocosms (MC4, 5): (1) *C. polykrikoides* in control mesocosms may have ingested diatoms, as has been observed recently in

laboratory experiments involving *S. costatum* cells (Yoo et al., unpubl. data); (2) *C. polykrikoides* might have had an allelopathic effect on diatom growth. Although this interaction remains speculative, the relationship between *C. polykrikoides* and diatoms should be examined, given that field observations have shown reversed horizontal distributions of *C. polykrikoides* and *S. costatum*. Also, Honjo (1993) has reported that the red tide alga *Heterosigma akashiwo* adversely affects the growth of *S. costatum*; and (3) the nutrients regenerated by *S. jeokjo* feeding on *C. polykrikoides* could support diatom growth. Cultures of fresh *P. micans* or *L. polyedrum* prey were added to *S. jeokjo* cultures when scaling up to 10-l volumes (approx. 1/10 (v/v) dilution) and these *S. jeokjo* cultures were introduced into mesocosms containing 60 l of seawater. If the *P. micans* or *L. polyedrum* cultures contained 100% of *f/2* medium (likely an overestimate), nutrient concentrations inside the mesocosms after addition of the *S. jeokjo* cultures may have approximated *f/120* medium. Therefore, the possibility that diluted *f* medium carried over into *S. jeokjo* cultures enhanced nutrient levels and thus diatom growth in experimental mesocosms cannot be discounted. Algal blooms dominated by *S. costatum* have not been reported to cause fish mortality, while *C. polykrikoides* red tides kill caged fish in the sea within 2 h (NFRDI, 1998). In the natural environment, replacement of the harmful alga *C. polykrikoides* by the non-harmful diatom *S. costatum* after introducing cultured *S. jeokjo* may increase the survival of caged fish in coastal waters.

The final mean abundances of total metazooplankton in the experimental, *C. polykrikoides* only control, and *S. jeokjo* only control mesocosms were as low as 0.33–0.53 indiv.  $\text{l}^{-1}$ . These values are common during *C. polykrikoides*-dominated red tides in the coastal waters off southern Korea (Jeong et al., unpubl. data). Grazing coefficients of the copepod *Acartia omorii* ( $0.53 \text{ indiv. l}^{-1}$ ) on *C. polykrikoides* ( $1000 \text{ cells ml}^{-1}$ ) and *S. jeokjo* ( $5 \text{ cells ml}^{-1}$ ) calculated according to Kim and Jeong (2004), and ingestion rate data for *A. omorii* feeding on *C. polykrikoides* (Kim, 2005) and *S. jeokjo* (Jeong et al., unpubl. data) are only 0.001 and  $0.013 \text{ day}^{-1}$ , respectively. Therefore, the effect of metazooplankton on *C. polykrikoides* or *S. jeokjo* populations was likely negligible.

#### 4.3. Biological control of *Cochlodinium polykrikoides* dominated red tides using mass-cultured *Strombidinopsis jeokjo*

Biological control of the outbreak and persistence of red tides using mass-cultured heterotrophic protistan grazers, if developed further, may have significant merits: (1) heterotrophic protistan grazers are isolated originally from natural seawater in areas where red tides occur and are consumed effectively by metazooplankton after ingesting the harmful algae (e.g., Stoecker and Egloff, 1987). Therefore, introduction of such grazers may be considered much safer than clay materials, NaOCl, or other chemicals; (2) heterotrophic protistan grazers grow rapidly when ingesting red tide organisms (Stoecker et al., 1981; Hansen, 1992; Jeong, 1999; Jeong and Latz, 1994; Jeong et al., 2004d, 2005a, 2007a; Kamiyama and Arima, 2001;

Tillmann, 2004). These grazers would thus be expected to reproduce quickly following their introduction into red tide waters, enabling them to dissipate large-scale red tide patches even at relatively low initial concentrations.

We have constructed an automated system producing 300 l day<sup>-1</sup> of *S. jeokjo* (30–40 cells ml<sup>-1</sup>) using *P. micans*, *L. polyedrum*, or *C. polykrikoides* as prey. Based on the results presented herein as well as several assumptions, the time for 10<sup>4</sup> l of *S. jeokjo* (30 cells ml<sup>-1</sup>) to eliminate *C. polykrikoides* cells in a red tide patch 100 m long, 10 m wide, and 1 m deep containing homogeneously distributed prey at a concentration of 1000 cells ml<sup>-1</sup> can be estimated. After *S. jeokjo* is introduced at a given location within a red tide patch, the grazer would be expected to move into adjacent areas of the patch as prey cells are eliminated. Therefore, unlike mesocosms that restricted movement of *S. jeokjo* from low to higher *C. polykrikoides* densities while feeding and consequently reduced its growth and ingestion rates due to food limitation, these rates may decline little in open, natural red tide patches if prey concentrations increase or remain similar throughout the patch. Growth and ingestion rates of cultured *S. jeokjo* feeding on natural *C. polykrikoides* populations at 1000 cells ml<sup>-1</sup> could thus be maintained at levels approximating 0.715 day<sup>-1</sup> and 51 ng C grazer<sup>-1</sup> day<sup>-1</sup> (72 cells grazer<sup>-1</sup> day<sup>-1</sup>), respectively, as measured in this study. If the red tide prey population does not grow, as in the control mesocosm, *S. jeokjo* should be able to dissipate the red tide patch within 6 days; however, if *C. polykrikoides* reproduces at its maximum growth rate observed in laboratory cultures ( $\mu = 0.4$  day<sup>-1</sup>; Jeong et al., unpubl. data), dissipation of the patch may require ~14 days. In Korean waters, red tides dominated by *C. polykrikoides* usually occur offshore near Koheung and then move onshore toward aquaculture farms (Jeong et al., 2000a). In most years, at least 2–3 weeks following the initial sighting of a bloom population are required for *C. polykrikoides* red tides to reach aquaculture farms and cause fish kills.

Results of the present study provide a possible means of controlling red tides using mass-cultured protistan grazers. Large-scale cultures of *S. jeokjo* may be useful for eliminating *C. polykrikoides* cells in fresh seawater supplied to large land-based, flow-through tanks in abalone aquaculture farms, where losses of USD \$15 million due to *C. polykrikoides* occurred in 2003. However, open natural environments are quite different from tanks, as well as the mesocosms employed herein, in their hydrographic, geochemical, and biological properties, including current, suspended material concentration, and the ratios of target prey and alternative prey. Small-scale trials are thus required evaluate the effectiveness of this method in open natural environments and any associated problems resolved before introduction of mass-cultured grazers can be considered for control of larger-scale red tides.

## 5. Conclusions

The present study showed that: (1) a large naked ciliate, *Strombidinopsis jeokjo*, reduced natural populations of *C.*

*polykrikoides* down to a very low prey concentrations within 2 days in mesocosms set up in nature; (2) introduction of cultured *S. jeokjo* caused a change in total diatom and heterotrophic dinoflagellate concentrations in experimental versus control mesocosms containing *C. polykrikoides* red tide waters, but had little effect on concentrations of phototrophic dinoflagellates excluding *C. polykrikoides* or ciliates excluding *S. jeokjo*; and (3) *S. jeokjo* cultured on a large-scale has the potential to control *C. polykrikoides* red tides near aquaculture farms, located in small ponds, lagoons, semi-enclosed bays, and large land-based, flow-through tanks receiving a constant supply of fresh seawater.

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